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MUTAGENICITY/ ANTIMUTAGENICITY OF PLANT EXTRACTS USED IN TRADITIONAL MEDICINE: A REVIEW

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ABSTRACT

Because of the immense potential of medicinal plants used in traditional systems, focus on plant research has increased all over the world and a large body of evidence has been collected. Many plant extracts have demonstrated potent cancer chemo preventive property and most of them are known to exert their effects by antioxidant mechanism either quenching reactive oxygen species (ROS), inhibiting lipid peroxidation or stimulating cellular antioxidant defense. From short term *in-vitro* and *in- vivo* studies as well as long term carcinogenicity studies with chemically treated animals confirmed that phytochemicals could possess antimutagenicity and anticarcinogenic effect. So, it is becoming essential to investigate the circumstance under which phytochemicals used in traditional medicine as potential prophylactic agent exhibit beneficial and harmful effects is very much importance. Free radicals, environmental pollution, tobacco smoke, formaldehyde, homologous recombination could be responsible to damage DNA. Various studies such as Ames test, Chromosomal abression test, Micro nucleus test and Sister Chromatid exchange could be used to study the Mutagenicity/ genotoxicity.

KEYWORDS: Mutagenicity, antimutagenicity, genotoxicity, DNA damage.

INTRODUCTION

Genotoxicity in a cell is referred to as the potential consequence of genetic damage that a cell can incur. The damage response mechanism is available in all cells and organisms. Substances causing genotoxicity in a cell are called genotoxic agents or genotoxins.[1]

Mutagenicity refers to the induction of permanent transmissible changes in the structure of the genetic material in cells or organisms. These changes (mutations) may involve a single gene or a block of genes. Genotoxicity is a broader term that refers to the ability of the compounds to interact with DNA or the cellular apparatus such as spindle apparatus and topoisomerase enzymes which regulates the fidelity of the genome.

Genotoxicity and mutagenicity testing is an important part of the hazard assessment of chemicals for regulatory purposes. To assess genotoxicity or mutagenicity, different end points must be taken into consideration. A compound can induce changes in chromosomal number (polyploidy or aneuploidy) or in chromosome structure (breaks, deletions, rearrangements) besides point mutation. However, aneuploidy can arise as a result of both genotoxic and non-genotoxic events. Since loss of chromosomes can be caused either by direct effects on the chromosome to produce an acentric fragment or by interfering with the site of attachment of the chromosome on the spindle. Due to the diversity of the end points it is clear that the potential genotoxicity or mutagenicity of a compound cannot be assessed by a single assay system. [2]

For the prediction of carcinogenicity and heritable mutation, genotoxicity studies have been traditionally used. Gene mutation in bacteria, an *in–vitro* test with cytogenetic evaluation, for chromosomal damage within mammalian cells and an *in-vivo* test for chromosomal damage using rodent hematopoietic cells are the batteries of test that has to be carried out for the registration of pharmaceuticals for human use. Significant level of safety of the drug can be confirmed when the drug shows negative result in three battery test and can be considered that the drug as nongenotoxic. In the standard test battery, when a drug gives positive result, it needs to be tested more extensively depending on their indications.

Prior to marketing approval, like other therapeutic agents, botanical drug products are required to provide genotoxic information. Prior to the marketing approval, genotoxicity tests are routinely required by the environmental protection agency for nontherapeutic agents such as pesticides and insecticides also.

For product marketing, genotoxicity testing is part of safety assessment which is required for food additives. In 1994, Dietary Supplement Health and Education Act were established. Safety studies might not be required for dietary ingredients that existed in the market prior to

that act (DSHEA). Safety testing is required for the new dietary ingredients that appeared after 1994.

Dietary supplement or therapeutic botanicals contain multiple chemical constituents which may be pharmacologically active with significant proportions of chemically undefined constituents. When compared to the individual chemical constituents, the genotoxic information obtained from studies using a whole herbal or multi-component herbal product is relatively lacking.

Testing date on the whole botanical may become more meaningful and significant because herbalist and practitioners of alternative medicine often believes that herbal mixtures offer "Combination" advantages of synergy in efficacy and mutual antagonism in toxicity. In over all safety evaluation of the botanical product a modest trend towards increasing inclusion of information a genotoxicity appeared in peak in the last four years because of significant awareness of the impact of genotoxicity. Importance of genotoxicity information has been recognized increasingly by the sponsors of botanical drugs and may have prioritized its acquisition in their strategic drug development programmes. Botanical drug sponsors should be encouraged to obtain genotoxic information early in their product development by performing the genotoxicity studies that are comparably cost effective, reproducible capacity with high statistical power. [3]

Approximately 60,000 years ago human started to use plant as medicine and today 65 % of the world's population relies on plant for their primary health care. Oldest form of health care includes the use of leaves, flowers, stem, berries and root of herbs for their therapeutic or medicinal value. Through scientific research many plants used as traditional medicines have been validated and ethno botanical information has been contributed to health care worldwide thorough the isolation of bioactive compounds for direct use in medicines To encourage the medicinal use of herbs, the scientific perspective deals with the search for various active components. The study which reveals the protective effect of plant materials against changes in the genetic material induced by chemical or physical influences is referred as antigenotoxicity assay. [4] In literatures the adverse effects of widely used plants are not well documented. One might expect that plants which are used in long term by humans to have at least low toxicity, through investigation it was reported that many plants that are used as food or drugs have mutagenic effects in the *in vitro* assays. Purpose of this study was to

investigate the potential mutagenic effects of plants used in Indian traditional medicines using *in vitro* assays. [5]

Need for the usage of plants as medicine

The complete cure for cancer remains elusive despite the advancement of medical research and technology. Radical surgery, chemotherapy and radiotherapy which can bring about undesirable physical and psychological distress to the patients are the current cancer therapies. It is necessary for the continual global efforts in search for novel anticancer compounds that possess high therapeutic efficacy and fewer side effects compared to the existing anticancer drugs in the market. The mutagenic effect of plant has not yet been evaluated for its safety for consumption to date. [6]

In developing countries medicinal use of herbs and spices have been increased gradually, which are generally considered safe and proved to be effective against various human ailments. High content of flavones, sulfur containing compounds and polyphenol derivatives has been reported to exhibit antioxidative end free radical scavenging abilities. [7]

Many alkaloids have demonstrated for outstanding pharmacological potentials which exhibit antimicrobial, anti plasmodial and antitumor activities. Despites many pharmacological activities and potential benefits to human health several flavonoids are described as mutagens. [8]

Chemoprevention property of medicinal plants

The scientific community has made immense progress in acquiring the knowledge needed to prove cancer curing the last three decades. To identify potentials cause of cancer particularly environmental factors such as diet and to provide insight regarding their mechanism of action, pioneering research helped to greater extent. The accumulation of multiple sequential mutations and alternations in invasive neoplasm results in carcinogenesis. To either arrest or reverse cancer development by interfering with one or more steps in the process of carcinogenesis, promising inhibitors of cancer were identified and systematically evaluated for their potentials as chemo preventive agents. [9]

To show the immense potential of medicinal plants used in traditional systems, focus on plant research has increased all over the world and a large body of evidence has been collected.

Using the modern scientific approaches, various medicinal plants have been identified and studied. Which revealed the potential of medicinal plants in the area of pharmacology? Prevention of cancer using specific agents to suppress or reverse the carcinogenic process is termed as chemoprevention.

Many plant extracts have demonstrated potent cancer chemo preventive property and most of them are known to exert their effects by antioxidant mechanism either quenching reactive oxygen species (ROS) inhibiting lipid peroxidation or stimulating cellular antioxidant defense. [10]

Role of phyto -constituents as medicine

Antioxidant, antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic, and vasodilatory and neuro protective properties can be offered by the plant extract or phenolic rich foods. To understand the potential health benefits of leafy vegetables, edible root, crops, grapes, apple, mangoes, onion, cocoa corn, and buck wheat it is very much interesting to understanding the effect of polyphenols, flavonoid content within human food. By oligomerization of polyphenols polymers, oligonol which delivers high level of oligomeric proanthocyanidine are produced. Bioavailability of monomeric and oligomeric phenolic which provide more beneficial effect are more than the polymeric form.[11]

Focus on plant research especially medicinal plants used in traditional systems has been increased all over the world. To show the immense potential of medicinal plants, various plants have been identified and studies used modern scientific approaches. In the area of pharmacology, medicinal plants revealed the potentials. Components of higher plants have demonstrated to possess potent anticancer chemo preventive property which plays an important role in the prevention of disease cancer. Phyto constituents present in higher plants are known to exert their biological activities by antioxidant mechanism. Antioxidant mechanism is performed either by quenching reactive oxygen species (ROS), inhibiting lipid per oxidation or stimulating cellular antioxidant defenses. [12]

Role of phyto constituents in producing genotoxicity

The isolated compounds from the plants such as Quercetin, furoquinoline alkaloids and isothiocyanates are considered to be mutagens. It is very difficult to speculate the

compounds that are responsible for mutagenic response detected with plant extracts because they are the complex mixtures of organic compounds. Solid scientific support is available for the plant used in traditional medicine with regard to efficacy. Finding in these type of studies raise question about the safety of these plants and their continued extensive use in health care system. [5]

Flavonoids, polyphenols are present in many foods. Flavonoids, stilbenes, lignans are the example of polyphenols, that are classified based on configuration of phenolic acid derivatives within their structure. These classifications can be further sub divides the groups due to the existence of stereoisomer, hydroxylation of phenolic rings. Polyphenols especially oligomers which constitute a small portion of total polyphenols are mainly responsible for the protection of plants from ultraviolet rays and pathogens. When the plant grows and ripens, the concentration of plant monomeric and oligomeric compounds diminishes because of continuing polymerization of polyphenols, bioactive components gives way to polymers with high molecular weight.

Flavonoids and other polyphenols which are classified based on the configuration of phenolic acid derivatives within their structure are present in many foods, further this group can be sub divided into various types based upon hydroxylation of phenolic rings, glycosylation, acylation with phenolic acid and existence of stero isomers. From ultra violet rays or pathogen a plant is descended by polyphenols which constitute a small portion of total poly phenols. As the plant grows and ripens the concentration of plant monomeric and oligomeric compounds diminishes because of continuing polymerization of poly phenols so that bio active components gives the way to polymers with higher molecular weight. Phenolic rich food wither in the form of extract or other purified preparations offers antioxidant, antibacterial, anti inflammatory, anti allergic, hepatoprotective, antithrombotic, antiviral, anti carcinogenic, vasodilators and neuroprotective properties. In understanding the effect of poly phenols/ flavonoids content within human food such as leafy vegetables, edible root, crops, graphs, apple, mangoes, onion, cocoa corn, buck wheat, red wine and tea, there is a compelling interest because of the potential health benefits from these compounds.[13]

Role of phyto constituents and its mechanism in antimutagenicity

From short term in vitro and in vivo studies as well as long term carcinogenicity studies with chemically treated animals confirmed that phytochemicals could possess antimutagenicity

and anticarcinogenic effect. With the intake of the plant materials, epidemiological studies also supported the chemo preventive effects in which phytochemicals exhibit genotoxic/ mutagenic effect by themselves or potentiate the effect of other xenobiotics. To investigate the circumstance under which phytochemicals used in traditional medicine or potential prophylactic agent exhibit beneficial and harmful effects is very much importance.

Apolycyclic aromatic hydrocarbon (PAH) like Benzo(a) pyrene is a strong mutagen and suspected human carcinogen can be used for genotoxicity studies. The principle exposure route for the entry of PAH which is ubiquitously distributed in the environment for humans is through the diet. Genotoxic or carcinogenic effect can be activated by CYP- 450 isoenzyme to a variety of mutagenic and carcinogenic electrophiles which can be covalently bind to DNA, RNA or protein. The presence of phytophenolic components including tannins, catechin, flavonones, isoflavones are responsible for the possible genotoxic effects of plant extracts. Genotoxicity might be related to hydrogen peroxide formation arising from auto oxidation of phenolic molecules. Flavonoids inhibit topoisomerase I and II enzyme which will interfere with the replication and transcription process inhibiting the relegation of DNA double strand breaks and enhancing the formation of cleavable DNA- enzyme complexes. Phenolic rich extracts could lead to accumulated DNA breaks and mutation thus contributing significantly to genotoxicity. The mechanism by which the plant extracts exhibit antigenotoxic effects either by inducing or inhibiting enzyme such as glutathione- s-transferase or CYP1A1 respectively as well as antioxidant and scavenging properties of polyphenolic contained in it. The most important mechanism in antimutagenesis and anticarcinogenesis is the scavenging of bio active molecule. The extract which exhibit potent antioxidant and free radical scavenging propensities ascribed to its polyphenolic richness more particularly to its flavonoid content. Overall activity of the plant extract may be due to synergic effects of a phenolic sub- classes which can be either used as dietary supplements or therapeutic agents. [14]

Reasons for Mutagenicity/ Genotoxicity/ DNA damage

Free radicals

By damaging the cellular antioxidant defense mechanism, ionizing radiations can induce oxidative damage to vital cellular molecules including DNA, proteins and lipids. Genomic DNA is the most important target in the living cells damaged by ionizing radiations. Damage

suffered by DNA includes strand break and cross link of the intra and inter strand type. Free radicals which are produced due to oxidative stress reported to produce similar types of damage in DNA. Lipid per oxidation is also found to be considered as a critical event of ionizing radiations which found to increase in dose dependent manner in rat liver mitochondria, micro some spleenic lymphocytes due to the reaction of hydroxyl radicals generated by ionizing radiations with poly unsaturated fatty acids. Malondialdehyde which is the product of lipid per oxidation forms adduct with cellular DNA. Antioxidant defense system present in the cells controls the deleterious effect of reactive oxygen species, enzymes and non- enzymatic radical scavengers can either directly detoxify ROS indirectly regulate their levels.

Effect of tobacco in genotoxicity

High incidence of many diseases including various type of cancer including lung, larynx, oral cavity, lip, bladder and pancreas, respiratory disease, cardiovascular disease. Gastro intestinal disorder as well as much other medical complication occurs due to hazards of tobacco smoke. The urine of the smokers has been found to mutagenic. Tobacco smoke can produce more chromosomal damage in somatic cells of smokers than in non- smokers. Passive smoking or environmental tobacco smoke has been seen to induce an induced risk in lung cancer with sufficient evidence in smoking; tobacco smoke has been included among those substances that cause cancer. Nicotine have widely adopted as specific biomarker for tobacco smoke in take. Measurement of nicotine and cotinine in urine distinguishes active smokers from unexposed non- smokers. [15]

Environmental pollutants

The main cause of environmental pollution is due to human and industrial activities which are the origin of the discharge of multiple chemical substances in the environment. Effects in the aquatic environment is reported due to fate of some pharmaceutical compounds as well as their occurrence which are genotoxic and suspected to be a possible cause of cancers epidemiological investigation have shown a link between genotoxic drinking water intake and a rise in cancer cases. Major concern for human health is represented by monitoring of water containment for potentially carcinogenic compound. From various sources like hospital, industrial or domestic discharge, genotoxic compounds can come in the aquatic environment which has a large chemical diversity. [16]

Environmental pollutants and drugs can cause cellular damage through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species which are derived from the metabolism of oxygen which includes superoxide radicals hydroxyl radicals, hydrogen peroxide radicals which are often generated or by products of biological reactions or from exogenous factors, some of the reactive oxygen species plays positive role in cell physiology as well as causes great damage to the cell membrane and DNA inducing oxidation that causes membrane lipid per oxidation , decreased membrane fluidity and DNA mutation which leads to cancer and other degenerative disease. [17]

Environmental conditions

On earth. Most extreme environmental conditions are there in Polar Regions. The organism living in the polar region have developed natural sun- protected strategies that enables their survival under direct and intense UV radiations during the gradual process of evolution. Avoidance of UV source , hiding under inert materials, producing photo protective compounds that especially screen un radiations such as scytonemins secreted by cyanobacteria, Flavonoids secreted by plants, melanin production by animals and human cells are the different reactions produced by every living organism when they exposed to UV radiations. Organisms which are native to habitats tend to have more developed UV tolerance than the organism from low intensity UV environment.

The evolution of terrestrial l\plant life was made possible by the development of ozone layer which is acting as UV screen in the stratosphere of the earth. Ozone layer absorb solar UV C which is in the range of 100- 280 nm and part of UVB radiations in the range of 280-315nm. UVA and UVB produce the cellular damage. In both isolated and cellular DNA, upon exposure to low doses of UVC or UVB radiations. Photoproduct distribution pattern s observed singlet oxygen together with smaller contribution of hydroxyl radical- mediated reactions through initially generated superoxide radicals produced / induced the oxidative damage to DNA.

High frequency of transition mutations at di-pyrimidine sequences containing cytosine is the hall mark of UVC and UVB mutagenesis. In the evolution of land plants development of phenolic polymer metabolism partly induced by UVB radiations has played a major role. Phenolic compound that possess photo protective properties have been subject of biological and medicinal interest. As a consequence of excess light or UV radiations change in

Flavonoids composition of plant leaves acts as chemo preventive agents against UV- induced damage in the organism. [18]

Formaldehyde

At ambient temperature formaldehyde (FA) is a gas with pungent odor is highly reactive which is widely used in commercial products such as carpeting drapery and garments and hence large amount of peoples are exposed to it. Formaldehyde is formed by off gassing of building material, furniture and upholstery which is exposed to human from indoor air. Morticians, pathologist and medicinal students are exposed to formaldehyde when they are using embalming fluid. Formaldehyde is a natural component of certain food which is also produced endogenously in animals as an intermediary metabolite.

At the first site of contact. Formaldehyde rapidly binds to thiols as well as rapid incorporation of carbon atoms occurs into various macro molecules because of its highly reactive nature. In proliferating cultured mammalian cells formaldehyde can induce various genotoxic effects which were indicated by numerous in vitro studies.

The primary DNA alterations which occurs after the exposure of formaldehyde are DNA-Protein cross links which arrests DNA replication and lead to the induction of other genotoxic effects such as sister chromatid exchange and micronuclei in proliferating cells. Formaldehyde can induce gene mutation at specific loci which are mainly attributable to chromosomal effects such as larger deletion and recombination. Micronucleus test and chromosomal aberration studies are thought to be the most sensitive genetic end points for the detection of Mutagenicity. In mammalian cells, the extend of DNA- Protein crosslink has been used as the most reliable biomarker. The DNA- Protein cross links are repaired by the nucleotide excision repair mechanism. [19]

Homologous recombination

One of the fundamental processes of like is homologous recombination which is responsible for the creativity of new genetic linkage in meiosis cells. In somatic cells, homologous recombination is one of the important DNA- damage repair pathway. In initiating recombination DSB (double strand break) is generally accepted which is a critical step. Via DNA damage repair or DNA strand replication, many types of DNA lesions such as ssDNA nicks and DNA adduct can be changed to DSB. Wide spectrum of mutagenic agents can

stimulate mitotic recombination, including those which cause single base change, deletion, strand break and frame shift. Induction of recombination is accepted as a cellular response to DNA insult and particularly suited as a broad indicator of genotoxicity or Mutagenicity. For artificial recombination substrate some transgenic organisms have been developed. Recombinant events can be easily detected by analyzing the expression of the reporter gene such as GUC and LUC. For the detection of the genotoxic/mutagenic effects of the various environmental stress, including IR,UVB, MMS, heavy metals and herbicides, the plant carrying substrate for the analysis of HRF have been frequently used. [19]

Viability, phagocytosis, aggregation and adherence of the microorganism is effected by non-self substances ability to later functional surveillance and internal mechanism of immune cellular components. Involvement of Na+/ H+ exchange and respiratory burst enzyme NADPH oxidases and Nitric oxide synthase in a cadmium induced lipid per oxidation and DNA damage in haemocytes of Mussels. [20]

Reason to carry out genotoxicity studies for plant extract

To treat many diseases, worldwide plants have been used especially in traditional preparations. Herbal plants that can be eaten raw can promote youthfulness and also improve the health status. To investigate the effects of medicinal plant and herbs on human tissue and DNA numerous studies have been conducted. Shortest life spans of the animals are due to highest rate of oxidative damage induced by free radicals. Greater spontaneous damage or inefficient DNA repair which shows the sign of premature aging in humans who have genetic disease. Decreased life span occurs in humans when they are exposed to various external causes of damage such as ultra violet light and cigarette smoke. By protecting the cells against DNA damage and by enhancing the efficiency DNA repair life span can be prolonged. For detoxifying the blood, to slow the aging process and to cure various diseases such as high blood pressure, diabetes and cancer, many cultures around the world have a long tradition of consuming various plants. At certain conditions, antioxidants such as vitamin C and E, have been shown to exert pro-oxidant effects in the presence of redox active elements such as iron. Therefore it is important to determine the antioxidant activity and also to evaluate the genotoxic properties of the plant. [17]

Through scientific research, there have been many validations of traditional medicines. Direct use of isolated bioactive compounds in medicine, ethno medical information's has

contributed to heath care worldwide. In the literature, the adverse effects of widely used plants are not well documented. One might expect plants used in traditional medicine to have low toxicity based on their long term use. In in-vitro assay, recent investigations have revealed that many plants used as food or in traditional medicine have mutagenic effects. Long term use of plants risen concern about the potential mutagenic hazard. [5] Therefore it is important to determine the antioxidant activity and also to evaluate the genotoxic properties of the plant.

Role of mutation in carcinogenesis

In cancer initiation and other stages of the carcinogenic process, mutation in somatic cells plays a key role. Mutagens are known to be potentially deleterious to human health and a large number of mutagens have been identified. Secondary metabolites of plants can be used as medicines, based on the fact that they contain natural substances whose consumption may provide health benefits and diminish illness. The detection and identification of natural mutagens and anti mutagens are important because interactions among the bio active compounds are complicated and ubiquitous. To enhance the health status of people, knowledge of source of natural antimutagens will help them to make selections of food or drink containing substantial amounts of a active components. Against human carcinogenesis and mutagenesis a wide range of evidences from epidemiological and laboratory studies have demonstrated that some plants eaten whole or substantial protective effects. Wide variety of antimutagenic/ antigenotoxic substances have proved to be present in several plant extract.[21]

Various methods to study genotoxicity

Chromosomal analysis during mitosis is one of the methods to study the mutagenicity. Chromatin breaks, dicentric chromosomes, multipolarity and polyploides are the different types of chromosomal aberrations that have to be screened after the treatment with either mutagen or herbs. Chromosomal aberration assay is an efficient and standard test for the chemical screening and in situ monitoring for genotoxicity of environmental substances and it is validated by the international programme on chemical safety (IPCS, WHO) and the United Nations environment programme (UNEP). [4]

For the measurement of total antioxidant activity of body fluids, food extracts and pure compounds. A number of assays have been introduced. Each methods act through a variety of mechanism and measurement of a range of end points at a fixed time point or over a range

relates to the generation of different radical. In the inhibition type of approach based on the extend of scavenging by hydrogen or electron- donation of a preformed free radical is the marker of antioxidant activity. Another approach of assays based on involving the presence of antioxidant system during the generation of the radical. [11]

To monitor the genotoxicity connected with smoke habits, the chromosomal aberration tests (CA), Micro nucleus test (MN) and sister chromatid exchange (SCE) have been used. Smokers exhibit frequently high levels of SCE, CA and MN in lymphocytes than non-smokers. Involuntary smokers have reported only margin or negative effects on SCE, CA and MN. For the estimation of DNA damage at the individual cell level in both in vivo and in vitro studies, comet assay is a well established genotoxicity test, which has been widely used to quantify DNA damage in isolated lymphocytes from subjects exposed to several environmental or occupational substances especially for estimation of oxidative dame in the DNA which is well known to be induced by tobacco smoke exposure. The comet assay is more sensitive than sister chromatid exchange. Lymphocytes of smokers either exhibit increased DNA damage or reduced capacity to repair DNA damage after exposure to different substances as H_2O_2, UV etc. Comet assay has been used to measure the susceptibility to DNA damaging by mutagens and the individual capacity to repair DNA damage. To detect the specific lesions, purified repair enzymes can be included. By using/including formanidopyrimidine glycosylase (FPG), a restriction enzyme which recognizes and remove the oxidized purines and some alkylated DNA product, Specific information about the oxidative DNA damage and its repair. Lesions are produced in the DNA as a result of removal of specific modified bases from DNA to create apurinic or apyrimidine site (AP- site) which are subsequently cleaved by its endonuclease enzyme (AP lyase) which can be detected by comet assay as additional strand breaks. In active smokers, cigarette smoking is the source of reactive oxygen species in peripheral blood lymphocytes where as passive smokers exposed to second hand tobacco smoke at work place. [15]

Micronucleus test

For detecting the genotoxic potential of test compound in vivo, micronucleus test is the most commonly performed assay. In many mammalian cells, chromosome fragment lag in anaphase and are not included in the daughter nuclei that are formed during the telophase. Instead, these fragments become membrane bound and form micro nuclei. Due to

chromosome breakage or spindle dysfunction, fragments of chromatin may occur. The most readily studied material is erythropoietic tissue from marrow and observations are commonly restricted to polychromatic erythrocytes which are formed when their precursors expel nuclei a few hours after mitosis. Micro nuclei become visible as small nuclear bodies which remain in the cytoplasm of erythrocytes but not expelled. The efficacy of the test compound as a genotoxic screen may be optimized using two or three daily test item doses followed by single sample after 24 hours of the lost dose. Failure or mistakes in repair process such that breaks either do not rejoin or rejoin process such that breaks that either do not rejoin or rejoin in abnormal configuration results in chromosomal aberrations expressed as breakage or breakage followed by reunion of both sister chromatids at an identitical site or breakage of single chromatids or breakage followed by reunion between chromatids which is the most common type of structural aberration. Clastogens (chemicals which cause chromosomal breakage) require a period of DNA synthesis to convert initial DNA damage into chromosomal alterations that becomes at mitosis. Exposure to phytohemagglutin (PHA) the lymphocytes in blood are stimulated to divide. After exposure to test compounds, at predetermined intervals, the lymphocytes are treated with colcemid (a metaphase- arresting substances). Then the cells are analyzed microscopically for the presence of chromosomal aberrations. After being converted to active intermediates by enzymes found in liver, many mutagenic chemicals act directly on DNA. An exogenous metabolic activation system(rat liver S9 homogenate) has to be included with the series of treatment to enhance the degree of conversion and ability of the assay to detect clastogenic, metabolic intermediates because human lymphocytes have only a limited capacity to metabolize some test compounds.[22]

In cancer radiotherapy and in the reduction of risk to exposed individual, protection against ionizing radiations has practical application. Most of the radiations induced biological damage arises from the interactions of free radicals with vital cellular biomolecules such as DNA, proteins and lipids. Antioxidants influence the indirect action of radiations by neutralization of ROS. Convenient strategy to protect against ionizing radiations- induced damage by modulating extra cellular and or intra cellular antioxidant. Antioxidant thiols were found to be decreased in cellular and blood levels during exposure to ionizing radiations. In reducing radio oxidation- mediated cellular injury, additional antioxidant thiols supplements may prove useful.

N- Acetyl-1- Cysteine (NAC) and reduced glutathione (GSH) are considered to be the most

natural of the thiol protectors which are approved for human use for various purposes. [5]

Ames Test

With Salmonella typhimurium strain TA 98 and TA 100, the Ames test was performed. In 20 ml of Nutrient broth 100 µl of bacterial stock was incubated for 16 hours at 37^0 C. 0.1 ml of overnight culture was added to 2 ml of top agar containing biotin and histidine along with 0.1 ml of plant extract, solvent control and positive control in two set of experiments. One set of experiment is carried out without metabolic activation and in second set of experiment 0.5 ml of metabolic activation mixture containing adequate amount of post- mitochondrial fraction (S9Mix) was added. Then top agar was poured over the surface of a minimal agar media and incubated at 37 ^{0} C for 48 hours. Numbers of reverse mutant colonies were counted after incubation. This assay was carried out in triplicate. By observing the background of bacterial growth which should be normally present. Absence of toxicity was examined. 4_ Nitroquinoline-1-oxide (4-NQO) at the concentration of 2µg/ml used for TA 98 and TA 100 strain without S9 mixture and Benzo[a]pyrene (B[a]-P) at the concentration of 20 µg/ml used for TA 98 and TA 100 strain without S9 mix used as positive control.

The plant extracts can be considered positive for mutagenic activity when it induces the revertants colonies numbering at least twice the revertants control number. The isolated compounds from the plants such as Quercetin, furoquinoline alkaloids and isothiocyanates are considered to be mutagens. It is very difficult to speculate the compounds that are responsible for mutagenic response detected with plant extracts because they are the complex mixtures of organic compounds. Solid scientific support is available for the plant used in traditional medicine with regard to efficacy. Finding in these types of studies raise question about the safety of these plants and their continued extensive use in health care system.

In bacterial test extracts that showed genotoxic effect also should show genotoxicity test in human blood. Before long term usage of traditional medicine, it is recommended to carry out thorough screening for potential harmful genotoxic effect. [5]

For the detection of strand break, cross links and alkali labile sites in nuclear DNA, single cell gel electrophoresis is widely used. For observation of DNA damage, comet assay was first demonstrated by Ostling and Johanson. The application of single cell gel electrophoresis

was initially restricted to isolated cells such as cultured cells of peripheral lymphocytes. Using comet assay, chemically induced DNA lesions in multiple mouse organ is detected. [10]

Biomarkers for genotoxicity

In human population studies cytogenic biomarkers are the best frequently used end point because of their sensitivity for measuring the exposure to genotoxic agents and their role as early predicators of cancer risks. For evaluating the effect of environmental exposure to genotoxic agents, the frequency of micro nuclei in peripheral blood lymphocytes in conjunction with cytokinesis – block assay is the most popular and effective biomarker. [23]

Reduction of genotoxicity

In reducing the genotoxic and carcinogenic effects induced by hazardous environmental chemicals, many studies have shown that the consumption of vegetables, fruits has been an effective strategy. Exposure to dangerous chemicals can lead to mutagenic and carcinogenic events although human body is equipped with self defense mechanism such as detoxification process through various enzymes. Due to their antioxidant and free radical scavenging properties, Flavonoids and phenolic compounds that are present in the plants possess many biological properties such as hepatoprotective, antibacterial and anticancer activities. [24]

Genotoxicity and carcinogenic effect induced by hazardous environment chemicals can be reduced by the consumption of vegetables and fruits. Self defense mechanism is present in the body to detoxify the dangerous chemicals which can lead to mutagenicity and carcinogenicity. Plants which contain many natural compounds such as flavonoids and phenolic compounds apart from their biological properties including hepatoprotective, antibacterial and anti cancer activities reported to have potential antimutagenic or anticarcinogenic effects. [34]

Health concerning in the field of human nutrition that have been centered around deficiency disorders of macro and micro nutrients with emphasis on the role of essential nutrients in health and disease, for the most part of this century. Various dietary constituents have been found to provide protection against any disease including cancer. Worldwide, for controlling the cancer incidence, any significant role by dietary intervention is encouraging and emerging as an acceptable approach. In the identification of potential agents which can wither abolish or delay the development of carcinogenesis, search and research helps. By preventing the

activation of carcinogen or by increasing detoxification or by blocking the interactions of ultimate carcinogen with cellular expansion of neoplastic cells, development of carcinogenesis can be delayed. [25]

Numerous epidemiological studies have shown possible correlation and association between reduced cancer risk and intake of vegetables in recent years. Considerable attention has been focused on increased dietary intake of fruits and vegetables. [25]

Genotoxicity studies of medicinal plants

In the literature the adverse effects of widely used plants are not well documented. One might expects plants used in traditional medicines to have low toxicity based on their long term use. One might expects plants used in traditional medicines to have low toxicity based on their long term use. In in-vitro assays recent investigations have revealed that many plants used as food or in traditional medicines have mutagenic effect, which raises concern about the potential mutagenic hazards resulting from long term use of such plants.

So far genotoxicity studies were carried out for the following plant extracts *Tulbaghia violacea* (Alliaceae), *Boophane disticha* (Amaryllidaceae), C. Macrowani, *Haroephyllum caffrum, Rhusgueinzil, Sclerocaryabirrea* (Anacardiaceae), *Heteromorphatrifoliata* (Apiaceae), *Acokanthera oblongifolia* (Apocynaceae), *C. rosens, Artemisia afra* (Asteraceae), *Senecio serratuloides, veemomia colorata* (Asteraceae), *Balanites maughramii, Kigelia africane* (Begoniaceae) *Afzelia quanzensis* (Caesal pinaceae), *Warburgia satutaris* (Canellaceae), *Diospyros wheteana, Euclea Divinorum* (Ebenaceae), *Euclea natalensis, Antidesma venosum, Crotom sylvaticus, Ricinus communis, Spirostachys Africana* (Euphorbiaceae) *ornithogalum longibracteatum* (Hyacinthaceae), Hypoxis colshicifolia (Hypoxidaceae) *Tetradenia riparia* (Lamiaceae) *Octteabullata* (Lauraceae*) Acacia sieberiana* (Leguminosae), *Erythrina caffra Merwilla netalensis* (Liliaceae) *Trichillia emetic, Turraea floribunda, Bersanaleceus* (Melianthaceae), *Syzygium cordatum* (Myrtacae) *Ochna serrulata* (Ochnaceae),*Oenothera biennis* (Onagraceae) *P.auriculata* (Plumbaginacea)e, *polygata virgata* (Polygalacaea), *Rhammus Prinoides* (Rhamnaceae) *Ziziphus mucronata, Prunus Africana* (Rosaceae) *Catunaregam spinosa*(Rubiaceae), *Gardenia volkensii, Dombeya rotundifolia* (Sterculiaceae), *Celtis Africana, C. aristata* (Ulmaceae), *Pouzolzia mixta* (Urticaeae) , *Siphonochitus aethopicus* (Zingiberaceae). [5] *Pereskia bleo* (Kunth) DC (Cactaceae), [6] *Strychnos pseudoquina* (

Loganiaceae), [8] *Parthenium hysterophorus* (Asteraceae) [39] *Piper methysticum* G (Piperaceae), [26] *Bauhinia Monandra lead* (Fabaceae), [27] *Toxidendron quercifolium* (Toxidendron genus), [14] *Brussels*, Sprouts, White cabbage, cauli flower, green cabbage, kohlrabi, broccoli, turnip, black raddish, [28] Ashwagandha [4] *Cecropia obtusifolia* extracts, [29] Hibiscus tiliaceus L. methanolic extract, [12] hawthorn extract against genotoxicity [24] Coriandrum sativum [30] Alepidea amatymbica and Alepidea natalensis, [31]

Toxicodendron quercifolium [14] Anacardium occidentale L [32] Matricaria chamomilla, Tilia cordata, Mentha piperita, Mentha pulegium, Uncaria tomentosa and Valeriana officinalis, [33] Phyllanthus amarus, [34] Passiflora alata [35] Ganoderma Extract, [36] Hoodia gordonii, [37] licorice flavonoid oil, [38] Monimiastrum globosum [14] Arabidopsis thaliana, [19] *Calendula officinalis* L. [39] Fenugreek, [40] green tea, [41] Rubia cordifolia L. [21]

DISCUSSION

Complex pathology of chronic disease such as cancer, cardio vascular disease, diabetes, hypertension, immune and neurodegenerative disorders as well aging processes can be modulated by polyphenols derived from the plant source. To identify the active functional ingredients in leafy food plant, traditional medicinal plants and bioprospective endemic plants scientific support is merging now. In addition to an analysis of the putative mechanism of action potential health claims require a basis of specific controlled safety and toxicological studies. [41]

Oxidative stress occurs in cell or tissue can lead to several human disease states, either age related or chronic such as diabetes, neurogenerative, cardio vascular disease and specially carcinogenesis, when the concentration of ROS generated exceeds its antioxidant capability. Research on naturally occurring protective antioxidant and the mechanism of their action has been focused with much attention. Secondary metabolites present in several plant extracts have been used. [10] Free radical scavenging activity, metal chelation, inhibition of oxidase and alteration of gene expression notably the antioxidant response elements which involve the Phase II detoxifying enzyme are the various multidimensional effects of phenolic compounds. [14]

Oxidative stress is caused due to increase in the intra cellular levels of ROS which is considered as a toxic insult, which interacts with macro molecules to induce the cell

membrane dysfunction, lipid peroxidation and DNA damage. DNA is an important target for ROS in the carcinogenic process. Antioxidant properties of the phytochemical material like flavonoids especially quercetin, kaemferol, Proanthocyanin can able to modulate DNA damage induced by hydrogen peroxide. [24] Important promoter of cancer in humans is the oxidative DNA damage. Many human diseases including cancer is induced by H_2O_2. Both in vitro and in vivo studies showed the involvement of H_2O_2 in carcinogenesis. Extensive oxidative damage is caused by the interactions of hydrogen peroxide with DNA through highly reactive oxygen and radical species.[21] Therefore it necessary to evaluate any medicinal agents which have to be consumed for longer time for the genotoxicity effect especially for the traditional medicines which are thought to be safe on prolonged usage.

In vitro and short term in vivo studies as well as long term carcinogenicity studies with chemically treated animals showed that phytochemicals could possess antimutagenic. Anticancerogenic effects. Phytochemicals exhibit genotoxic. Mutagenic effects of other xenobiotics under some experimental conditions. So, it is paramount importance to investigate the circumstances under which phytochemicals exhibit beneficial or harmful effects. [14]

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