This book is a consolidation of the exhaustive information on recent applications of Biotechnology and life science in the areas of Disease Diagnosis & Therapeutics, Phytoremediation, Plant science and omics based approaches. As this book covers topics from various sections of Biotechnology and life science, hence it will be proven as handy resource for a broad range of researches, students, and biotech professionals from both academia and industry, hailing from different backgrounds.
ADVANCES IN
BIOTECHNOLOGY
AND
LIFE SCIENCES

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Co-Editors: Prof Sudha Srivastava, Dr Shalini Mani
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Biotechnology is a broad discipline in which biological processes, organisms, cells or cellular components are exploited to develop new technologies or products. Beyond academics research in microorganisms, plants and animals, the studies in Biotechnology has reached to different applications related to pharmaceutics, disease diagnosis, therapeutics, phytoremediation, medicinal herbs and computational biology as well. Hence, it is worth analyzing and summarizing the impact of related academic and industrial research and developments in different branches of Biotechnology and life sciences, to directly benefit the humanity. Based upon the similar objectives, this book is an amalgamation of the detailed knowledge on recent applications of Biotechnological and life sciences in the areas of Disease Diagnosis & Therapeutics, Phytoremediation, Plant science and Computational approaches. As this book covers topics from various sections of Biotechnology and life sciences, hence it will be proven as handy resource for a broad range of researchers, students, and biotech professionals from both academia and industry, hailing from different backgrounds.

The editors are thankful to Hon’ble Vice Chancellor, Jaypee Institute of Information Technology, Noida for his constant support and guidance. Editors are also thankful to all the authors for their timely and quality contribution for the successful publication of this book.

Finally, the editors solicit suggestions for improvement of this book from the researchers, academicians and any other person, who may be interested in promoting this discipline.

Jaypee Institute of Information Technology, Noida

Pammi Gauba

21st Oct, 2020

Sudha Srivastava

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CHAPTER-1

Applications of Machine learning techniques in predicting the early detection of psychiatric disorders

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ABSTRACT: Schizophrenia, bipolar disorder, and major depression disorder are among the most prevalent psychiatric disorders affecting more than 300 million people globally with a burden of 4.3% according to World Health Organization (WHO). It is expected to be the leading cause of disease in high-income countries by year 2030. The early detection of disease may be treated effectively hence, there is need for a prediction model for an early detection for those that are suffering from mental disorders to minimize the impact on public health as well as reducing the escalation of the disease. Several reports reveals that datasets from various databases are explored to identify the most appropriate methods for the early detection of psychiatric disorders based on machine learning algorithms. The frequently used machine learning algorithms used in the early detection includes support vector machines (SVM), Random Forest, logistics regression, k-means clustering etc. These algorithms have major advantages as well as disadvantages subjected to data types leaving the research space and challenges for developing the novel model for effective early detection. In the review article, we will discuss about the concepts of the machine learning algorithms that are used in the psychiatric disorder prediction and their application in the disease prediction. Currently, the application of empirical to hybrid methods and technologies such as the deep learning methods have been applied for the effective prediction.

Keywords: MDD; machine learning; support vector machines; random forest, psychiatric disorder

INTRODUCTION

The psychiatric disorders place a large emotional, health, and financial burden on patients, their families, and the society. According to the Global Burden of Disease Study in 2017, psychiatric disorders is the fourth largest cause of disability-adjusted life years (DALYs) worldwide [1]. The major grounds of impairment in both males and females is Depression. The World Health Organization (WHO) conducted a survey in 2016, which states that the most of the patients are effected in both the high income and low income countries, while the females have 50% more chance of being
affected by depression than males [2]. Depression is also found to be most prevailing psychiatric disorder causing disability globally and affects more than 300 million people worldwide, mainly women [3]. Many robust techniques are developed that uses the probabilistic and the statistical techniques such as machine learning algorithms [4]. Machine learning is one of the fastest growing field of computer science with multidisciplinary applications. It helps in detecting the patterns in the data automatically. A large amount of unprecedented health data is generated globally, so machine leaning algorithms paves the best way to solve the classification problems, access the hidden patterns in the big data, extracting similarities and dissimilarities between distinct data sets and deals with the vast range of variables. ML techniques have been a lot of advantages in dealing with the ‘big data’ that processes the large scale of data, whose importance was discovered even before the ‘big data’ era emerged [5]. Machine learning can be used to create new hypothesis, predict the drug efficacy, and to predict the heterogeneity of the symptoms of the psychiatric disorders. In this review article, we discussed all supervised and unsupervised machine learning algorithms and how they help on the early prediction of psychiatric and neurological disorders [6].

---

**Figure 1: The different types of machine learning methods**

The machine learning techniques are used for analyzing data, prediction of the target features as well predict the meaning of the given data. The machine learning techniques are primarily divided in to two types: supervised learning and unsupervised learning as given in figure 1 [7].

**Supervised Learning**
Supervised learning generates functions which deals with labels in the inputs to get the desired output. The supervised learning deal with the labeled data which consists of the set of features called attributes. These attributes describes a data instance, which is further used to predict the labels and unknown instances. It can be a discrete value such as numbers or the continuous variable such as characters or string. Both the values represents either the classification or the regression problem [8]. The supervised learning also deals with the function approximation to predict the accurate, error free results using the training and the test dataset [9]. The supervised algorithms can further be applied for the early prediction of the psychiatric illness by using the patient’s data, even before the medical professionals [10]. The different supervised machine learning algorithms used for the early prediction of psychotic disorders are Support vector machines (SVM), Random Forest, k-means clustering. These algorithms have applications in the early prediction of diseases such as depression, post-traumatic stress disorder (PTSD), schizophrenia-spectrum disorder, and bipolar disorder disease [11]. The table 1 represents the advantages and the disadvantages of the various machine learning algorithms.

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<tr>
<th>ML-based algorithms</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Supervised learning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Support vector machine</td>
<td>This model gives more accuracy precision than other models. It does maximal margin separation of two classes.</td>
<td>To examine large dataset, it does not perform well and generates noise.</td>
</tr>
<tr>
<td>Random Forest</td>
<td>It does not over fit and interacts with the predictor variables handling continuous and categorical predictors</td>
<td>Interpretation between attributes and target is complex. In complex datasets, Random Forest prediction rate is comparatively slow.</td>
</tr>
<tr>
<td>Logistics Regression</td>
<td>It function fast with training dataset, shows resistance to over fitting</td>
<td>It does not work well when the decision boundary is non-linear</td>
</tr>
<tr>
<td>Unsupervised learning</td>
<td></td>
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</table>
**SUPPORT VECTOR MACHINE**

Support vector machines (SVM) is a supervised learning algorithm which was first found by Vapnik et al. in 1992, who named it as maximum margin classifier. They used it for solving problems such as perceptron, radial bias functions and polynomials [12]. It is a training algorithm that maximizes the width of the margin between the training classes and classify the points to make them separable [13]. The SVM model is used for solving a wide range of the classification problems such as classification and regression analysis in psychiatric and neurological diseases [14]. It is a widely used robust learning tool and based on statistical methods [15]. The SVM classifiers are divided in to linear and non-linear SVM functions, the non-linear function use the kernel-based method for mapping in higher dimensional feature space to get the maximum margin between the classes [16]. The subjects can be optimized by using the hyperplane which divides it in to two different classes with maximal margin space, it can be described using an equation:

$$ w^T x_i + b = 0,$$

Where, $x_i$ can be called as the column vector of the $i^{th}$ subject, $w$ is the weight vector that divides the hyperplane and $b$ denotes the offset.

Both the parameters lie on the different sides of the hyperplane and maintains the maximum space between the two classes [17, 18]. Karen-Inge et al. utilized the target information equivalence algorithm and used it with the SVM classifier with 10-fold cross-validation to optimize the prediction of the non-remitting PTSD. The datasets consisted of the telephone based interviews of around 4743 participants, who suffered traumatic exposure [19]. Akinci et al, developed the eye pupil detection system used the SVM algorithm to distinguish whether patients had bipolar disorder or not. Based on the pattern recognition of the position and the radius information of the eye pupils, the SVM algorithm was utilized with ellipse-fitting algorithm and the prediction accuracy was 96.36 % for the results [20, 21]. Similarly, Pavol et al., applied the SVM classifier to detect the disease patterns in
patients with a first episode of schizophrenia-spectrum disorder. The classifier successfully demonstrated the high accuracy of 62.34% and distinguished between the diseased and the non-diseased patients even before the onset of the illness based on the MRI data [22].

**RANDOM FOREST**

Random forests are also called as random decision forests is a ranking algorithm, which consists of large number of decision tress used for solving the classification and the regression problem. It is an ensemble of the unpruned classification and regression trees [23]. The random forest classifier uses the training dataset using the randomly selected features at each node to create a tree, this is also called as bagging or bootstrap aggregating [24]. It even works well with the meta-data, can deal with large number of variables and solve multi-class problem [25]. The random forest algorithm uses the mean square error formula to calculate the each note from the branch to solve the regression problem which is represented by

\[ R = \frac{1}{N} \sum_{i=1}^{N} (f_i - y_i)^2 \]

Where, the number of the data points is represented by \( N \), \( f_i \) and \( y_i \) represents the value returned and the actual data point value \( i \), respectively. While, the classification problem of the random forest is represented by the Gini index, which is

\[ G = 1 - \sum_{i=1}^{c}(P_i)^2, \]

Where \( P_i \) the relative frequency and \( c \) is the number of the class [26]. Reece et al. developed the various machine learning models such as using the random forest to predict the emergence of the depression and post-traumatic stress disorder (PTSD), using the depression history of 204 individuals. The random forest classifier predicted the results with highest precision rate of 88.2% with only 1 false positive for every 10 depression diagnoses [27]. Kautzky et al. used the random forest with five-fold classification for feature selection and classification to find the accuracy of the attention-deficient hyperactivity disorder (ADHD) medicated by single nucleotide polymorphisms (SNPs) with 82 % precision and accuracy [28]. Similarly, Stephanie et al. utilized around 12,229 dataset information from Patients Like Me (PLM) data base where the patients suffering from major depression disorder with suicidal ideation (MDSI) were analyzed using the
random forest classifier which used the risk factor as the feature selection and predicted that around 50% of the patients suffering from MDSI were younger patients below the age of 35 years [29].

LOGISTIC REGRESSION

It is widely used classification algorithm for the multivariate analysis of the regression data in biomedical field. It uses the regression function to calculate the probability of a new binary outcome [30]. It is majorly used for statistical discrete data analysis.

Mendez et al. showed that patients suffering from the bipolar disorder (BD) have achieved the syndromic recovery using the logistic regression models with the maximum accuracy, but still there was impact on the functioning and the academic performance of the adolescents [31]

UNSUPERVISED LEARNING

The unsupervised learning does not require supervision, determines groups in the data, and analyses data to put labels on it. While, in supervised learning, they train and predict data using labeled feature selection methods. The unsupervised learning performs its functions by handling data without the help of target attributes such as reducing the dimensionality in order to reshape them. The unsupervised learning can be used with the supervised learning methods to analyze data as collecting labelled data is a tedious task for supervised learning [32].

K-MEANS CLUSTERING

The various types of unsupervised learning algorithms are k-means clustering, categorization and neural networks. The clustering is one of the most used unsupervised leaning algorithm in machine learning for data analysis in terms of feature selection, grouping methods, distance functions and cluster formation and validation. The k-means clustering algorithm uses Euclidean distance functions in a given metric spaces, to form clusters and optimizes squared errors. The clusters are formed as centroids, which collects all the nearby points to the nearest possible centroids and new assembled clusters are formed, hence minimizing the squared Euclidean distances [33]. The k-means clustering has various advantages and disadvantages such as they are simple and scalable, they can form compact
clusters using the suitable datasets and keep them well-separated [34]. Their disadvantages are that they have difficulty in dealing with clusters of arbitrary sizes, shapes and density. They also show sensitivity towards noises and initialization, formation of poor cluster descriptors [35]. The algorithm uses the squared error function which helps in minimizing the objective function, which can be described as

\[ J(V) = \sum_{i=1}^{c} \sum_{j=1}^{c_i} \left( \| x_i - v_j \| \right)^2, \]

where, \( \| x_i - v_j \| \) can be described as the squared error Euclidian distance between \( x_i \) and \( v_j \), \( c_i \) denotes the \( i^{th} \) cluster in cluster centers and \( c \) denotes the cluster centers [36]. Fuente-Tomas et al. developed a clustering algorithm based model that allocated the Bipolar disorder (BD) based patients according to the severity of the disease using the cluster-based severity classification model. It may help the clinicians in developing personalized medicine and precise decisive-making. The paper have reduced the dimension of the different four variables including the socio-demographic, BD characteristics, laboratory results and use of the psychometric instruments [37].

The machine learning algorithms have various applications in diseases such as depression, post-traumatic stress disorder (PTSD), schizophrenia, ADHD, bipolar disease.

Table 2: The ML techniques and data types for the detection and diagnosis of psychiatric illness

<table>
<thead>
<tr>
<th>Mental Health Application</th>
<th>ML Technique(s)</th>
<th>Data Type</th>
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<tr>
<td>Bipolar disorder</td>
<td>SVM [21], Random forest [27], Logistic Regression [30]</td>
<td>Case-study [30], telephone based interviews [21], Database [27]</td>
</tr>
<tr>
<td>Major depression disorder</td>
<td>Regression, Random Forest [27], SVM [20]</td>
<td>Database such as PubMed, Embasse [20], Survey [27]</td>
</tr>
<tr>
<td>Post-traumatic stress disorder (PTSD)</td>
<td>Random Forest [27], SVM [19]</td>
<td>Interview, Survey, Database [27], ED records [19]</td>
</tr>
<tr>
<td>Schizophrenia-spectrum disorder</td>
<td>SVM [22]</td>
<td>MRI [22]</td>
</tr>
<tr>
<td>ADHD</td>
<td>Random Forest [28]</td>
<td>PET, EEG, MRI [28]</td>
</tr>
</tbody>
</table>
RESEARCH CHALLENGES

The researchers face a lot of challenges while performing the data analysis for early prediction of computational psychiatry. Some of them can be described as

1. **Reproducibility**: the data generated in one research centers will be different in another research Centre. The ML tools can reduce the extent of the errors product in the data such as in ADNI datasets, but still the improvement in the accuracy and the implementation is required [38].

2. **Data availability and management**: The various neuroimaging techniques such as MRI, etc. are only available to very less people in developing countries, yet their availability is getting increased day by day in developed countries [39].

3. **Data heterogeneity and incomplete data**: The models developed are limited in their quality, which can be improved by using the training datasets and maintaining the collaboration between the researchers and the clinicians for the maximum usefulness [40]. The ML techniques also proves to be challenging in the real world applications as the factors can change such as the results appeared in the lab can show different results in real-time data analysis, or when applied to large datasets [41, 42].

CONCLUSION

The review article describes the machine learning techniques which are focused on the supervised learning as well as unsupervised learning algorithms in the field of psychiatric disorders. The study of mental wellness using different Machine Learning approaches are primarily centered on supervised learning for classifications. Based on or review article, depression, Bipolar disorder, Alzheimer's disease, schizophrenia, ADHD are the major diseases of the psychiatric disorders, where the various machine learning techniques have been utilized such as SVM, Random Forest, Logistics Regression, k-means clustering. Each of these machine learning algorithms have their own advantages and the disadvantages. The applications of some of these algorithms is discussed in the Table 2. The researchers uses the different types of ML algorithms alone or by using different classifiers together predict the maximum accuracy of the results. The performance of all these models depends on different
properties of data and purpose of the study. We also covered some of the research gaps found, while reviewing the studies on psychiatric disorders. The future scope of the study lies in developing an automation process where the large amount of data set such as ‘big data’ can be processed in real-time data analysis to get results with maximum accuracy and precision. Hence, further research is required in the mental health with the ML becoming more accessible to the researchers and the clinicians.

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[27] Reece, A.; Reagan, A.; Lix, K.; Dodds, P.; Danforth, C.; Langer, E. Forecasting The Onset And Course Of Mental Illness With Twitter Data. Scientific Reports 2017, 7 (1).


ABSTRACT: Colorectal cancer is one of the deadliest diseases happening to do any population across the world. In the disease chart of the world health organization it is the mostly diagnosed third rank harmful disease. The disease appears with selective symptoms like a change in bowel habit is a more common symptom, ventral pain, alter in stool form, rectal bleeding, blood within the stool, and eventually intestinal obstruction. Sedentary lifestyle heavy uses of alcohol and abnormal lifestyle aside from genetic decomposition could be the associated factors of colorectal cancer. Emergence of the disease usually observed in the small intestine and large intestine in an inner membrane which become malignant and spread across the other part. The available treatment for cancer like surgery, radiotherapy, chemotherapy, targeted therapy, immunotherapy often damage healthy cells & tissues isn't so effective. The tremendous growth in the multidisciplinary area like genomics proteomics clinical science and soft computing technologies have offered some hope to succeed in the good treatment if the available data of bioscience are analyzed intelligently and effectively. In this review, we are focusing on latest soft computing methods, its application and challenges in colorectal cancer gene data analytics.

Keywords: Colorectal Cancer, Machine Learning, Deep learning, Belief Network, and Evolutionary computation.

INTRODUCTION:

The new cancer cases approximately more than 1.4 million per annum in the world, colorectal cancer is second largest cause of cancer death. It also referred to as bowel cancer and colon cancer is the growth of cancer from the rectum (parts of the large digestive tract). The disease appears with selective symptoms like a change in bowel habit is a more common symptom, ventral pain, alter in stool form, rectal bleeding, blood within the stool, and eventually intestinal obstruction. Most colorectal cancers are due to over age and factors of lifestyle, and genetic deformation. Other hazard factors include diet, fatness, smoking, and scarcity of physical activity. The cancer treatments depend on respective factors, like size of cancer, location of cancer, and stage of
cancer. The available treatment of cancer is chemotherapy, radiotherapy, and surgery which aren't so effective. In screening phrases and early detection the extensively conducted gFOBT (guaiac-based fecal occult blood test) has excellent precision but low sensitivity, especially for the detection of colorectal adenomas. However, screening-based randomized trials using gFOBT have demonstrated significant reductions in mortality from colorectal cancer [2-3]. Basically Fecal immunochemical test (FIT) for human hemoglobin in stool evolves and is increasingly being used. FIT is more sensitive to colorectal cancers diagnosis and its precursors as opposed to gFOBT [4-5]. Soft Computing is a branch of artificial intelligence or computational intelligence that may analyze various complicated medical statistics by using employing one-of-a-kind varieties of optimization techniques to decorate the diagnosis and detection of cancerous nodules. Its most prominent methodologies are Artificial Neural Networks (ANNs), Fuzzy Logic (FL) and Genetic Algorithm (GA). Due to the notability of soft computing approach, it is often used in medical and healthcare fact for accurately detection of diseases and to achieving better consequences in contrast to conventional approaches. Soft computing techniques have the strength to evolve itself well regulated with the problem area. Between the exploration and exploitation processes a good balance is another aspect. The soft computing approaches greater effective, reliable, and efficient made by these aspects. The above characteristics make soft computing methods extra suitable and capable for providing the data on health care [6].

**Soft computing approach:** Soft computing technologies supported by technical advances will certainly minimize the lack of adequate fitness treatment within the market, make it available omnipresent in the most cost-effective way and could serve as a great tool for practitioners to embrace as true [7]. The best soft computing techniques integration models are pattern recognition, Bayesian networks, reasoning models, deep learning, evolutionary computation, fuzzy logic, differential algorithms, optimization algorithm, perception, and Bayesian networks [8]. Soft computing approach integrating the fuzzy set theory, artificial neural network, genetic algorithms, and other intelligent methods which are extensively utilized to deal with unsure and unique problems. Using soft computing, the wisdom of individual is often replicate in computer
in the same artificial matter. Soft Computing methods divided into mainly four parts describing below:

![Diagram of Soft Computing Methods]

**Figure 1. Branches of soft computing methods**

**A. Artificial neural network (ANN):** An Artificial Neural Network is going to be implemented for the decision-making process among all techniques of Soft computing approach. ANN is beneficial to development of algorithms for complex pattern recognition [9]. The working style of ANN is just like human brain nervous systems, which accept the inputs from different sources and produce the appropriate output reaction. The ANN extricates the hidden statistics available in the data without previous knowledge and uses them to address problems. ANN mainly formed by three separate layers input layer, output layer and one or more than one hidden layers. These layers of ANN having neurons and to exchange signal from one layer to another, these are connected to the neurons in the consequent layer. The input layer’s node use to provide inputs in the ANN. For creation of ANN model, a person ought to choose the quantity of nodes within the input layers, output layers and hidden layers. In the input and output layers, the wide variety of nodes must be set equal to the wide variety of input and output variables. It is also important to agree on the right number of hidden layers and nodes, because of the fact that the network memorizes the relationship between inputs and outputs with so many hidden layers and nodes, rather than understanding it [10].

The cellular neural, self-organizing map, MLP networks and multi-layer recurrent are commonly used ANN configurations. The essential parameters of ANN are weight and bias. The interconnected neuronal relationships decide by and the degree of freedom determines by bias term and weight.
term. The calculation of ANN structure may be summing by the outputs of each layer and via transfer function it converts to the subsequent layer [11].

**B. Fuzzy control:** This has been used to overcome deep management problems for over 20 years. Additionally, by using the principles of fuzzy logic many instrumentation issues are being solved. An evolutionary fuzzy control model used hybrid learning methodology which combines the fuzzy model with evolutionary algorithms and integrates the optimization of evolutionary algorithms collectively with the abilities of fuzzy systems to solve complicated issues. Fuzzy-genetic control system is an advance version of a fuzzy control model which is capable in reduces the working system pressure with ideal pressure value. This advance version of Fuzzy-system, has acquired information throughout its training, which allow it to interrupt distant from the precise operative situations and improve itself positive, among positive limits, no longer encountered throughout the training stage [12]. Among the most highlights of fuzzy logic control system (FLCs) is their capability to create satisfactory control selections induction through human-like linguistic descriptions which represented through fuzzy regulations based on heuristics, experience and knowledge and are often used to govern a given systems. One of the most widely applied schemes is neuro-control approach. The Adaptive Neuro-Fuzzy System (ANFIS) provide the best result for control of robotic operators as compared to the ordinary control strategies. The ANFIS can construct an input-output mapping by using a hybrid learning procedure, based on both human knowing and prescribed input-output data pairs [13].

**C. Machine learning:** Machine learning algorithms are used for disease prediction. There are two sorts of machine learning algorithms. One is supervised learning that is used to predict output by training known and labeled; another is unsupervised learning that does not need knowledge of output class and data is unlabeled. Machine learning algorithms are used for disease prediction in healthcare services (HCS) [14]. There are two sorts of machine learning algorithms, one is supervised learning that is used to predict output by training known & labelled and another is unsupervised learning that doesn't need knowledge of output class & data is unlabeled [15]. Machine learning techniques are a neural network, Support Vector Machine (SVM), Neuro-
Fuzzy Network, Fuzzy c-means, K-Nearest Neighbor (KNN), and Decision tree [16].

**D. Expert system:** The expert system referred to as a expertise-primarily based system, is based on computational framework to be able to make smart choices by means of emulating the choice making competencies of human professionals. The expert system is knowledge based computational framework to be able to make brilliant decision by means of imitate the judgement creating competencies of human specialist. These systems are regulation based from input to output clearly define the steps involved in progressing. The Expert systems can modify their choice and create new selections on the premise of external features. The application of this expert systems in the area of engineering design, online healthcare framework for problem diagnosis, robotics, legal matters, and financial loan/credit decisions. In expert systems knowledge acquisition is one of the main issues [17]. In expert system the key elements are user interface, inference engine, and knowledge base. For an Expert Systems Development the Key participants are Domain Expert, Knowledge Engineer, and End User etc. The improved decision quality, reduce cost, consistency, reliability and speed are the key benefits of an Expert System [18].

**Soft computing in healthcare:** The soft computing technique was also implemented in health care data to successfully diagnose the disease and achieve the superior results compared to traditional approaches. According to problem domain the Soft computing approaches having ability to adapt itself. We can say that it is a good balance between exploitation processes and exploration. The soft computing technique more capable, dependable and productive made by these aspects. On the basis of above mentioned characteristics for health care data the soft computing approaches more capable and suitable [6]. In the area of healthcare system there are so many applications of AI and soft computing technique. In the medical diagnosis by using clinical and multi-omics data it provides confirmation of early detection of cancer and infectious disease. Image data and deep learning methods are widely used for cancer diagnosis and medical screening. Convolution Neural Networks (CNNs) is the most commonly used deep learning methods. For healthful life style and early disease prognostication the health management of population provide the
Patient oriented information system through digitization of disease management protocols. For Administration and regulation in healthcare the Big Data ensures the quality based result for hospital management and disease monitoring [19]. Neural Network (NN) Based Systems provide to develop a Probabilistic Neural Network for estimation of risk factor of cardiac surgery. To improvement in characterization of urinary bladder tumours the nonlinear Hebbian learning methods are commonly used. For determine the risk factor of coronary heart disease the FCM based Fuzzy Expert System are suggested by human expert [20].

Applications of soft computing techniques: Soft computing techniques used in different areas like in wireless communication, Robotics, Healthcare system, Consumer Appliances and Data mining etc. The Soft computing approach is applicable in communication with wireless system, which covers wide range of networking optimization, Handoffs, useful resource allocation and prognostication. In Communication Systems to gain the answers which have now not been capable of remedy by way of Hard Computing, Soft Computing can be effectively applied. For using the data compression and equalizer the Artificial Neural Network and Fuzzy Logic combined together which provide a Neuro-Fuzzy approach. In Robotics system, based on human thinking and behaviour, Robotics is an emerging field. To develop useful real world applications the Expert System strategies and Fuzzy Logic integrate in a manner. The Soft Computing method is applicable in building clever vehicles and offer efficient surroundings to machines and drivers in the transportation area. In the healthcare sector, today the computer technology is more applicable in Health care environment. The Soft Computing strategies offer better and developing aids with the assist of advancement in computer system technology, that assist the physician in many instances, fast identification of sicknesses and analysis in actual time. In the field of data mining who is also known as knowledge discovery. The purpose of Data mining is for solving issues in a specific area [21]. There are two main applications of soft computing techniques. The first one is Medical applications where soft computing strategies are utilized by numerous clinical packages like medical photograph registration using of genetic algorithm, to resolve prognostic problems within the medical domain using system learning techniques,
diagnosing the cancer by using Artificial neural networks (ANN), and fuzzy Logic in numerous disease. Another is Genetic algorithms which are section of fuzzy computing system & artificial intelligence and that they may be especially used to clear up various optimization cases encountered in actual life styles packages [22]. In relation to healthcare system such as skin cancer, a neural network trained using a clinical images dataset by researchers and examined its overall performance in opposition to 21 board-licensed dermatologists on biopsy-demonstrated clinical images. Hence an artificial intelligence technique became able to classifying the cancer. In other hand an AI also benefited to clinical neuroscience. Similarly, a machine learning algorithm strategy outlined to evaluate the headway to dementia [23].

**Major challenges in soft computing application in colorectal cancer genomics:** It is still very hard to define the transient data in the process of clarification of disease and still many false-positive cases being reported. It is still a challenge to handle over-fitting and under-fitting. Classifying and optimizing the heterogeneous biological data computation and resolving the curse of dimensionality is still a challenge. In a systematic review it is recently identified that for deep learning model the temporality and irregularity of information using electronic health evidence data reduce the model performance if record data not maintained properly. An ANN based monitoring method developed by Fisher et al (2016) for evaluating Parkinson’s disease motor symptoms. The explanation of state of disease descriptors, due to the inherent subjectivity, it is reported to detecting disease states [24]. While the utility of soft computing methods in the distribution of healthcare system has a hopeful ability, many demanding situations both ethical & technical exist. It is a major challenge for soft computing methods that is basically lead and maintained by a computer expert who haven't any knowledge of medical sciences [1]. As per modern-day prediction the machine learning models like Support Vector Machine, Neural Network and linear regression are conditional methods which are not easy to recognize. Model robustness in cloud computing for resource provisioning is another challenge. By the using of appropriate model architecture and its inner parameters the more robust resource prediction model can create. [25].

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CONCLUSION:

Immeasurable work is done in the cancer disease several reports already published. But it is still very hard to define the transient data in the process of clarification of disease, false-positive cases, Classifying and optimizing the heterogeneous biological data computation, and resolving the curse of dimensionality. Therefore, it is required for more advancement in soft computing methods like artificial intelligence, deep learning, and machine learning aside from efficiency data pre-processing procedure. The benefits of soft computing approach to hassle-fixing lies inside the adaptability and simplicity to therapeutically domain. Healthcare distribution has through the years emerged as complex and tough. Due to problem-fixing approach the soft computing technique can address this requirement with their smart performance which consolidate learning, thinking and capacity to act independently. Hence the therapeutic domain provide a fruitful ground for soft computing methods analysts to check their procedures and numerous occurrences; soft computing methods effectively solved the issues with results proportionate to that of human doctors. The medical facility shipping turns into extra luxurious, the stakeholders will become much more appearance to answers that could substitute the pricey factors inpatient care and soft computing techniques will be prominent in these situations. In any case, cold innovative technology cannot supplant the human components in patient care, and a model that consolidates each innovative technology and human care needs to be explored.

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Small Millets: Grains full of Nutrients yet largely underutilized

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ABSTRACT: Millets are small seeded grains of grass family Poaceae. These are broadly divided into two main groups as major and small millets on the basis of their production. Major millets include pearl millet and sorghum while small millets include barnyard millet, kodo millet, brown top millet, guinea millet, proso millet foxtail millet, finger millet and little millet. These crops stand at the junction of being exploited as potential candidates for our increasing need of sustainable food chain development. They offer superior agronomic traits such as ability to grow in less fertile soil and resistance against drought and pest. In addition, from a nutritional point of view they are rich in vitamins, minerals, have prebiotic and probiotic functional properties and possess a number of active secondary metabolites with potential therapeutic effects. However, despite these numerous prospects these crops are marginalized and are only grown in certain local communities or used as bird feed especially small millets. Therefore, it becomes necessary that we harness this untapped resource and emphasize on bringing them in mainstream agriculture.

Keywords: small millets; antioxidant; anti-cancerous; antimicrobial; secondary metabolites; gluten-free.

1. Introduction

Cereals are an important reservoir of chemical energy that can sustain the basic nutritional needs of mankind; both directly and indirectly as animal feed. They belong to a large family of monocotyledonous grasses called Gramineae. They are mainly cultivated for their grains while the other parts of the plant may be used as animal feed. Different geographical and climatic conditions have favored the growth of many different types of cereals with certain additional nutritional characteristics. However, as a whole generalized grain content of cereals is 70 percent carbohydrates, ten percent protein, and three percent lipids. The importance of cereals can be underlined by the fact that they are consumed as staple food throughout the world. Of these wheat, maize and rice are primarily produced in bulk occupying nearly half of the world harvested area while five others namely barley, sorghum, oats, millets and rye are also produced for their nutritional value but to a much lesser extent.
2. Millets:

Millets are cereals with small-seeded grains primarily cultivated in dry tropical, subtropical and temperate regions of the world. They are typical flowering plants of C-4 type. The kernel of millet grain consists mainly of a husk, endosperm, bran, and embryo. Husk is the nonedible part of millets while bran is the component that is edible but usually removed during milling for food uses. Endosperm is primarily characterized by the presence of storage carbohydrates with varying amounts of minerals, proteins, and lipids. The main fatty acids in millets include palmitic, linoleic and oleic acids constituting 85% of total fatty acids. Embryo is a distinct tissue constituting approximately two percent proportion of the millet kernel. Millets are broadly divided into two groups on the basis of their production as major and small millets. Major millets include Sorghum and Pearl millet and Small millets are of six main types including Kodo millet, Barnyard millet, Foxtail millet, Little millet, Proso millet and Finger millet. There are also other millets such as teff and fonio and other lesser-known millets such as Browntop millets and crap grass (Taylor, 2019).

3. Small millets:

Small millets, also known as “nutri-cereals”, not only offer diversity to the food we consume but are also rich in carbohydrates, proteins, lipids, vitamins and minerals that can serve as a source of a balanced diet. Table 1 presents a comparison of the nutritional aspects of small millets in comparison to other major cereals. As can be seen from the table that millets are in no way inferior to other cereal grains and in fact, they possess some important essential minerals, dietary fibers in much higher quantity as compared to wheat, rice and maize (in bold).

<table>
<thead>
<tr>
<th></th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Dietary fibers</th>
<th>Carbohydrates</th>
<th>Minerals</th>
<th>Calcium (mg)</th>
<th>Iron (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearl</td>
<td>12.4</td>
<td>11.6</td>
<td>5.0</td>
<td>12.0</td>
<td>67.5</td>
<td>2.3</td>
<td>42</td>
<td>8.0</td>
</tr>
<tr>
<td>Finger</td>
<td>13.1</td>
<td>7.3</td>
<td>1.3</td>
<td>19.8</td>
<td>66.8</td>
<td>2.7</td>
<td>344</td>
<td>5.0</td>
</tr>
<tr>
<td>Foxtail</td>
<td>11.2</td>
<td>12.3</td>
<td>4.3</td>
<td>14.0</td>
<td>60.9</td>
<td>3.3</td>
<td>31</td>
<td>2.8</td>
</tr>
<tr>
<td>Little</td>
<td>11.5</td>
<td>7.7</td>
<td>4.7</td>
<td>12.2</td>
<td>67.0</td>
<td>1.5</td>
<td>17</td>
<td>9.3</td>
</tr>
<tr>
<td>Barnyard</td>
<td>11.1</td>
<td>6.2</td>
<td>2.2</td>
<td>13.7</td>
<td>65.5</td>
<td>4.4</td>
<td>20</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Table 1: Nutritional composition of small millets in comparison to other major cereals (gram/100gram) (Source: National Institute of Nutrition data 2012).

<table>
<thead>
<tr>
<th></th>
<th>Kodo</th>
<th>Rice</th>
<th>Wheat</th>
<th>Maize</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.4</td>
<td>13.7</td>
<td>12.8</td>
<td>14.9</td>
</tr>
<tr>
<td>Protein</td>
<td>8.3</td>
<td>6.8</td>
<td>11.8</td>
<td>11.1</td>
</tr>
<tr>
<td>Fiber</td>
<td>1.4</td>
<td>0.5</td>
<td>1.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Ash</td>
<td>15.0</td>
<td>1.5</td>
<td>12.9</td>
<td>10.5</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>65.9</td>
<td>76.9</td>
<td>71.2</td>
<td>66.2</td>
</tr>
<tr>
<td>Fat</td>
<td>2.6</td>
<td>0.6</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>27</td>
<td>10</td>
<td>41</td>
<td>10</td>
</tr>
<tr>
<td>Iron</td>
<td>0.5</td>
<td>0.7</td>
<td>5.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Besides, small millets also have various health beneficial attributes such as anti-oxidant, anti-cancerous properties, anti-diabetic and anti-ulcerative activities and many more discussed further.

4. Anti-oxidative effect of millets:

Oxidative stress refers to a state of the cell when the loss of electrons in the system becomes more prominent than electron gain leading to a redox imbalance of the cell. The effect can be long lasting. Oxidative stress is mainly caused by damage and defence of cells in response to environmental stresses such as drought, salinity, etc. The oxidative stress in plants is mainly due to reactive oxygen species and free radicals. These molecules exert their effect in plant cells by oxidizing cellular compounds. Reactive nitrogen species also have role in oxidative stress however these are not as well understood.

Molecular O$_2$ is a free radical but it is not very reactive as the spin of the two free electrons of the two oxygen atoms is either same or parallel. This makes it very difficult for other molecules to have anti-parallel spin to react with these oxygen atoms. However, energy input can activate these oxygen molecules. In a biochemical system there are a number of factors that can provide energy to these systems such as UV light, electron transport chain, etc. resulting in a number of reactive oxygen species such as hydroxyl radical, hydrogen peroxide, singlet O$_2$, superoxide radical and nitric oxide, etc collectively referred to as reactive oxygen species (Demidchik, 2015). A flowchart of the formation of different radicals in response to energy is depicted in Fig 1.

Free radicals and oxygen species can create havoc in cell. These can react with different components of the cell including lipids, protein and DNA. Lipid peroxidation can lead to membrane protein damage, decreased
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Fluidity and increased leaking of while DNA damage in the form of deletion, alkylation, and oxidation of bases can alter the overall cellular regulatory machinery of a cell. The process once initiated can lead to a chain of events that can ultimately leads to the death of the cell (Gill et al, 2010).

\[
\text{Singlet oxygen} \xrightarrow{\text{Dioxygen}} \text{Superoxide radical} \xrightarrow{e^-} \text{Peroxide ion} \quad H^+ \quad \text{Peroxide} \quad 2H^+ \\
\text{Perhydroxyl radical} \quad \text{Hydrogen peroxide} \quad \text{Hydroxyl radical} \quad \text{Fe}^{2+}
\]

Fig1. Generation of Reactive oxygen species (ROS) (Gill et al, 2010)

Plants are also equipped with compounds that can quench these ROS and free radicals. Plant innate defense mechanism against these radicals are either enzymatic or non-enzymatic. Superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalases, glutathione S-transferase (GST) is some enzymatic free radical scavenger. Vitamin C and E, phenols, glutathione and proline are some non-enzymatic defenses. It is also noteworthy that these antioxidants are localized in specific compartments that prevent oxidative damage. For example, phenols are mainly present in the outer layers of plants where they shield the plant from the high energy radiations. This ability of phenols is mainly due to the presence of electron- rich pi bonds in their structure that can particularly absorb these radiations before they damage the cell (Gill et al 2010).

In millets, antioxidant activity is exhibited by a number of compounds including phenolics, dietary fibers, vitamins and many more. A brief summary of antioxidant compounds in millets and their mechanism of action are presented in Table2. Dietary fibers and phenolics are more concentrated in the outer layers of the grains.
<table>
<thead>
<tr>
<th>Name of compound</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td>The -OH group of phenols can donate hydrogen atoms to free radicals that are electron deficient to form a stable product that is comparatively less reactive than the free radical.</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>The presence of multiple –OH groups and &gt;C=O groups function as free radical scavenger by stabilizing the radical-flavonoid conjugate by electron delocalization and conjugation.</td>
</tr>
<tr>
<td>Tannins</td>
<td>The procyanidin-o-quinone group of tannins can undergo coupling reactions to form oligomeric compounds while retaining their –OH groups that by redox cycling can form stable products with reactive oxygen species.</td>
</tr>
<tr>
<td>Xylo-oligosaccharides</td>
<td>-OH group of polyphenols linked to oligosaccharides by ester linkages, &gt;C=O of sugar moieties and degree of polymerization is primarily responsible for anti-oxidant activity.</td>
</tr>
<tr>
<td>Insoluble fibers</td>
<td>Antioxidant activity attributed to phytochemical composition</td>
</tr>
<tr>
<td>Protein and Peptides</td>
<td>Proteins can bind in a more complex fashion to specific oxidants, chelate metal ions and reactive oxygen species with oxidative damage potential</td>
</tr>
<tr>
<td>Carotenoid</td>
<td>Quench free radicals and single oxygen</td>
</tr>
</tbody>
</table>
5. Hypoglycaemic effect of millets:

The amount of glucose released from starch and the amount absorbed in the body is mainly dependent on the activity of two enzymes; α-amylase and α-glucosidase. Therefore, targeting these enzymes to control blood glucose levels in the body is widely employed. In the current scenario, market is flooded with synthetic inhibitors of these enzymes. However, natural inhibitors present in food are largely underutilized. They also offer added advantage of being non-toxic, easily available, and cost effective with potentially nil side-effects. A number of studies indicate the activity of millet phenolics on these enzymes. A study by Pradeep et al., suggested that millet based phenolics can inhibit these two enzymes. They also evaluated the effect of processing such as germination, open steaming and microwave on phenolic activity. The group reported a decrease in phenolic extract enzyme inhibitory activity with heat treatment while germination increased the activity in comparison to raw. Further, a comparison of the IC\textsubscript{50} value for barnyard, foxtail and proso millets for α-amylase and α-glucosidase were as follows: Foxtail > Proso > Barnyard suggesting that the enzyme inhibition activity of barnyard millet is highest among the three. Further, it was also observed that processing showed a similar trend (Pradeep et al, 2015).

6. Anti-inflammatory effect of millets:

Millets are known to exert anti-inflammatory activity in cells. Dietary phenols and lipids are considered one of the primary factors that regulate this activity in food materials. Therefore, studies on isolated phenols and lipid fractions from millets can give a potential insight into this role of millets. Since, T cells are involved in immune regulation a study was conducted to analyze the effects of pearl millet polyphenol and lipid fractions on T cell activation. The rationale behind the study included an evaluation of the mitogen-activated protein kinase and free calcium ion concentration of T cells which are upstream regulators of T cell proliferation in response to stimuli. Both phenolic and lipid extracts showed
inhibition of MAPK phosphorylation and sustained high calcium levels in the cell thus suggesting their possible role in immune suppression of T-cells activation. However, the effect was more profound in phenolic extracts in contrast to lipid extracts (Nani et al., 2015). A schematic of the mode of action of extracts is depicted in Fig 2.

Fig 2. A schematic representation of the events in T cell activation and proliferation and the observed effect of extracts from pearl millets (lipid and phenols) (Adapted from literature Nani et al., 2015).

A similar study on the role of polyphenols of foxtail millets in immune regulation has been reported by Shi et al. They studied the anti-inflammatory response of bound polyphenols of millet bran (BPIS) on HT-29 cells induced by lipopolysaccharide. The results suggested that BPIS suppress the synthesis of cytokine IL-1β, IL-6 and IL-8 the primary modulators of inflammation in cells. The reported activity of these extracts is mediated via decreased levels of Akt and Akt phosphorylation which in turn affect NFKβ-p65 expression level in cells. NFKβ-p65 is involved in the synthesis of inflammatory cytokines. The role of miRNA-149 has been implicated in the turn down of Akt activity. It was also shown that miRNA-149 expression levels increased in response to reactive oxygen species which in turn is upregulated by the presence of phenolic extracts (Shi et al., 2017).
7. Anti-hypertensive effect of millets:

Hypertension is one of the leading causes of heart related disorders. A study by Wei et al. evaluated the role of millet-based diet on hypertensive effects of a high salt diet on the myocardial tissue of male Sprague-Dawley rats over a period of seven weeks. It was found that a millet-based diet can reduce the effects of hypertension. It was suggested that high salt diet induced oxidative stress on myocardial cells. Therefore, malondialdehyde (formed as a result of degradation of polyunsaturated lipids by reactive oxygen species) levels increased; which in turn resulted in a cell damaging JAK/STAT pathway via angiotensin II (Ang II) binding to angiotensin type 1 receptor (Ang1) resulting in myocardial hypertrophy as observed in mice fed with high salt in comparison to control. However, the effects were reversed in millet+high salt diet. Similarly, it was also found that the pathways involved in inhibiting oxidative damage induced hypertrophy were down-regulated in high salt fed mice which were significantly enhanced by millets addition in the diet. Superoxide dismutase (SOD) is an enzyme involved in scavenging reactive oxygen species from cells and PI3K/Akt pathway cross talks with JAK/STAT pathway to determine the ability of myocardial to resist apoptosis (Wei et al, 2018).

8. Anticancerous effect of millets:

Bound polyphenols of millets have been shown to possess anti-cancerous activity against a number of cancerous cell lines especially against human colorectal cancer. A study by Shi et al, showed that the inner shell bound polyphenols (BPIS) of foxtail millets bran have the potential to induce apoptosis in HCT-116 cell line; a human colon cancer cell line without any significant damage to the normal cells. It was also proposed that the said activity of the polyphenols is mediated by means of generation of reactive oxygen species (ROS) which in turn decreased the membrane potential of mitochondria. As a consequence, an apoptotic event begins involving cleavage of pro-caspases to active forms especially caspase-9 and caspase-3. In addition, a decrease in NF-κB signaling is also induced by ROS generation. Xenograft analysis of HCT-116 cells on nude mice models also gave promising results with tumor size significantly decreasing after seven injections of 1 mg BPIS per gram of mice bodyweight each at an interval of two days compared to control (Shi et al., 2015). Peroxidase named as FMBP isolated from foxtail millet bran also showed anti-proliferative
activity against human colon cancer cell lines DLD-1 and HCT-116 by generation of ROS and inhibition of transcription factor Nrf2. Nrf2 is involved in the expression of enzymes that regulate the balance of ROS in the cell specifically catalase and glutathione. However, normal cells remained unaffected. The increased ROS production led to STAT3 blockage and its downstream products thus acting as a potential anti-cancerous agent (Shan et al., 2015). Supercritical fluid extraction (SFE) of Proso and Barnyard millet using liquid CO₂ and methanol led to isolation of bioactive compounds identified as vanillin with apoptotic activity against the cell line HT-29 associated with colon cancer. Vanillin at low concentration around 200µg/mL inhibited cell cycle progression through G₀/G₁ phase while at higher concentrations of 1000µg/mL arrested G₂/M progression. It was also observed to induce nuclear DNA fragmentation (Ramadoss et al., 2019).

Studies also indicate that beside colon cancer, millets-based diets can also serve as potential threat to myeloid leukemia in humans. Sen and Dutta, studied the role of protease inhibitors isolated from finger millets (ragi) and their apoptotic potential in K562 myeloid leukemia cell line. These protease inhibitors are bifunctional and inhibit both trypsin and α amylase and IC₅₀ value was determined to be 20µg/mL for them on myeloic cancer cell line K562 (Sen and Datta, 2012).

These studies point to the fact that millets are an important reservoir of naturally occurring substances with anti-cancerous properties and a detailed investigation of these compounds and their mode of action can yield fruitful results against a number of cancer types.

9. Anti-cataractogenesis activity:

Cataract is emerging as one of the leading causes of blindness especially in diabetic individuals. It has been observed that diabetes induced cataract leads to the accumulation of sorbitol in eye lens by the action of an enzyme aldose reductase (AR) via polyol pathway. A study by Chethan et al., found that the polyphenols from finger millets seed coats act as potent inhibitors of AR activity. The activity of crude polyphenol extract prepared by HCl-methanol extraction of seed coat on AR activity showed an IC₅₀ of 60.12µg/mL. Therefore, the crude extract was further analysed by HPLC. Table 3. indicates the activity of different fractions on AR activity.
Correlation analysis of enzyme inhibition and antioxidant activity of polyphenol components showed strong positive correlation. Therefore, it can be inferred that the enzyme inhibition may be due to proton abstracting ability of polyphenols. The study also indicated that the mode of action of quercitin, the most potent inhibitor of AR activity is non-competitive and thus have potential clinical significance (Chethan et al., 2008).

<table>
<thead>
<tr>
<th>Polyphenol component</th>
<th>Percent composition</th>
<th>AR IC&lt;sub&gt;50&lt;/sub&gt; (µg/mL)</th>
<th>DPPH radical scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>quercetin</td>
<td>5.6%</td>
<td>25.2</td>
<td>56.8</td>
</tr>
<tr>
<td>Protocatechuic acids</td>
<td>15.3%</td>
<td>42.7</td>
<td>77.63</td>
</tr>
<tr>
<td>trans-cinnamic acids</td>
<td>3.6%</td>
<td>68.1</td>
<td>96.7</td>
</tr>
<tr>
<td>synringic</td>
<td>4.0%</td>
<td>172.1</td>
<td>155.6</td>
</tr>
<tr>
<td>p-coumaric</td>
<td>4.4%</td>
<td>162.3</td>
<td>112.01</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>12.6%</td>
<td>97.3</td>
<td>26.9</td>
</tr>
<tr>
<td>p-Hydroxy benzoic acid</td>
<td>17.9%</td>
<td>No activity</td>
<td>183.7</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>3.8%</td>
<td>No activity</td>
<td>176.5</td>
</tr>
<tr>
<td>Ferulic acids</td>
<td>32.8%</td>
<td>No activity</td>
<td>189.1</td>
</tr>
</tbody>
</table>

Table 3. Activity of different fractions of crude polyphenol extract of finger millet seed coat separated by HPLC on aldose reductase inhibition (Chethan et al., 2008).

10. Bioactive peptides from millets:

Bioactive peptides are short chains of amino acids that are of plant or animal origin and have some regulatory function when released in vitro or in vivo. Several bioactive peptides with beneficial roles have been described in millets. Some of these functions include their role as anti-microbial agents, antioxidants, anti-hypertensive, anti-cancerous and many more.
A study by Xu et al., reported the presence of novel antifungal peptides against *Trichoderma viride*, *Botrytis cinerea*, *Alternaria alternate*, and *Fusarium oxysporum* in foxtail millets seeds. The effect of these peptides was also studied on the morphology of *Alternaria alternate*. These peptides were shown to damage the cell wall of the fungi resulting in cell wall thickening, deformed cells, cytoplasm retraction in hyphae and mycelium death. So they proposed that the said activity of these peptides may be mediated by inhibition of enzymes involved in fungal cell wall synthesis (Xu et al., 2011).

The ability of bioactive peptides to chelate metal or donate hydrogen electron is believed to be responsible for their antioxidant activity. Further it has shown that shorter peptides are more efficient as antioxidants due to their easier accessibility to such systems in contrast to larger peptides. Amino acids such as histidine, leucine, glycine and proline are commonly found in peptides with antioxidant activity. A study led by Agrawal et al., identified two novel peptides with significant antioxidant activity from finger millet seeds. Protein extracts were prepared from these seeds which were further digested in vitro by pepsin and trypsin enzyme. Trypsin digested hydrolysate showed better hydrolysis. Therefore, it was further fractionated by chromatographic techniques and analysed for antioxidant activity. The results indicate that the action of intestinal enzymes can release bioactive peptides from millet-based food. These peptides can further be explored for their bioavailability and their incorporation in food as functional ingredients (Agrawal et al., 2019). Amadou et al also identified three peptides sequences released by in vitro fermentation of foxtail millet seeds using *Lactobacillus paracasei*. These peptides also showed considerable antioxidant activity in vitro. Table 4 gives a brief outline of the identified peptides.

<table>
<thead>
<tr>
<th>Millet</th>
<th>Method of production</th>
<th>Peptide sequence</th>
<th>Properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foxtail millet</td>
<td>Fermentation by <em>Lactobacillus paracasei</em></td>
<td>SGYYMH, LGTFQN</td>
<td>Antioxidant, resistant to trypsin action</td>
<td>Amadou et al., 2013</td>
</tr>
</tbody>
</table>
Finger millets | Digestion by trypsin | TSSSLNMAVRGGLTR, STTVGLGISMRSASVR | Antioxidant | Agrawal et al., 2019

Table 4. Bioactive peptides identified from millets.

11. Conclusion and future prospects:

India is one of the largest producers of millets in the world. Millets are a reservoir of organic compounds that offer a number of health benefits. They had been shown to possess antimicrobial, antioxidant, anti-cancerous, anti-diabetic and a number of other health related properties. However, in our country, the main focus is on the cultivation of pearl and finger millets which is also declining with a decrease in the total area of cultivation under these millets. Other small millets are also cultivated in certain regions however on a very small scale and mainly to be used as bird feed. Under such a scenario, it is possible that without realizing the true potential of millets we in the near future may totally neglect them. Therefore, it is necessary to create awareness among the consumers about millets as alternate sources of energy and work in the direction of bringing them in mainstream agriculture.

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REFERENCES:


CHAPTER-4

Diversity of Cotton leaf Curl Virus and its Management Strategies

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ABSTRACT: Begomovirus infection is one of the biggest threat to the agricultural crops worldwide. This virus resides in alternative host when the prominent crops are not available. Cotton plants are the worst affected candidate of begomovirus infection which causes disease called as Cotton Leaf Curl Disease (CLCuD). Due to the infection of the CLCuD, significant loss of cotton crops has been observed. In this chapter, we will discuss several characteristics of cotton leaf curl virus (CLCuV) and various measures to control its infection.

Keywords: Gossypium; Cotton Leaf Curl Disease; Cotton Leaf Curl Virus; Bemisia tabaci; Begomovirus; Geminiviridae; Preventive measures.

Introduction:

Cotton plant (Gossypium sp) is a shrub and is belongs to the Malvaceae family. Cotton is an angiosperm, dicotyledonous, perennial plant but it is cultivated annually. After the harvest, the plant is plough and destroyed. There are approx. 1500 species of cotton known so far. Most widely cultivated cotton species are as follows:

- Gossypium hirsutum.
- Gossypium arboreum.
- Gossypium herbaceum.
- Gossypium barbadense.
Cotton is a natural fiber which is mainly composed of cellulose. The fiber is twisted and ribbon like in shape. Cotton is cultivated in the areas with warm climate. Growth rate of cotton plants is mainly depends on temperature. The optimum temperature for the cotton crop development lies in the range 16ºC to 35ºC. Temperature below or above this range may cause delay in the crop development.

Healthy plant grows up to 1 to 1.5 meter in height. Leaves are broad heart shaped, having 3 to 5 lobes and coarse veins. Plants have many branches with one as main central stem.

Diseased plant which is infected with CLCuV becomes dwarf. Leaves become thick gives leathery texture, look wilted, veins swelling and enation are significant. Young leaf becomes yellowish. Characteristic vein darken symptom can be seen under light. Diseased plant gives bushy appearance.
Fig 02: Picture of healthy cotton leaf.

Fig 03: Picture of diseased cotton leaf infected by the CLCuD.

This virus is vectored by whitefly (Bemisia tabaci) in persistent circulative manner. Bemisia tabaci conventionally known as the whiteflies or silver leaf whiteflies are agricultural pests. This species thrives in temperate habitat world-wide and cannot survive in cold environment.
Female *B. tabaci* lays 100-150 eggs on either side of leaf. The color of eggs is yellow/orange and are minute cigar in shape which is laid in groups. Hatching occurs in two weeks with nymphal instars those are pale yellow in color are also known as crawler. From pupae, adult formation takes place after 1 week is white in color. White flies gives rise to 11 generations every year.

**Family Geminiviridae:**

The virus family Geminiviridae are group of plant viruses having very wide host range, from monocots to dicots. These viruses are responsible for significant economic damage to many important crops like
tomatoes, beans, squash, cassava and cotton. Presently there are currently 322 species well known.

Table 01: Following are the known genera of the family **Geminiviridae**
till date, these are:

<table>
<thead>
<tr>
<th>Genera</th>
<th>Transmitted by</th>
<th>Infected host in general</th>
<th>Type species</th>
<th>Types of genome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Becurtovirus</strong></td>
<td>Leafhopper</td>
<td>Dicots</td>
<td><em>Beet curly top Iran virus</em></td>
<td>Monopartite</td>
</tr>
<tr>
<td><strong>Begomovirus</strong></td>
<td>Whitefly</td>
<td>Dicots</td>
<td><em>Bean golden mosaic virus-Puerto Rico</em></td>
<td>Either monopartite or bipartite</td>
</tr>
<tr>
<td><strong>Capulavirus</strong></td>
<td>Aphid</td>
<td>Dicots</td>
<td><em>Euphorbia caput-medusae latent virus</em></td>
<td>Monopartite</td>
</tr>
<tr>
<td><strong>Curtovirus</strong></td>
<td>Leafhopper</td>
<td>Dicots</td>
<td><em>Beet curly top virus</em></td>
<td>Monopartite</td>
</tr>
<tr>
<td><strong>Eragrovirus</strong></td>
<td>Not Identified</td>
<td>Monocot</td>
<td><em>Eragrostis curvula streak virus</em></td>
<td>Monopartite</td>
</tr>
<tr>
<td><strong>Grablovirus</strong></td>
<td>Treehopper</td>
<td>Dicots</td>
<td><em>Grapevine red blotch virus</em></td>
<td>Monopartite</td>
</tr>
<tr>
<td><strong>Mastrevirus</strong></td>
<td>Leafhopper</td>
<td>Monocots</td>
<td><em>Maize steak virus</em></td>
<td>Bipartite</td>
</tr>
<tr>
<td><strong>Topocuvirus</strong></td>
<td>Treehopper</td>
<td>Dicots</td>
<td><em>Tomato pseudo curly top virus</em></td>
<td>Monopartite</td>
</tr>
<tr>
<td><strong>Turncurtovirus</strong></td>
<td>Leafhopper</td>
<td>Dicots</td>
<td><em>Turnip curly top virus</em></td>
<td>Monopartite</td>
</tr>
</tbody>
</table>

**Begomovirus:**

The name *Begomovirus* is obtained from BGMV (Bean Golden Mosaic Virus). This genus consists of approximately 300 species which makes it largest genus among the **Geminiviridae** family. Begomovirus is transmitted by the host insect vector *Bemicia tabaci*.
Fig 05: Diagramatic representation of Geminiviruses.

Fig 06: Diagramatic representation of bipartite genome DNA-A.
Begomovirus are typically geminate with two incomplete icosahedral symmetry (twin quasi-isometric), ~38nm long and ~32nm in diameter consisting ~22 capsomers per nucleocapsid. Begomovirus can be monopartite as well as bipartite. The monopartite begomovirus consist of
single ssDNA which is identified as DNA-A. DNA-A component consists of 6 ORF’s in a conserved harmonization associated with the virion strand (V1 & V2) and complementary strand (C1, C2, C3, & C4).

While the bipartite begomovirus consists of a pair of ssDNA identified as DNA-A and DNA-B. DNA-A component comprise of 6 ORF’s in a conserved disposition linked with virion strand (AV1 & AV2) and complementary strand (AC1, AC2, AC3 & AC4). The DNA-B component codes for the movement protein and nuclear shuttle protein BC1 & BV1 respectively. The common region (CR) disperse the bidirectional transcriptional unit of both DNA-A & DNA-B in the highly conserved non-coding region.

The monopartite are usually identified with the satellite molecules (α, β and δ) along.

Fig 09: Diagramatic representation of Betasatellite.
Fig 10: Diagramatic representation of Alphasatellite.

Fig 11: Diagramatic representation of Deltasatellite from new world.
Specifications of these satellites are:

A. Alphasatellite: Related to the Rep encoding complex of nanovirus but its contribution towards infection is not clear.
B. Betasatellite: It contains sequences which is non-homologous to virus but TAATATT↓AC seq. which is located in the loop within the intergenic region. Betasatellite modulates virulence by clamping down the host gene silencing.

C. Deltasatellite: This does not codes for any protein

**Lifecycle of Begomovirus:**

When silver leaf whitefly (*B. tabaci*) interacts with the cotton plant leaf, the transfer of CLCuV geminate particles takes place. The ssDNA of CLCuV is the transferred into the cytoplasm. From there, CLCuV ssDNA is then enters the nucleoplasm via ssDNA-NSP complex. This complex is formed when CLCuV ssDNA interacts with the nuclear shuttle protein. CLCuV ssDNA undergoes replication by the involvement of viral replication protein (REP) and geminivirus replication accessory protein (REn) which gives rise to the dsDNA replicative form (RF). RF acts as a template for the replication and transcription which may be or may not be methylated. This replication undergoes by rolling circle mechanism. Transcription activator protein (TrAP) and viral replication protein (REP) helps dsDNA (RF) undergo transcription for the synthesis of the viral m-RNA. CLCuV m-RNA codes for several viral proteins like REP, REn, AC1, AC2, AC3 etc.
Fig 14: Schematic representation about the life cycle of Begomoviruses. 
(Adopted from: Hashmi F. et. al.; doi: 10.13140/RG.2.2.14344.16643.)

Also, the ds-DNA (RF) when turn into ss-DNA, interact with NSP to form ssDNA-NSP complex which shuttles the viral ssDNA in between the nucleus and the cytoplasm. In the cytoplasm ssDNA-NSP complex interacts with the movement protein (MP) which transports ssDNA-NSP complex towards the periphery of the cell and helps to move across the cell wall which causes infection in the surrounding cells.
Fig 15: Diagramatic representation of GroEL molecular chaperon.

When ssDNA of CLCuV interacts with the coat protein (CP), forms the capsid of the geminate virus particle. This protein checks the virus transport through the intestinal wall of Bemicia tabaci into the hemocoele where coat protein binds with a molecular chaperon GroEL analogue produced by endosymbiont bacteria to form CP-GroEL complex. The CP-GroEL complex provides protection to the CLCuV DNA from degradation.

Table 02: List of various sequences of CLCuV isolates from GenBank.

<table>
<thead>
<tr>
<th>Name of Virus isolates</th>
<th>Genbank ID</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accession</td>
<td>Authors</td>
<td>Title</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>GU112081</td>
<td>Venkataravanappa V, Reddy CL, Jalali S, Reddy MK</td>
<td>Molecular characterization of distinct bipartite begomovirus infecting bhendi (Abelmoschus esculentus L.) in India</td>
</tr>
<tr>
<td>GU112004</td>
<td>Venkataravanappa V, Reddy CL, Jalali S, Reddy MK</td>
<td>Molecular characterization of distinct bipartite begomovirus infecting bhendi (Abelmoschus esculentus L.) in India</td>
</tr>
<tr>
<td>FJ210467</td>
<td>Nawaz-ul-Rehman, M.S. and Fauquet, C.M.</td>
<td>Diversity of begomoviruses in cotton</td>
</tr>
<tr>
<td>EU384575</td>
<td>Rehman, M., Mansoor, S. and Fauquet, C.</td>
<td>Symptomless Reservoirs of Begomoviruses</td>
</tr>
<tr>
<td>AY705380</td>
<td>Reddy RC, Muniyappa V, Colvin J, Seal S</td>
<td>A new begomovirus isolated from Gossypium barbadense in Southern India</td>
</tr>
<tr>
<td>Accession Number</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Accession Number</td>
<td>Authors and Descriptions</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------------</td>
<td></td>
</tr>
<tr>
<td>AF363011</td>
<td>Radhakrishnan,G., Malathi,V.G. and Varma,A. Fulfilling Koch's postulates and molecular characterization of cotton leaf curl</td>
<td></td>
</tr>
</tbody>
</table>

Fig 16: Phylogenetic relationship among the various isolates.

General approaches to overcome the CLCuD:

Following are the general approaches to get rid of CLCuD:

A. Removal and destruction of the infected plant debris.
B. Removal of the weed host and other plants that acts as an alternative host to begomovirus.
C. Performing crop rotation by planting non-host crops.
D. Cultivation of the disease resistant variety like HHH-223, H-1117, H-1226, etc.
E. By spraying 2 to 3 times with monocrotophos [Dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate].

**Modern Scientific approach towards CLCuD:**

Following are the general scientific approach those are adopted for the research purpose towards CLCuD:

A. RNA silencing: This technique involves suppression of gene expression by sequence specific deterioration. This technique is known as PTGS (*Post Transcription Gene Silencing*) in plants, RNAi (*RNA Interference*) in animals and quelling in fungi. Crucial molecules are Dicer and AGO (Argonaute). Gene silencing follows three different pathways:

(i) siRNA silencing: When dsRNA or hairpin RNA (hpRNA) interacts with DICER forms ~22nt RNA fragments called as siRNA duplexes. DICER is encoded by DICER1 gene and is called as endoribonulease dicer or helicase with RNase motif. These siRNA duplexes interacts with AGO (Argonaute), DICER and TRBP (Trans-activation response RNA Binding Protein), leads to the formation of RISC (RNA induced silencing complex) and the passenger strand got cleaved. This RISC when binds to the target RNA forms siRNA/mRNA complex which leads to the degradation of the target mRNA and as a result, gene silencing takes place. Later, the siRNA/RISC complex undergoes recycling back to RISC and siRNA undergoes RdRP (RNA dependent RNA polymerase) mediated amplification. Thus, gene silencing via siRNA takes place.
(ii) Silencing of mRNA by miRNA: By the transcription of miRNA gene, synthesis of a hairpin like Pri-miRNA takes place which when undergoes through the influence of DROSHA changes to the Pre-miRNA. On the nuclear membrane, there’s present a protein calles as Exportin5 which helps Pre-miRNA to travel across the nuclear membrane and to enter the cytoplasm.
Inside the cytoplasm, Pre-miRNA got to interact with the DICER which removes the hairpin like structure and this newly modified structure is known as miRNA. This miRNA comes under the influence of DICER, Argonaute and TRBP (Trans-activation response RNA binding protein) and this leads to the formation of the miRISC and the passenger strand got degraded. With the target mRNA, there’s occur an incomplete complementary binding of the guide strand. Finally, there’s occur translational repression or the target mRNA cleavage.

Fig 18: Diagramatic representation of miRNA mediated gene silencing.
(iii) DNA methylation and suppression by transcription:

Suppression by transcription is similar to the previous approaches, but the significant difference is due to methylation of the RNA duplexes on the ribose of the last nucleotide by a methyltransferase enzyme HEN1 takes place just before the formation of RISC. HEN1 methylates both siRNA and miRNA.

B. Virus Induced Gene Silencing (VIGS): RNA silencing technique used in plants which uses viral vectors that carries a fragment of the gene of interest with the aim to develop ds-RNA that initiates the targeted gene silencing. The technique involves agro-infiltration of VIGS vector which is carrying the gene of interest into seedlings at 2 to 3 leaf stage.

Fig 19: Diagramatic representation of VIGS.
C. Crispr/Cas9: By the use of the short stretch of guide RNA, Crispr/Cas9 system allows to manipulate any genomic sequence. Crispr system depends upon Crispr RNA (cr-RNA) which anneal to the Transactivating-Crispr RNA (tracr-RNA) which leads to the creation of the guide RNA (g-RNA) which directs sequence specific silencing and cleavage of the foreign DNA through Cas-protein.

Fig 20: Diagramatic representation of gene silencing by CRISPR/Cas9.

Conclusion:
In this chapter, we have discussed about the cotton plant and associated leaf curl disease and various methods to control begomovirus infection and also the techniques associated with them. Also, modern biotechnological approaches based on RNAi has been applied to the wide range of species of insects and viruses thus brings high surmise for the future work and is powerful and safe if applies properly. The Crispr/Cas9 based approach provides wide and durable viral resistance, since it is easy to target conserved region of viral genome and are customizable according to the target virus.

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References: (Vancouver style)


CHAPTER-5

EFFECT OF DIFFERENT LIGHT COLOUR SOURCES ON GROWTH AND DEVELOPMENT OF PLANTS

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ABSTRACT: Plants are an integral part of our community as they provide us with plethora of useful substances starting from food, clothes to biofuel. Therefore constant efforts are being made to enhance the yield of plants by focusing on the factors affecting their growth and development. Light plays an important role in growth and development of plants and affects the quality of plant. Plant yield is reported to significantly increase on exposure to red light supplemented with blue light. There is maximum absorption and thus maximum photosynthetic yield in presence of blue and red visible light spectra. Plants also absorb cyan, orange, yellow, UV and far-red light which also has impact on photosynthetic rates, yield and growth. Hence it can be concluded that white light might be more suitable for plant growth. Different crops, vegetables and floricultural plants have shown the effect of different types of light on growth, weight, leaf area and development. This study highlights the use of different colours and its effect on plant growth.

Keywords: Far-red; Light emitting diodes [LEDs]; Photoreceptors; Photosynthetic.

INTRODUCTION
Photiosynthesis is a fundamental process in plants through which it converts light energy into chemical energy which in turn is used to drive all the metabolic and physiological processes in plants. Plants do not absorb entire wavelength of light (solar radiation), they are very selective in absorbing defined ranges of wavelength according to their requirements through phototropin, cryptochrome and phytochrome receptors. The perceived light signals lead to varied physiological and metabolic processes by affecting the transcription, translation and thus mRNA stability in plant cells [¹].

The most important part of the light spectrum for plant growth and development is 400nm to 700 nm which is known as photosynthetically
active radiation (PAR). These radiations are absorbed by chloroplast, a semi-autonomous organelle characteristic of a plant cell which is responsible for energy transformation and photosynthesis. Various research studies has shown that morphological characteristics of chloroplasts are unstable, hence it is able to adapt to different environmental conditions and different or changes in light conditions affects the ultrastructure of the organelle leading to changes in efficiency of photosynthesis \[2\]. Chlorophyll a (absorbance peaks at 430nm and 662nm) and chlorophyll b (absorbance peaks at 453 and 642nm) are the major photosynthetic pigments in chloroplast and absorb maximum in blue and red regions of light spectra.

Red light is important for the development of the photosynthetic apparatus and accumulation of starch whereas blue light is important in the formation of chlorophyll, chloroplast development, stomatal opening and photomorphogenesis. Plants also have other photosynthetic pigments, known as antenna pigments (such as the carotenoids Xanthophyll, phycobilisomes etc.), which participate in light absorption and increase the spectrum of absorption thus playing a significant role in photosynthesis.

![Absorption spectrum of different plant pigments](image)

**Fig.1 - Absorption spectrum of different plant pigments** \[3\]

With ever increasing world population and majority of it residing in urban settings it is becoming the need of the hour to establish indoor
farming or urban agriculture as an alternative approach for providing food in these areas thus reducing the pressure on rural agriculture and decompensate land loss \[4\]. Indoor farming is a type of urban agriculture in which the entire produce is grown in controlled and specified environmental conditions including temperature, humidity, soil factors, pesticides, insecticides, irrigation etc. all regulated according to the need of the plant species in concern.

Light is one of the major environmental factors plays an important role in plant growth and has direct effects on plant morphology and physiology. In the beginning of indoor farming high intensity sodium lamps, fluorescent lamps, metal halide lamps and other similar artificial lighting sources were used to provide as near as possible natural photon flux density to the plant, but soon there disadvantages started to pile up, the photon flux density was not accurate and reduced with time \[4\], they could not be used as point source lighting due to high surface temperatures, some high intensity discharge lamps provided unsuitable spectra which limited their use in plant production. With recent advances in lighting technology and advent of light emitting diodes (LEDs) the concept of indoor farming has been revolutionized. Light emitting diodes harbours a number of advantages over traditional lighting sources. They are small, light-weight, have narrowly centred spectrum, solid-state construction, low energy consumption and heat output, superior safety, longevity etc. \[5\].

Application and importance of light emitting diodes in plant growth studies is immense as it has made possible to study the effect of individual or combined light wavelengths on plant cells or tissues in vitro. LEDs can be precisely manipulated to emit light of certain specified wavelength hence making it possible to study the effect of that light on plant. The lighting boards can be manufactured in such a way that one or more light colours can be incorporated while defining the entire and individual photon flux density of the board and light colour respectively \[1,5\]. This study highlights the use of different colour lights or LED and its effect on plant growth.

1. **EFFECT OF LEDS ON PLANT GROWTH,**
Leaves of the plants readily absorb red and blue light with minimum reflectance therefore plants grown in only these two lighting sources (solely or combined) appear greyish or purplish to the eyes (Fig 2a, 2b) making it difficult to visualise any disease or infection the plant might be facing. However for green light plants have minimum absorbance and maximum reflectance and it is the reason due to which plants appear green to us. Therefore supplementing green or white light along with red and blue improves the visual efficiency to the human eyes resulting in better analysis of the plant condition. Also studies show that illuminating plant with green light has significant effects on the physiological growth of the plant. Kim et al. showed that supplementing green light along with red and blue promoted growth in lettuce [5].

Fig 2. Cannabis Plants under different light condition a) Blue light  b) Red light  c) Dual (red plus blue) light d) white light [6]
Red light is of immense importance to plants as it falls both under the absorption range of chlorophylls and phytochromes \[^5\]. Enhanced photon flux density of red light can be provided by LEDs than sun and increased absorption by chlorophyll leads to rise in plant yield and phytochromes leads to phototropism, root growth, seed germination, elongation of hypocotyls and cotyledons etc. by absorbing red as well as far-red light. It is also reported that red light is the basal component of lighting spectra and normal plant growth and photosynthesis is observed in solely red light is provided to the plant \[^9\].

Plants respond to red light with phytochrome Pr (inactive) and to far red light by phytochrome Pfr (active). These two forms are interconvertible, Pr absorbs red light at 660nm and converts to Pfr whereas Pfr absorbs far-red light at 730nm and converts to Pr form. Ratio of R: FR (known as Phytochrome stationary state; PSS) is received by plant is significant and guides the conversion of pigments and thus changes observed in the plant. Kalaitzoglou et al. showed that PSS decreasing PSS results in increase in total dry weight (PSS>0.80), increase in petiole angle, increase total leaf area per plant \[^11\].

M.Adil et al. reported that dry weight, wet weight, secondary metabolite concentration and other parameters such as protein content and antioxidant enzyme expression varied widely with light conditions. They supplied 33 µmol ·m\(^{-2}\)·s\(^{-1}\) PPFD (photosynthetic photon flux density) to callus and showed that compact callus texture was obtained in 100% Blue light which on sub culturing produced microshoots, granular texture of callus was obtained in fluorescent white light whereas non-regenerative watery callus was obtained in red and dichromatic (red+ blue = 1:1) lightening. They also reported that highest content of flavonoids and phenolic content was reported from plants grown in dichromatic light \[^1\]. In secondary metabolite production similar result was obtained by Zhang et al. they cultivated two different genotypic varieties of strawberry (\textit{Fragaria × ananassa}):
‘Toyonaka’ (8x) and ‘Tokun’ (10x). They showed that anthocyanin production was affected by genotype as Toyonaka produced more anthocyanin than tokun but they also concluded that light treatments also play a major role in metabolite production. Both the genotypes produced higher amounts of anthocyanin in dichromatic light (red+blue = 1:1, 100 µmol · m⁻²·s⁻¹) treatment [8].

Pham et al. studied leaf chlorosis, epinasty, carbohydrate content and growth of tomato under different ratios of red and blue light and white light. A uniform, continuous photon flux density of 200 ± 5 µmol · m⁻²·s⁻¹ was provided throughout the experiment. It was observed that fresh and dry weight of plant increased with increasing percentage of red PPFD. Treatments with 100%, 75% and 50% red light had higher dry weight whereas in contrast lowest dry weight gain was observed in monochromatic blue light condition indicating that this light condition inhibited plant growth. Similarly leaf area of plants exposed to sole blue light and white light has smaller surface area than other treatments further proving that these light conditions had strong adverse effects on the tested tomato plants. They also showed that starch content was maximum in monochromatic red light almost comparable to continuous white light. From the results obtained on content of H₂O₂ and O₂⁻ the researchers concluded that oxidative damage by these species might not be directly caused by leaf chlorosis as monochromatic blue light showed low content of H₂O₂ and O₂⁻ but highest degree of leaf chlorosis [13].

Table 1: Effect of red light and blue light on growth of plants and secondary metabolites production
Silvestri et al. used commercially available LED lamps provided different spectrum of blue, red and green light in different combination with one dominant wavelength and observed increase in shoot length under higher percentage of red light in both the hazelnut varieties being tested. They also showed that chlorophyll and anthocyanin contents were also strongly influenced by the light treatments given. Lowered chlorophyll content was seen in plants
grown under red LEDs whereas highest anthocyanin and phenolic content was observed under red illumination [10].

Apart from growth and secondary metabolite production different light colours also affect the flowering in plants. Flowering is a phenomenon which is majorly controlled by the phytochrome photoreceptor and thus is turn is affected by the red and far red light, though low intensity blue light might also affects flowering in some plant species. Based on the exposure required by a plant to flower they are

characterized as short day and long day plants, but contrary to the perception that this exposure refers to the exposure to light, the flowering in plants is affected by the length of dark period they encounter. Any interruption in this dark period leads to changes in flowering. Park et al. studied the effect of this interruption on 10 hours short day plant *Dendranthema grandiflorum*. They interrupted the 14 hours long night period for 4 hours with two light colours (combination of blue, red, white and far-red) on rotation of 2 hour each at low intensity of 10 μmol·m$^{-2}$·s$^{-1}$ and studied its effect not only on flowering but also on morphogenesis and gene expression. For flowering they concluded that day to initiation of formation of visible flower buds was reduced in treatments with far-red and blue light irrespective of the order in which they were given and thus showed a synergetic effect in chrysanthemum [14]. Since controlled environment culture has majorly benefitted the floriculture industry studies have been done not only on production of flowers but also to study the effects of different lighting post harvestation also. With similar approach Aalifar et al. studied and showed that blue light increases the vase-life of Carnation (*Dianthus caryophyllus* cv. ‘Moon light’) cut flower by 5 days post-harvest as opposed to white light whereas red light even further reduced the vase-life than was seen under white light. This improvement in vase life seen as blue light reduced the concentration of oxidative damaging species such as H2O2 and malondialdehyde by increasing the concentration of antioxidant enzymes [15]. Yoshida et al. also showed that anthesis was achieved earlier in plants treated with red light of different peaks (630nm, 660nm, 685nm) compared to blue lights with different
peaks (405nm, 450nm, 470nm) in both everbearing strawberry (*Fragaria × ananassa* Duch. ‘HS138’ and woodland strawberry (*Fragaria vesca* L.) [16].

Apart from vegetables and floricultural plant species studied have also been conducted on crop plants of economic importance. Gerald et al. showed that in winter barley (*Hordeum vulgare*) at photosynthetic photon flux density of ~216 µmol ·m⁻²·s⁻¹ addition of far red light to the daylight fluorescent spectra reduced the lag period of initiation of flowering and enhanced the rate of development apart from increasing the dry weight of the plant [15]. Monostori et al. also showed that flowering is delayed when wheat (*Triticum. aestivum* ssp. *aestivum* cv. ‘Mv Kikelet’) plants were only subjected to blue light compared to those grown in white or red light [18]. Hence it is correctly established that exposure of red and far light is necessary for flowering.

Thus it is evident that quality, quantity (flux density), photoperiod and colour of light have a range of effects and responses in plants. Every plant species react to its environmental surrounding uniquely and thus shoe different responses in the form of morphogenesis, flowering, growth and development. Several studies have been done to understand the effect of light on these responses if it difficult to generalize the possible results and outcomes of different lighting treatments on plant species as individual plant species of different genera responds uniquely and thus differently.

### 2. ACKNOWLEDGEMENT

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**ABREVIATIONS**

UV- Ultraviolet Radiation
LED- Light Emitting Diodes
mRNA- Messenger Ribonucleic Acid
Pr & PFr- Two photo-interconvertible forms of Phytochromes, Pr absorbs red light whereas Pfr absorbs far-red light. R- Red light
Fr- Far red light PSS-

**Phytochrome** photostationary state PPFD- Photosynthetic photon flux density

**REFERENCES:**


CHAPTER-6
MICROBES AS A SOURCE OF SUSTAINABLE ENERGY
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ABSTRACT: The quick declining concentration of fossil fuels has triggered exploration of new alternative fuels which replaces the traditional non-renewable energy sources. Biofuels have gained importance within last few years as an alternative source of fuel which reduces our dependence on fossil fuels and also counters the expanded deposition of greenhouse gasses in the atmosphere, which has prompted considerable climatic changes. One such approach is the use of microorganisms to change substrates into biofuels and high value-added products, and at the same time taking advantage of various microbial biomass components to produce other products of interest, as a coordinated process. This way maximizes the economical value of the entire process, with the desired reduction of the waste streams produced. It is expected that this coordinated system makes the biofuel production monetarily economical and competitive in the near future. The rapid advances seen in the development of these technologies will almost undoubtedly facilitate the efficient and reliable production of systems for novel biofuels.

KEYWORDS: Biofuel; micro-organisms; microalgae; yeast

INTRODUCTION

In an effort to combat climate change, to aid energy independence, and to counteract diminishing supplies of fossil fuels, there has been a resurgence of research on renewable and carbon-neutral energy sources. Biofuels production captures the energy of the sun as chemical energy in the bonds of biologically produced materials. All routes to biofuels hence start with photosynthesis, and it is at that point where they diverge.

Heterotrophic microbes have been used in industrial processes for centuries, the alcohol fermentation with Saccharomyces cerevisiae being the oldest example. A more recent example is the production of bioplastics by Escherichia coli. These two model organisms are being used to develop most of the new microbial fuels. Although heterotrophic microbes require organic feedstocks, fuels produced by these organisms approach carbon neutrality, because the feedstocks, in contrast to petroleum, are synthesized
by recent plant based carbon dioxide fixation. However, the organic feedstock is the major cost driver for using heterotrophic microbes to produce fuels. Therefore, to avoid any waste of organic carbon, the pathways and fermentation processes leading to a particular fuel must be as efficient as possible.

**BIOFUELS**

Biofuels have been around longer than vehicles have; yet ordinary gas and diesel have since a long time ago kept them on the fringe. Spikes in oil costs, and now worldwide efforts to prevent the most exceedingly terrible impacts of environmental change, have lent new urgency to search for clean, renewable fuels.

Our road travel, flights, and shipping account for about one-fourth of the world's ozone harming substance outflows, and transportation today remains intensely reliant on petroleum derivatives. The idea behind biofuel is to replace conventional fuels with those produced using plant material or different feed stocks that are renewable. [1]

There are different methods for making biofuels, but they generally use chemical reactions, fermentation, and heat to break down the starches, sugars, and other molecules in plants. The resulting products are then refined to deliver a fuel that cars or different vehicles can use. [2]

**WHAT IS BIOFUEL MADE FROM?**

A variety of materials, or feedstocks, can be utilized to make biofuels. In spite of the fact that corn and sugarcane are well-established ethanol feedstocks, the process of developing the harvests, making composts and pesticides, and preparing the plants into fuel expends a great deal of energy—so much energy that there is debate about whether ethanol from corn really gives enough of an environmental benefit to merit the speculation.

So researchers and new businesses are exploring different materials that can possibly fill in as fuel without the accompanying concerns about nourishment supply and ecological effect. Cellulosic ethanol, for instance, uses corn stover, wood waste, or other plant material that would not be used generally. Other potential biofuel feed stocks include grasses, algae, animal waste, cooking oil, and wastewater sludge, however research continues to
find the most proficient and cost-effective approaches to change them into usable fuel. [1]

ADVANTAGES OF BIOFUELS

1. **Cost Benefit:** As of now, biofuels cost the equivalent in the market as fuel does. Nonetheless, the overall cost benefit of utilizing them is a lot higher. They are cleaner fuels, which means they produce less emissions on burning. Biofuels are versatile to current engine designs and perform very well in many conditions. This keeps the engine running for longer, requires less maintenance and cuts down generally pollution check costs. With the expanded interest of biofuels, they have a capability of getting to be less expensive in future also. In this way, the utilization of biofuels will have a potential of becoming cheaper in future as well.

2. **Easy To Source:** Gasoline is refined from raw petroleum, which happens to be a non-renewable resource. Although current reservoirs of gas will support for a long time, they will end soon. Biofuels are produced using a wide range of sources, for example, manure, waste from crops and plants grown specifically for the fuel.

3. **Sustainable:** Most of the non-renewable energy sources will end up in smoke one day. Since the greater part of the sources like manure, corn, switch grass, soybeans, waste from crops and plants are renewable and are not likely to run out at any point in the near future, making the use of biofuels efficient in nature. These crops can be replanted over and over.

4. **Reduce Greenhouse Gases:** Fossil fuels, when burnt, produce huge amount of greenhouse gases for example carbon dioxide in the atmosphere. These greenhouse gases trap daylight and cause planet to warm. The burning of coal and oil increases the temperature and causes global warming. To lessen the effect of greenhouse gases, individuals around the globe are using biofuels. Studies suggest that biofuels reduce greenhouse gases up to 65 percent.

5. **Economic Security:** Not every nation has enormous reservoirs of crude oil. For them, bringing in the oil places a huge dent in the economy. If more people start moving towards biofuels, a country can reduce its reliance on fossil fuels. More jobs will be made with a developing biofuel industry, which will keep our economy secure.
6. **Lessen dependence on Foreign Oil:** While locally developed crops have decreased the country's reliance on fossil fuels, numerous specialists believe that it will require a long time to solve our energy needs. As prices of crude oil is touching the sky, we need some alternative energy solutions to reduce our dependence on fossil fuels.

7. **Lower Levels of Pollution:** Since biofuels can be produced using renewable resources, they cause less pollution to the planet. However, that is not the only reason behind why the use of biofuels is being encouraged. They release lower levels of carbon dioxide and other emissions when burnt. In spite of the fact that the generation of biofuels makes carbon dioxide as a byproduct, it is frequently used to develop the plants that will be converted into the fuel. This enables it to become something near a self-supporting system. [2]

**CLASSIFICATION OF BIOFUEL**

Biofuels are generally classified into two categories:

1. **Primary Biofuels:** Essential biofuels are natural biomass, for example, creature animal waste, forest residue, crop residue, firewood and wood chips, kindling and wood chips where the natural matter is used in a natural form. These fuels are utilized to supply cooking fuel, heating or power generation needs in little and huge-scale industrial applications.

2. **Secondary Biofuels:** Secondary biofuels are adjusted primary biofuels which have been processed and delivered in the structure solids (charcoal), fluids (ethanol, biodiesel, bio-oil) or gases (biogas, synthesis gas). These energize have a wide scope of uses which incorporates transport and high-temperature modern procedures. Optional biofuels are additionally ordered into three generations: first, second and third generation biofuels predominantly based on feedstock utilized. [1]

The original biofuels are delivered from eatable harvests like sugars, grains or seeds and require a relatively straightforward procedure for generation of product. Bioethanol and biodiesel are notable first-generation biofuels[1]. Bioethanol is for the most part delivered from sugar-containing boring harvests or oat crops. Fermentation of sugar happens by catalysts created by yeasts. The yeasts mostly convert glucose to ethanol which can be utilized
as fuel [2]. Biodiesel is delivered from straight vegetable oils or oleaginous plants by transesterification forms. The vegetable oil is changed over into fuel which can be utilized legitimately as a fuel by modified engines. Trans esterification utilizes enzymatic catalyzers or acids, soluble and methanol or ethanol and produces glycerine and fatty acids (biodiesel) as the buildup [3,4,5]. The promising part of this fuel is its effortless and economic conversion into fuel while the most widely recognized concern is that as the creation limit increments, so, therefore, its opposition with agribusiness for arable land utilized for nourishment generation. This expanded weight can prompt nourishment deficiencies uncommonly in creating nations. [6]

The second generation biofuels are delivered from non-palatable yields like lignocellulosic materials (straw, grass), timberland deposits, wood handling waste and so on [6]. Lignocellulosic biomass is bounteous in nature however just a little segment of it very well may be used. Hypothetically, these wellsprings of biomass can give a very decent measure of vitality [7]. Bioethanol, biobutanol, syndiesel, and so forth are delivered from lignocellulosic materials [8]. The principle favourable position of this fuel is that as it is created from non-palatable harvests, it is effectively accessible and limits the immediate nourishment versus fuel rivalry related with original biofuel. Notwithstanding, the modern scale up of second generation biofuel encountered the fundamental obstacle because of innovative issues which included significant expense, medium yield, trendsetting innovations and offices to help transformation process. [9]

**BIOFUEL PRODUCTION BY MICRO-ORGANISMS**

1. **MICRO ALGAE**

Microalgae, perceived as one of the most established living life forms, are alluded to as minute photosynthetic living beings, which live in fresh and saline water. They convert water and carbon dioxide to natural carbon by methods for photosynthesis and produce a colossal measure of cell vitality inserted with sugar, protein, and lipid. Microalgae require inorganic parts for their improvement which comprise their cell divider. Basic components incorporate nitrogen and phosphorous. Nitrogen is routinely provided as nitrate however alkali and urea are likewise utilized. Then again, phosphorous is given in fairly excess sum in light of the fact that not all phosphorous is bio-accessible. Microalgae development is not only
dependent on macro-nutrient (C, N, P, O) but they also require major ions (Mg$^{2+}$, Ca$^{2+}$, Cl$^{-}$ and SO$_{4}^{2-}$) and micro-nutrients (Fe, Mn, Zn, Co, Cu and Mo). [11-12]

Because of quick exhaustion of petroleum derivative overall on account of extreme utilization of vitality sources, microalgae development is seen a deductively appropriate answer for an ecological well-disposed power source. The microalgae development is a spotlight for biofuel producing because of its various positive angles, for example,

1. They do not interfere with human and animal food chains.
2. They have a very rich source of carbohydrate-protein-lipid content.
3. They require less water and can grow in wastewater, freshwater or saline water.
4. They can grow 365 days per year in the presence of sunlight.
5. They can be cultivated in any water containing territory like the sea, ponds, rivers, waste dump area, municipal waste seepage or in wet uncovered terrains.
6. They develop sustainable oxygen generation system.
7. They consume carbon dioxide by taking it up for photosynthesis respiration.
8. Microalgae have short harvesting cycle and produce a high amount of biofuel [13-19].

Biodiesel is an alternative fuel gotten from inexhaustible sources. It is biodegradable and non-dangerous in nature. Biodiesel got from microalgae can be an incredible elective source to the present diesel emergency. Stains with high development rate and oil substance ought to be chosen for biodiesel creation. The biodiesel creation forms FAME (unsaturated fat methyl esters). The biodiesel from microalgae can be either gotten straightforwardly from transesterification of microalgal biomass or created by a two-advance procedure wherein at first lipids are removed and afterwards trans esterified. The procedure of direct transesterification is quick and practical innovation.

Biogas or biomethane is formed due to anaerobic digestion of organic matter. It is mainly composed of methane (55-70%) and carbon dioxide (25-
Microalgae produce biogas in a low yield on account of the affectability of microalgae cells to bacterial debasement and low carbon and nitrogen proportion which prompts the development of alkali.

Hydrogen is a promising future vital energy source since it doesn't transmit ozone-depleting substances and discharges water as a result. Microalgae straightforwardly produce hydrogen from sunlight and water only in anaerobic conditions. Directly, generation of hydrogen is by steam transformation, photo fermentation and photolysis of water-interceded by photosynthetic algae.

Bioethanol can be a substitution for fossil-derived petrol. At present, bioethanol is created from the aging of sugars (like corn) and with new innovation, hydrolysing cellulose and hemicellulose to sugars. Microalgae is a superior alternative than previously mentioned segments as (1) it has high starch content, (2) it lacks lignin, so the processing becomes easier, (3) it has a very simple composition and biomass can be utilized readily, (4) microalgal cells consists of large amount of polysaccharides, which can be converted to sugar and (5) microalgae can be genetically engineered to produce ethanol. [17]

2. BACTERIA

A large number of microbes are available for the production of biofuel including microalgae, fungi, yeast, bacteria. While algae have the ability to produce biofuel feedstocks directly from carbon dioxide and sunlight; yeast is widely used for ethanol production; bacteria have the ability to use both pentose and hexose sugar. [20] Production of biofuel from bacteria requires significant genetic manipulations for economically relevant yields and rates which includes engineering biofuel pathways into genetically tractable model organisms. [21]

Bacteria such as *E. coli* and *Clostridia sp.* are used for the production of bioethanol because of their ability to use both pentose and hexose sugars. *E. coli* produces ethanol by an endogenous process in anaerobic conditions which gives a yield of 0.26 g ethanol/g of glucose while the maximum possible theoretical yield is 0.51 g ethanol/g of glucose. During the process, one mole of glucose is metabolized into two moles of formate, two moles of acetate and one mole of ethanol. The last step involves the reduction of acetyl-coA into ethanol by AdhE. [22]
Some cellulolytic bacteria secrete monomeric cellulases into the medium while some secrete large cellulosome organelles. *Thermoanaerobacter* and *Thermanaerobacterium sp.* can be engineered for high titer ethanol production in non-cellulolytic *Thermoanaerobacterium saccharolyticum* [23] while *Cellvibrio japonicas* have genes that express ethanol production. [24] *Clostridium thermocellum* and *T. saccharolyticum* produce ethanol at titers upto 38 g/L. [25] *Clostridium cellulosolicum* is the only cellulolytic strain engineered to produce isobutanol.[26]

Non-cellulolytic bacteria like *Geobacillus thermoglucosidasius* is capable of cellobiose metabolism. It has thermophilic nature which helps in biomass breakdown and fuel isolation. Theoretically, it can yield >90% ethanol. [27]
3. YEAST

Compared to other types of microorganisms, yeasts particularly *Saccharomyces cerevisiae* is the common microbes used in ethanol production due to its high ethanol efficiency, high ethanol tolerance and ability of fermenting wide range of sugars. Different types of yeast strains have been used in fermentation for ethanol production including hybrid, recombinant and wild-type yeasts. Yeasts can directly ferment simple sugars into ethanol while other type of feedstocks must be converted to fermentable sugars before it can be fermented to ethanol. [24]

Yeast is a genuine little processing factory, capable for changing plant sugars into fuel. It is not impossible to see our cars driven solely by alcohol in the near future. Bioethanol has been identified as mostly used biofuel worldwide since it altogether adds to the reduction of crude oil consumption and environmental pollution.

It can be produced from various types of feedstocks, for example, sucrose, starch, lignocellulose and algal biomass through fermentation process by microorganisms. The enchantment works during an anaerobic procedure (without air) called fermentation. Under the activity of yeast enzymes, sugars (specifically glucose) contained in cellulose or starch is transformed into ethanol.

The aim is to produce second-generation ethanol on an industrial scale. The regular procedures involved in ethanol production are pretreatment, hydrolysis and fermentation. Production of bioethanol during fermentation depends upon several factors, for example, temperature, sugar concentration, pH, fermentation time, agitation rate, and inoculum size. Immobilizing the yeast cells can improve the productivity and efficiency of ethanol. [24]

**CONCLUSION**

The past few years it has been seen a blast in the production of fuel which were derived from isoprenoid alcohol and fatty acids biosynthesis in engineered microbial hosts. With a huge advancement in metabolic modeling results into the continued discovery of new metabolic networks,
also the possibilities of new routes toward biofuels biosynthesis now appear almost limitless.

Biofuels can be produced from biomass by thermochemical means or by fermentation with microbes. Both seem to be about equally land-efficient. Production maturity will be reached more rapidly by new and microbially produced biofuels.

The use of biotechnology for future production of biofuels will include microbial as well as technological / chemical production methods and often a mixture of both. Bioethanol is the start of these strategies for mixing gas. Biofuel production needs to incorporate biomass of entire plants to arrive at high return. This will likewise diminish rivalry with food creation and nature preservation.

Microbial biofuels have extraordinary development potential in process steps such as substrate separation, fermentation, pretreatment, energy coupling and others.

FUTURE PROSPECTS

Biofuels will change our interaction with the environment in a general sense if we are wise and careful, we can shape this tremendous transition to simultaneously improve the world's economic and environmental security and provide more prominent prosperity for rural areas around the globe[6]. We need to devise suitable measurements and strategies to guarantee that biofuels achieve their potential for economic and environmental security. Hence, inappropriate and misleading measurements, for example, net energy must be disposed off. This is a crucial time of transition. Our society needs to contemplate these interrelated issues [25].

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ABSTRACT: A radioactive material when breaks down, it releases energy into the environment which can harm a human body in two ways either by killing the cells directly or by causing mutations to DNA which if not repaired can turn cancerous. Leafy green vegetables in Japan were found to contain up to 22,000 becquerels/kg of iodine-131. Eating a kg of such vegetables would give half the amount of radiation typically received by the average person from the natural environment in a year. Uranium is another radioactive element found in the environment. Depleted uranium (DU) is a waste product of uranium enrichment and is utilized to make tank armor and bullets, and is about 40% less radioactive than natural uranium. It was utilized as armor-piercing ammunition in worldwide military conflicts which results in a lot of known medical problems such as the Gulf war Syndrome as of late as the Balkan Syndrome. Since uranium compounds have poor solubility and lack of information on speciation makes impossible the use of radio ecological models for exposure assessment, biomonitoring be the only way used for assessing exposed persons. The toxicity of uranium includes nephrotoxicity, genotoxicity, and developmental defects. Also, at the molecular level, it raises the biological plausibility of adverse effects on the brain, on reproduction, including estrogenic effects, on gene expression, and on uranium metabolism. The US government is allowed to control the development, regulation and removal of nuclear materials and facilities in the United States under the atomic energy act of 1954. As the damage are irreversible, and possibly cumulative, efforts in the present needs to be more vigorous to limit environmental heavy metal contamination and exposure. This study explored the sources and impact of these radioactive elements.

KEYWORDS: Biomonitoring; Genotoxicity; Nephrotoxicity; Speciation.

1. INTRODUCTION

Radioactive metals are naturally and synthetically produced metals that can produce alpha (α), beta (β), and gamma (γ) rays. These metals can be beneficial for human beings, such as in production of power, nuclear battery, material building, malignant growth treatment but their impact on human health is very harmful. Any atom which has abundant nuclear energy is unstable in the environment and is known as a Radionuclide. Some of the radionuclides that are discharged in very less amount to the
environment are uranium, thorium, potassium, radium, etc and transferred through water or air. (Fig1) Naturally occurring radioactive metals found in trace level in soil, rock, water, plants, and animals are Uranium, Thorium, and radium and These three are responsible for generation of gamma ray and hence there is a direct relationship between concentration of primary radioelement’s and gamma ray exposure. The most abundantly present radioactive element found in different parts of India is Uranium. Some of the other radioactive elements found are Potassium-40, Carbon-14, Radium, and Thorium.

![Diagram](image)

**Fig.1:** Pathway through which radioactive metals enter into the environment and humans.

### 2. SOURCES OF RADIOACTIVE METALS

In recent years it has been observed that, there is quick and increasing advancement of the nuclear industry and the broad utilization of radioactive isotopes. The radioactive materials are released into the environment from different sources such as nuclear industry in a form of waste. The activities submitted either intentionally or incidentally through carelessness or simply
ignorance are one of the major sources of radioactive metals. Below mentioned are the sources of radioactive metals or materials

2.1 **Hospitals:** In nuclear medicine department of a hospital certain radioactive metals are used in various imaging for example, Technetium-99m (Tc-99), Tc-99 is produced due to nuclear reactor operations. It is used in nuclear medicine and medical diagnostics.

2.2 **Nuclear fuel cycle:** Radioactive materials are released into the environment in each part of the nuclear fuel cycle from mining and milling through fuel fabrication, reactor operation and also, reprocessing of spent fuel to the furthest limit of cycle activities as waste management.

2.3 Mining results in releases of naturally occurring radioactive materials (NORM). For instance, the release of the trace quantities of uranium and thorium in coal, when it is burned in power stations.

2.4 **An industrial or research accident:** The major accidents involve emission of radionuclides from nuclear reactors and reprocessing plants in a abundant amount which leave the site contaminated for years at times.. For example, Chernobyl accident. It took place on April 26, 1986 in the Ukraine where a low power engineering experiment was being conducted at the Chernobyl nuclear power plant (NPP). The reactor became unstable which resulted in a nuclear excursion, thermal explosions and fires. The radioactive material released from the accident carries a mass around 6000–8000 kg.

2.5 **Military activity:** This is one of the main sources of global radiological contamination. For example, a nuclear weapons test. Some of the Radionuclide released in atmosphere during nuclear weapon testing is shown in table 1.

2.6 **Releases from reprocessing plants:** One of the major sources of environmental contamination is reprocessing plants and especially during Pu production, the radioactive waste solutions were often discharged into water streams directly. Along with the consistent releases, infrequent accidental releases have also happened throughout the long term. In the most recent decades,
numerous safety measures were acquainted with improved reprocessing, and the current releases in normal working conditions are at levels that ought not to be of concern.

2.7 Releases which occur as a result of a crime: For example, the Goiania accident where thieves, unaware of its radioactive content, stole some medical equipment and as a result a number of people were exposed to radiation. The Goiania accident was a radioactive contamination accident that occurred on September 13, 1987, in Goiania where two thieves stole a medical equipment in which the radiation source was a small capsule containing about 93 grams of highly radioactive caesium chloride salt made with a radioisotope, caesium-137 enclosed in a canister made of lead and steel.

2.8 Phosphate fertilizer industry: Over the top and continuous utilization of nitrogen and phosphorous fertilizers for a considerable length of time have changed over the farming soils into virtual chemical time bombs. The way potassium and nitrogen play an important role for plant growth, phosphorus also is an important for the same role but the phosphate fertilizer industry is a major global concern.

Table 1: Radionuclide released in atmosphere during nuclear weapon tests. [1]

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half-life</th>
<th>Global release (10^{18} Bq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3H</td>
<td>12.33 a</td>
<td>186</td>
</tr>
<tr>
<td>14C</td>
<td>5730 a</td>
<td>0.213</td>
</tr>
<tr>
<td>54Mn</td>
<td>312.2 d</td>
<td>3.98</td>
</tr>
<tr>
<td>55Fe</td>
<td>2.73 a</td>
<td>1.53</td>
</tr>
<tr>
<td>89Sr</td>
<td>50.6 d</td>
<td>117</td>
</tr>
<tr>
<td>90Sr</td>
<td>28.5 a</td>
<td>0.622</td>
</tr>
<tr>
<td>91Y</td>
<td>58.5 d</td>
<td>120</td>
</tr>
</tbody>
</table>

3. ENTRANCE INTO HUMAN BODY
There are various pathways such as Ingestion, inhalation, and skin through which radionuclides enters into human body.

3.1 **Ingestion:** Radionuclides can be present in any food item we consume or even in water. At times during medical therapy or in diagnostic procedure, we intentionally ingest some radionuclides. Radionuclides such as iodine-131, phosphorous-32, yttrium-90 and I-131 MIBG are used for the treatment of many benign and malignant disorders. At times, radionuclides ingested by people can remain in their body for long periods of time while others are quickly eliminated, often within hours.

3.2 **Inhalation:** Radionuclides remain suspended in the atmosphere and can later enter our lungs. Usually radioactive particles are exhaled, but at times some remain in lungs and produce radiation which strikes lung tissue. Radioactive gas radon is one of the important natural sources of radiation. When these Radon atoms decay and produce other radioactive atoms, they get trapped in the lungs. According to the United States Environmental Protection Agency, these “radon decay products” are responsible for several thousand lung cancer deaths each year in the United States.

3.3 **Through the Skin:** Radionuclides can be absorbed through the skin’s surface, or can enter the body through a break in the skin. During medical therapy and diagnosis, radionuclides are injected which is another pathway for them to get into the skin.

4. **IMPACTS**

The impact of radioactive metals is very dangerous for the environment, humans and living organisms. Limiting their use in form of fertilizers, medicinal therapies and diagnostics is very important. Their harmful impacts should be analyzed in order to avoid or decrease their dangerous and toxic effects on humans and environment.

4.1 **Human Beings:** Exposure to Radioactive metals radiations can cause various acute and long term health effects. Even the minor exposure can contribute to the Cancer risk. The presence of these toxic elements causes various diseases and harm the gastrointestinal, pulmonary tract and kidney.
The impact of some radioactive metals on human beings is shown in the Table 2.

**Table 2: Various impacts of radioactive metals on human body.**

<table>
<thead>
<tr>
<th>Radioactive metals</th>
<th>Impact on human body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>Itai-itai disease(Spinal and leg pain, coughing, anemia, and kidney failure)</td>
</tr>
<tr>
<td>Uranium</td>
<td>Kidney damage</td>
</tr>
<tr>
<td>Thorium</td>
<td>lung disease, cancer of the lung or pancreas, change in genetic material when inhaled its dust</td>
</tr>
<tr>
<td>Radium</td>
<td>lung and bone cancer, anemia, broken teeth, cataracts, reduced bone growth</td>
</tr>
<tr>
<td>Plutonium</td>
<td>Lung disease and cancer.</td>
</tr>
<tr>
<td>Polonium</td>
<td>lung cancer, Damage to DNA</td>
</tr>
<tr>
<td>Strontium</td>
<td>Leukemia, anemia, bone cancer</td>
</tr>
</tbody>
</table>

➢ **CADMIUM:** ‘Itai-Itai’ disease was discovered in Japan during minting of currency by using cadmium as a metal. When this metal was mixed with water, people get infected by this disease with symptoms mainly pain in spinal cord and bone joints together with coughing and kidney failure.

➢ **URANIUM:** is a chemo toxic and has radio toxicity, hence it is considered as a disease causing element. The geochemical pathways lead these toxic elements into food crops, soil, water, air and ultimately the human body tissues via the food chain.

➢ **THORIUM:** Thorium can easily get stored in bones leading to bone cancer. Inhaling massive amounts of this metal can lead to metal poisoning and can even be lethal. Inhaling thorium dust can even lead to change in the genetic material of body cells.

➢ **RADIUM:** It can increase the risk to many cancer specially lung and bone cancer. It also harms eyes and teeth. A large portion of the radium originates from uranium mines in Democratic Republic of Congo and Canada.

➢ **PLUTONIUM:** It is the most dangerous if inhaled as it emits alpha particles. These particles can kill lung cells leading to diseases and
cancer. The United States produces plutonium in the largest amount nearly 502 tons.

- **POLONIUM:** There are various harmful metals but one of the most harmful metals to humans is Polonium as it spreads around the body, it leaves a chain of reactive radicals, because it has the ability to take electrons from any molecule in its path. The alpha particle radiation and cause Damage to DNA which can result in apoptosis or “cell suicide.” DNA damage at even a low level can cause genetic changes that affect the ability of the cells to reproduce.

- **STRONTIUM:** It causes anemia and can even lead to cancer as well as damage to the genetic material present in cells. Strontium-90 which is also known as "bone seeker” works like calcium in the human body and gets stored in bone and blood- forming tissue (bone marrow).

### 4.2 Into the environment:

The standard method of misusing the stone minerals either from the surface mining or, heap leaching or, underground mining, satisfying the great demand, has eventually resulted in major environmental and social risks. Environmental radioactivity is caused by to actinides and non-actinides both. Non actinides such as radon and radium cannot be ignored.

#### Table 2: Various impacts of radioactive metals on environment.

<table>
<thead>
<tr>
<th>Radioactive metals</th>
<th>Impact on environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plutonium</td>
<td>contaminate the soil, affects surface water and emits alpha radiation which enters inside cells</td>
</tr>
<tr>
<td>Thorium</td>
<td>contaminates water and oxygen</td>
</tr>
<tr>
<td>Uranium</td>
<td>Ruins the surface water and ground water quality, contaminates the soil and air quality, its mining results in higher levels of radium</td>
</tr>
<tr>
<td>Radium</td>
<td>Contaminates soil, plants absorb it and animals that eat them which will lead to accumulation of radium, impacts food chain</td>
</tr>
<tr>
<td>Element</td>
<td>Environmental Contamination</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Polonium</td>
<td>environmental contamination</td>
</tr>
<tr>
<td>Americium</td>
<td>tendency to remain in soil and water at generally shallow depths, contaminates plants (grain plants mainly) and animals</td>
</tr>
<tr>
<td>Curium</td>
<td>fix to soil particles, environmental contamination</td>
</tr>
</tbody>
</table>

- **Plutonium**: It is the most dangerous radioactive metal for the environment. The nuclear waste is harmful for thousands of years. Plutonium is even more dangerous than uranium as one of its isotope, Plutonium-239, which is found in the spent MOX fuel, is much more radioactive than the depleted Uranium-238 in the fuel. The sources of plutonium in the environment are atomic batteries in pacemakers, nuclear power plants and bomb safety trials.

- **Thorium**: In India, a large amount of thorium ore is found in the form of monazite in the Tamil Nadu coastal areas. The people living in these areas are exposed to a naturally occurring radiation.

- **Uranium**: Its mining and processing can affect the surface water and ground water quality to a great level. Uranium mining results in higher levels of radium. Plants and animals are affected. This entire process ultimately harms the food chain and thus the ecosystem.

- **Polonium**: Although Polonium occurs naturally, it has gotten substantially more accessible for going into water, food, living cells and tissue since the mining blast which started not long after the Second World War. Due to volatile characteristic of polonium, environmental contamination of 210Po which is usually released from the coal power plant, steel-making industry and refractory material industry has been an exposure problem for the people.

- **Americium**: When it is released in the environment, has the tendency to remain in soil and water at generally shallow depths and harms the food chain ultimately. Shellfish such as shrimp are known to consume americium-241 in their shells.
➢ **CURIUM:** Improper disposal of curium can result in contamination of environmental. Curium which is found in nature in the form of its oxides has the tendency to fix to soil particles, thus contaminating and generating the radiation.

3. **CONCLUSION:**

With increasing population growth, the demand for food production has also increased. Mineral fertilizers play an important role as a nutrient source for enhanced food production, but it has harmful impacts. The amounts of these undesirable toxic materials can be hazardously high if the handling does not include sufficient cleaner production methodology. Numerous countries have implemented severe laws on the maximum permissible levels of the toxic elements in fertilizer products as they have negative and harmful impact on the environment. Depending on their origin, inorganic fertilizers such as superphosphates and rock phosphates can lead to a modification of the natural geobiochemical equilibrium which eventually affects human health immensely. Using toxic metals into agricultural soils is a big problem because they do not degrade and remain in the soil indefinitely. Kids and embryos are particularly delicate to radiation exposure. The cells in them divide rapidly, giving greater chance to radiation to disturb the cycle and cause cell harm. The danger from a specific radionuclide depends on the energy and type of radiation, type of exposure (internal or external) and how long the radionuclide remains in body. Cadmium has been recorded as one of the most possibly risky components found in phosphate fertilizers. Austria, Finland and Sweden upheld limits for Cd in fertilizers at the international level. As a result of this, the average Cd concentration of fertilizers in Sweden fell from 80 mg/kg to about 8 mg/kg P2O5. WHO has built up a radiation program to secure patients, laborers, and the general population against the health risks of radiation exposure under planned, existing and emergency exposure situations. This programme covers activities which are related to the risk imposed due to radiation and its management. National and international co-operation is essential for proposing the right and adequate strategy in order to prevent the environment and humans from radioactive metal toxicity.

4. **ACKNOWLEDGEMENTS:**
This Book Chapter and the research behind it would not have been possible without the exceptional support of all those who are not mentioned as authors in the chapter but their enthusiasm, knowledge and exacting attention to detail have been an inspiration and kept my work on track. I am also grateful for the insightful comments offered by the teachers at my university. I am grateful of the liberality, generosity and skill of everyone who helped me improve this study in countless ways and saved me from numerous mistakes; those that inevitably remain are altogether my own responsibility.

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CHAPTER-8

EPIGENETICS IN HUMAN HEALTH AND DISEASE

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ABSTRACT: Epigenetics is the study of heritable changes in gene expression (active versus inactive genes) that do not involve changes to the underlying DNA sequence — a change in phenotype without a change in genotype — which in turn affects how cells read the genes. DNA methylation and chromatin remodelling are the two elementary mechanisms that mediate epigenetic phenomenon in all living beings, which can be studied by various lab techniques and mechanisms such as bisulfite sequencing, ChIP technique and microarray. Many of these epigenetic changes are necessary to direct normal cellular development and differentiation in the developing organism. However, developmental abnormalities may occur in response to inappropriate epigenetic signalling that occurs secondarily due to still poorly understood causes. With identical genetic heritages, two twins may evolve differently depending on their respective environments. There is growing evidence that our genes “listen” to the environment in complex ways that affect our health and behaviour. Thus, in addition to genetic and stochastic influences on epigenetic processes, epigenetic variation can also arise as a consequence of environmental factors. Different environmental factors such as chemical pollutants, dietary components, behaviour, physical activities, working habits, smoking and alcohol consumption etc are known to affect the epigenetics of human genome and may lead to different disease related to our life style. Diets with low vitamin B12 and folate lead to reduced levels of DNA methylation as they are required for methionine and SAM synthesis similarly selenium epigenetically modulates DNA and histone to activate methylation-silenced genes. Smoking tobacco decreases DNA methylation but hypermethylated genes such as p53 and p16 and increases acetylation due to degradation of HDAC. Alcohol is an antagonist of folate metabolism and effects DNA methylation. When exposed to toxic levels of arsenic it causes hyper/hypomethylation of blood DNA. And these modifications are responsible for human diseases, including Fragile X syndrome, Angelman’s syndrome, Prader-Willi syndrome, and various cancers. Although epigenetic modifications are influenced by the environment, most of these changes tend to be re-established each generation; however, this does not happen at some loci in the human genome. Thus, epigenetics is expected to help explaining how gene expression is modulated by lifestyle and environmental factors, and to bring a more complete understanding of individual responses to environmental cues and acquired risk factors.
1. INTRODUCTION

Epigenetics is the study of heritable changes in gene expression (active versus inactive genes) that do not involve changes to the underlying DNA sequence — a change in phenotype without a change in genotype — which in turn affects how cells read the genes.[1] Epigenetic change is a regular and natural occurrence but can also be influenced by several factors including age, the environment/lifestyle, and disease state. Epigenetic modifications can manifest as commonly as the manner in which cells terminally differentiate to end up as skin cells, liver cells, brain cells, etc. Or, epigenetic change can have more damaging effects that can result in diseases like cancer. At least three systems including DNA methylation, histone modification and non-coding RNA (ncRNA)-associated gene silencing are currently considered to initiate and sustain epigenetic change.

1.1 Difference between Genetics and Epigenetics.

<table>
<thead>
<tr>
<th>GENETICS</th>
<th>EPIGENETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Refers to the study of heredity and the variation of inherited characteristics.</td>
<td>• Refers to the study of inheritable changes in the organism caused by the modification of gene expression.</td>
</tr>
<tr>
<td>• Studies the structure, interaction, function and alteration of genes of a particular organism.</td>
<td>• Studies modification of gene expression of a particular organism.</td>
</tr>
<tr>
<td>• Covers genomics, transcriptomics, proteonomics, heredity, evolutionary genetics and genomics diseases.</td>
<td>• Covers gene regulation, interaction of gene and environment and the interaction of protein and environment.</td>
</tr>
<tr>
<td>• Environmental influences do not change the genotype and there is no inheritance of acquired characteristics.</td>
<td>• Often reversed. Eg- Genomic imprinting.</td>
</tr>
<tr>
<td></td>
<td>• Environment of a cell is important in determining its properties or fate in developing organism.</td>
</tr>
</tbody>
</table>
1.2 Different types of Epigenetic phenomenon

DNA methylation and posttranslational modifications of histones have been considered to be the two major epigenetic mechanisms that affect phenotype, but there are many other epigenetic mechanisms, including genomic imprinting, X-chromosome inactivation, small and noncoding RNAs and nucleosome remodeling pattern regulation.[3]

Epigenetic changes in DNA and chromatin structure are heritable, although they express this characteristic in a dynamic manner. During the division process, the cell remembers the epigenetic mark but, at the same time, such mark can also be modified by environmental factors. Typical epigenetic marks consist of those phenomena that determine the specificity of each tissue and the difference between germ cells and somatic cells as well as between malignant cells and normal cells. Such epigenetic phenomena do not involve alterations in the DNA nucleotide sequence but yet are responsible for the genome function and stability as well as for the prevention of gene disruption and translocations, such as those seen in cancer disease.[3]

2. Epigenetic Mechanisms

Mechanisms involved in epigenetic modifications are DNA Methylation, Histone modifications and non-coding ribonucleic acid regulations. Together these mechanisms determine the architecture of chromatin, genetic loci’s accessibility to transcriptional machinery, and gene expression levels.[4]

2.1 DNA Methylation

DNA methylation is a modification mechanism in which the methyl group is added to the 5th position of a cytosine residue which forms 5-methylcytosine. This process is catalysed by a family of enzymes called the DNA methyltransferases or Dnmt's which relocate the methyl group from SAM (S-adenyl methionine). [5]
Figure 2: DNA Methylation
Dnmt1 acts upon hemi methylated or methylated DNA and retains the methylation pattern from the parent strand to the newly synthesized daughter strand during the semiconservative replication of DNA.[5]

Figure 3: DNA methyltransferase 3a and its regulatory factor (left) and DNA methyltransferase 1 bound to a short piece of DNA (right).
(Source-https://pdb101.rcsb.org/motm/139)
2.1.1 Site of Methylation

The modification takes place on cytosine that precedes guanine in 5' to 3' direction or the CpG islands. These are regions on DNA that have a high prevalence of CpG sites i.e. at least 500 bp to 1500 bp long with a CG: GC ratio exceeding 0.6. These are found in certain regulatory regions in particular regard to the promoter region of housekeeping genes.[5]

Figure 4: The presence of a dense hypermethylation change completely its molecular environment. White dots, unmethylated CpGs; Black dots, methylated CpGs.


They occur in low abundance throughout the genome presumably due to frequent methylation eventually resulting in a risk of losing CpG dinucleotides as a consequence of mutation. The human genome comprises of ~30,000 CpG islands (CGIs).[5]
Figure 5: A part of the sequence of the human APRT gene is given. CpG dinucleotides are emphasized in red. The five exons are in blue. The start (ATG) and stop (TGA) codons are indicated (bold blue).


2.1.2 Basic Mechanism of DNA Methylation

There are three categories of enzymes the writers, erasers, and readers that are involved in the establishment, recognition, and removal of DNA methylation. Writers are the group of enzymes that promote or catalyze the attachment of methyl group onto the 5th carbon of cytosine. Readers identify and attach to the methyl group and eventually influence the expression of the gene. Finally, the erasers carry out the process of demethylation where 5-methyl cytosine gets converted back to cytosine by various mechanisms. [5]

2.1.2.1 Writing DNA Methylation

Dnmt1, Dnmt3a, and Dnmt3b are the three enzymes that are involved in the direct catalysis of methyl group addition onto the DNA. Despite of the fact that these enzymes have structural similarities their functions and expression patterns are highly uncommon. [5]

2.1.2.1 De-novo Methylation

De-novo methylation is carried out on the cytosine residue of the CpG islands, where Dnmt3a and Dnmt3b relocated the methyl group from SAM (S-adenyl methionine) to the 5th position of the cytosine. This
leads to the formation of 5- methyl cytosine and the methyl donor SAM gets converted to SAH (S-adenyl homocysteine).[5]

---

**Figure 6: De-novo methylation Mechanism**

2.1.2.1.2 **Maintenance Mechanism**

Dnmt1 preferably binds to hemi methylated DNA and methylate’s during replication. When DNA undergoes semiconservative replication, it is the parent strand that withholds the original pattern of methylation, Dnmt1 in assistance with the replication foci precisely mimics the original pattern of DNA methylation by adding methyl group on the newly synthesized strand. Moreover, it is also capable of repairing DNA methylation. Thus, Dnmt1 is also known as maintenance Dnmt.[5]

---

**Figure 7: Maintenance methylation Mechanism**
2.1.2.2 Erasing DNA Methylation

The process of demethylation requires a series of enzymatic reactions to take place to revert 5mC to the naked cytosine. Demethylation occurs through a series of chemical reactions which include deamination or oxidation reaction, and further, the product is recognized by base excision repair pathway to substitute the modified base with cytosine.[5]

5- methyl Cytosine, the end product of DNA methylation can be amended at 2 sites by chemical reactions and these two sites are – the amine group and the methyl group.[5]

Deamination reaction takes place at the amine group of 5mC in the presence of AID (activation-induced cytidine deiminase) efficiently converting 5mC to thymine. This creates a G/T mismatch in the DNA sequence and instigates base excision repair pathway to rectify the bases.[5]

The modification at the methyl group of 5mC occurs by the addition of the hydroxyl group. This chemical reaction is mediated by Tet enzymes (ten-eleven translocation enzyme) and the results in the formation of 5hmC (5-hydroxymethyl Cytosine), which is again acted upon at two sites by two separate mechanisms that convert 5hmC back to cytosine. The first is repetitive oxidation of the methyl group by Tet enzymes that results in the formation of 5-formyl Cytosine (5fC) and later on 5- carboxy Cytosine (5caC). Second is deamination of 5hmC by AID (activation-induced cytidine deiminase) resulting in the formation of 5-hydroxymethyl Uracil (5hmU).[5]
Figure 8: DNA Demethylation Mechanism

Ultimately, the products of all the above-stated mechanisms – thymine, 5-hydroxymethyl Uracil, 5-formyl Cytosine, and 5-carboxy Cytosine are recognized and split off to substitute cytosine mediated by TDG (thymine DNA glycosylase) by base excision repair pathway.[5]

2.1.3 Effects of DNA Methylation
The process of methylation is required for silencing of retroviral elements, regulation of tissue-specific gene expression, aging, genomic imprinting and also X-chromosome inactivation.[38]
2.1.3.1 **Genomic Imprinting** - an epigenetic process that marks the DNA in a sex-dependent manner, resulting in differential expression of a gene depending on its parent of origin. Some genetic diseases caused by an error in a specific gene and chromosomal imprinting can be Prader-Willi syndrome and Angelman syndrome.[38]

2.1.3.2 **X-chromosome Inactivation** - is the process in which one of the two copies of the females gets randomly and permanently inactivated in all the cells leaving behind the egg cells. This phenomenon is also known as lyonization. Here the aim is to ensure that unlike males, females also have only one functional copy of X-chromosome in each cell.[38]

2.1.3.3 **Gene Expression** – refers suppression of transcription of gene because of methylated DNA as site of transcription are not identified by RNA polymerase makes DNA methylation a mechanism to control the expression of genes.[38]

---

**Figure 9:** Epigenetic regulation of gene expression. Epigenetic alterations such as DNA methylation and/or histone modifications

2.1.3.4 Ageing- suppression of DNA methylation is seen during the formation of zygote and increases during development, but there is a loss of methylation during aging. As the age increases hypermethylation of genes is seen because the biological clock of the body acts as a biomarker in the aging process. And this peculiar pattern of methylation leads to heritable changes.[38]

Figure 10: In young individuals, the cells within each cell type have a similar pattern of gene expression, determined in large part by each cell having similar epigenetic information. During aging, the epigenetic information changes sporadically in response to exogenous and endogenous factors. The resulting abnormal chromatin state is characterized by different histone variants being incorporated, altered DNA methylation patterns, and altered histone modification patterns, resulting in the recruitment of different chromatin modifiers.

(Source- Sangita Pal and Jessica K. Tyler,” Epigenetics and Ageing”, Science Advances, 29 Jul 2016.)

DNA methylation being the most frequent epigenetic modification mechanism has proven to be most correlated with many human illnesses, for instance, various types of cancers, autoimmune
disorders (muscular dystrophy, systemic lupus erythematosus), neurological disorders (fragile X syndrome, Alzheimer, Parkinson, Huntington, and schizophrenia).

2.2 Histone Modifications
Histone modification is an essential post-translation modification that regulates gene expression by chromatin remodeling and involves acetylation, methylation, phosphorylation, and ubiquitination. Unlike DNA methylation, histone modification is capable of modifying the availability of nucleotide sequences to the transcriptional machinery without altering it. Histone modifications are generally catalyzed by specific enzymes that act, predominantly, but not exclusively (e.g. some types of histone phosphorylation), at the histone N-terminal tails involving amino acids such as lysine or arginine as well as serine, threonine, tyrosine, etc. 

2.2.1 Histone Acetylation
It is the most widely studied modification mechanism which takes place on the conserved lysine of the N-terminal tail of all 4 histones (H2A, H2B, H3, and H4).

![Figure 11: The structure of chromatin. DNA is wound around nucleosomes, which are composed of eight histone molecules with](image-url)
two copies of histones H2A, H2B, H3 and H4. Each histone molecule has a long tail rich in lysine residues (K), which are the sites of enzymatic modification, such as acetylation, thus changing the charge of the molecule and leading to DNA unwinding.

Histone acetylation and deacetylation being an essential part of the modification process are catalyzed by enzymes called Histone Acetyltransferase (HAT) and Histone Deacetylase (HDAC).[8] Histone Acetyltransferase (HAT) - These enzymes assist the transfer of acetyl group from the acetyl donor acetyl-CoA to the lysine residue.[8]

![Figure 12: Structure of histone acetyltransferase](http://www.rcsb.org/pdb/explore/explore.do?structureId=5trm)

Histone Deacetylase (HDAC) - These enzymes perform the function of histone deacetylation and leads to the removal of acetyl group from acetyl lysine as it is not naturally liable.[8]

![Figure 13: Structure of histone deacetylase](image)
2.2.1.1 Mechanism

The site for the mechanism for acetylation and deacetylation is the NH3+ groups of Lysine amino acid residues, which are located on the tails of histone comprising of the nucleosome of packed dsDNA. The enzymes that assists the process are known as Histone Acetyltransferases (HATs); it facilitates the transfer of acetyl group from Acetyl Coenzyme-A (Acetyl-CoA) to the NH3+ group on Lysine. For the process of deacetylation factors such as Histone Deacetylases (HDACs) catalyze the removal of the acetyl group with a molecule of H2O.[8]

![Figure 14: Mechanism of acetylation of lysine located on the tail of histone protein](image)

The formation of the nucleosome is dependent on the positive charge of histones and the negative charge of DNA, the process of acetylation interrupts the association resulting in the weakening of the binding of the nucleosomal components. This makes the DNA more accessible to the transcription factors thus make it more prone transcription and leading to increased gene expression.[8]
Figure 15: Histone acetylation alters chromatin structure. Shown in this illustration, the dynamic state of histone acetylation/deacetylation regulated by HAT and HDAC enzymes. Acetylation of histones alters accessibility of chromatin and allows DNA binding proteins to interact with exposed sites to activate gene transcription and downstream cellular functions.


2.2.2 Histone Methylation

Histone methylation is a procedure in which a methyl group is transmitted to the amino acids of histone protein. This process reduces the chemical attraction between the histone core and the DNA, so transcription increases, because it allows the DNA to uncoil from the nucleosome thus making it accessible to the transcription factors and RNA polymerase. It is an important process for gene expression.[9]
In general lysine and arginine residue of histone H3 and H4 are methylated. Methylation involves different types of enzymes depending on the amino acid being methylated, when occurs on lysine histone methyltransferase enzyme promotes methylation while on arginine protein arginine methyl transferase does the same. [9]

Figure: 16. Structure of histone methyl transferase
(Source: https://en.wikipedia.org/wiki/Histone_methyltransferase#/media/File:Histone_Methyltransferase_front_view.tiff)

2.2.2.1 Mechanism
The site of methylation on histones is only lysine (K) and arginine (R) but is more frequently it is observed on lysine residue of histone H3 and H4. [9]
Lysine and arginine residues both contain amino groups, which confer basic and hydrophobic characteristics. Lysine can be mono-, di-, or trimethylated with a methyl group replacing each hydrogen of its NH3+ group. With a free NH2 and NH2+ group, arginine is able to be mono- or demethylated. This demethylation can occur symmetrically on the NH2 group or asymmetrically with one methylation on each group. Methylation of an arginine residue requires a complex including protein arginine methyltransferase (PRMT) while lysine requires a specific histone methyltransferase (HMT). [9]
2.2.3 Histone Phosphorylation
Histone phosphorylation also a post-translational modification takes place at various sites such as H3S10, S28, H4S1, and H2AS1. It can
cause transcriptional activation of immediate early genes, also chromatin compaction during mitosis and comprises an essential step in DNA repair. This process is catalyzed by histone kinase and the removal of the phosphate group is catalyzed by histone phosphatase. It makes histone core more negative thus leading to stronger binding of nucleosomal components, making the DNA less accessible to transcription factors.\textsuperscript{[10]}

The most studied site of histone phosphorylation is the serine 10 of histone H3 (H3S10). Other than serine, threonine and tyrosine can also phosphorylate.\textsuperscript{[10]}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure19.png}
\caption{Phosphorylation of Serine (S), Threonine (T) and Tyrosine (Y)}
\end{figure}

(Sources- Bart Ellenbroek and Jin Youn, “Environment Challenges the brain”, Gene- Environment Challenge in Psychiatry, pp.107-139,2016.)

Histone phosphorylation in particular phosphorylation of H3S10 has a role in DNA damage response and DNA repair. The rapid process of phosphorylation of H3S10 by protein kinase at double strand break (DSB) site, is one of the first and most easily detectable DNA damage signalling post translational events.\textsuperscript{[10]}

\subsection*{2.2.4 Histone Ubiquitination}
Ubiquitin commonly binds it’s the last amino acid to the lysine residue on the nucleosome, which leads to isopeptide bond formation between the carboxyl group (COO\textsuperscript{−}) of ubiquitin glycine and epsilon
amino acid of the substrate. There are three types of enzymes involved in histone ubiquitination, which are ubiquitin-activating (E1) which is ATP dependent, ubiquitin-conjugating (E2) and ubiquitin-ligase (E3).[26] The basic functions of ubiquitination are antigen processing, apoptosis, DNA transcription, and neural and muscular degeneration.[11]

2.2.4.1 Mechanism
For ubiquitination to start ubiquitin protein needs to be activated which is a two-step reaction performed by E1. After activation of ubiquitin protein E2 catalyses the transfer of ubiquitin protein from E1 to active site cysteine of E2 via (trans(thio)esterification) reaction. Here E2 binds with activated ubiquitin and E1. In the final step, E3 plays the role of catalyzes and created isopeptide bonds between lysine and C-terminal of ubiquitin. For deubiquitinating enzyme deubiquitinating enzymes (DUB) removes ubiquitin from the nucleosome. [11]

Figure 20: Schematic diagram illustrating the sequential reaction steps of ubiquitin activation and transferring involving a cascade of E1/E2/E3 enzymes
2.2 **RNA Interference**

RNA silencing is an unprecedented gene regulatory mechanism which restricts the level of transcription either by inhibiting transcription process (transcriptional gene silencing [TGS]) or by simulating a sequence-specific RNA degradation process (transcriptional gene silencing [TGS]).[12] The basic function of RNAi includes the protection of genome against invasion by mobile genetic elements such as viruses and transposons as well as harmonized functioning of the developmental program of eukaryotic organisms.[12]

2.3.1. **Mechanism**

The first step, also referred to as RNAi initiating step, involves the binding of RNA nuclease also known as the DICER enzyme to larger dsRNA (double-stranded RNA) and its cleavage into discrete 21 to 25 nucleotide RNA fragments (siRNA). In the next step, these siRNA join a multinuclease complex, RISC (RNA-induced silencing complex), which degrades the homologous single stranded mRNAs.[12]

One of the most intriguing features of RNA interference is its catalytic nature. Only a few molecules of dsRNA are adequate to degrade a continuously transcribed target of mRNA for a long period. Despite the fact that conversion of the long dsRNA into many small siRNA results in some degree of amplification, it is insufficient to bring about such a continuous mRNA degradation. Such mutations in the gene encoding RNA-dependent RNA polymerase (RdRP) affect RNAi, it was proposed that this type of polymerase might replicate siRNAs as epigenetic agents.[12]
3. Epigenetics and Lifestyle

Lifestyle includes various factors such as diet, behaviour, stress, physical activity, working habits, smoking and alcohol consumption. Individual genetic background and environmental factors are intertwined to lifestyle in determining the health status of individuals. There are increasing evidences showing that the environmental and lifestyle factors influence epigenetic mechanisms, such as DNA methylation, histone modification and microRNA expression. Variation in epigenetic mark is also associated with various types of human diseases such as cancer, cardiovascular diseases and neurodegenerative diseases.[13]
Figure 22: Environment-Epigenetic interaction

<table>
<thead>
<tr>
<th>FACTORS</th>
<th>EXAMPLE</th>
<th>EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional</td>
<td>Folate and</td>
<td>Required for methionine and SAM synthesis and maintenance of DNA methylation.</td>
</tr>
<tr>
<td></td>
<td>Vitamin B12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polyphenols</td>
<td>Reverse adverse epigenetic regulation by altering DNA methylation and histone modification</td>
</tr>
<tr>
<td></td>
<td>Selenium</td>
<td>Epigenetically modulate DNA and histone to activate methylation-silenced genes.</td>
</tr>
<tr>
<td>Smoking</td>
<td>Tobacco</td>
<td>Overall decrease in DNA methylation but hypermethylates genes such as p53 and p16. Increased acetylation due to degradation of HDAC. Can lead to various diseases like cancer, emphysema, COPD, impotency, female infertility etc</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Alcohol</td>
<td>Leads to significant reduction in SAM levels, thereby contributing to DNA hypomethylation.</td>
</tr>
<tr>
<td>Consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environmental Pollutants</td>
<td>Arsenic</td>
<td>Significant DNA hypermethylation of p53 and p16 promoter regions.</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------</td>
<td>-----------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Aromatic Hydrocarbons (Benzene)</td>
<td>Increases risk of acute myelogenous leukaemia, characterized by global hypomethylation and gene specific hypermethylation/hypomethylation.</td>
</tr>
</tbody>
</table>

| Psychological Stress | | Hypermethylation of the glucocorticoid receptor gene was found among suicide victims with a history of abuse in childhood, but not among controls or suicide victims with a negative history of childhood abuse |

**TABLE 1. Various factors and their effects on epigenetic mechanisms**


4. **Techniques to study Epigenetic Mechanism**

4.1. **Bi-Sulfite Sequencing**

Bisulfite genomic sequencing is regarded as a gold-standard technology for detection of DNA methylation because it provides a qualitative, quantitative and efficient approach to identify 5-methylcytosine at single base-pair resolution. The basic principle behind this technique is that the amination reaction of cytosine and 5-methyl cytosine (5mC) proceeds with very different consequences when treated with sodium bisulfite.\[14\]

In this method the double stranded DNA is first denatured and then treated with bisulfite. After which unmethylated cytosine residues are converted to uracil whereas 5-methyl cytosine (5mC) remains unaffected. After PCR amplification, uracil residues are converted to thymine. DNA methylation status can be determined by direct PCR sequencing or cloning sequencing.\[14\]
Figure 23: Bisulfite sequencing
(Source- Yuanyuan Li. and Trygve O. Tollifsbol, “DNA Methylation detection: Bisulfite genomic sequencing analysis”, Methods Mol Bio, pp.11-21, 2011)

4.2 ChIP Technique
Chromatin immunoprecipitation (ChIP) has become the technique of choice to investigate protein–DNA interactions inside the cell. ChIP has been used for mapping the localization of post-translationally modified histones and histone variants in the genome, and for mapping DNA target sites for transcription factors and other chromosome-associated proteins.[15]
For the process to begin DNA and proteins are commonly reversibly cross-linked with formaldehyde (which is heat-reversible) to covalently attach proteins to target DNA sequences. Chromatin is
subsequently fragmented, either by enzymatic digestion with micrococcal nuclease, or by sonication of whole cells or nuclei, into fragments of 200–1,000 base pair (bp), with an average of 500 bp. The lysate is cleared by sedimentation and protein–DNA complexes are immunoprecipitated from the supernatant (chromatin) using antibodies to the protein of interest. Immunoprecipitated complexes are washed under stringent conditions to remove non-specifically bound chromatin, the cross-link is reversed, proteins are digested and the precipitated ChIP-enriched DNA is purified. DNA sequences associated with the precipitated protein can be identified by end-point polymerase chain reaction (PCR) or quantitative (q)PCR.[15]

Figure 24: The chromatin immunoprecipitation (ChIP) assay and various methods of analysis.

(Source- Philippe Collas, “The current state of Chromatin Immunoprecipitation”, Molecular Biotechnology, pp.87-100, 14 Jan 2010)
4.3 Microarray

mRNA is an intermediary molecule which carries the genetic information from the cell nucleus to the cytoplasm for protein synthesis. Whenever some genes are expressed or are in their active state, many copies of mRNA corresponding to the particular genes are produced by transcription. These mRNAs synthesize the corresponding protein by translation. So, indirectly by assessing the various mRNAs, we can assess the genetic information or the gene expression. This helps in the understanding of various processes behind every altered genetic expression. Thus, mRNA acts as a surrogate marker. Since mRNA is degraded easily, it is necessary to convert it into a more stable cDNA form. Labeling of cDNA is done by fluorochrome dyes Cy3 (green) and Cy5 (red). The principle behind microarrays is that complementary sequences will bind to each other.[16]

The unknown DNA molecules are cut into fragments by restriction endonucleases; fluorescent markers are attached to these DNA fragments. These are then allowed to react with probes of the DNA chip. Then the target DNA fragments along with complementary sequences bind to the DNA probes. The remaining DNA fragments are washed away. The target DNA pieces can be identified by their fluorescence emission by passing a laser beam. A computer is used to record the pattern of fluorescence emission and DNA identification.[16]
Figure 25: Microarray technique

5. CONCLUSION

Epigenetics is paving a new pathway of scientific understanding in which the long-standing issue in the dichotomous debate on nature vs. nurture may no longer be relevant. And neither may the independent study of psychology or biology. In fact, there is clear evidence confirming a complex interplay between the experiences that occur throughout the lifespan of an individual and the environmental factors that contribute to development. These developmental experiences have consequences for the individual, his or her offspring, and generations of relations to come.
Environmental and physiological events influence the genotypes and phenotypes in complicated chemical interactions. These interactions have both protective and destructive features and possibilities. Epigenetic research points to these powerful interactions as overlapping factors that have contributed to a new understanding of development and pathology. This understanding comes from the growing number of studies implicating that early adversity has a long-term effect on the Behavioural development of humans.

Over the last decade there have been important medical advances that have allowed for the identification of the epigenetic mechanisms that play a role in gene regulation. This information has been essential to the understanding of epigenetic systems, their replication, and subsequent mutations. However, there are still several questions yet to be answered.

6. REFERENCES


CHAPTER-9

VITAMIN D DEFICIENCY IN DIABETES

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ABSTRACT: Diabetes is a group of metabolic disorders. On the basis of various factors like age at onset, degree of insulin resistance, β cell dysfunction it can be classified into two types – Type 1 Diabetes (T1DM) and Type 2 Diabetes (T2 DM). Recently T2 DM has been reported to account for more than 85% of Diabetic cases. It has been estimated by the IDF that around 425 million people were having diabetes in 2017 and the number has been increasing till now. Thus, there is a huge need for prevention and treatment of diabetes. In this regard, Vitamin D supplementation seems to be a promising agent as it improves insulin action as well as enhances insulin sensitivity. It has also proven to be a good antioxidant in various studies. Thus, our study aims to create awareness among researchers/clinicians that Vitamin D could pave a way for a therapeutic approach in the treatment and management of Type 2 Diabetes Mellitus.

INTRODUCTION

Type 2 diabetes (T2DM) is a metabolic disorder that is increasing both national and worldwide [Wang et. al, 2017]. According to reports by WHO, estimated 90% of all cases of diabetes constitutes T2DM. Globally, 15 million (approx.) people suffer from Type 2 Diabetes and this number might double by 2025 [Tao et. al, 2017]. The various factors like systemic inflammation, defects in insulin signalling pathway and dysfunction in pancreatic beta cells are involved in both insulin resistance and T2DM development [Wang et. al, 2017]. Nowadays, there has been an association of vitamin D deficiency and its associated metabolic disorders [Wang et. al,2017]. On the basis of suggested evidence, it has been reported that Vitamin D plays an important role in regulation of various sequential events that are responsible for enabling the pancreatic -cells to secrete insulin, and thereby to control blood glucose level.

1. Vitamin D: The Sunshine Vitamin

Vitamin D also known as the sunshine vitamin, is a fat-soluble prohormone. Sources of vitamin D include fortified foods, dietary supplements and plant sources like mushrooms. However, its main source is exposure to sunlight.
It is an essential micronutrient that is not synthesized in our body but has to be supplied through dietary sources for the survival of an organism. It was earlier classified as vitamin, but evident studies indicated that it can be produced in our body with the help of ultraviolet radiation from sunlight, thus violating the definition of a vitamin. Thus, vitamin D is now thought to be a prohormone as it is produced in one part of the body and transported through blood circulation to its target site distant from it to exert its action [Anandbaskar et. al, 2018]. It is also defined as steroid or secosteroid according to the nomenclature by The International Union of Pure and Applied Chemistry’s Commission. [Demer et. al, 2018]. Its active form is 1,25-hydroxycholecalciferol, which helps in maintaining the bone-mineral homeostasis. Vitamin D is known to have a crucial role in immune responses, muscle function, cell growth, protein function, and cardiovascular function. [Anandbaskar et. al, 2018, Yague et. al, 2020]

1.1 History of Vitamin D

Although rickets was prevalent from ancient times, there was lack of knowledge regarding its etiology and treatment, until the discovery of vitamin D. According to studies, it was observed that prevalence of rickets was more in London compared to that in the tropical zones like India and China in 1980s. It leads to the observation that sunlight exposure was essential for rickets prevention. Mellan et al, 1919 experimentally found that cod liver oil could cure rickets in dogs that were unable to retain sunlight. It concluded that probably Vitamin A or similar substance was responsible for improved symptoms of rickets in dogs or deficiency of vitamin A can lead to rickets. In the year 1922, McCollum et al. revealed that a new fat-soluble vitamin (which they named as vitamin D) distinct from vitamin A, was responsible for the cure of rickets in dogs. Various researchers like Windaus, Thiele, Schenck and Werder followed its discovery and identified the structure and properties of vitamin D, which is derived from both plant and animal sources. [Anandbaskar et. al, 2018]

1.2 Sources of Vitamin D3

Sources of Vitamin D for humans include exposure to sunlight, from their diet and from dietary supplements (Figure 1). There is major contribution of sunlight exposure in vitamin D supply accounting 80% in comparison to
diet and dietary supplements. Factors that affects synthesis of Vitamin D are genetic, environmental as well as lifestyle. [Pilz et. al, 2019]

There are two major forms of vitamin D-
1. Vitamin D2 or Ergocalciferol (Manufactured through UV irradiation of ergosterol from yeast)
2. Vitamin D3 or Cholecalciferol (Through UV irradiation of 7-dehydrocholesterol from lanolin)
However the both forms of Vitamin D differ only in side chains.

Vitamin D2 or Ergocalciferol
Vitamin D3 or Cholecalciferol

(Source: Pubchem 5280793)
(Source: Pubchem 5280795)

### Table 1: Sources of vitamin D

<table>
<thead>
<tr>
<th>Sources of Vitamin D</th>
<th>Form of Vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sunlight</td>
<td>Vitamin D3</td>
</tr>
<tr>
<td>2. <strong>Animal Sources</strong></td>
<td></td>
</tr>
<tr>
<td>Fish (Salmon, Mackerel, Tuna)</td>
<td>Vitamin D3</td>
</tr>
<tr>
<td>Cod-liver oil</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td></td>
</tr>
<tr>
<td>Egg yolk</td>
<td></td>
</tr>
</tbody>
</table>
Milk and milk products

3. Plant Sources
   Fungi
   Mushrooms

Vitamin D2

4. Fortified foods
   Milk and milk products
   Orange juice

Vitamin D3

5. Vitamin D supplements

Vitamin D2 and Vitamin D3

According to the 2011 report by the Institute of medicine (IOM) committee, there should be 400 IU dietary Vitamin D in infants, 600 IU in the age group of 1 to 70 years, and for individuals above 70 years it is 800 IU [Anandbaskar et. al, 2018]

1.3 Biosynthesis of Vitamin D

On exposure to sunlight, 7-dehydrocholesterol in the epidermis of skin is converted to previtamin D3, which is immediately converted to vitamin D3. However, to prevent Vitamin D intoxication previtamin D3 and vitamin D3 degraded on excessive exposure to sunlight and gets converted into inactive photoproducts like lumisterol and tachysterol [Holick et. al, 2007]. The rate of formation of vitamin D3 is affected by both UV B intensity and skin pigmentation. Vitamin D made in the skin or ingested in the diet can be stored in and then released from fat cells. There is incorporation of Vitamin D2 and D3 from dietary sources into chylomicrons which are then transported into venous circulation by the lymphatic system. [Silva et. al, 2018]. Vitamin D is inert in nature; thus, it undergoes 3 main metabolic steps including 25-hydroxylation, 1α-hydroxylation, and 24-hydroxylation for its activation. These hydroxylation steps are performed by cytochrome P450 mixed-function oxidases (CYPs). The location of these enzymes is either in the mitochondrion (e.g. CYP27B1, and CYP24A1) or in the endoplasmic reticulum (e.g. CYP2R1). Among these CYPS, CYP2R1 is physiologically more important as its deficiency can cause rickets. [Hollis et. al, 2007; Heaney et. al, 2011].There is Vitamin -D binding protein (DBP) in the circulation, with which vitamin D binds and then is transported to the liver (Figure 2), where it is converted to 25-hydroxyvitamin D [25-(OH) D] by Vitamin D-25 hydroxylase or CYP2R1,a
cytochrome P450 enzyme. Major circulating form of vitamin D measured by clinicians for determining vitamin D status is 25-hydroxy vitamin D. Although it is biologically inactive form that must be converted in the kidneys by 25-hydroxyvitamin D-1αhydroxylase (1-OHase) or CYP27B1 to the biologically active form — 1,25-dihydroxyvitamin D [1,25(OH)₂D] [Carlberg et. al, 2016]. There is extrarenal conversion of 25(OH)D to 1,25(OH)₂D in various tissues such as macrophages, adipocytes, pancreatic β cells, breast, colon and prostate which expresses CYP27B1.[Holick et. al, 2007]. There is degradation of both calcitriol(1,25(OH)₂D) and its precursor 25(OH)D to biological inactive compound i.e calcitroic acid that is excreted with bile by the action of 25(OH)D-24-hydroxylase (CYP24A1). Various factors mediate the gene expression of CYP27B1 in kidney. Parathyroid hormone stimulated by low level of vitamin D and calcium affects activation of CYP27B1 and increases the calcitriol activation.[Christakos et. al, 2017]. However, calcitriol regulates its own synthesis via negative feedback mechanism and decreases the synthesis and secretion of PTH. PTH is also involved in stimulation of skeletal fibroblast growth factor-23 (FGF-23) synthesis [Brown et. al, 2015; Christakos et. al, 2017] and inhibition of enzyme CYP24A1. FGF-23 can decrease (1,25(OH)₂D) levels in serum by inhibiting CYP27B1 and stimulation of CYP24A1.[Holick et. al, 2007]. There is an important role of DBP in influencing downstream gene transcription as it is involved in delivery of vitamin D active form i.e 1-25 dihydroxy vitamin D ligand to tissues thus increasing its chances of availability to VDR, its transcription factor. [Keane et. al, 2018].
2. MOLECULAR ACTION OF VITAMIN D

Vitamin D regulates gene transcription via VDR (Vitamin D receptor). VDR is the member of steroid receptor family. Vitamin D mediates both genomic and non-genomic effects. Genomic actions are mediated, when the active form of vitamin D i.e. 25-hydroxy vitamin D or calcitriol binds to vitamin D receptors. VDRs are the ubiquitous receptors that are present in the target cell's nuclei, containing a ligand binding domain and a DNA binding domain. This interaction results in conformational changes in receptor thus allowing VDR to partner with another protein called retinoid X receptor (RXR) [Khammisa et. al, 2018]. The resulting complex then binds to particular regions of DNA, known as vitamin D response elements (VDRE), and regulates the activity of vitamin D-responsive genes. The complex helps in controlling calcium and phosphate absorption and other processes by turning these genes on or off. VDR was first identified in 1974. VDR is known to be present in more than 38 tissue types like brain, prostate, breast, immune cells, skeletal muscle cells, pancreatic beta cells but it is highly expressed in tissues (kidneys, intestine and bone) [Battault et. al, 2013] [Keane et. al, 2018] [Khammisa et. al, 2018]. VDRE sequence consists of two direct imperfect repeats of hexanucleotides (GGTCCA) with
a spacer of three nucleotides. This motif sequence is known as DR3 (Direct Repeat 3) sequence. [Haussler et. al, 1998; Rosen et. al, 2012]. Some vitamin D regulated genes also consist of DR4 and DR6 configuration [Rosen et. al, 2012; Haussler et. al, 1998; Gill et. al, 1993]. VDR functions as a transcription factor for more than 200 genes and thus exerts its biological actions by binding to its respective ligand [Rehim et. al, 2019]. VDR other than binding to RXR and forming VDR-RXR dimer can also bind with other nuclear receptors like thyroid, Vitamin A, PPAR-γ and other orphan receptors. The high affinity binding to VDRE is due to heterodimerization of 1,25(OH)2D3-VDR and RXR. Thus, Vitamin D is involved in regulation of gene transcription in many systems and tissues via VDR-RXR dimer. [Rosen et. al, 2012].

Figure 2: Vitamin D signalling via VDR and its effect on different organs

2.1 Biological actions of Vitamin D

Vitamin D exerts its action with the help of its active form i.e calcitriol [1,25(OH)2D] by acting on its target organs that are kidneys, small intestine and bones [Buyuker et. al, 2019]. Recent studies have mentioned that VDRs
have also biological effect as they exist in variety of cells such as pancreas, large intestine, small intestine, muscles and nerves which are not involved in bone mineral homeostasis. It also contributes to the regulation of cellular differentiation and growth, such as the induction of differentiation of leukaemia cells, the induction of apoptosis of breast cancer cells, and the inhibition of proliferation of a large variety of cancer cells. [Carlberg et. al, 2016]. However, the two main functions of circulating active form of vitamin D is involvement in increased efficiency of intestinal Ca\(^{2+}\) and PO\(_4\)\(^{2-}\) absorption and secondly formation of mature osteoclasts from preosteoclasts [Battault et. al, 2013]. All the Vitamin D mediated actions may be divided either as Genomic or Non-genomic actions.

**A. Genomic Action**

Genomic actions are mediated via VDR. The heterodimerization of active form of vitamin D (1,25(OH)2D) i.e calcitriol with RXR results in complex formation of (1,25(OH)2D)-VDR-RXR. This complex in nucleus bind to VDRE in the promoter region of Vitamin D responsive genes. Classical functions are mediated through genomic action via interaction between Calcitriol-VDR-RXR resulting in upregulation and downregulation expression of target genes. [Christakos et. al, 2017]

Vitamin D is mainly recognized for its function in maintenance of calcium homeostasis by promoting calcium absorption in the kidney and intestine. Thus, it plays an important role in maintaining Ca++ homeostasis by regulation of several Ca++ sensitive signalling components that acts to reduce Ca++ levels [Bikle et. al, 2010]. It acts by upregulation of TRPV5, TRPV6, calbindin D-9k, calbindin D-28k, parvalbumin, Na+/Ca++exchanger (NCX) and plasma membrane Ca++-ATPase 1b (PMCA1b) and downregulation of the L-type Ca\(_v\)1.2 and Ca\(_v\)1.3 channels. [L.d 2001] that are mainly responsible for epithelial calcium transport. [Haussler et. al, 2013; A.V. Perez et. al, 2008].

1,25(OH)2D3 also plays a key role in triggering apoptosis by inhibiting the anti-apoptotic proteins and/or by activation of pro-apoptotic proteins. Vitamin D is involved in upregulated expression of Bcl-2-associated X (BAX) and downregulated expression of B-cell lymphoma (BCL) protein 2 (BCL2) and B-cell lymphoma-extra-large (BCL-XL) thus promoting apoptosis. [Umar et. al, 2018]
Vitamin D has an important role in protecting oxidative stress by promoting antioxidant defences. It contributes its role in protection from oxidative stress as it induces the expression of many enzymes that are involved in ROS detoxification. Vitamin D is known to regulate the expression of NF-E2-related factor-2 (NRF2), a transcription factor by either its increased expression, nuclear translocation, or decrease in KEAP1-mediated degradation. NRF2 can be a potent regulator for vitamin D to exert its antioxidant effects as it is the master regulator of expression of antioxidant enzymes.[Jeon et. al, 2018]Nrf2 binds to antioxidant response element (ARE) to mediate its action by enhancing the expression of large number of genes.Nrf2 upregulates the expression of Fos and JUN, which in turn increase the VDR and RXRα expression thus enhancing cells response in case of low levels of vitamin D [L. Bobilev et. al, 2011].Nrf2 also involved in upregulation of many antioxidant and detoxifying enzymes like catalase, glutamate cysteine ligase (GCL) that synthesizes the redox buffer GSH, glutathione S-transferase, Glucose 6-PO4 dehydrogenase, haem oxygenase 1 (HO1), NAD(P)H quinone oxidase 1 (NQO1), peroxiredoxins, Superoxide dismutase 1 (SOD1), SOD2 and thioredoxin (TRX).Vitamin D also regulates the expression of Klotho, which is an anti-ageing gene. Klotho can suppress oxidative stress by upregulating the expression of peroxiredoxins (Prx-2 and Prx-3) and thioredoxin reductase 1 (Trxrd-1) [E. Zeldich et. al, 2014]. Thus, Vitamin D with the help of both Klotho and Nrf2 prevents oxidative stress by removing ROS via regulation of expression of many antioxidant systems. [Berridge et. al, 2015].

It has been evident from various studies that vitamin D is involved in upregulated expression of GLUT 4 as well as its mobilization in adipocytes and monocytes [Manna and Jain et. al, 2012; Tamilselvan et. al, 2013]. In a study by Tamilselvan et al, 2013, it has been shown that Vitamin D is involved in increased glucose uptake via upregulated expression of glucose transporters in myotubes [Tamilselvan et. al, 2013]. It was also found in a study in 3T3L1 adipocyte cell lines that 1,25(OH)2D3 is directly involved in upregulation of GLUT-4 expression [Manna and Jain et. al, 2012].The other components of insulin signal transduction are also affected by Vitamin D [Manna et. al, 2017,2018].Manna et al, 2018 reported that calcitriol increases GLUT-4 dependent glucose uptake via promoting phosphorylation of IRS-1 in murine C2C12 myotubes [Manna et. al, 2018]. It has also been reported that Calcitriol increases insulin sensitivity via
SIRT-1/AMPK signalling pathway and induces GLUT-4 localization in the diabetic mice [Manna et. al, 2017]. In L6 adipocytes, Tamilselvan and coworkers (2013) showed that Vitamin D increases IRS [Tamilselvan et. al, 2013]. In a study by He et al 2019, it has been reported that Calcitriol is a potent activator for PI3/Akt pathway in the diabetes mellitus. It was found that Vitamin D via promotion of Akt, IRS-1 and ERK signalling pathways improved IST [Insulin signal transduction]. It was demonstrated in 2018 by Benetti and colleagues, on supplementation of vitamin D there was improvement in insulin signalling pathways in high-fat diet induced T2DM mice [Benetti, 2018]. IRS-1 phosphorylation is also promoted by Vitamin D supplementation [Elseweidy et. al, 2017]. It was reported that intake of Vitamin D in diabetic animals showed improved glucose homeostasis by activating IRS-1 phosphorylation and promoting IST [Elseweidy et. al, 2017].

There have been studies supporting the role of vitamin D in hepatic lipogenesis and gluconeogenesis mediated via Vitamin D regulated pathways such as AMP-activated protein kinase (AMPk)-calmodulin and Akt/Notch signalling. Calcium/Calmodulin protein kinase beta (CaMKK) or serine/threonine kinase 11 pathways activates AMPk by phosphorylating it [Carling et. al, 2008]. This hepatic AMPK activation promotes anti-diabetic activity by inhibiting gluconeogenesis and lipogenesis and increases glycolytic activity and lipid oxidation [Long et. al, 2006]. In addition, hepatic AMPk activation is also involved in inhibition of Foxo1 [Barthel et. al, 2002] that results in reduced levels of hepatic ER stress and alleviation of insulin resistance and hepatic steatosis [Kamagate et. al, 2010; Li et. al, 2011]. In a study by Leung et al, it has been observed that high doses of calcitriol were able to improve the abnormal hepatic glucose and lipid metabolism in insulin resistant models without any toxic effect. Another confirmatory study by Lin et al., which showed that elevated levels of cytosolic 1,25(OH)2D3 in HepG2 cells leads to stimulation of Ca2+/CaMKK/AMPK pathways, thus supporting the regulatory role of calcitriol on glucose and lipids [Leung et. al, 2016].

B. Non-Genomic Action

On the other hand, recent studies have indicated that there are many non-genomic actions of Vitamin D other than Calcium and phosphate homeostasis. The non-skeletal effects of 1,25(OH)2D includes role in cell
proliferation and differentiation, regulation of hormone secretion and a key role in immune response. [Battault et. al, 2013]. Non-genomic actions are mediated by activation of various signalling molecules or transcription factors. Activation of signalling molecules such as phosphatidylinositol-3 kinase, phospholipase C (PLC), Ca2+-calmodulin kinase II (CaMPKII), protein kinase A (PKA), mitogen-activated protein kinases (MAPK)s, SRC, Protein kinase C (PKC)), p21 RAS is mediated via non-genomic action. [Dwivedi, P.P et. al, 2010; Hossein et. al, 2013; Pike et. al, 2014]. The transcription factors (i.e., SP1, SP3, and RXR) acts as target for these signalling molecules which then interact with VDRE on the promoter region of vitamin D-responsive genes. The non-genomic actions also include production of secondary messengers (like cyclic AMP, Ca2+, fatty acids, and 3-phosphoinositides) and has role in opening of Ca2+ and Cl- channels. [Hii, C.S. et. al, 2016].

3. VITAMIN D STATUS

Due to a short half-life of 4 hour, the active form i.e. 1,25-hydroxyvitamin D is not used to determine the vitamin D status of an individual. That’s why the 25 hydroxy vitamin D is considered as the best indicator for estimation of vitamin D status of a person as it has a longer half-life of 3 weeks. There is classification of vitamin D status[Table 2] regarding the optimal levels of 25-hydroxy vitamin D required for maintaining good health. [Anandbaskar et. al, 2018]

Table 2: Classification of Vitamin D status on the basis of serum 25 (OH) D levels

<table>
<thead>
<tr>
<th>Concentration of 25 (OH) D (ng/ml)</th>
<th>Classification on the basis of serum 25 (OH) D levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>Severe Deficiency</td>
</tr>
<tr>
<td>20-32</td>
<td>Insufficiency</td>
</tr>
<tr>
<td>54-90</td>
<td>Normal</td>
</tr>
<tr>
<td>&gt;100</td>
<td>Excess</td>
</tr>
<tr>
<td>&gt;150</td>
<td>Intoxication</td>
</tr>
</tbody>
</table>

ISBN: 978-81-948426-2-0
Referred range is when serum 25-hydroxyvitamin D levels are above than 150 ng/ml. However, there are lesser chances of toxicity by exposure to sunlight as it is a self-regulated process. It can occur due to overconsumption of fortified food products or dietary supplements. Biomarkers of Vitamin D toxicity are elevated urine calcium levels and decreased parathormone levels. Symptoms include nausea, vomiting, abdominal pain, polyuria and bone pain. Discontinuation of vitamin D intake, calcium-low diet, diuretics aid in the treatment of its toxicity. [Anandbaskar et. al, 2018]

4. DEFICIENCY OF VITAMIN D

Vitamin D deficiency has become a global epidemic and is highly prevalent in all age groups, including children, adolescents, adults, pregnant and lactating women. India also faces the problem of vitamin D deficiency despite being the country with abundant sunshine. The high prevalence of hypovitaminosis can be due to multiple causes. Since sunlight is the major contributor to the body’s vitamin D stores, lack of adequate exposure to sunlight, use of sunscreen and increased melanin content of the skin are the major causes of vitamin deficiency. Lack of intake of vitamin D-rich foods or defects in intestinal absorption of vitamin D could also be the reason for being Vitamin D deficient. Vitamin D deficiency has many associations as vitamin D is complicately linked with maintenance of calcium homeostasis. Defects in mineralization of the skeleton results in manifestation of either as rickets or osteomalacia in children and adults respectively. There are also evident associations of its deficiency with increasing risk of prostate, colon and breast cancer. [Anandbaskar et. al, 2018; Kamboj et. al, 2018]

Several studies have documented that Vitamin D is deficient in the majority of healthy population not only in India but it is prevalent all over the world. It is becoming a major health problem occurring in all age groups from children to elders. In India factors like skin complexion, vegetarian dietary habits, less intake of Vitamin D fortified food products and lack of awareness might be the reason behind increasing rise of deficiency.

<table>
<thead>
<tr>
<th>Vitamin D Deficiency in India</th>
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<tbody>
<tr>
<td>Vitamin D Deficiency in India: Prevalence, Gupta et. al, 2014</td>
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<tr>
<td>Casualties and Interventions</td>
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<tr>
<td>---------------------------------------------------------------------------------------------</td>
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<tr>
<td>Prevalence of Vitamin D deficiency in a Pediatric hospital of Eastern India</td>
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<tr>
<td>Vitamin D status in Indian Population: Major health concern</td>
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<tr>
<td>Prevalence of Vitamin D deficiency in north-west Punjab population</td>
</tr>
<tr>
<td>Current Scenario of Prevalence of Vitamin D Deficiency in Ostensibly Healthy Indian Population: A Hospital Based Retrospective Study</td>
</tr>
<tr>
<td>Prevalence of Vitamin D deficiency and associated risk factors among children residing at high altitude in Shimla district, Himachal Pradesh, India</td>
</tr>
<tr>
<td>Vitamin D levels and deficiency with different occupations: a systematic review</td>
</tr>
<tr>
<td>Is vitamin D deficiency a public health concern for low middle income countries? A systematic literature review</td>
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<tr>
<td>Prevalence of hypovitaminosis D in India &amp; way forward</td>
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<tr>
<td>Vitamin D deficiency in India</td>
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<tr>
<td>Pandemic of Vitamin D Deficiency: Cardiometabolic Concern or Skeletal Biochemical Abnormality?</td>
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<tr>
<td>Modern India and the tale of twin nutrient deficiency- Calcium and Vitamin D- Nutrition Trend data 50 years- Retrospect, Introspect and Prospect</td>
</tr>
</tbody>
</table>
Deficiency of Vitamin D can cause various skeletal disorders like osteoporosis, osteomalacia, rickets etc. and various non-skeletal disorders. Its deficiency has generally been associated with the increasing risk of fractures, loss in bone mass and muscle weakening. [Kamboj et. al, 2018]. It is reported in various studies that Vitamin d deficiency is also associated with various disorders such as cancer, cardiovascular disease, and diabetes mellitus. [Rehim et. al, 2019].

5. VITAMIN D AND DIABETES MELLITUS

Type 2 diabetes (T2DM) is a metabolic disorder characterized by hyperglycemia. It results due to defects in insulin secretion or action or both. [Wang et. al, 2017] It is a matter of serious concern as it is increasing at alarming rate both national and worldwide. According to reports by WHO, it has been estimated that among all diabetic cases 90% are of Type 2 DM and around 15 million people (approx.) globally suffered from Type 2 DM [Tao et. al, 2017]. It has also been estimated that this number might increase double by 2025 [Tao et. al, 2017]. Defects in insulin signaling pathway, systemic inflammation, and dysfunctioning of pancreatic beta cells, all these factors contribute to both insulin resistance and T2DM development. [Wang et. al, 2017].

Recently, Vitamin D deficiency has been reported frequently and it has been associated with pathogenesis of a number of diseases including metabolic disorders [Wang et. al, 2017]. The relation between Vitamin D deficiency and insulin resistance has also been proposed in many studies [Tao et. al, 2017]. Recently, evident from many studies indicated a link between Vitamin D deficiency and T2DM as VDRs were detected in various cell types including pancreatic beta cells and insulin responsive cells such as adipocytes [Nakashima et. al, 2016; Abbas et. al, 2017]. Thus, on the basis of various studies it has been suggested that Vitamin D acts as a regulator of numerous sequential events that are responsible for enabling the pancreatic beta cells to secrete insulin and thereby controlling the blood glucose level.

There have been many studies that indicate the link between Vitamin D deficiency and Type 2 Diabetes. Thus, highlighting the fact that vitamin D deficiency plays a crucial role in development of Type 2 Diabetes. Several population studies have been reported that establish the association between
status of vitamin D and its effect on Insulin secretion and sensitivity with differing results [Al Shoumer et. al, 2015; Mousa et. al, 2017; Wimalawansa et. al, 2018; Kumar PS et. al, 2019]. The effect of Vitamin D supplementation on T2DM has also been studied [Haroon et. al, 2015; Gulseth et. al, 2017; Ashinne et. al, 2018; Pittas et. al, 2019; Safarpour et. al, 2020]. There can be a contribution of Vitamin D deficiency in the onset of diabetes as the vitamin D receptors (VDRs) and 1 alpha hydroxylase enzyme is present on the pancreatic β cells. It has been indicated and evident in studies that glucose tolerance and resistance to insulin can be improved by vitamin D supplementation. It has been reported in various animal studies that there is a crucial link between Vitamin D3 concentration and normal functioning of insulin and pancreatic beta cells. It has been evident from studies that vitamin D deficiency can lead to the increased chances of Type 2 diabetes mellitus [Rehim et. al, 2019]. There has been an indirect effect on secretion of insulin via calcium effect. Vitamin D involves stimulation of expression of insulin receptor thus improving action of insulin and has a direct effect on cytokines by improvement in systemic inflammation [Mitri et. al, 2014]. Vitamin D supplementation promotes beta cell survival and results in restoring insulin secretion. Insulin secretion is indirectly dependent on calcium concentration. Thus, vitamin d regulates secretion of calcium by regulating the function of calcium binding protein, calbindin, that is present in beta cells of pancreas. Insulin sensitivity is also regulated by vitamin D by the stimulation of expression of insulin receptors. The expression of peroxisome proliferator-activated receptor delta (PPARδ), also results in enhanced insulin sensitivity. It has been shown in animal models that vitamin D can modulate expression and activity of cytokine resulting in protection from β- cell cytokine induced apoptosis [Nakashima et. al, 2016]. Vitamin D helps to prevent diabetes by decreasing the levels of both Ca2+ and ROS which are associated with the onset of diabetes. Vitamin D regulates ROS level as it has the ability to control the cellular antioxidants expression .It reduces the onset of diabetes as it is involved in maintaining large no. of cellular processes. [Berridge et. al, 2017]

5.1 Vitamin D in Insulin Secretion

On the basis of preclinical studies, it has been shown that Vitamin D plays an important role in insulin secretion, Ca++ level, and pancreatic beta cell
survival. It has been evident from several studies that there is a contribution of vitamin D deficiency in impairment of glucose mediated secretion of insulin in rat pancreatic beta cells [Norman et. al, 1980; Kadowaki et. al, 1984; Mitri et. al, 2014]. It was also reported that Vitamin D supplementation restored the glucose-mediated secretion of insulin [Norman et. al, 1980; Cade et. al, 1986; Mitri et. al, 2014].

In pancreatic beta cells, both VDR and CYP27B1 are expressed. Thus, Vitamin D via VDR exerts direct effect on beta-circulation [Johnson et. al, 1994; Bland et. al, 2004]. It was presented in a study that after glucose load the insulin secretion was impaired in the mice with non-functional VDR. This impairment also related to decrease in synthesis of insulin by the pancreatic beta cell thus resulting in reduced amount of stored insulin [Zeitz et. al, 2003]. VDRE was found to be present in the promoter of insulin gene in pancreatic beta cells thus supporting the direct action of calcitriol in insulin secretion [Altieri et. al, 2017]. It is of great interest that VDRE induces numerous genes other than activating transcription of insulin gene, that are involved in cytoskeletal organization, intracellular junctions and cellular growth of pancreatic beta cells [Wolden-Kirk et. al, 2013].

Calcium plays an important role in insulin mediated action in target tissues: muscle and adipose tissue. Optimal intracellular levels of Ca ++ are necessary for maintenance of proper insulin action as insulin secretion is a calcium-dependent process. Defect in insulin signalling, being related to downregulation of expression of glucose transporters as a result of alterations of intracellular Ca++ in target tissues, may contribute to peripheral insulin resistance. Calcitriol mediates its action on insulin sensitivity by regulation of extracellular Ca++ concentration and its flux through cell membranes [Mitri et. al, 2014]. It has been reported that increased Ca++ concentration due to vitamin D deficiency may decrease GLUT-4 activity thus resulting in insulin resistance. [Reusch et. al, 1991; Draznin B et. al, 1989].

Vitamin D regulates the Ca++ flux in pancreatic beta cells by downregulating the expression of the L-type Ca++ channels leading to alteration in Ca++ signalling. Exocytosis mechanism of insulin secretion in pancreatic beta cells is stimulated by increased levels of cytoplasmic Ca++ that is mediated via Vitamin D rapid non-genomic action. This action was mediated via stimulation of two signalling pathways. 1) Activation of PKA
that phosphorylates different proteins involved in the role of L-type voltage-dependent calcium channels related to increase of secretion of insulin 2) Activation of synthesis of IP3 and PLC, that contributes to release of Ca++ from endoplasmic reticulum and diacylglycerol (DAG) that in turn activates PKC. Phosphorylation of the KATP channels and L-type voltage-dependent Ca++ channels is mediated via activated PKC. All of these events results into depolarization of cytoplasmic membranes and opening of L-type and T-type Ca++ channels, increasing intracellular Ca++, which in turn activates insulin secretion [Altieri B et. al, 2017]. Elevated Ca++ levels and PKC mediated mobilization of secretory vesicles together stimulates insulin secretion [Doyle et. al, 2003]. It was also shown that elevated Ca++ levels lead to activation of CaMKII (Ca++/Calmodulin dependent protein kinase II) thus resulting in secretion of insulin. CaMKII is serine threonine kinase that is present in insulin secretory vesicles. It mainly functions by promoting the phosphorylation of proteins that are involved in both mobilization and exocytosis of insulin vesicles. It has also been proposed that via CREB (cAMP-responsive Element-binding Protein ) elevated Ca++ levels might stimulate the expression of insulin gene. CREB is an important transcription factor that plays crucial role in maintenance of efficient insulin gene transcription, glucose sensing, survival of pancreatic beta cells and insulin exocytosis [Dalle et. al, 2011]. Vitamin D is also involved in regulation of calbindin-D28k expression. Calbindin-D28k is a cytosolic protein that promotes insulin secretion by regulating intracellular Ca++ level [Johnson et. al, 1994; Berrdige et. al, 2017]. Upregulation of expression of parvalbumin, calbindin D-9k, the Na+/Ca++ exchanger (NCX), and the plasma membrane Ca2+-ATPase 1b and the Ca2+ pumps is mediated via Calcitriol. All of these proteins contributes to low resting levels of Ca++ [Bouillon et. al, 2008; De Viragh et. al, 1989; Wasserman, R.H. et. al, 2004].

5.2 Vitamin D in Insulin Signalling and sensitivity

Calcitriol is not only involved in pancreatic beta cell function, but also in insulin responsive tissues such as adipose tissue, skeletal muscle and liver [Altieri et. al, 2017]. There are a number of ways in which calcitriol could affect insulin sensitivity. It has been shown in various studies that Vitamin D action mediated via VDR increases insulin sensitivity by promoting the expression of IRs (Insulin receptors) on target tissues as well as PPAR-δ.
In target insulin-responsive tissue, Vitamin D is able to activate IRs. In insulin responsive cells, 1,25(OH)2D3 interacts with VDR which in turn heterodimerize with RXR thus forming 1,25(OH)2D3 complex. This complex binds to VDRE in the promoter region of the human insulin receptor gene. This in turn results in enhanced transcriptional activation of insulin gene and increased number of IRs. The increased number of IRs helps in maintenance of proper functioning of insulin signalling pathway [Maestro et. al, 2000; 2002]. Thus, calcitriol improves insulin sensitivity via stimulation of IR expression [Maestro, B et. al, 2000; 2002;2003]. It is evident from this data that there is involvement of vitamin D deficiency in the onset of insulin resistance due to a decrease in the number of IR [Berridge et. al, 2017].

Calcitriol improves insulin sensitivity via activation of PPAR-δ (Peroxisome proliferator-activated receptor). PPAR-δ is a transcription factor that is involved in metabolism of fatty acid in adipose tissue and skeletal muscle. PPAR-δ stimulation via 1,25(OH)2D3 decreases FFAs-induced insulin resistance in skeletal muscle [Dunlop et. al, 2005]. 1,25(OH)2D3 also exerts its action on insulin sensitivity via regulation of intracellular Ca++. Elevated Ca++ concentration enhances translocation of GLUT4 to the cell membrane in muscle cells and glucose uptake [Wright et. al, 2004].

Vitamin D deficiency is also linked to elevated levels of PTH associated with insulin resistance [Reis et. al, 2007; Chiu et. al, 2000]. In insulin responsive tissues such as skeletal muscle and adipose tissue, PTH may increase the concentration of free intracellular Ca++ [Baczynski et. al, 1985; Ni, Z et. al, 1994]. It has been suggested that PTH may lead to insulin resistance via downregulation of expression of GLUT 1 and GLUT 4 in cell membranes thereby reducing uptake of glucose [Teegarden et. al, 2009]. Thus, insulin resistance is promoted by PTH via reduced glucose uptake in adipose tissue, liver and skeletal muscle [Sung et. al, 2012].

It should be of great emphasis that Vitamin D exerts its indirect action on insulin resistance via RAAS (renin–angiotensin–aldosterone system). RAAS mainly functions by regulation of Ca++ levels in skeletal muscle cells and by exerting inhibitory effects on insulin action in peripheral tissues. This in turn promotes glucose transport through membrane via GLUT4 recruitment [Wright et. al, 2004; Wei Y et. al, 2008; Muscogiuri|
et. al, 2008]. Moreover, activation of angiotensin II results in ROS generation via NADPH activating NF-κβ. Insulin resistance is triggered in skeletal muscle via activation of NF-κβ [Wei et. al, 2008]. In VDR null mice, expression of renin and production of angiotensin II have been elevated and calcitriol supplementation inhibits the biosynthesis of renin [Kong et. al, 2003; Li et. al, 2002]. Thus, Calcitriol via inhibition of RAAS may improve insulin sensitivity [Angellotti et. al, 2017].

6. CONCLUSION

We can say that Vitamin D has many beneficial effects that has been the point of attraction to many health professionals, scientists as well as clinicians. According to the recent studies there has been an association between vitamin D deficiency and onset of insulin resistance. There is involvement of both genomic and non-genomic actions of vitamin D in the maintenance of insulin sensitivity. Thus, keeping in mind the beneficial effects of vitamin D we can say that there is direct effect on insulin signalling as well as it also helps in reducing oxidative stress by promoting anti-oxidant activities. Along with that, studies also suggested that Vitamin D helps in improving mitochondrial metabolic activity and ATP levels. Interestingly, mitochondria activity is also crucial for proper insulin secretion and action as activation of PPAR-δ and exocytosis mechanism both are energy dependent processes. Thus, Vitamin D supplementation may be used as potential upcoming therapy for treatment of Type 2 Diabetes.

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ASSOCIATION OF VITAMIN D DEFICIENCY WITH CANCER PHENOTYPE

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ABSTRACT: Cancer is a disease where cells in a specific part of our body divide in an uncontrollable manner. Malignancy is a gathering of sicknesses including anomalous cell development with the possibility to attack or spread to different pieces of the body. These diverge from benevolent tumors, which don't spread. Potential signs and indications incorporate a bump, strange dying, drawn out hack, unexplained weight reduction, and an adjustment in solid discharges. These cancerous cells can invade, sometimes spread and destroy surrounding healthy tissue including organs. In 2015, about 90.5 million individuals had cancer. As of 2019, around 18 million new cases happen annually. It caused about 8.8 million passings (15.7% of passings) To halt this disease new therapies need to be invented. In this chapter we have discussed role of Vitamin D3 as a fat-soluble vitamin which shows a cytotoxic effect on cancer cells through different cell signaling pathways involved in cell proliferation. The deficiency of vitamin D3 may lead to various diseases, one of them is cancer. Hence, for this purpose, specific drugs like vitamin D3 are repurposed with analogues of Vitamin D to prevent types of cancer. Also, many clinical trials have been an epidemiological evidence for the evolution of Vitamin D3 as an anti-cancer agent. [Y. Krishnan et.al, 2017]

INTRODUCTION

These are the following factors: The Cell development and division missing the correct signs, Consistent development and division even given opposite signs, Evasion of customized cell demise, Boundless number of cell divisions, Advancing vein development, Attack of tissue and arrangement of metastases. Tobacco use is the reason for about 22% of malignancy deaths. Another 10% are because of stoutness, terrible eating routine, absence of physical action or extreme drinking of alcohol. Other components incorporate certain diseases, presentation to ionizing radiation and natural pollutants. In the creating scene, 15% of tumors are because of
contaminations. [K. Deeb et al., 2017] These elements demonstration, at any rate halfway, by changing the qualities of a cell. Typically, numerous hereditary changes are required before disease develops. Approximately 5–10% of malignancies are because of acquired hereditary imperfections from an individual's parents. Epidemiological investigations demonstrate that nutrient D deficiency could have an aetiological function in different human malignancies. Preclinical examination demonstrates that the dynamic metabolite of nutrient D, 1α,25(OH)2D3, otherwise called calcitriol, or nutrient D analogs may have potential as anticancer specialists on the grounds that their organization has antiproliferative impacts, can enact apoptotic pathways and restrain angiogenesis. Moreover, 1α,25(OH)2D3 potentiates the anticancer impacts of numerous cytotoxic and antiproliferative anticancer operators. Here, we plot the epidemiological, preclinical and clinical investigations that help the advancement of 1α,25(OH)2D3 and nutrient D analogs as protection and helpful. [T. Cohen et al., 2017]

1. Vitamin D

Vitamin D is the name given to a gathering of fat-solvent prohormones (substances that generally have minimal hormonal movement without anyone else however that the body can transform into hormones). mol. Vitamin D enables the body to utilize calcium and phosphorus to make solid bones and teeth. Skin presented to daylight can make Vitamin D, and Vitamin D can likewise be acquired from specific nourishments. Vitamin D insufficiency can cause a debilitating of the bones that is called rickets in kids and osteomalacia in grown-ups. The two significant types of vitamin d that are critical to people are Vitamin D2, or ergocalciferol, and Vitamin D3, or cholecalciferol molecular weight of the Vitamin D3 is 384.64 g/. Vitamin D2 is made normally by plants, and Vitamin D3 is made normally by the body when skin is presented to bright radiation in daylight. the two structures are changed over to 25-hydroxyvitamin D in the liver. 25-hydroxyvitamin D at that point ventures out through the blood to the kidneys, where it is additionally changed to 1,25-dihydroxyvitamin D, or calcitriol, the dynamic type of Vitamin D in the body. The most precise technique for assessing an individual's Vitamin D status is to gauge the degree of 25-hydroxyvitamin d in the blood [B. Imanishi et al., 2011]. The vast majority get probably a portion of the Vitamin D they need through
daylight introduction. Dietary sources incorporate a couple of nourishments that normally contain Vitamin D, for example, greasy fish, fish liver oil, and eggs. In any case, most dietary Vitamin D originates from nourishment strengthened with Vitamin D, for example milk, squeezes, and breakfast oats. Vitamin D can likewise be gotten through dietary enhancements.

1.1 History of Vitamin D in cancer

The main perception of a backwards connection between daylight presentation and in general disease rate and mortality in North America was distributed just about 80 years ago. Afterward, in 1980 and 1992, the main epidemiological contemplates connecting low daylight presentation and high danger of colon and prostate diseases were accounted for, separately, which recommended that Vitamin D as a substitute for day light introduction may ensure against colon and prostate disease risk. From that point forward, numerous epidemiological investigations have upheld and expanded the UVB–nutrient D–disease speculation in 18 distinct kinds of cancers. The theory has been additionally upheld by examines demonstrating the immediate relationship between vitamin D and malignant growth hazard. A few lines of populace-based examinations uncovered an opposite relationship between serum 25-hydroxyvitamin D (25(OH)D) levels and high danger of colon, breast, prostate, gastric, and other cancers. Besides, there are solid confirmations from few cell culture and creature studies to help the antitumorigenic impacts of nutrient D. In that capacity, it is currently turning out to be apparent that insufficiency of vitamin D.

1.2 Metabolism of Vitamin D3 In Human Body

In response to the sunlight exposure, photochemical synthesis occurs of vitamin D3 where pro-vitamin D3 is converted to pre-vitaminD3. It is obtained from the isomerization of pre-Vitamin D3 in the epidermal basal layers or by absorption of natural and fortified foods and supplements which binds to vitamin D-binding protein (DBP) in the blood stream and is transported to the liver. It is hydroxylated by the enzyme 25-OHase in liver. The resultant 25-hydroxycalciferol (25(OH)D3) is 1α-hydroxylated in the kidney by 25-hydroxy Vitamin D3 1α-OHase. This yields the active ketosteroids 1α,25(OH)2D3(calcitriol), which has different effects on various target tissues. The synthesis of 1α,25(OH)2D3 from 25(OH)D3 is
stimulated by PTH and suppressed by Ca2+, Pi and 1α,25(OH)2D3 itself. The rate-limiting step in catabolism is the degradation of 25(OH)D3 and 1α,25(OH)2D3 to 24,25(OH)D3 and 1α,24,25(OH)2D3, which occurs through 24-hydroxylation by 25-hydroxyvitamin D 24hydroxylase (24-OHase), encoded by the CYP24A1 gene. 24,25(OH)D3 and 1α,24,25(OH)2D3 are consequently excreted [B. Imanishiet.al,2011].

1.3 Vitamin D3 Deficiency in Different Diseases

Vitamin D3, otherwise called cholecalciferol, originates from invigorated nourishments, creature nourishments (greasy fish, cod liver oil, eggs and liver), supplements, and can be made inside when your skin is presented to bright (UV) radiation from the sun. Fundamentally, these two are not the equivalent. Many accept that Vitamin D ought to be delegated a hormone, with some considering it the overlooked neurosteroids. The wellbeing results of being inadequate go a long ways past rickets and what happens with some other nutrient. Also, in contrast to different nutrients, it tends to be made by your body when presented to sun and the dynamic structure in your body, called calcitriol, has likenesses to different hormones (estrogen, cortisol, and testosterone). The genuine perils of overabundance presentation to the sun and skin malignant growth have been extraordinarily pitched and brought about individuals concealing and utilizing sunscreen when in the sun. We have likewise had a move in investing less energy outside in light of expanded work hours and increasingly stationary lives. Accordingly, Vitamin D levels started dropping without most medicinal services experts acknowledging it. Specialists have been concentrating on the results of Vitamin D inadequacy and have discovered a disturbing number of medical problems outside of its job with rickets. These incorporate skeletal ailments like osteoporosis, certain malignant growths, cardiovascular illness, immune system maladies, contaminations, provocative entrail sicknesses, mental scatters, subjective disarranges, stoutness, as well as mortality. These sums depend on what is expected to keep up the blood levels that every rule council has built up as perfect. The higher the blood level that you have to keep up, the more Vitamin D you should keep up that level. Levels of Vitamin D: (a) Adequate 30-60ng/ml (b) Deficiency-Below20ng/ml (c) Insufficient-21-29ng/ml.
2. Vitamin D3 as anti-cancerous agent

Cholecalciferol (Vitamin D3) is 25 hydroxylated at C-25 to shape 25-hydroxycholecalciferol (25(OH)D3). This is 1α-hydroxylated at C-1 by 1α-OHase to yield 1α,25(OH)2D3 (calcitriol). 1α,25(OH)2D3 is a secosteroid that is comparable in structure to steroids yet with a ‘broken’ B-ring (meant second B-ring) where two of the carbon particles (C-9 and C-10) of the four steroids rings are not joined. Numerous Vitamin D analogs hold these secosteroid structure with changed side chain structures around the C-24 position, which makes them less hypercalcemic and less prone to debasement by 24-OHase [170,171]. A few structures of Vitamin D analogs alluded to in the content are shown; paricalcitol (19- nor-1α(OH)2D2), ILX23-7553 (16-ene-23-yn-1α,25(OH)2D3), OCT (Maxacalcitol, 22- oxa-1α,25(OH)2D3) and EB1089 (Seocalcitol, 1α-dihydroxy-22,24-diene-24,26,27-trihomo-Vitamin D3. Vitamin D3 receptor modulators (VDRMs, right) are non-secosteroidal in structure. A portion of the delegate mixes portrayed are LY2108491, LY2109866 and LG190119. Paradigm for improvement and clinical interpretation of 1α,25(OH)2D3 as an anticancer specialist [D. Skrajnowska et.al,2019]. Foundation of in vitro and in vivo test frameworks is essential to creating 1α,25(OH)2D3 or Vitamin D analogs that target Vitamin D3 digestion and flagging. These frameworks permit the instruments of activity of
1α,25(OH)2D3 to be concentrated alongside novel analogs (likewise in blend with cytotoxic medications) in different transformed cell types and their organic impacts (tumor and typical tissues) in creatures. Significantly, concentrates on the pharmacokinetics and pharmacodynamics of medication activity will empower the advancement of better planned clinical dosing plans for clinical preliminaries that will reflect the exposures dynamic in preclinical models where ideal natural impacts of 1α,25(OH)2D3 are exhibited and are feasible in human tumors in clinical therapy [D. Skrajnowska et. al, 2019].

Figure 2: Development Of 1α,25(OH)2D3 And Vitamin D Analouges As Anticancer Drugs [D. Skrajnowska et. al, 2019].

2.1 Key- cancer related signalling pathway

Key cancer regulated pathway have been discussed below:

2.1.1 Nongenomic action of Vitamin D3

Non genomic actions intervened by 1α,25(OH)2D3 are fast and not dependent on interpretation. Be that as it may, nongenomic signalling may in a roundabout way influence interpretation through cross-talk with other flagging pathways. Although there is no concurrence on how the nongenomic actions are started, information recommend that these impacts start at the plasma layer and include a non-traditional membrane receptor portrayed in intestinal caveolae and a 1α,25(OH)2D3-film related rapid response steroid restricting protein (1α,25D3-MARRS) isolated from chick.
intestinal basal-sidelong membrane. The most all around depicted nongenomic impact of 1α,25(OH)2D3 is the quick intestinal retention of Ca2+. Authoritative of 1α,25(OH)2D3 to the proposed membrane receptor can bring about the initiation of numerous signalling cascades. Activation of these signaling cascades, for example, protein kinase C (PKC), can result in the rapid opening of voltage-gated Ca2+ channels and an increase in intracellular Ca2+ which may subsequently activate the Raf–mitogen-activated protein kinase extracellular signal-controlled kinase (MEK)–mitogen-activated protein kinase (MAPK)–extracellular signal regulated kinase (ERK) course in skeletal muscle cells [K. Deeb et. al, 2017].

2.1.2 Anti-tumor effects of Vitamin D3 signalling

1α,25(OH)2D3 has been analyzed preclinically for its restorative viability in chemo-preventive and anticancer activity. A chemoprevention study utilized Nkx3-1; Pten freak mice to reiterate prostate carcinogenesis, and demonstrated that 1α,25(OH)2D3 organization postponed the beginning of prostate intraepithelial neoplasia (PIN) and would be advised to against tumor action when regulated to mice with beginning period (PIN) instead of cutting-edge stage prostate malady. Moreover, contemplates utilizing model systems of squamous cell carcinoma (SCC), prostate adenocarcinoma, diseases of the ovary, bosom and lung indicated that the organization of 1α,25(OH)2D3 or Vitamin D3 analogs had critical anticancer impacts [T. Cohen et. al, 2017].

2.1.3 Antiproliferative effects of 1α,25(OH)2D3

Cell-cycle bother is key to 1α,25(OH)2D3-interceded antiproliferative movement in tumor cells. Movement through the cell cycle is controlled by cyclins, and their relationship with CDKs and CDK inhibitors (CKIs). Articulation of the CKIs p21 and p27 represses multiplication, to a limited extent by inciting G1 cell-cycle capture and withdrawal from the cell cycle (G0). CDKN1A and GADD45A contain a useful VDRE and are immediate transcriptional targets of 1α,25(OH)2D3–VDR [T. Cohen et. al, 2017].

2.1.4 Apoptosis

Not withstanding the antiproliferative impacts of 1α,25(OH)2D3, there is expanding proof that 1α,25(OH)2D3 applies against tumor impacts by regulating key go between of apoptosis, for example, curbing the statement
of the counter apoptotic, professional endurance proteins BCL2 and BCL-XL, or prompting the declaration of proapoptotic proteins, (for example, BAX, BAK and Awful). It has been accounted for that 1α,25(OH)2D3 downregulate BCL2 articulation in MCF-7 bosom tumor and HL-60leukaemia cells and upregulates BAX and BAK articulation in prostate malignant growth, colorectal adenoma and carcinoma cells. In expansion to control the statement of the BCL2 family, 1α,25(OH)2D3 may likewise legitimately enact caspase effector atoms, despite the fact that it is muddled whether 1α,25(OH)2D3- incited apoptosis is caspase-subordinate [R. Scatena et. al, 2014].

2.1.5 Angiogenesis

1α,25(OH)2D3 restrains the expansion of endothelial cells in vitro and diminishes angiogenesis in vivo [106–108]. Vascular endothelial development factor (VEGF)- actuated endothelial cell tube arrangement and tumor development are repressed in vivo by 1α,25(OH)2D3 administration to mice with VEGF-overexpressing MCF-7 xenografts [86]. 1α,25(OH)2D3 can build VEGF mRNA levels in vascular smooth muscle cells109 and upregulate mRNA levels of the intense enemy of angiogenic factor thrombospondin 1 (THBS1) in SW480-ADH human colon tumor cells [R. Scatena et. al, 2014].

2.2 Clinical trials of Vitamin D3 as anti-tumour agent

Interestingly VDR mice show hyper-proliferation and expanded mitotic movement in the dropping colon suggesting a job for 1α,25(OH)2D3-intervened motioning in tumor suppression. Zinser and colleagues demonstrated that VDR ablation mouse expanded concoction carcinogenesis is in mammary, epidermis and lymph not in ovary, uterus, lung or liver. Nonetheless, mice inadequate in key individuals from the Vitamin D blend and catabolic pathways don't create unconstrained malignant growth. The original finding by Garland of higher death rates from colon disease in the upper east and lower rates in the south, south west and west in the United States prompted the significant death at presentation to bright UV-B or day light, which prompts Vitamin D union, can lessen the danger of colorectal malignancy. A few epidemiological perceptions have demonstrated a relationship between low serum 25(OH)D3 levels and expanded hazard for colorectal, breast and prostate
malignant growths. Giovannucci et al. revealed that an expansion of 25 nmol/L in anticipated 25(OH)D3 level is related with 29% decrease in malignant growth-related mortality and a 17% decrease in disease rate, recommending that high 25(OH)D3 levels may be related with a diminished danger of certain tumors. A meta-investigation of case–control and companion contemplate found that people with ≥ 33 ng ml for each ml 25(OH)D3 had a half lower frequency of colorectal malignant growth. Furthermore, patients with beginning time non-little cell lung malignant growth with high 25(OH)D3levels and high VITAMIND consumption at the hour of conclusion and commencement of treatment had improved by and large and repeat free endurance. In this way, these information recommend that low degrees of 25(OH)D3 are a significant hazard factor for malignancy rate.

3. CONCLUSION

We understood a profound progress has been made in the complexity of Vitamin D3 biomolecule in transformed cells, the influence of calcitriol on the nutrient utilization of tumours still unclear. While 1,25(OH)2D3 appears to be involved in complex and metabolic programmes in cancer cells, such as AMPK activation and autophagic signalling, as well as G6PD induction and, thus, likely PPP improvement. Several uncharted territories still remain regarding our understanding of the role of calcitriol in regulating the metabolism of cellular energy. It is, for instance, becoming it is increasingly clear that other important and non-essential amino acids also play a crucial role in maintaining cancer cell survival and proliferation, despite glutamine taking centre stage in tumor cell-specific amino acid metabolism. It has yet to be thoroughly investigated whether 1,25(OH)2D3 affects the use of these amino acids in tumour cells. In addition, further studies are required to explain whether calcitriol’s metabolism-modulating activities are responsible for primary effects or rather a secondary result of the calcitriol-mediated regulation of different tumour suppressors, oncogenes, and other energy-/glucose-related signalling networks. However, our clinical understanding of how calcitriol and its analogues are responsible these cancer hallmarks should enable the combination of these effects with demonstrated findings and newly intervened chemotherapeutics, with the aim of achieving corresponding synergy.

4. REFERENCES
The role of vitamin D and VDR in carcinogenesis: Through epidemiology and basic sciences


Abstract: Fermented food products are used worldwide. Fermented food products treat many problems related to health. The aim of study is to provide knowledge to consumers about consumption of fermented food products. Collection of data was done by using a questionnaire. The data that was collected was analysed which helped to conclude people attitude about consumption of fermented food products. 40 respondents were taken and 69.6% were men and 30.4% women and respondents were mainly of age 19-25 years. Respondents were aware of fermented food products consumption .97% of people were familiar by word fermented food products and 56.4% people consumed fermented food products widely.92.3% respondents got health benefits on consumption of fermented food products which resulted in reduction in risk of heart disease. 25.6% respondents did had any side effects on consuming fermented food products. 86.8% people consumed fermented food products in future. 78.4% people had attitude that fermented food products improved quality of life and 76.9% people recommended fermented food products to others which led to conclusion about health benefits of fermented food products.

Keywords: Fermented food products; respondents; questionnaire.

1. Introduction

1.1 Fermentation is process wherein yeasts or bacteria convert carbohydrates to alcohol or organic acids in absence of oxygen. Fermented food products are extensively used all across the globe. These are also used to cure and prevent many health-related problems. Food fermentation has five main purpose-

- Diet enrichment by development of flavors diversity.
- To enrich food substrates with aromas and textures.
- Preservation of major amount of food by lactic acid, alcohol fermentation.
- Reduction of cooking time and fuel use in making food products.
- Improvement of shelf life of products.
Fermentation is used widely to produce many food products of daily use, for example, kefir, sauerkraut, kimchi, kombucha, idly and bread. Fermented foods have unique flavor that is tasty and aromatic. [1]

1.2 Types of Fermented Food products

There are many types of fermented food products such as:

- **KEFIR** - Kefir is a fermented dairy product, and fermentation is done by bacteria and yeast. Kefir is made from milk of goat, sheep, and has taste of yoghurt.

- **KOMBUCHA** - Black tea and sugar is used to make Kombucha, and it is also a fermented drink. Kombucha is fermented by symbiotic culture of bacteria and yeast.

- **SAUERKRAUT** - Fermented green or red cabbage is used to make sauerkraut. Sauerkraut is enriched with fiber, Vitamin A, vitamin C, vitamin K and B vitamins, and is rich in iron, copper, calcium, manganese, sodium and magnesium.

- **KIMCHI** – Kimchi, a Korean dish, which is prepared by vegetables such as cabbage and spices that include ginger and garlic that have been lactose fermented.

- **BREAD** - Bread is a staple food, and is made from dough of flour and water by baking.

- **IDLY** – Idly, a traditional breakfast usually used in south Indian households, and it is prepared by batter consisting of fermented black lentils and rice. [3]

1.3 Health benefits of fermented food products

- **Improvement of digestive health** - Fermentation helps in restoring friendly bacteria balance in gut by producing probiotics and treats digestive problems like irritable bowel syndrome (IBS).

- **Boosting immune system** - Gut bacteria have great effect on immune system. Due to high probiotic content in fermented food products they boost immune system and reduce risk of common cold.

- **Reduction of lactose intolerance** - Fermentation helps in breaking down nutrients in food which makes them easier to digest.
Intestinal pH balance - Foods that are fermented naturally consist of Lactobacilli bacteria which produce lactic acid which in turn helps to keep healthy level of pH of large intestine.

Antihypertensive activity - Fermented food products lower risk of heart disease.

Anticarcinogenic effect - Fermented food products have anticarcinogenic effect. Anticarcinogenic effect is shown by lactic acid bacteria by initiation of cancer prevention or suppressing cancer initiation.

Synthesis and availability of nutrients - Fermented food products increase the vitamins and minerals availability for body to absorb.

Mental health - Probiotic strains like Lactobacillus helveticus and Bifidobacterium longum cause reduction in symptoms of anxiety and depression.

Weight loss - Probiotic strains like Lactobacillus rhamnosus and Lactobacillus gasseri cause decreased weight loss[5].

1.4 Applications of fermentation

Fermentation processes are exceedingly used in:

- Preservation of fruits and vegetables;
- Preservation of food by organic acid and alcohols at times of scarcity;
- To provide texture to foods;
- Reduction in toxicity;
- To decrease cooking time[2].

2. Methodology

To conduct survey, google form was created and questions were based on-

- Knowledge of students about fermented food products;
- Attitude towards fermented food products consumption;
- Side effects on consumption of fermented food products;
- Frequency of consumption of fermented food products.

The questions were of multiple choice and data was collected from students aged 19-25 years and represented in percentage and presented in tables and
3. Results and discussion

Among the sample size of 40 about 97% of population was familiar with word fermented food products out of which only 56.4% consumed fermented food products. Respondents were asked to determine how they heard about fermented food products. Internet was found to be most common source of providing information about fermented food products to respondents.

Table 1: Frequency and percentage values of respondents for question How they heard about fermented food products? [4]

<table>
<thead>
<tr>
<th>Source</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social Media</td>
<td>13</td>
<td>34.2%</td>
</tr>
<tr>
<td>Other</td>
<td>9</td>
<td>23.7%</td>
</tr>
<tr>
<td>College courses</td>
<td>7</td>
<td>18.4%</td>
</tr>
<tr>
<td>Television programmes</td>
<td>6</td>
<td>15.8%</td>
</tr>
<tr>
<td>Newspapers</td>
<td>2</td>
<td>5.3%</td>
</tr>
<tr>
<td>Retailers</td>
<td>1</td>
<td>2.6%</td>
</tr>
</tbody>
</table>

Table 2: Frequency and percentage values of respondents for question, which fermented food products they consume mostly? [4]

<table>
<thead>
<tr>
<th>Form of fermented food products</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All of the above</td>
<td>22</td>
<td>56.4%</td>
</tr>
<tr>
<td>Pickles</td>
<td>8</td>
<td>20.5%</td>
</tr>
<tr>
<td>Dosa</td>
<td>4</td>
<td>10.3%</td>
</tr>
<tr>
<td>Idli</td>
<td>3</td>
<td>7.7%</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>2.6%</td>
</tr>
</tbody>
</table>
Survey had question about consumption frequency of fermented food products with maximum percentage of respondents (41%) consuming fermented food products 1-2 times per week and 25.6% respondents did not experienced any side effects on consuming fermented food products. 92.3% people had benefit from consuming fermented food products. 76.9% people recommend fermented food products to others showing that respondents are highly satisfied with product and its health benefits and even 78.4% people think that fermented food products can improve quality of life.

Table 3: Demographics of respondents (N=40)[4]

<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>Total(n)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Consumption frequency of fermented food products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 times per week</td>
<td>16</td>
<td>41%</td>
</tr>
<tr>
<td>1-2 times per month</td>
<td>9</td>
<td>23.1%</td>
</tr>
<tr>
<td>Sometimes</td>
<td>9</td>
<td>23.1%</td>
</tr>
<tr>
<td>Once a month</td>
<td>4</td>
<td>10.3%</td>
</tr>
<tr>
<td>Rarely</td>
<td>1</td>
<td>2.6%</td>
</tr>
<tr>
<td><strong>Awareness of health beneficial properties of fermented food products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36</td>
<td>92.3%</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>7.7%</td>
</tr>
<tr>
<td><strong>Health benefits on consuming fermented food products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All of the above</td>
<td>15</td>
<td>41.7%</td>
</tr>
<tr>
<td>Improvement of digestive health</td>
<td>12</td>
<td>33.3%</td>
</tr>
<tr>
<td>Boosting of immune system</td>
<td>5</td>
<td>13.9%</td>
</tr>
<tr>
<td>None of the above</td>
<td>4</td>
<td>11.1%</td>
</tr>
<tr>
<td>Awareness of side effects on consuming fermented food products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Yes</td>
<td>29</td>
<td>74.4%</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td>25.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Side effects on consuming fermented food products</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None of the above</td>
<td>18</td>
<td>54.5%</td>
</tr>
<tr>
<td>All of the above</td>
<td>6</td>
<td>18.2%</td>
</tr>
<tr>
<td>Bloating</td>
<td>6</td>
<td>18.2%</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>Itching</td>
<td>1</td>
<td>3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Awareness of term fermented food products</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>38</td>
<td>95%</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>5%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of people in household consuming fermented food products</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All of the above</td>
<td>27</td>
<td>71.1%</td>
</tr>
<tr>
<td>Adults</td>
<td>6</td>
<td>15.8%</td>
</tr>
<tr>
<td>Children</td>
<td>3</td>
<td>7.9%</td>
</tr>
<tr>
<td>Old aged people</td>
<td>1</td>
<td>2.6%</td>
</tr>
<tr>
<td>None of the above</td>
<td>1</td>
<td>2.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quality of fermented food products</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>19</td>
<td>48.7%</td>
</tr>
<tr>
<td>Excellant</td>
<td>17</td>
<td>43.6%</td>
</tr>
<tr>
<td>None of the above</td>
<td>3</td>
<td>7.7%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intention of consuming fermented food products in future</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommendation of fermented food products to others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>76.9%</td>
</tr>
<tr>
<td>No</td>
<td>9</td>
<td>23.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Role of fermented food products in improving quality of life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
</tbody>
</table>

![Graph representing percentage of awareness of term fermented food products](image)

**Fig. 1:** Graph representing percentage of awareness of term fermented food products
Fig.2: Graph representing percentage of awareness of fermented food products

Fig.3: Graph representing percentage of number of people in household consuming fermented food products
Fig. 4: Graph representing percentage of form of fermented food products consumed.

Fig. 5: Graph representing percentage of consumption frequency of fermented food products.
Fig. 6: Graph representing percentage of awareness of health beneficial properties of fermented food products.

Fig. 7: Graph representing percentage of health benefits on consuming fermented food products consumption.
Fig. 8: Graph representing percentage of awareness of side effects of fermented food products consumption

Fig. 9: Graph representing percentage of side effects on consuming fermented food products
Fig. 10: Graph representing percentage of quality of fermented food products

Fig. 11: Graph representing percentage of intention of consuming fermented food products in future
4. Conclusion and Future Prospects

In recent years market of fermented food products has considerably developed in India. 97% of people were aware about fermented food products it means people have proper knowledge and internet has increased
awareness widely among people. People consumed fermented food products frequently about 1-2 times per week which means they are aware of benefits and side effects of fermented food products.

25.6% respondents did not experience any adverse reactions after consumption so fermented food products are safe for health but some people experienced headache, gas or bloating. 78.4% people think fermented food products improve quality of life and 76.9 % people recommend fermented food products to others, which show immense benefits of fermented food products. Thus, on conducting survey statistical results were in favor of fermented food products market and thus fermented food products are used widely all across the world.

References

CHAPTER-12

Transdermal therapeutics for the treatment of cancer

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ABSTRACT: There are various classic therapies available in the market for the treatment of cancer such as: Chemotherapy, UV radiation therapy and others. Since, these therapies are highly expensive, invasive and carry various side effects, scientist all over the world are exploring new harmless, non-invasive, economic, patient compliant and targeted therapies for cancer. Currently, there is major focus on transdermal delivery of the drugs to target the site, in a less invasive, targeted and slow release manner. The treatment methods using natural products are shown to be much more effective for the treatment of basal cell carcinoma. So far, it has been observed that patches developed (one of the transdermal therapeutics) with the natural ingredients, are more effective and rarely cause mild side effects such as: mild itching and redness etc. The transdermal patches are directly placed over the skin to treat skin cancers. The ointments or the gels are soothing form of the treatment but, as they barely penetrate the skin, syringe or the injectable polymer are being used for such cases. The herbs such as: Gingko, Kava kava, Grape fruit, St John wort etc. have been worked upon to be used as therapeutic agents with patches and gels. Present chapter discusses various approaches, ingredients and effectiveness of the currently developed dermal therapeutics for the treatment of cancer.

Key words: Transdermal Patches; Skin Cancer; Natural Products

1. INTRODUCTION

1.1 Cancer

Among all the diseases prevailing worldwide, cancer is the second leading cause of death (Hossen et al, 2019). In case of cancer, abnormal division of cells takes place, which further leads splitting of cancer cells invading neighboring tissues and organs (Sharifi-Rad et al, 2019). Cancer can appear at any part of the body and based on its appearance at different locations, it is distinguished in several types such as: Blood Cancer, Lung Cancer, Colon Cancer, Rectum Cancer, Prostate Cancer, Skin Cancer, Breast Cancer, Uterus Cancer, Thyroid Cancer, Lymphatic System Cancer and many others (Hassanpour et al, 2017).
There are many therapies prevailing in the market for the treatment of cancer which includes: chemotherapy, precision medicine, radiation therapy, surgery, stem cell transplant, hormone therapy, immune therapy, and targeted therapy (Koul, 2019). But, these methods of treatment carry many side effects such as: constipation, fatigue, nausea, weight loss, hair loss, lack of appetite and diarrhea etc. because, while they destroy the abnormal cells, they also affect the normal cells (Pearce et al, 2017). Other than these various side effects, most of these therapies are invasive, unaffordable and they also lack patient compliance (Arruebo et al, 2011). So, scientists and doctors throughout the world have been looking for harmless, economic, non-invasive and patient compliant and effective therapies. Present chapter discusses such therapeutics where, the treatment of cancer (and few other diseases) can be done using transdermal therapeutics, made up of the natural polymers such as Natural rubber, Gums, Natural rubber, Shellac, Waxes, Chitosan, Edible gums along with natural products as therapeutic agents, including crude forms or pure components of Turmeric, Ginger, Cloves and Curcuma etc (Sreedhar et al, 2018) and few other modern drugs, in the form of dermal patches.

1.2 Transdermal patches

Transdermal patch is defined as desensitized sticky patch which is placed above the skin to deliver the drug in a controlled and determined rate (Duppala et al, 2016). This therapy is a recent advancement in the treatment of cancer (and many other diseases like diabetes and pain relief etc). A transdermal patch can deliver an exact dose of drug through the skin into the bloodstream with a predetermined rate of release to reach to site of action.

Transdermal patches are a noninvasive form of dermal drug delivery system. The continuous controlled release of the drug from this system, leads to the early recovery of the diseased tissue and when the required amount of dosage is utilized, the patches can be removed easily (Ghulaxe et al, 2015). Self-contained dosage of active and inactive components of dermal patches when applied over the integral part of the skin, delivers the drug to the skin (Tanwar et al, 2016). Transdermal delivery system enhances the delivery of the drugs to target the site in a less invasive, targeted and slow release manner (Hossen et al, 2019). This method of treatment is found to be less harmful as, these methods do not require high
dosage of the drugs. As mentioned before, mild side effects have been observed with these patches in rare cases and they are: itching, irritation and redness caused on their application on the dermis.

2. Skin structure

The main interests in dermal absorption and delivery of the drugs through the skin are: transport of drugs through the skin, surface effects, targeting of deeper tissues, controlling the unwanted absorption etc. To understand the mechanism of action of these transdermal therapeutics, we need to understand the structure of the skin first. Skin is known to be the largest organ of our body which covers the surface our body (Escobar-Chavez et al, 2012).

Structural and biochemical features of the human skin facilitate the drug penetration, and contribute to the barrier function, as well. They also decide the rate of drug access into the body via the skin (Davies 2018). Simple schematics of skin structure is shown in figure 1.

![Figure 1: Skin Structure](image)

The epidermis is the first layer of the skin which contains three types of cells namely the Squamous cells (comprise most of the epidermis), Basal cells (round cells) and the Melanocytes (Davies 2018). Stratum corneum (SC) is the topmost layer of the skin and forms the rate-controlling barrier for diffusion for almost all compounds. The stratum corneum functions as a
barrier to prevent the loss of internal body components, particularly water, to the external environment (Escobar-Chavez et al., 2012). The second layer is dermis which contains skin vasculature, nerves, blood vessels, sebaceous, and sweat glands (Davies 2018). Further hypodermis is the deepest layer with subcutaneous tissue. It acts as heat modulator and shock absorber (Escobar-Chavez et al., 2012). Other than these, there are various skin appendages present in the skin which includes: hair, sebaceous glands and the sweat glands (Escobar-Chavez et al., 2012).

Skin condition & Skin age, are also the major factors affecting transdermal drug delivery. The skin of adults and children allows the penetration of the drug easily as compared to the older ones. On the other hand, skin, of the children show many side effects as kid’s skin is more sensitive than the adults. Some physicochemical factors and environmental factors such as hydration of skin, temperature and pH of the skin, sunlight, cold season, and air pollution are also responsible for affecting the drug penetration (Patel et al., 2012).

There are three concern routes which promotes the delivery of drug molecule across the skin layer. First is through the skin appendages like: through the sweat gland and the hair follicles. Second is the transcellular route, which involves the permeation through the coenocytes in the stratum corneum. Third is the intercellular route, which allows the entry of drugs through the continuous lipid matrix (Rastogi et al., 2012).

3. Components of Transdermal Drug Delivery Systems

Basic components required for the formation of the transdermal drug delivery system includes: Polymer matrix, Drug, Permeation Enhancers and other excipients. Polymer matrix are those polymers which are used to hold the drug for the treatment. These polymers can be of both natural and synthetic type. The natural polymers can be obtained from the food gums, fruit peels, cellulose derivatives and other sources. Whereas, the synthetic polymers used can be chemically synthesized for example: silicone nitrile butyl rubber etc. For the success of the delivery of the drug and these polymers have to be chosen with care.

Other major components of these patches is the permeation enhancer. Permeation enhancers are the compounds in the form of surfactants or solvents, which enhance the skin permeability for the drug transport. The
other excipients used in the system formation are the adhesives, backing membranes which stick the system together (Rastogi et al, 2012).

4. Modes of transdermal drug delivery

Major modes for transdermal drug delivery are based on active and passive technologies. Active mode of skin penetration of drugs is based on physical or instrumental triggers. In case of passive transdermal drug delivery, the stratum corneum is not disrupted to facilitate delivery whereas, in case of active technologies, it is. Several such technologies are described as below.

4.1 Active technologies

4.1.1 Iontophoresis: It involves permeation of the drug applying low ampere of current to the skin, to a limited targeted area using the electrodes, which are attached to the formulation to be administered (Hardainiyan et al, 2014).

4.1.2 Ultrasound: It involves delivery through high frequency wave, which forcefully drive drug to the skin rupturing the lipid molecules present in the stratum corneum and therefore, increases permeability of the drug to the target tissue. The frequency applied is in the range of 20 – 100 kHz which cannot be heard by humans (Prausnitz et al, 2008).

4.1.3 Thermal Ablation: It involves microsecond heat pulses which disrupt the selective area of the stratum cornea for the transport of drug to the specific site without causing any rupture of the deeper skin cells (Jijie et al, 2017). The temperature, duration, and localization of thermal energy applied to the skin, are the critical design parameters. Skin should be heated well above 100°C and possibly up to many hundreds of degrees Celsius. (Rathod et al, 2010).

4.1.4 Electroporation: It involves the strategy of applying high voltage pulse for a short duration to the skin which induces the skin permeability by formation of the pores for the drug delivery (Hardainiyan et al, 2014).

4.1.5 Microneedles: It involves micro sized tips loaded with drug. The tips disrupt the barrier between the skin and the affected site. It is a non-painful technique. It provides direct access of the drug to the epidermis without causing damage to the blood vessel (Jijie et al., 2017).

4.1.6 Magnetophoresis: It involves application of the magnetic field
which acts as an external driving force to enhance the diffusion of a diamagnetic solute across the skin. The effect of the magnetic field enhances with increased applied strength that leads to the passive diffusion of drug substance (Hardainiyan et al, 2014).

4.2 Passive technologies

There are majorly 3 major types of popular passive technologies: nano carrier based, vesicular and dendrimer based. Details for these three are given as below.

4.2.1 Nano-carrier based delivery system

The dermal passage of the nano-carriers through skin is via two delivery routes. One of them is through the skin appendages such as the hair follicles and the other is through the intercellular tissues the corneocytes (Jijie et al, 2017). Such delivery systems improve the bioavailability, therapeutic efficiency of drugs by targeting the specific affected site (Rahman et al, 2020) and are described as follows.

4.2.1.1 Lipid and polymer based nano carrier

It is known to be the first nanostructured system used for the transdermal delivery and includes a diverse variety of formulation (Jijie et al, 2017) as follow:

i. Solid lipid nanoparticles: These particles are a combination biodegradable and biocompatible matrix of solid lipid. The stability to the matrix is provided by the surfactant (Sanchez-Moreno et al, 2016). They have different formations as the liquid core of lipid nanoparticles is replaced by a lipid solid (Jijie et al, 2017). This colloidal system is very small in size ranging from 50nm to 100nm. These are highly flexible in nature which provides transport of highly insoluble drug within lipid matrix (Krishnan 2020).

ii. Nanostructured lipid nanoparticle: These are the formulations of the blended solid and liquid lipids that do not possess the crystalline structure (Krishnan 2020). This matrix of solid and liquid lipids is preserved by the surfactants (Jijie et al, 2017). These particles are preferred over the solid lipid nanoparticles as they can do better controlled drug delivery, have enhanced drug holding capacity and the absorption properties (Rahman et al, 2020).
iii. **Nano emulsions:** These are thermodynamic dispersions of water in oil or oil in water (Krishnan, 2020). It is an attractive mode of drug delivery as, they release the drug to the specific site, enhances the drug permeability to the skin, are low viscous, and induces very less pain with avoiding the allergic reaction (Rahman et al, 2020). They provide long duration stability to the drugs due to their small size and the surfactants used in the formation (Escobar-Chavez et al, 2012).

### 4.2.2 Vesicular Carriers

i. **Liposomes:** Liposomes are composition lipids such as: cholesterol and phospholipids (Krishnan, 2020). The phospholipids present are the major component used as they carry hydrophobic tail of fatty acids and hydrophilic head of phosphate group (Hossen et al, 2019). Such hollow bilayer structure provides transport of the hydrophilic drug inside the core and hydrophobic drug between the layers (Escobar-Chavez et al, 2012).

ii. **Niosomes:** Niosomes are formed to overcome the drawbacks of the liposomes. This liposome category is with anionic surfactants, and the hydration process of these depends upon the presence or absence of cholesterol (Krishnan, 2020).

iii. **Ethosome:** Ethosomes are novel form of liposomes with noninvasive lipid based vesicles. It has the ability to transport both hydrophilic and hydrophobic drugs deeper to the skin layer under the stratum corneum and regulates the blood circulation (Rahman et al, 2020).

iv. **Transfersomes:** Transfersomes are advanced liposomes which allow the delivery of drug into the skin which liposomes are unable to process as, they penetrate in the stratum corneum only (Krishnan 2020).

### 4.2.3 Dendrimers

Dendrimers are symmetrically arranged layered structure with a central core, that gives rise to repeated units in a form of branches (Krishnan 2020). Structure of Dendrimers is distinguished into three parts: the core, the branches dendrites and the surface active groups. These surface active groups categorize the dendrimers as hydrophilic or hydrophobic (Hossen et al, 2019).

The ionisable group present in the dendrimers provides it high surface density which makes them efficient to attach drugs by electrostatic forces.
This property provides the drugs with better solubility, increased transport through biological membranes (Escobar-Chavez et al, 2012).

5. Types of Transdermal Patches

There are several types of transdermal patches and majorly known patches have been described as below:

a. **Single layer drug-in-adhesive patches:** In such patches the single adhesive layer of the patch, sticks to the skin and regulate the drug release, as well (Ghulaxe et al, 2015).

b. **Multilayer drug-in-adhesive patches:** Such patches are beneficial for prolonged drug delivery in which, the adhesive and the drug reservoir layer store the drug and release it in the required time (Al Hanbali et al, 2019). They have removable liner layer and a permanent backing layer (Ghulaxe et al, 2015).

c. **Vapour transdermal patches:** In such types of patches, the vapour release is promoted. They are new type of patches in the market. These patches can release oils for up to 6 hrs (Ghulaxe et al, 2015). They contain only a single adhesive layer. Vapour patches are being used for anti-depressant medications (Al Hanbali et al, 2019).

d. **Micro-reservoir transdermal patches:** These types of patches are combination of matrix dispersion with a drug reservoir (Al Hanbali et al, 2019). The drug is not loaded between the layers in this type of patches but, a separate drug layer is provided (Ghulaxe et al, 2015).

e. **Matrix system:** It contains a drug layer of a semisolid matrix containing a drug solution or suspension. The adhesive layer in this patch surrounds the drug layer partially overlaying it (Ghulaxe et al, 2015). It is a multilayer transdermal patch with several backing and adhesive layers (Al Hanbali et al, 2019).

6. Advantages and disadvantages of transdermal patches

We have been discussing majorly the advantages throughout the chapter and certain disadvantages also associated with these. One of them is having mild side effects such as: causing itching and oedema etc. They are also not found to be compatible to ionic drugs but, such kind of technical issue can be handled by further research. Otherwise, they are found to be safe, patient
compliant and provide constant release and are really good in chronic conditions. Table 1 summarizes the advantages and disadvantages of the patches.

**Table 1: Advantages & disadvantages of transdermal patches**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safe, convenient and pain-free self-administration for patients</td>
<td>Itching, edema, erythema etc. may be seen due to patches</td>
</tr>
<tr>
<td>Provide a constant rate of release of medicine to maintain concentration level of drug</td>
<td>Not compatible with ionic drugs.</td>
</tr>
<tr>
<td>Useful in drugs possesses short half-life to avoid frequent dosing administration.</td>
<td>Cannot carry heavy dosage drug</td>
</tr>
<tr>
<td>Drug input can be terminated at any point of time</td>
<td>Only used in chronic conditions</td>
</tr>
<tr>
<td>Inexpensive and economic</td>
<td>Variability in barrier</td>
</tr>
<tr>
<td>Easier to use and provides large area of application</td>
<td>Drug sometimes gets dumped</td>
</tr>
</tbody>
</table>

7. **Natural gums for patches**

It has been, mentioned earlier that natural obtained gums are found to be harmless in comparison to the synthetic gums, many times they also carry medicinal properties as well and can even compliment the anticancer component used for the preparation of patches. Following is a compilation of such gums and their associated plants in table 3 and systematically arranged in figure 2 as well.
<table>
<thead>
<tr>
<th>No</th>
<th>Source</th>
<th>Gum</th>
<th>Phytochemical</th>
<th>Mode of Action</th>
<th>Cancers cured</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Acacia Senegal</em></td>
<td>Acacia Gum</td>
<td>Alkaloids, Glycosides</td>
<td>Decrease in number of tumors, colonic mRNA levels</td>
<td>Colorectal, Breast, Cervical</td>
<td>Elnour et al, 2020 Aloqbi 2020</td>
</tr>
<tr>
<td>3</td>
<td><em>Sterculia urens</em></td>
<td>Karaya Gum</td>
<td>Galacturonic acid, glucuronic acid, L-rhamnose and other residues</td>
<td>Inhibits DNA synthesis, Retards tumor growth</td>
<td>Colorectal</td>
<td>Alange et al, 2017</td>
</tr>
<tr>
<td>4</td>
<td><em>Sphingomonas elodea</em></td>
<td>Gellan Gum</td>
<td>Polysaccharides, Carboxylic sugar etc.</td>
<td>Inhibit oral cancer progression</td>
<td>Oral cancer</td>
<td>Tsai et al, 2017</td>
</tr>
<tr>
<td>5</td>
<td><em>Anogeissus latifolia</em></td>
<td>Ghatti Gum</td>
<td>Galactose, Mannose, Xylose, Galacturonic</td>
<td>Colon ulceration removed</td>
<td>Colon</td>
<td>Maronpot et al, 2013</td>
</tr>
<tr>
<td>6</td>
<td><em>Pistacia atlantica</em></td>
<td>Mastic Resin Gum</td>
<td>Phenol and flavonoid</td>
<td>Reduces cancer cells proliferation, Reduce viability, cancer cell death</td>
<td>Severa l cancers</td>
<td>Rahman, 2018</td>
</tr>
</tbody>
</table>
Figure 2: Natural Gums- Origin and Synthesis Methods

7.1 Acacia gum: Acacia gum is obtained from the stems and branches of Acacia Senegal. It is composed of high molecular weight polysaccharides. It is a gum which easily gets dissolved in the water and forms solutions in accordance with various concentration parameters without becoming highly viscous. It has strong anti-oxidant properties (Singh et al, 2017)

7.2 Tamarind gum: Tamarind is commonly called as Imli (Tamarindus indica). The seed of this plant is a polysaccharide content and has proved to
be beneficial for several dosage forms and drug delivery. The gum obtained from the tamarind seed possesses high viscosity, pH tolerant factors and adhesive quality. It binds the cell surface and intensifies the contact between drugs and the adsorbing biological membrane (Mali et al, 2019).

**7.3 Karaya gum:** *Sterculia urens* tree is the source of Karaya gum. The gum is a composition of diverse feature such as it hydrophilic in nature, biocompatible with high consistence and gel forming adhesion skills however it suffers from uncontrolled rates of association, pH scale dependent solubility and microorganism related contamination (Sethi et al, 2019).

**7.4 Gellan gum:** Gellan gum is obtained by microbial fermentation of the *Sphingomonas paucimobilis* microorganism. The composites or the mixed formation of these gums produces soft gels with elastic property whereas, the pure forms produces hard and transparent gels which are more biocompatible, nontoxic, heat tolerant and stable with a confined rigidity. It is not only, used for drug delivery but also, is beneficial for many biological applications such as: the pharmaceutical and the medical field for dental purpose and many others (Muthukumar et al, 2019)

**7.5 Ghatti gum:** Gum Ghatti is obtained from the *Anogeissus latifolia* tree. This tree is the member of the *Combretaceae* family. Gum Ghatti is an anionic polysaccharide. Several investigation have been done on the gum ghatti based hydrogels such as the Gum ghatti/poly(acrylamide-co-acrylic acid), gum ghatti/poly(acrylamide-co-acrylonitrile), gum ghatti-cl-poly(acrylamide). The adhesive, consistency, preservative & viscous nature of this gum it have been proved out to be compatible for the drug delivery purpose (Xie et al, 2017).

**7.6 Mastic resin gum:** This gum is collected from the Baneh or Daraban tree (*Pistacia atlantica*). It is commonly known as “bneshta tal”. Such gums are resistant to antimicrobial agents. The mastic gum resin has been used in traditional Kurdish medicine for treating various disorders such as topical wound and gastric ulcer. Majorly the gum contains volatile oil with α-pinenes, sabinene, and limonene which makes it efficient for drug delivery for various targeting process. It is also used in the formation of many chewing gums through the natural process (Rahman 2018).

**7.7 Guar gum:** It is obtained from the endosperm of Guar plant
(Cyamopsis tetragonoloba). It has different characteristics property such as non-toxicity, biodegradability, biocompatibility and bioactivity. It is hydrophilic in nature with polysaccharide contents. Many reports say that such gums are also beneficial for oral controlled drug delivery other than the dermal approach. Guar gum based delivery system is as pH-responsive drug delivery system (Xie et al, 2017).

7.8 Tragacanth gum: It is non-mutagenic, non-allergenic, non-carcinogenic, non-teratogenic and non-toxic gum. Singh et al.2016 were the first to report that tragacanth gum is efficient enough for drug delivery and wound dressing when applied in the form of gels which was being prepared by the radiation method. Due to its controlled drug delivery, optimal swelling property, wound healing process it is found appropriate for drug delivery (Xie et al, 2017).

8. Few studies using natural products based parches and few other Transdermal formulations.

Here the authors are discussing few research studies who have used transdermal formulations with special focus on dermal patches are discussed. The summary of these and various others are mentioned in table 4.

<table>
<thead>
<tr>
<th>No</th>
<th>Drug/Plant Extract/Component</th>
<th>Patch Components</th>
<th>Patch/formulation Properties</th>
<th>Pharmacological Properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Turmeric oil</td>
<td>Polyvinyl alcohol as backing membrane and HPMC-50CPS</td>
<td>Flexible, clear and elastic</td>
<td>Anti-colon, cervical and many other types of cancer</td>
<td>Vishwakarma et al, 2012</td>
</tr>
<tr>
<td>2</td>
<td>Zingiber cassumuna r powder</td>
<td>Chitosan HPMC Glycerine</td>
<td>Moisture Uptake Swelling Ability</td>
<td>Enhances the cell cytotoxicity</td>
<td>Suksaeree et al, 2014 Taechowisan et al 2018</td>
</tr>
<tr>
<td>3</td>
<td>Oil extracted from Khardal seeds</td>
<td>Crude extracts oil, chitosan, Polyethylene glycol.</td>
<td>Slight opaque, Pale colour, Jelly formation, Numerous</td>
<td>Mortality rate of brine shrimp was increased with Podina,</td>
<td>Saleem and Idris 2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Zanjabeel rhizomes, Podina (mint) leaves and Sirka (vinegar)</td>
<td>physical properties</td>
<td>Zingiber decreases tumor count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Glucosamine</td>
<td>Gellan gum/ Glucosamine/Cl iquino1 1- ethyl- 3-(3dimethyl amino-propyl) carbodimide</td>
<td>Soft, Flexible, Transparent, Stable</td>
<td>Induces apoptosis in the tumor cells of mice</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Curcumin</td>
<td>Curcumin, HPMC grades, DMSO, PVA</td>
<td>Drug content, moisture absorbing ability, thickness and drug release</td>
<td>Inhibits angiogenesis growth, Tumor associated genes</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Aqueous extracts of neem and bitter gaurd</td>
<td>HPMC, PEG, Aqueous extracts of plants</td>
<td>Flexible, Elastic, Controlled, drug release, Flat</td>
<td>Antidiabetic May be Anticancer as well, via Apoptosis induction, DNA fragmentatio n.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Camellia sinensis</td>
<td>Extract cream and study using Franz Diffusion cell</td>
<td>in vitro penetration</td>
<td>Many pharmacological uses</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Camellia sinensis</td>
<td>Percolation using Dulbecco’s modified eagle’s medium</td>
<td>Accurate thickness and drug release</td>
<td>Cancer cell survival in Caco-2 colorectal cancer cell line</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Carvedilol</td>
<td>With resin gum</td>
<td>Rate control</td>
<td>Long-term hypertension</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Cissus quadrangularis plant extract</td>
<td>Aqueous extracts of the plant, HPMC, DMSO, Dibutyl</td>
<td>Thickness, folding endurance, Moisture</td>
<td>May be able to as Inhibit cell proliferation</td>
<td></td>
</tr>
<tr>
<td>Phthalate, Chloroform and Methanol content, Weight uniformity, the MG63 cell lines</td>
<td></td>
<td></td>
<td></td>
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<td>---</td>
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</tr>
<tr>
<td><strong>12</strong></td>
<td><em>Hibiscus rosa sinensis.</em></td>
<td>Leaves extract, Potassium Dihydrogen Ortho phosphate, Sodium hydroxide, Anhydrous calcium carbonate, Ethanol LR, Chloroform LR, Carbopol–940/Pectin/Sodium alginate, Tween–80</td>
<td>Uniform weight, thickness, moisture content drug release.</td>
<td>Diabetic patients</td>
<td>Dhanalaks hmi et al, 2019</td>
</tr>
</tbody>
</table>

| **13** | Piper and beetle leaf extract | HPMC, PVP –K 90, Propylene glycol, PEG Paraben | Appropriate physical appearance and mechanical properties | MCF-7 cells viability was increased with increase dose in the extract | Boontha et al, 2019 |

In 2012 Vishwakarma et al, formulated a patch containing mixture of turmeric oil, polyvinyl alcohol as backing membrane, HPMC-50CPS as dispersion polymer and poly ethylene glycol as plasticizer. The solvent was allowed to evaporate and after 24hrs of drying the patch was collected and evaluated for weight thickness, drug releases, moisture content and stability. The study showed the best result highlighting that turmeric oil may be incorporated into the dermal patch for their suitable and convenient use as it is flexible, clear and elastic. Turmeric has been found to be important for various types of cancers as later reviewed by Korrapati et al, in 2016 discussing its importance against colon, cervical and many other types of cancer as, it has numerous anti-oxidants, anti-inflammatory, anti-fibrotic activity and is an immunity booster.
Suksaeree et al (2014) worked on the formulation of *Zingiber cassumunar* powder to obtain crude oil and compound D. *Zingiber cassumunar* Roxb., Thai is herb, is used pain relieving medicinal product. It is also used in healing the inflammation caused in a great number of conditions involving the joints and muscles. Due to its therapeutic values ginger has proved to be a wonderful uplifting peppery green eucalyptus aroma, and is highly regarded for its therapeutic properties in massage. Also, similar to ginger are the anti-inflammatory and analgesic actions, though it has an overall cooling, rather than warming effect. The blended patch was a formulation of chitosan mixed with HPMC using glycerine as an adhesive agent. *Zingiber cassumunar* oil was further mixed in the polymer blended solution. The patch was formulated to study the difference between blank and herbal blended patches based on the controlled release of the drug through the patches. \textit{In vitro} skin permeation study was carried out in a Franz diffusion cell using new born pig as a separating membrane and resulted that the blended patch can entrap compound D to control its release. Cytotoxicity of crude extracts of *Zingiber c. oil* has been reviewed by Taechowisan et al (2018) against Monkey kidney line and Murine fibroblast cell line by MTT assay and resulted that the extract enhances the cell cytotoxicity in concentration dependent manner. Thus, it can be expected that this extract based patches might be beneficial for cancer treatment, as well.

Saleem & Idris (2016) formulated an Unani Transdermal Patch for antiemetic therapy. The researchers introduced the incorporation of ingredients, namely, Khardal (*Brassica nigra*), Zanjabeel (*Zingiber officinale*), Podina (*Mentha arvensis*), and Sirka (Vinegar). The patch was prepared by solvent evaporation technique with lactic acid solution of crude extracts oil along with chitosan, Polyethylene glycol. The composition was measured accordingly to prepare 2 patches, which were later evaluated for thickness, weight and moisture etc. \textit{In vitro} permeation study of patch was carried out through egg shell membrane for 24 hours as it resembles human stratum corneum due the presence of keratin. The study resulted out that the drug release in maximum in 1 hour and slows down further.

A study was done by Tsai et al (2017) on preparation and characterization of Gellan gum/ Glucosamine/Cliquinol film for oral cancer patch treatment. The film was fabricated by mixing the three ingredients along with EDC (1-ethyl-3-(3dimethyl amino-propyl) carbodimide). \textit{In vivo} experiment was
carried out with Syrain golden hamster aged 4 weeks which was induced with DMBA oral carcinogenesis. The entire mucosal surface of the left cheek of the animal was treated for 14 weeks to remove the tumor and the film was placed over the wound. Epidermal growth receptor (EGFR) was selected as a marker for evaluating the cancer growth which showed that the release activated apoptosis in the tumor cells without the inhibition of EGFR as it was increased in the cytoplasm of both oral squamous and oral cancer cells.

Curcumin is a natural molecule obtained from *Curcuma longa* which is used in the treatment of arthritis and many others including cancer. Karpagavalli et al (2017) formulated a patch because the oral dosage of curcumin creates instability in the gastrointestinal tract. The prepared was prepared by solvent casting method using 2 grades of HPMC, DMSO and PVC. The solvent was kept to dry for 24 hrs further the patch was extracted and evaluated within 3 days of casting. The evaluation procedure was carried out on the basis of moisture content, weight, thickness and the *in vitro* release through Franz diffusion cell. The study resulted that the patch has determined thickness, weight, moisture content and good folding ability and controlled drug release.

Chauhan et al (2018) worked on the formulation of herbal antidiabetic transdermal patch of neem and karela extracts. The patch was prepared by solvent casting method using HPMC, PEG solution and aqueous extracts of neem and karela. They figured out that herbal drugs in the form of extracts can be used in formulating transdermal patches at an appropriate concentration of drug. Former in 2017 Roy et al in their review explained the effectiveness of *Azadirachta* in treatment of skin cancer, buccal cancer, mammary cancer, prostate cancer and gastric cancer as well. The team reviewed that the ethanolic extract of *Azadirachta indica* causes prostate cancer cell death by apoptosis induction. It acts in a dose dependent manner and increase the fragmentation of DNA.

Ramadon et al (2018) worked on the Green Tea leaves extract cream and *in vitro* penetration study using Franz diffusion cell. Green tea contains a group of phenolic compounds in the form of catechin group which make it able for many pharmacological uses. The researchers focused on
transfersomes based cream to enhance the penetration of EGCG and the ability of the cream formulated was tested by in vitro penetration study using Franz diffusion Cells. Further, Esghaei et al (2018) reported that administration of this *Camellia sinensis* based creams prevents cancer cell survival in Caco-2 colorectal cancer cell line. The extract was prepared by percolation using Dulbecco’s modified eagle’s medium. These were prepared in ranges from 50µg/ml- 800µg/ml to identify the effects on 2 cancer cell lines 1st Caco-2 colorectal cancer cell line, 2nd L929 mouse normal fibroblast. Cytotoxicity of the cell lines was determined using the MTT assay & Aquaporin (Cell survival biomarker) after 48hr treatment and resulted that Caco-2 Cells growth was inhibited when treated with higher concentration extract with decreased level of aquaporin and had less effect in normal cell line.

ati et al, 2018 approached for the formulation of carvedilol transdermal patch with resin gum as rate control. Carvedoil is a drug being prescribed for long-term hypertension treatment. The dosage shows no therapeutic effect when administered orally as it converts in inactive form when undergoes first pass metabolism so to overcome its disability they moved on to apply the drug dermally which resulted out with a better controlled rate.

Das and his team in the year 2018 formulated a transdermal patch of *Cissus quadrangularis* plant extract. The aqueous extracts of the plant were prepared using Maceration method. The patch was prepared by solvent evaporation method with composition of aqueous extracts of the plant, HPMC, DMSO, Dibutyl Phthalate,Chloroform and Methanol The prepared formulation was evaluated for different Physico-chemical characteristics such as: thickness, folding endurance, moisture content, and weight uniformity which resulted that the folding endurance was found to be consistent and the weight uniformity was good and within range. Suresh et al (2019) studied the anticancer activity of the plant methanolic extracts against the human osteoscarcoma cells (MG63 lines) resulting out that the extracts from the aerial part of the part inhibits the cell proliferation, decreases the cell viability, inhibits inhibition of the MG63 cell lines. With the result of the study it can be inferred that the plants extracts based patches can prove beneficial for the cancer cell lines.

Dhanalakshmi et al 2019 worked on the fabrication and evaluation of herbal transdermal film from *Hibiscus rosa sinensis*. It is used as ingredient for the
formulation of transdermal patch due to their numerous therapeutic values. The patch was formulation of Leaves extract, Potassium Dihydrogen Ortho phosphate, Sodium hydroxide, Anhydrous calcium carbonate, Ethanol LR, Chloroform LR, Carbopol–940/Pectin/Sodium alginate, Tween–80 Carbopol was used as an adhesive layer as it is a gelling agent. The study was successful as when the patch was applied over the diabetic patients the drug release was in controlled rate due to the uniformity of the patch.

Boontha et al (2019) stepped towards the preparation of transdermal patches of the *Piper betle* L (Piperaceae) leaf extract. They evaluated the antioxidant activity by DPPH radial scavenging assay and anticancer effects of *Piper betle* L (Piperaceae) leaf extract on Breast cancer cell lines (MCF-7 cells) by sulforhodamine B (SRB) method in which the cells were exposed to a leaf extract containing medium for 48 hrs. Later they were stained with SRB for 30 min. The patch was formulated by casting method containing HPMC, propylene glycol & paraben. The study revealed that the extract exerted cytotoxic activity on MCF-7 cells as cell viability was decreased when increasing dose of the extract.

9. Conclusion and future prospects

The above discussion leads to the conclusion that the new therapeutics in the form of patches have appeared to be an efficient delivery system for cancer and other diseases. It is capable of targeting tumor and other diseased sites directly or through permeation routs. This is more patient compliant method and is a bliss for those patients who cannot take the oral medication for various reasons. It also decreases the graph of severe side effects as less amount of drug is needed as, it bypasses systemic circulation and hepatic degradation of the drug, in majority of the cases. Use of natural components such as: fruit peels, leaves, fruits, stems and root extracts combined gums or other natural or synthetic polymers, for fabricating such systems, can result into further harmless and more effective therapeutics. This arrangement has been assumed to improve quality of life and survival of the cancer patients being less harmful and more effective. The advent of new delivery systems can already be seen in the form of transdermal patches available in the market, for various ailments. The future is bright for transdermal therapeutics as it limits dosage of the drug at the specific site which is difficult to get using systemic administrations and is considered the main clinical failure of chemotherapy in cancer treatment. More research is
required in developing transdermal therapeutics to get the appropriate effectiveness for different diseases as its implementation is not just limited to cancer but is open all various diseased conditions.

References


Yati K, Pamungkas ST. The formulation of carvedilol transdermal patch with resin gum as rate control. Pharmaciana. 2018;8:1:135-144.
CHAPTER-13

Synbiotics: Properties, Actions and Combinations
(A Review)

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Abstract- Human Gut is extremely dominated by the variety of Bacterial Species, hence termed as “Microbiome”. Gibson and Roberfroid (1995) speculated the term “Synbiotics”, Combination of health promoting bacterial species or Probiotics and Non-digestible dietary fibers or Prebiotics, which can potentially lead to the Synergistic outcomes in the form of Health benefits. For being called as Probiotic and Prebiotic, it is important that, Both Bacterial specie and the Fiber must possess some defined properties like Non-Pathogenic Nature, Genetic stability, Bile acid Tolerance (For Probiotics), and Ability for Micro-flora Modulation, Selective Metabolic action, Varying Viscosity, No-side effects (For Prebiotics). As the small variation of the bacterial population in the “Microbiome” can lead to several types of illnesses such as Diarrhea, Hypercholesterolemia etc, Synbiotics are expected to be potentially helpful in the re-establishment of Balance of Bacterial population count in the Gut. It has been found that, the Synbiotics and the Probiotics has the efficacy to Modulate the Health by their different actions such as, Immune system Modulation, Enhancement of Epithelial Barrier, Anti-Microbial activity etc. In recent studies done by the researchers across the globe, it has been found that the Synbiotic Combinations can show their action on many health issues such as Colon Health Cancer, Ventilator associated Pneumonia, Non alcoholic fatty liver diseases. Despite of so many health benefits, there are still not enough evidences to confirm such benefits. As the Synbiotics is a subject of debate for the researchers and the research work is going on across the globe.

Keywords: Synbiotics; Actions; Gastrointestinal tract; immune-modulation; Combinations;

1. Introduction:

The Gut microbiome is home to a big number of microorganisms and so, it is called “Micro-biota” [1]. The human Gut is prominently dominated with variety of Bacterial Population such as “Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria”, among these, two phyla Firmicutes and Bacteroidetes give almost 90% of the total bacterial population of the Gut Microbiome [1, 2]. Due to the prominence of bacterial population, Gut Microbiota or Microbiome has been identified as Modulator of health and also has been designated as an “Essential Organ” for the balanced functioning of the metabolism [3, 4], to particular extent. In the experimental studies, it has been also found that, GIT or Gastrointestinal
Tract contains compounds which are highly rich in nutritional value, resulting in high colonization of Microbes which are health modulating and health promoting [5]. Even the small variation in the population of Microbes can cause some certain type of illnesses and disease such as Diarrhea, Hypercholesterolemia etc [5, 6]

*Lactobacillus, Bifidobacterium and Saccharomyces* are some bacterial species which have shown positive impacts on health by re-establishing the balance of microbes through their health promoting or Probiotic action and some Non-digestible soluble ingredients that can selectively stimulate the growth and metabolism of health promoting bacteria in gastro-intestinal tract designated as Prebiotic. Probiotics and Prebiotics [7], by their simultaneous potential activity can lead to the synergistic effects and result in the formation of “Synbiotic” Combination [7]. The concept of Synbiotics revolve the Combination of Probiotics and Prebiotics that can beneficially affect the host by improving the survival and implantation of living microbes in Gastro-intestinal Tract which can selectively stimulate the growth and activate metabolism of specified bacteria and hence results in improvement of host welfare in the form of Synergistic effects [7].

**Table 1: List of Novel Prebiotics and Probiotics.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Prebiotics</th>
<th>Probiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Low Molecular weight Polysaccharides</td>
<td><em>F. Prausnitzii</em></td>
</tr>
<tr>
<td>2.</td>
<td>Oligosaccharides</td>
<td><em>L. plantarum</em></td>
</tr>
<tr>
<td>3.</td>
<td>Ulvans</td>
<td><em>L. Rhamnosus.</em></td>
</tr>
<tr>
<td>4.</td>
<td>Beta-Glucans</td>
<td><em>L. Acidophilus</em></td>
</tr>
<tr>
<td>5.</td>
<td>Inulin type fructants</td>
<td><em>B. coagulans</em></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td><em>L. Lactis</em></td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td><em>L. bulgaricus</em></td>
</tr>
</tbody>
</table>

Source: Pandey, K. R. et al 2015
Gibson and Roberfroid (1995) speculated the importance of Prebiotics and proposed the term called “Synbiotics” and said that the mixtures of Probiotics and Prebiotics that benefit the host by showing the improvement in the survival and implantation of live microbial dietary supplements in the GIT, by selective stimulation of the growth and/or by activation of the metabolism of one or a limited number of health-promoting bacteria and thus leads to the well-fare of the host [7, 8, 9]. On the other hand, “united nations food and agriculture organization”, in their guidelines, has recommended that the term of Synbiotics can be only used if the given benefit to health is Synergistic [10].

In present day, the combination of strains of *Bifidobacterium* and *Lactobacillus* genus bacteria with the oligosaccharides (fructo-oligisaccharides), inulin etc taken from the various natural resources make some symbiotic combinations giving effects on diarrhea, obesity and also on serious issues like cancer and cardiovascular issues [11]. Synbiotics has increased the genus count of *Lactobacillus* and *Bifidobacterium* species, which has directly contributed to the maintenance of the intestinal microbiota and also became a pioneer in the improvement of immune system modulation [7, 11].

There are several factors which can affect the Gut from the conditions of Delivery of infant and manner of feeding as it is known that before the birth, micro-organism are absent in gut where as they instantly colonize it after the birth whereas this balance can be affected by physiological factors and stresses and this imbalance in population as said early can lead to obesity, inflammatory bowel disease and the balance naturally establish at age of 2-3 years but still it can be affected by ageing, diet and medicine consumption [12].

2. **History:**

“Syn” stands for the synergistic or synergetic effects or “an effect between two or more substances, factors or entities that produces greater effect than the sum of their individual effect” [7]. Gibson and Roberfroid (1995) found the synergic effects shown by the combination of Probiotics and Prebiotics and they termed the combination as Synbiotics and according to the Food and agriculture organization by united nations said, the term ‘Synbiotics’ can be only used if net health benefit is synergistic [8, 10]. In the therapeutic Prospect, these synergic or synergistic effects help in balancing the composition and metabolism of ‘human gut microbiota’ [7]. But still
there is a lack of enough evidence for the recommendation of Synbiotics as a beneficial thing for health. It has been claimed that the combination of aloe which is an anti topical inflammatory agent and carrageenan minimize the mortality value of Probiotic bacteria by making a thick protective layer during the gastric transit after the passage from gastric mucosal barrier. In 1999, Gallaher performed a series of experiments to analyze the effect of combination of Prebiotic (oligo-fructose) and Probiotic (Bifidobacterium animillus) on the carcinogenic substances [13]. In 2015, Alexander and Evan had given a process to manufacture the improved spectrum of Synbiotic. The process is done by the fermentation of beans to obtain a Synbiotic composition having a long life. This study was contexts for the problem because, during traditional processes the raw beans are fermented and the cooking of the fermented product results in loss of vital components [14].

There are some studies which demonstrate the potential of Synbiotics and their synergistic effects on health. Hedin, Whelan and Lindsay (2007), in their study, demonstrated the effect of Synbiotics in inflammatory bowel disease [15]. Similarly, Kukkonen, savilahti and others demonstrated the effects on the allergies in 2007 [16]. Lee and Salminen in 2009 suggested in their study that Synbiotics also exhibits the benefits which are associated with the specified consumption of Probiotics [17]. These benefits includes production of cobalamin, thiamine and riboflavin etc and a study also indicates in the direction of synergistic influence on liver related brain dysfunction and colorectal cancer by Pool-Zobel BL 2005 And Hylla, Gostner, Dusel and others in 1998 [17, 18].

In a study by Yukako kojima and others from Tsurumi University strained the 40 Probiotic and 12 Prebiotic strains from the source Lactobacilli and isolated 5 type of strains and saccharides like arabinose, xylose and xylitol. The Synbiotic combination can inhibit the growth of Candida albicans which is a dimorphic fungus and Porphyromonas gingivalis which is a gram negative pathogenic bacterium. The aim behind this investigation was to explain the use of Synbiotic combination to fight off the infections in the mouth [19].

3. Properties:

For attribution and evaluation of a compound as Prebiotic or any Bacteria as a health promoting bacteria or Probiotic, it is must that both of the Probiotic and Prebiotic must show a defined set of Properties or Characteristics,
Pammi Gauba, Sudha Srivastava, Shalini Mani

which makes them eligible for benefitting health Synergistically [17], when act in a combined (Probiotic+Prebiotic) fashion. WHO/FAO has given clear guidelines which are actually used to evaluate the bacteria as Probiotic, which can potentially benefit the health [7, 17, 20]. Swennen et al in 2006 has given the ideal characteristics of Prebiotic, a non digestible dietary fibre which can potentially show the characteristics such as, Selective metabolic action of Microbes, Ability of Micro-flora Modulation, Easy storage and also No-adverse effects or No Side-effects. The ideal characteristics of Probiotics and Prebiotics are given in the following table. [7, 21]

Table 2: Novel Properties of the Probiotics and Prebiotic fibres.

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Prebiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infectious or Non-Pathogenic</td>
<td>Low-dose action</td>
</tr>
<tr>
<td>Nature</td>
<td></td>
</tr>
<tr>
<td>Genetic Stability</td>
<td>No Harmful or No side-effects</td>
</tr>
<tr>
<td>Anti-genotoxicity</td>
<td>Variable viscous nature</td>
</tr>
<tr>
<td>Bile-Acid tolerance</td>
<td>Easy storage</td>
</tr>
<tr>
<td>Short Generation time</td>
<td>Ability of Micro-flora modulation</td>
</tr>
<tr>
<td>Effective adhesion</td>
<td>Selective metabolic action</td>
</tr>
<tr>
<td>Survival</td>
<td></td>
</tr>
</tbody>
</table>


4. General Probiotics and Prebiotics;
4.1 Probiotics:

*F. prausnitzii:* It is an extremely oxygen sensitive bacteria, and covers over the 5% of fecal microbiota of human which play key role in maintenance of the intestinal health whereas decrease in level of this bacterium has been found in the patients of colorectal cancer, celiac and inflammatory bowel disease studies done by Martin and others in 2017 on properties like anti-inflammatory nature has opened a hope for new possibilities in which they had tried to represent a brief profile of some of the properties like lytic activities, adhesion and resistance to antibiotics in which they involved the strains of *faecalibacterium* collected from humans, also there correlation
between this bacterium and IDB and IBS like diseases has been found. As they discussed, the above properties can open the possibility for murine models to test for proper determination of health benefits on hosts [22].

*L. plantarum*: A health promoting bacteria found in GIT of humans and majorly employed in the Food-Industries, it is a Lactic acid G+ Bacteria which has some properties like anti-obesity, anti-inflammatory and also anti-diabetic and has many medical field applications. Whereas on the other hand it helps in regulation of lipid metabolism in adipose tissues and it can also increase anti-proliferative activity [23].

*L. acidophilus*: it is also a health promoting bacteria and one of the prominent commercially employed Probiotics found in various products based on the milk such as yogurt and supplements and it can help in controlling intestinal infections and reducing cholesterol levels of the body whereas it can also act as anti-carcinogens which makes it one of the best Probiotics [24].

*L. lactis*: This bacteria has characters like antioxidant activity, antidepressant whereas one of the strains of this bacteria *Cermoris LL95* has a resistance in its nature from various levels of concentrations of Bile acids [25]. Study done by B. Ramahlo and others (2019) suggested that the above strain of the bacteria has efficiency in exertion of free radical scavenging effect and found that the above strain ac improves the depressive and anxiety like behaviour although molecular mechanism of the above studies has not been found yet [26].

### 4.2 Prebiotics:

Low Molecular Polysaccharides: In studies, These Polysaccharides have shown observable increase in the production of propionic acid and acetic acid which are one of the major organic acids. The above Prebiotic is generally extracted from the source like alginate and agar seaweeds [27].

Alginates: it is an anionic polysaccharide commonly found in blue green algae also called Alginic acid. It is an irregular blockwise chain structure containing alpha-L-guluronic acid and 1,4 linked beta-mannuronic acid residues which both are uronic acids (monosaccharides) of 6 carbon structure [28].

### 5. Actions of Probiotics and Synbiotics:

There are several mode of actions are shown by Probiotics where they directly works with Prebiotic, as Prebiotics act as substrates for them. Some of them are following.
1. Enhancement in barrier.
2. Immune system modulation.
3. Production of Anti-Microbial Substance.
4. Feed for Aquaculture.

5.1 Enhancement in barrier (epithelial barrier): The intestinal barrier is the main defence mechanism which plays a vital role in the maintenance and protection of integrity. This defence line consists of mucous layer, peptides which are antimicrobial, secretory IgA and the epithelial junction adhesion complex. The disruption in the barrier can lead to serious intestinal disorders like inflammatory bowel disease, as the bacterial and food antigens reach the submucosa induce inflammatory response. The several studies concluded that the Probiotic bacteria can contribute for the maintenance of barriers, however the mechanisms which function in the process are not fully understood yet [29]. Some studies concluded that the integrity of the intestinal barrier can be reinforced by the enhancement in the gene expression. It has been found that Lactobacilli can modulate the process of regulation of several genes which encodes the adherence junction proteins like E-cadherin, beta-cadherin [29, 30]. Also, Lactobacilli influence the abundance of PKC (protein kinase C) isoforms. In recent studies, it has been found that the E. coli can prevent the mucosal barrier and also restores the integrity in cells like T84 and Caco-2. This function is shown by enhanced expression and redistribution of tight junction proteins such as PKC and ZO-2 which directly results in reconstruction of the tight junctions [30].

Where, VSL3 activates the p38 and extracellular regulated kinase signalling pathway, which directly plays a role in the protection of epithelial barrier and also enhance the tight junction protein expression invivo and invitro. The characteristic of inflammatory bowel disease which is cytokine induced epithelial damage can be prevented with the use of Probiotics and it can also contribute in the process of reinforcement of mucosal barrier [31]. p40 and p75 are the peptides which are secreted by Lactobacillus rhamnosus GG which can prevent the cytokine induced cell apoptosis by the activation of anti apoptotic protein kinase B in phosphatidylinositol-3-kinase-dependent pathway and by inhibiting the pro apoptotic p38/mitogen-activated protein kinase(MAPK) leading to the Promotion of cell growth in Intestinal epithelial barrier [31, 32].
5.2 Immune system modulation: Probiotic bacteria show the immunomodulatory effects in the body as bacteria can interact with the epithelial cells, monocytes, lymphocytes etc which directly affects the modulation in the system. Whereas, the acquired immune system is dependent on antigens B lymphocytes and T lymphocytes and the innate immune system is related to the pathogens of the body, or in other words it depends upon pathogen-associated-molecular patterns (PAMP). The initial response is given by pattern recognition receptors which join or bind with PAMPs. There are some pattern recognition receptors like TLR (toll like receptors), CLR (c type lectin receptors) and also NOD like receptors transmit the signals on interaction [33, 34, 35].

Here, IECs is a term given to the cells which interact with Probiotics where Dendritic cells (DC) are encountered by Probiotics play an important role in both types of immunities. Both DC and IEC interact and respond to gut microorganisms through pattern recognition receptors [35]. In the recent experiments, active components of Probiotics have been determined. In experiment, matrix assisted laser desorption ionization time of flight spectrometry analysis of bifidobacterium animalis subsp is coupled by two dimensional gel, where the secretion of proteins by the help of Lactis BB 12 has shown the 74 type of distinct proteins. In these proteins, 31 type of proteins are expected and predicted to show the physiological role on the outside of cell and on surface of cell, solute binding proteins, amino acids and cell wall metabolizing proteins etc, where some other proteins perform a mediated interaction with epithelial cells of human host body. There are some functions such as formation of fimbriae, adhesion to collagen, attachment to mucin and intestinal cell and immunomodulatory responses. The above observations suggested the importance of Probiotics in the immune system modulation process.

The fascinating effects of Probiotics are also shown by modification in the bacterial genome. In Probiotic named L. acidophilus NCFM, the deletion of phosphoglycerol transferase gene which perform the mediation of biosynthesis process lipoteichoic acid has reduced the response of IL 12 and TNF but it has enhanced the production of IL10 in the dendritic cells (DC) and the control in co stimulatory function of (DC) which further resulted into the inactivation to induce the CD 4+ T cell activation [36].

The treatment of rats with these bacteria resulted in decrease in the Dextran sulphate sodium and CD 4+CD45RBhigh T Cell induced colitis.
Consequently, decreased mucosal inflammation was co-linked with regulation of IL 10 and CD4+FoxP3+ T regulatory cells [36].

**5.3 Production of antimicrobial substances:** The formation of the low molecular weight compounds and production of and production of antibacterial substances is one of the health beneficial activities performed by Probiotics [37].

Acetic acids and lactic acids are one of the organic acids which are considered as compounds, performing inhibitory activity of Probiotics against the Gram negative bacteria. In this activity the organic acid dissociates inside the cytoplasm of the bacterial cell and results in lowering in the pH and also the ionized organic acid leads to the death of pathogens when they are accumulated. Where the “bacteriocins” produced by gram positive bacteria show a narrow spectrum of activity, in other words they act against only bacteria which are related to them. The bacteriocins act on the bacteria by affecting its cell wall [38].

Some studies reveal that production of bacteriocins helps in the growth of the prevalence of strains as well as also help to directly inhibit the growth of microorganisms in gastrointestinal tract (GIT) [38].

The intestinal bacteria also produce a variety of fatty acids which are health promoting and certain strains of intestinal *Bifidobacteria* and *Lactobacilli* give rise to formation of conjugated linoleic acid (CLA) which is an anti carcinogenic in nature. In an experiment, the anti obesity effect of CLA producing *L. plantarum* has been observed and the capability to modulate the composition of fatty acid of liver and adipose tissue of host upon the oral administration of *Bifidobacteria* and *Lactobacilli* producing CLA has been demonstrated in murine model [39, 40]. In the recent studies it has been found that Probiotic bacteria can also produce the de-conjugated bile acids, having the higher rate of antimicrobial activity as compared to the bile acids synthesized by host but the reason behind the resistance of Probiotics from their bile acids is not found yet [41].

Some researches show that lactobacillus can produce the substances like benzoic acid, methylhydantoin etc which are anti fungal in nature [42]. In 2001 Magnusson and Schnurer concluded that *Lactobacillus corynformis* can produce the proteinaceous compounds which can also perform antifungal properties [43]. In a study, Dal Bello et al has shown the identification and chemical characterization of four type of antifungal substances such as lactic acids, phenyllactic acids, and two cyclic dipeptides.
which are cyclo(L-Leu- L-Pro) and cyclo(L-Phe- L-Pro), produced by *L. plantarum* FST 1.7. The antifungal culture from Probiotics can reduce the growth of *Fusarium culmorum* and *Fusarium graminearum* which are found in breads [44].

5.4 Feed for animals and aquaculture: There are some similarities in the Probiotics and the competitive exclusion products (which contain a variety of microbes which are considered as friendly for the animals) Probiotics are always expected for the improvement of microbial balance in the gut. Probiotics are effective in certain situations like in newborn animals, the Probiotics helps in boosting the health of the host animal, but still the strong evidence of mechanism involved in the Probiotics as animal feed is not found yet [45].

Because of the initiatives of organizations for ending the use of antibiotics, there was a requirement of a strategy by which modulation of the gastrointestinal system can be maintained and Probiotics are considered as an alternative for the antibiotics. On the other hand, Synbiotics (Prebiotic + Probiotic) provides a perfect equilibrium which is more favourable, then one component. As being a synergistic way, Synbiotics selectively stimulate the growth and implant the microbes fed in the GIT by the activation of metabolism of a limited number of bacteria [45, 46].

6. Combination:
The “Synbiotic” Combination results can vary as per the type of strain of the bacteria and as per the type of Non-digestible dietary fibres. There are “N” numbers of Strains and Non-digestible dietary fibres which have been discovered in the several studies that can potentially give Synergistic effects and Results. Some of the combinations which have been studied by the researchers in the recent times are as follows:

Table 3: Probiotic and Prebiotic Combinations involved in the study and description of the outcome of the study

<table>
<thead>
<tr>
<th>Probiotic Strain involved.</th>
<th>Prebiotic Fibre involved</th>
<th>Outcome of the study</th>
<th>Year of the study.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. plantarum</em></td>
<td>Fructo-oligosaccharides</td>
<td>High bacterial diversity was found among the infants in their microbiota.</td>
<td>Chandal, D. S. et al 2017.</td>
</tr>
<tr>
<td>Bifidobacterium breve and Lactobacillus casei</td>
<td>Inulin</td>
<td>The administration of this combination resulted in decreased concentration levels of Glutathione and free sulphydryl groups, Malondialdehyde and Hydrogen Peroxide. It has shown Positive influence on the oxidative stress marker which was involved in the study</td>
<td>Kleniewska, P. 2017.</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis IVS-1 and Bifidobacterium lactis BB-12</td>
<td>Galactooligosaccharides</td>
<td>It has been found in this study that Prophylactic Synbiotics can gut microbiota and prevent from the problems such as incidence of enteritis and ventilator-associated pneumonia</td>
<td>Shimizu, K. et al 2018.</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis IVS-1 and Bifidobacterium lactis BB-12</td>
<td>Galactooligosaccharides</td>
<td>In this study, More activity of the Probiotic has been observed than the Prebiotic Substrate involved in the study but the Probiotic and Prebiotic combination didn’t show any Synergistic results.</td>
<td>Krumbeck, J. A. 2018.</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>Fructooligosaccharides</td>
<td>The Synbiotic helped in</td>
<td>Yao,</td>
</tr>
<tr>
<td><strong>Lactobacillus paracasei N1115</strong></td>
<td>arides</td>
<td>the alleviation of High fat diet induced steatosis and the releasing process of Tumor necrosis factor α and also slowed down the Progress of cirrhosis. Reduced the serum total triglyceride and Cholesterol levels. It was found that these findings can be related to the transcriptional repression of inflammatory factors and can improve intestinal barrier functioning and Histological integrity, as discussed in the Actions. It has been found that Synbiotics can be effective in the treatment of Non-alcoholic fatty liver disease.</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td><strong>Lactobacillus plantarum M BTU-HK1</strong></td>
<td>acacia gum</td>
<td>In this study, the Synbiotic and Probiotic, both had shown the effects such as reinforcement of immunoglobulin levels and modulation of the Phagocytosis. Synbiotics also showed the stimulatory effects on Splenocyte viability. This treatment has also shown a reduction in the levels of</td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th><strong>Lactobacillus rhamnosus GG</strong></th>
<th>Turmeric Powder</th>
<th>As per the aim of the study, the suppressive effect on the Allergic Inflammation in a Murine Model of House Dust Mite-Induced Asthma by the Synbiotics and Probiotics has been studied. In this study, it has been found that, Synbiotic Intervention can suppress the development process of airway hyperresponsiveness in the response to Methacholine and also can down regulate eosinophilia, IL-5, CCL-17, and IL-13. In the response to T cell, CD4+ Th2 cells and CD4+...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pammi Gauba, Sudha Srivastava, Shalini Mani</td>
<td>tumour necrosis factor α. The Bacterial procarcinogenic fecal enzyme activities were found to be lowered by the Synbiotic treatment, Which proves the role of Probiotics and Synbiotics in the Prevention of Colon cancer incidence.</td>
<td>Ghiamati Yazdi, F. 2020.</td>
</tr>
</tbody>
</table>
7. Conclusion:

From 1995 to the current time, Since Gibson and Roberfroid coined the term of Synbiotic, there have been a lot of advancements globally. Several Probiotic and Synbiotic supplements are being manufactured on the Commercial scale. Due to the properties of the Probiotics and Synbiotics such as Non Pathogenic nature, genetic stability, selective activity, viability and activities such as Intestinal barrier or epithelial barrier enhancement, immune-system modulation, anti-cancer nature, antimicrobial nature, the expectation of the scientific community from this field are really very high. Meanwhile, Several studies on the effectiveness of the several different combinations has been done by researchers, which showed several promising results such as effects on Asthma, Colon Cancer incidence, Non-alcoholic fatty acid diseases, ventilator associated pneumonia etc. still there is a lack of strong evidences to counter the many anti-thesis related to the effectiveness and the results of these supplements. Many researches and debates are going on across the world, it is expected that there will be a lot of advancements in the scope of the Probiotics, Prebiotics and Synbiotics in Near Future.

8. Acknowledgement:

I am thankful to Dr Kuldeep Kumar (Head of the Department), Department of Biotechnology, Multani Mal Modi College, Patiala and Dr Mandeep Singh Sibian (PhD) for their Guidance and Support while writing this Chapter. I am also thankful to Miss. Smile, Post graduation student,
Department of Biotechnology, Multani Mal Modi College, Patiala for the help and guidance while writing this Chapter.

9. References:


Drakes, M., Blanchard, T., & Czinn, S. Bacterial Probiotic modulation of dendritic cells. *Infection and immunity*, 2004;72(6), 3299–3309.


CHAPTER 14

Phytoextraction of Precious Metal

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Abstract: As the global economies and world population continue to grow at an increasingly fast step and because of advancements in technology and science, the demand for noble materials, including silver, gold, platinum-group metals and other rare earth elements, is rising rapidly. This demand comes with a great price; the mining of such noble metals causes high risks to human health and ecosystems. Conventional mining activities can cause pollution on the surface water, groundwater, soils and air. Such activities further cause tremendous amounts of waste that are generated every day, generally from industrial and commercial activities, demolition and construction and domestic households, leaving a traditional of non-recycled metals in landfills or in another disposal facilities. Phytoremediation offers a ‘natural’ way of addressing these environmental issues ranging from phytoextraction to green phytodegradation processes for agricultural industrial and municipal residues and effluents. Phytoextraction is an important element in the paradigm of ‘sustainable development’, whose purpose is to achieve goals that include in the particular safe disposal of waste and the recovery of materials, for example, precious metals, that are present in all types of waste from various sources. Using phytoextraction technology, precious metals can be recovered from materials that are much more economically beneficial as compared to conventional technology.

Keywords: Economies; Phytoremediation; Phytoextraction; Phytodegradation; Precious Metals

1. INTRODUCTION

Plants accumulate in their tissue all the macro- and micro-elements which are essential for its growth and reproduction like nitrogen, phosphorus, potassium, iron, copper and also some other elements for which no definite role has been established, such as chromium, nickel, arsenic, tin and precious metal like gold, silver, palladium, platinum, etc. These elements can occur in plants over wide ranges of concentration, depending on the plant species, their growth rates, and soil and environmental factors. The accumulation of metals in plant’s body has number of great applications. Thus, the accumulation of metals in the plant’s body has the number of great applications. Thus, the analysis of plant material for zinc, lead, mercury and arsenic constitutes an elegant means of monitoring atmospheric releases of these pollutants. Similarly, extensive concentrations
of cadmium(Cd) have been found in various species of plant growing near metal conglomerate. First-ever gold presences in plant tissues were detected in early 1900 when production of gold beads from hardwood trees was done using the method of fire-ashing of fire-ashing[1]. Since then, concentrations of gold have been reported in a wide range of plant species collected from different mineral-rich areas throughout the world. Indeed, it has been shown that under some particular conditions, plants display overt symptoms of phytotoxicity caused because of metal accumulation.

2. Precious metals

Precious metals are naturally occurring, rare metallic chemical Earth elements of high penny-pinching value. Chemically, the precious metals are less reactive than most of the elements. They are generally ductile and have a high lustre point. Historically, these precious metals were treated as currency but now they are regarded mainly as industrial and investment commodities. The currency code of gold, silver, palladium, and platinum is an ISO 4217.

Silver and gold are the most known precious metals which are also known as coinage metals. Though both them are known for their commercial uses, they tend to be used in jewellery, coinage and art. Other examples of precious metals are platinum, osmium, ruthenium, palladium, rhodium and iridium, which altogether are also called a platinum group of metals. Out of this group, platinum is known to be the most popular traded element. The demands of precious metals are different not just because of its practical uses but also because of the value they store and the way they work as investments.

2.1 Sources of Precious metals in soil
2.3 Effect of precious metal to environment

Generally, precious metals are not known to be essential for plant metabolism, and hence it may be classed as a "ballast" element in plants. In some studies, it is reported that the precious metals cause some impact to the plant that uptake it. In one study it was reported that the accumulation of Pt(II) via plant tissues affected vital processes, with stimulatory effects at very low concentrations and toxic effects at high concentrations. It also disturbs the plant morphology, biomass accumulation, the functioning of photosynthetic equipment and membrane integrity[2]. In another study it was shown that the uptake of gold nanoparticles by plant causes some positive and negative effect on the plant. These particles enter into the roots of the plant by a size depended on mechanism there they affect the biomass/growth or causes a reduction in cell growth by causing imbalance at molecular, biochemical and physiological levels generating oxidative stress. They also cause higher inhibition of reactive oxygen species which shows the free radical scavenging activities of this gold nanoparticles. There was also increased in antioxidant enzyme activities and presides the stress.
microRNA. Gold nanoparticles have also increased the quantity and quality of vegetables and fruits[3].

3. Methods of remediation of metals

4. Advantage of Phytoremediation over mechanical and chemical treatments

- Phytoremediation is more cost-effective than traditional ex-situ and in-situ processes.
- Plants are easy to handle and monitor
- Higher possibility of the reuse or recovery of valuable metals
- One of the least harmful method as it only uses naturally occurring organisms and do not cause any harm to the environment
- Maintains the fertility of soils as it preserved the top-soil
- Improves soil’s yield, health and increase plant phytochemicals
- Use of plant prevents metal leaching in soil and also reduces soil erosion

5. Phytoremediation
Phytoremediation is defined as the use of plants and their associated soil microorganisms to reduce the harmful effects or the concentrations of contaminants in the environment. Phytoremediation is mainly accepted as a cost-beneficial environmental re-installing technology. Phytoremediation is a substitute for engineering methods that are usually more expensive and are destructive to the soil. However, phytoremediation is limited to the root area of the plants [4,5]. This technology also has a restricted application where the concentrations of contaminants are harmful to plants and soil. These technologies are available for different contaminants in various environments. These involve different actions such as in situ degradation or removal (i.e., volatilization or extraction) and stabilization of contaminants.

5.1 Types of phytoremediation
i. Phytoextraction/phytoaccumulation,
ii. Phytostabilization,
iii. Phytostimulation,
iv. Phytovolatilization,
v. Phytodegradation

Different techniques of phytoremediation

6. Phytoextraction
Phytoextraction is a novel approach in which plants are used to uptake pollutants like heavy metals from the soil by its roots through the absorption process. The metal absorbed is either stored or gets accumulated in leaves and stems i.e. the harvest-able portion of the plants. Plants that are used for
this purpose are called hyperaccumulators. They have a high tolerance to contaminants like heavy metal, are efficient of absorbing a huge amount of metal and also are capable of treating multiple heavy metals simultaneously in comparison to other plant species[6]. The harvested part, that has accumulated heavy metal, can safely and easily be degraded by ashing, dying and composting. Some of the extracted metal from the ash can be retrieved by generating recycling revenues[7].

6.1 Processes involve in phytorextraction of metal from soil

There are four process that happen when a plant extract heavy metal from soil and water:

i. **Dissolution**- In this process plant roots secrets phytosiderophores, carboxylates and organic acids that mediate the dissolution or chelation of metals that has less fraction of phytoavailability, as an ion in solution to be mobile in the plant. Once the metal is mobile it gets transported to the root cell wall[8].

ii. **Root Absorption**- After a metal gets absorbed it binds to the root cell wall and then gets transported into the root. Some plants the store the metal through chelation or sequestration. Many specific transition metal
ligands contributing to metal detoxification and transport are up-regulated in plant when metal are available in the rhizosphere[9].

iii. **Root-to-shoot transport** - The root-to-shoot transport of heavy metals is strongly regulated by gene expression. The genes that code for metal transport systems in plants, are expressed in both hyper-accumulating and non-hyper-accumulating plants[9].

iv. **Storage** - Systems that transport and store heavy metals are the most critical systems in a hyper-accumulator, because heavy metals damage the plant before they are stored. Often in hyper accumulators the heavy metals are stored in the leaves[8].

7. **Quantification of phytoextraction efficiency**

The efficiency of phytoextraction can be quantified by calculating Bioconcentration Factor (BFC) and Translocation Factor (TF):

\[ BFC = \frac{C_{ht}}{C_{soil}} \]
Where $C_{ht}$ (C harvested) = Conc. of metal in plant and $C_{soil}$ = Conc. of metal in soil.

TF indicates the efficiency of plants in translocating the accumulated metal from roots-shoots.

$$\text{TF} = \frac{C_{\text{shoot}}}{C_{\text{root}}}$$

Where $C_{\text{shoot}}$ = Conc. of metal in shoot

$C_{\text{root}}$ = Cont. of metal in root

Both BCF and TF are important in screening hyperaccumulators for phytoextraction of heavy metals.

### 8. Phytoextraction of precious metal

<table>
<thead>
<tr>
<th>Precious metal</th>
<th>Plant used</th>
<th>Concentrations accumulated (mg/kg)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold</td>
<td><em>B. juncea</em></td>
<td>39.00</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td><em>Z. mays</em></td>
<td>20.00</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td><em>H. annuus</em></td>
<td>50.00</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td><em>T. repens</em></td>
<td>29.76</td>
<td>[12]</td>
</tr>
<tr>
<td>Silver</td>
<td><em>B. juncea</em></td>
<td>4.60</td>
<td>[13]</td>
</tr>
<tr>
<td>Palladium</td>
<td><em>A. thaliana</em></td>
<td>62.00</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td><em>B. coddii</em></td>
<td>0.71</td>
<td>[15]</td>
</tr>
<tr>
<td>Platinum</td>
<td><em>B. coddii</em></td>
<td>0.22</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td><em>A. thaliana</em></td>
<td>0.27</td>
<td>[16]</td>
</tr>
</tbody>
</table>
9. Discussion

The study was initiated to uncover different plants that can be used for phytoextraction method to remove an economically feasible amount of some precious metals (gold, silver, palladium and platinum) from the soil. Plants generally do not accumulate gold as they have less fraction of phyto-availability. Anderson and Moreno[10] carried out first reported phytoextraction of gold outside a controlled laboratory working with Z. mays and B. juncea in the area of Fazenda Brasileiro mine in Bahia, Brazil that carries 0.6 g t⁻¹ gold ore. B. juncea accumulated an average concentration of about 39 mg kg⁻¹ gold and Z. mays accumulated an average concentration of 20 mg kg⁻¹ after the field was treated with sodium cyanide (NaCN).

Wilson et al.[11] worked with H. annuus and K. serrata in combination with sodium cyanide (NaCN), thiourea [SC(NH2)2], ammonium thiosulphate [(NH4)2S2O3], and ammonium thiocyanate (NH4SCN) to improve the accumulation of gold from the Magistral mine in Sinaloa State, Mexico. In K. serrata the average concentration of gold was increased up to 9 mg kg⁻¹ and in H. annuus average concentration of gold was increased up to 21 mg kg⁻¹ in the plant stem, 16 mg kg⁻¹ in root and 15 mg kg⁻¹ in leaves.

Piccinin et al.[12] used the same method for the extraction of gold from the low-grade ore of 1.75 g t⁻¹ gold using native Australian flora and with sodium cyanide treatment (NaCN). 29.76 mg kg⁻¹ mean concentration of gold was accumulated in plant tissue of T. repens.

Harumain et al.[14] shows that in comparison to commercially accessible 3% palladium on carbon catalyst, the least amount of palladium nano-particle concentration (12-18 g kg⁻¹) in dried biomass of A. thaliana for catalytical activity was achieved. To enhance this method species that are best for ‘in the field’ application were also used and cyanide treatment was also done. These plants were capable of accumulate palladium nano-particles from both mine-collected and synthetic tailing. The best result was shone in synthetic tailing with the highest accumulation of 0.82 g kg⁻¹ in willow.
Nemutandani et al.[15] conducted an experiment to see the efficiency of *B. coddii* to phytoextraction palladium, platinum and nickel. The average concentration of platinum in plant’s leaves was 0.22 mg kg\(^{-1}\) and in roots, it was 0.14 mg kg\(^{-1}\). whereas the mean concentration of palladium was about 0.18 mg kg\(^{-1}\) in roots and 0.71 mg kg\(^{-1}\) in leaves. This study shows that *B. coddii* has the capability to accumulate nickel, palladium and platinum simultaneously.

Gawrońska et al.[16] conducted an experiment to show the accumulation, distribution and toxic effect of platinum on *A. thaliana*. *A. thaliana* was treated with platinum from range 0.025 µM to 100 µM. Results show that the concentration of platinum in root was 0.10 - 0.42 mg kg\(^{-1}\) and that in rosette was 0.018 - 0.21 mg kg\(^{-1}\). Toxic effect of platinum was also observed in plants biomass accumulation, morphology and function of photosynthetic parts at higher concentration (25-100 µM).

10. Conclusion

Phytoextraction came out to be a very promising technology for removal of precious metals from metaliferous for the sake of preventing environment and also for approaching commercialization. We all know that the current (2020) economic trend is projecting numbers that are not desirable. By using phytoextraction, which is way more cost-effective than traditional technologies, we will be able to meet the need of the hour and full fill one of the major factor that researches and investors are looking for. After the recovery of precious metal and converting it to a commercially acceptable form, will help to enhance our economy too.

11. Acknowledge

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12. References

CHAPTER-15

Medical Devices: Advancements and Regulations
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ABSTRACT: Since early 2000’s, medical devices industry has seen an increment in manufacturing, development and sales. With advancements in medications and patient treatment, including a push into medical tourism, helpful aids to the normal medication is a need. Manufacturing medical devices and their regulations for creating and granting marketing rights is currently gaining importance for regions. Medical devices regulatory approval processes are very exhaustively created and presented by countries like USA and regions of European Union. These associations in the form of ICH groups have laid down certain guidelines that are followed across the member countries, thereby reducing pressure on R&D units for reaping benefits out of their investment.

However, other regions and the upcoming markets have a varied regulatory system and the guidelines for approval differ in different regions and even across countries. So there exists an urge to adopt unanimous regulatory system i.e. harmonization and also for the Health authorities to develop certain guidelines that could ease up the whole process, which ultimately will reduce cost and bring relief to patient.

This review depicts and covers all aspects of a device development since its inception to approval and marketing stage. Wherein all the aspects such as disadvantages of not adopting unanimity in regulatory system are covered. Breakthrough technologies like Mobile applications and company's benefit-risk assessment for launching products taking risk are presented as cases.

Keywords- Medical devices, Regulatory Approval, ICH guidelines

1. INTRODUCTION

Among all the therapeutic areas concerned in life sciences, Medical device market is the most rapidly developing segment among its class in Life science products and Healthcare sector [1]. At whatever point, whether a device is developed or manufactured, or even before it gets approval for marketing and reach the patient, it must have a formal regulatory approval from the designated Health Secretariat or the Health Ministry to certify its safety standards and efficacy parameters [2, 3]. This Health authority-based testing and approval is done in labs with
proper equipped specialists found globally. Since early 1980s, the regulatory landscape pertaining to all segments of life sciences have changed significantly [4]. From couple of nations in starting, at present more than 50+ nations, which have implemented a more control-based stringent regulation for devices sector that can promise for better patient compliance, keeping in track with new innovative technologies which drives the upcoming future of life science sector to new heights[5]. The importance of regulatory agencies in medical devices is summarized in Fig. 1.1 (A).

![Fig. 1.1 (A) essence of regulatory affairs in public healthcare](image)

Before proceeding, the fundamental definitions of Medical Device and the respective portfolio of their country wise and what elemental Medical Device Harmonization actually means can be discussed.

1.1 What is a medical device?

'Medical Device' implies to any apparatus, instrument, mechanical assembly, execute, machine and even reagent for in-vitro use, programming, planned by the maker to be utilized, alone or in a group with, for individuals/patients, for benefits [6].
This definition is not obsolete, this is the elemental definition, but the actual definition of Medical devices varies from country-to-country, which both widens the scope of instruments and help manufactures classify their products justifiably [7].

1.2 Safety of medical devices

The most important details a product must have in its vision pertaining to its safety [8]:

• Absolute security can't be ensured
• It can be treated as hazard administration issue
• It is firmly lined up with device performance
• It requires shared obligation among the stakeholders

However, Medical device safety standards and QMS are described in subsequent sections.

1.3 Medical device safety and risk management

Medical device safety is similar or say analogue to Drug safety. It can be taken into consideration only in relative terms. This embraces the definition of risk and helps in classification of these devices. Classification of devices is not a standard procedure (Points of salient differences from pharmaceuticals) but varies from Authority to authority [9].

All Medical devices convey a specific level of risk and could lead to a therapeutic concerned condition. Numerous of such therapeutic risks can't be identified until the point when broad market experience and user feedback, is taken into consideration; the Post market surveillance [10,11].

For different devices, its elemental or component failure can be likewise, irregular. The whole criteria tackling the security lie with foreseeing of the possible risk that can affect the well-being. There are factors like economy, medical needs and healthcare providers that influence the medical device industry, refer to Fig. 1.1 (B) for more.

Hazard means the seriousness or possibility for an occurrence of serious event, the threat associated. It can be measured by having these
following points mentioned: [12]

(1) The risk;
(2) the possibility of adverse event occurrence;
(3) The effect of risk involved

Fig 1.1 (B). Major factors impacting Medical Device Industry
The most critical assessment or the necessity is "Basic standards of well-being and execution of medical device"[14]. It is briefly explained in table 1.

<table>
<thead>
<tr>
<th>Label or Certification</th>
<th>Meaning</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>Conformite Europeene</td>
<td>Important for medical devices approval, Status of device in EU</td>
</tr>
<tr>
<td>ISO 14791</td>
<td>International Organization for Standardization</td>
<td>Quality and Efficacy of product</td>
</tr>
<tr>
<td>ISO 27001</td>
<td>International Organization for Standardization</td>
<td>Data Security and Compliance</td>
</tr>
</tbody>
</table>

Table 1.1: Certifications pertaining to life science industry
It has information of hazardousness that can be caused by a medical device. The rationale, in this manner, boost profit thereby limit chance. Producers and the developers in medical device additionally utilize the managing method [15].

2. Medical Device Development

World Health Organization has a proper plan of how medical devices are developed. Fig 2.1 has the steps in brief [16].

![Fig 2.1: Steps in Life span of a Medical Devices](image)

**Steps:**

1. Conception and Development

It includes developing the strategy, design, formulating usage and a prototype. Strategy building includes creating the need and doing economic availability and finding out possibilities for product sales.

This includes- Product need and Offered solutions, identifying market for the product, Identifying resources, and Allocating budget [17].
2. Fabricate

Creation of a Prototype:

Development of a tester product, which is not commercialized, but is just a design. Proper approval must be taken for making it and GLP must be followed [18].

3. Bundling and naming

Creating the batch version, main step of manufacturing and labeling appears. Each product should be similar and efficacious without a lag [19].

4. Advertising

Marketing phase, one of the most critical stages where generally companies are stuck [20].

5. Sale

After commercialization, thorough proper supply chain and logistics, selling of the product in the market and mending money [21].

6. Use

Use by the end user or patient for treatment or as an aid/ technology to ease the work [22].

7. Disposal

Proper and safe disposal includes least harm to other living and non-living with proper environmental consideration taken care with [23].

2.2 Quality check for medical devices
<table>
<thead>
<tr>
<th>Practice</th>
<th>To be applied on</th>
<th>Stage of product development</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good Manufacturing Practice (GMP)</td>
<td>Manufacturing facility</td>
<td>Manufacturing batch formulations</td>
<td>Reduction in batch to batch variation</td>
</tr>
<tr>
<td>Good Clinical Practice (GCP)</td>
<td>Clinical Trials</td>
<td>Clinical Stage</td>
<td>Proper Implementation</td>
</tr>
<tr>
<td>Good Laboratory Practice (GLP)</td>
<td>Lab based correct approach</td>
<td>Development phase</td>
<td>Dose estimation Correct Analytical approach</td>
</tr>
<tr>
<td>Good Distribution Practice (GDP)</td>
<td>Distribution and supply chain</td>
<td>Marketing phase</td>
<td>Maintain proper storage and channelizing conditions</td>
</tr>
<tr>
<td>Good Vigilance Practice (GVP)</td>
<td>Safety post marketing of products</td>
<td>Post marketing phase</td>
<td>Drug safety and validation</td>
</tr>
</tbody>
</table>

Medical device manufacturing stakeholders (Manufacturers, vendors) need to apply in prior with appropriate quality frameworks for their products which clearly defines about Safety, efficacy and effectiveness. Compliance and audit can occur at any of the given stage is explained briefly in the given Table 2.1 [24, 25]. The act depends on ISO9001:2000 and empowers the manufacturers or administrations to execute and keep up a quality management framework. Numerous nations perceive this standard as an approach to achieve Good Manufacturing
Practice following the most critical restoration pathways for development and validation [26].

2.3 Regulatory guidelines for Medical devices

It is interesting to look at, how medical devices enterprises are regulated across various nations. The USA is one of the greatest life science and healthcare markets. One study found that "32 of the 46 health care innovation organizations for medical devices with more than $1 billion in yearly income are based out of the United States". Food and Drug Administration (FDA) oversees and manages all healthcare and life science-based devices, manufactured or moved in the United States [27]. USFDA is the subsidiary of the health secretariat in the US and manages approval and technical guidelines for granting access to drugs, biological, medical devices, and cosmetics and health supplements in and out of the country. For any company, allowed to bring their product in the market, it must have a proper bod from USFDA. Similarly, particularly to the devices verticals, which is huge and have greater scope, there exists an independent functional body called Center for Devices and Radiological Health (CDRH), which maintains that all the devices in the nation are compliant [28].

Regulations in the landscape of medical devices occurs in twostages:

- Premarket (Development and manufacturing phase)
- After approval or Post marketing

Regulations in Development and Manufacturing phase: Stringent health authority laid USA has a proper and dedicated classification system for the devices that are either manufactured or moved in the country. This system takes two major things into consideration [29]:

1. Risk involved with the usage/handling
2. Intended usage of the product

The devices are thereby divided into three separate classes i.e. Class I, II and III, which is in technically different to European Union’s four classes-based classification. Medical devices are divided as accordance with the hazard they posture to end user and are classified into Class I, II, and III in increasing order of both parameters.

Regulatory stringency and approval time and complexity increases from going down the classes to III. All the devices that are classified as in
Class I category area b stained from obtaining a proper registration or notification process. But on the other hand, all devices falling under Class II must have a PMN (Premarket Notification) or 510(k) approved from the FDA [30, 31].

Similarly, the regulations are getting tougher and longer with Class III or the most risk-based products. They require a Premarket Approval (PMA) before they can get into the market. Regulatory approvals are normally important for hazardous devices. The way toward accepting this archive is extensive and costly.

If the manufacturers are having a product and it is fairly similar in the way of working to an old or the Predicate device, then also the HA approves exemption from having a proper pre-market approval, rather a shorter pathway of Pre-market notification or 510k can serve them this purpose. For obtaining an approval, the latter product or simply, gaining thought about being significantly proportionate, the new product must have an indistinguishable proposed utilize and innovative attributes from the predicate; clinical information exhibiting wellbeing and viability are generally not required.

Similarly, if the manufacturer or the developer of a new device is not sure, how to classify the product as per HA guidelines and move ahead with the registration/ notification process, the sponsor can also apply for a formal HA based classification process by the pathway called 513 g, which directs HA to formally check the status of product and put them into a specific class [32].

Studies must be done for the medical device to prove its efficacious and safe nature by conducting trials or post approval safety studies. Any device must be properly classified as per the FDA guidelines and approved before making into the market. Accordingly, the health authority has also given proper and wide definitions and the classification approach for the device which is shown in upcoming sections. Classes and important endorsement methods for the medical device are exhibited in the table underneath [33].

The complexity of regulatory process is basic for potentially hazardous devices. The routetoward creating this PMA file takes a lot of time and is also expensive. As soon as a PMA application is filed, FDA strictly conducts audits and views all the Clinical studies data. Another way that producers has devised or set up for the high-peril devices is available aim
at purchasing by the general society with FDA's assent way incorporates introducing a 510(k) cautioning.

A basic breakup of medical devices based on technique laid down by the USFDA. Classes and the associated pathways necessary to obtain approvals [34].

Premarket approvals are large, and require some clinical information preceding starting with the approval. FDA may take any of the accompanying activities on a 510(k) in the wake of directing its clinical audit:
- discover the device significantly proportional to original so a freedom approval is granted;
- confirming exclusion of the product from 510k
- ask for extra data (with the last freedom choice pending audit of that data).

CONCLUSION:
Technology in updating Medical devices is advancing and improving by the day and hence the Regulatory authorities require to keep in pace by increasing the stringency in the guidelines. Regulatory authorities are certainly very important as mentioned in the 67th WHA report that regulatory system strengthens medical products as regulation ensures better products so as to maintain public health and also increases access to safe and effective medical products. Medical device development is a major area of concern for these authorities which include manufacturing, repackaging, relabelling and importing of medical devices.

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