Review Article

Review on Genotoxicity, its Molecular Mechanisms and Prevention

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ABSTRACT

Genotoxicity has become a major problem for the cause of many cancers. In this article we discuss about the basics of genotoxicity, the chemicals which cause these genetic damage and also their mechanism of action. Some *in vitro* and *in vivo* methods for measuring the extend of genotoxicity have also been discussed such as chromosomal aberration test and micronucleus assay. Finally a brief account on the drugs being used in present days, and also some plant products which show anti mutagenic effects have been emphasized.

Keywords: Genotoxicity, Mutagen, Mutations, Aberrations.

INTRODUCTION

All chemicals that produce DNA damage leading to mutation or cancer are described as genotoxic. Toxicological studies have undergone a significant evolution during the past decade, with much greater emphasis being placed on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity. The mutations in somatic cells are not only involved in the carcinogenesis process but also play a role in the pathogenesis of other chronic degenerative diseases, such as atherosclerosis and heart diseases, which are the leading causes of death in the human population[1,2]. Micronucleus test and chromosomal aberration test are used for studying antimutagenic activity of a drug. One of the best ways to minimize the effect of mutagens and carcinogens is to identify the anticlastogens/ antimutagens (substances which suppress or inhibit the process of mutagenesis by acting directly on the mechanism of cell) and desmutagens (substances which somehow destroy or inactivate, partially or fully the mutagens, thereby affecting less cell population) in our diets and increasing their use. Nature has bestowed us with medicinal plants. There is a need to explore them for use as antimutagenic and anticarcinogenic food or drug additives.

In genetics, genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer. While genotoxicity is often confused with mutagenicity, it is important to note that all mutagens are genotoxic, however, not all genotoxic substances are mutagenic. The alteration can have direct or indirect effects on the DNA: the induction of mutations, mistimed event activation, and direct DNA damage leading to mutations[3]. The permanent, hereditary changes can affect either somatic cells of the organism or germ cells to be passed on to future generations. Cells prevent expression of the genotoxic mutation by either DNA repair or apoptosis; however, the damage may not always be fixed leading to mutagenesis [4].

To assay for genotoxic molecules, researchers assay for DNA damage in cells exposed to the toxic substrates. This DNA damage can be in the form of single and double strand breaks, loss of excision repair, cross-linking, alkali-labile sites, point mutations, and structural and numerical chromosomal aberrations. The compromised integrity of the genetic material has been known to cause cancer. Consequently, many sophisticated techniques including Ames Assay, *in vitro* and *in vivo* Toxicology Tests, and Comet Assay have been developed to assess the chemicals' potential to cause DNA damage that may lead to cancer.

Anti mutagen is described as an agent that reduces the apparent yield of spontaneous and /or induced mutations. Mechanisms of anti mutagenesis have been classified into two major processes one is desmutagenesis: in which factors act directly on mutagens or inactivate them. The other is bio-antimutagenesis in which factors act on the processes of mutagenesis or repair DNA damages that result in a decrease in the mutation frequency. Gemcitabine used as a mutagen with anti-metabolites activity, it exerts its effect by prohibiting DNA chain elongation. Antimutagenesis are considered as one of the most feasible ways for inhibiting the negative effects of environmental genotoxicants including carcinogens. Nowadays a large number of anti-mutagens of plants origins are known [5]. Evaluation of genetic toxicity is an important component of the safety assessment of chemicals, including pharmaceuticals, agricultural chemicals, food, additives and industrial chemicals. Up to the present time, genotoxicity has been regulated mainly on the basis of qualitative outcomes of hazard identification assays, i.e. decisions are often based on classification as positive or negative for genotoxic potential. Most human carcinogens are identified by epidemiological studies. These studies are necessarily

long term, as no effect is expected to be observed until decades after the carcinogenic event or events [6].However convincing, these studies are costly and exposure levels and effects are difficult to quantify. A few multiple generation mutation assays have been carried out using rodents:

- Dominant lethal
- Mouse spot test
- Heritable translocation test

These tests must be carried out on a large scale, and tend to be insensitive; in order to detect a 1% increase (which is a very strong effect) in carcinogenicity in a human population, one would need to perform an animal study to such a large scale as to cost over 25 million dollars. Genotoxicity tests can be defined as in vitro and in vivo tests designed to detect compounds that induce genetic damage by various mechanisms. These tests enable hazard identification with respect to damage to DNA and its fixation. Fixation of damage to DNA in the form of gene mutations, larger scale chromosomal damage or recombination is generally considered to be essential for heritable effects and in the multi-step process of malignancy, a complex process in which genetic changes may play only a part. Numerical chromosome changes have also been associated with tumour genesis and can indicate a potential for aneuploidy in germ cells. Compounds that are positive in tests that detect such kinds of damage have the potential to be human carcinogens and/or mutagens. Because the relationship between exposure to particular chemicals and carcinogenesis is established for humans, whilst a similar relationship has been difficult to prove for heritable diseases, genotoxicity tests have been used mainly for the prediction of carcinogenicity. Nevertheless, because germ line mutations are clearly associated with human disease, the suspicion that a compound might induce heritable effects is considered to be just as serious as the suspicion that a compound might induce cancer. In addition, the outcome of genotoxicity tests can be valuable for the interpretation of carcinogenicity studies [7].

Mutations are changes in the DNA sequence of a cell's genome and are caused by radiation, viruses, transposons and mutagenic chemicals, as well as errors that occur during meiosis or DNA replication. There is no consensus among genetic toxicologists regarding the classification of mutations.

Three groups of mutations can be distinguished:

1. Single point mutations or Gene mutations: These are small changes in the DNA at the level of the bases and genes, which are invisible under a light microscope. It again includes –

a) Base pair substitutions

b) Addition or deletion of bases

2. Structural chromosomal aberrations

3. Genome mutations

ANTI-MUTAGEN

Anti-mutagen is described as an agent that reduces the apparent yield of spontaneous and induced mutations. Mechanisms of anti-mutagenesis have been classified into two major processes one is desmutagenesis: in which factors on mutagens or inactivate them. The other is bio-antimutagenesis in which factors act on the processes of mutagenesis or repair DNA damages that result in a decrease in the mutation frequency. Gemcitabine used as a mutagen with anti-metabolites activity, exerts its effect by prohibiting DNA chain elongation[8].

Molecular Mechanisms involved in production of chromosomal aberrations

One of the endpoints of genotoxicity is gene mutations. Mutagenic chemicals cause predominantly gene mutations, which are generally not lethal but can form a major threat to the integrity of chromosomes and viability of cells. Fortunately, cells are equipped with several DNA repair systems. Depending on the specific classes of DNA lesions, one or more DNA repair pathways become active[9]. Four of the 5 major DNA repair pathways are involved in the repair of DNA lesions leading to gene mutations: direct repair, base excision repair (BER), nucleotide excisions repair (NER) and mismatch repair[10]. The 5th major repair pathway involved is single/double strand break repair.

a. Direct repair

Direct repair acts by removing or reversing the DNA lesions by a single enzyme reaction in a basically error-free manner and with high substrate specificity. This mechanism does not require a template, since the damage they restore only occurs in one base and there is no involvement of incision of the sugar-phosphate backbone or base excision. These lesions can occur due to alkylating agents. Direct repair is carried out by specific enzymes called alkyl guanine-DNA methyl transferases (AGMT), which remove the alkyl group from the guanine residue of DNA and transfers it to one of its own cysteine residues. Next to AGMT, in bacteria and yeast, photolyases can directly reverse UV-induced DNA damage[11-13].

b. Base excision repair (BER)

Base excision repair (BER) is a cellular mechanism that repairs damaged DNA throughout the cell cycle. This mechanism protects cells from the deleterious effects of endogenous DNA damage induced by hydrolysis, reactive oxygen species and other intracellular metabolites, and is also responsible for the removal of many lesions induced by ionizing radiation and strong alkylating agents. The main enzymes involved in BER are DNA glycosylases and AP endonucleases. The DNA glycosylases are involved in excision of the damaged base, where after the remaining a-basic site is further processed by AP endonucleases. BER is divided into short-patch repair (where a single nucleotide is replaced) or long-patch repair (where 2-10 nucleotides are replaced [14, 15].

c. Nucleotide excision repair (NER)

Nucleotide excision repair (NER) is a repair pathway that is involved in the removal of several kinds of DNA lesions which mainly originate from exogenous sources like UV light or genotoxic chemicals producing bulky adducts and DNA cross-links [16, 17]. NER consists of two different sub-pathways: global genome repair (GGR) and transcription-coupled repair (TCR). These two sub pathways are only different in the first step of DNA damage recognition. The first pathway (GGR) eliminates DNA damage present in the genome overall. The DNA recognition is accomplished by a complex of protein factors (XPC-HR23B and XPE). The second pathway (TCR) removes lesions from active genes. Hereby, the primary trigger in the DNA damage recognition is a stalled RNA polymerase II, which is accompanied by Cockayne syndrome (CS) proteins[18, 19]. The next stages involved in DNA repair are mostly studied for GGR, but are identical in the TCR pathway. After binding of the XPC-HR23B complex to the damaged DNA in GGR, several other proteins are bound such as a complex called transcription factor IIH (TFIIH) and the endonuclease XPG. TFIIH contains two DNA helicase activities with opposite polarity (XPB and XPD) that unwind the DNA duplex. After binding of the replication protein A (RPA), the damage is verified by XPA, where after the endonucleases XPG and ERCC1/XPF cleave the 3, and the 5, of the DNA lesion. This results in the release of a fragment, containing the DNA damage, of 27-30 nucleotides. The remaining gap is filled in by a complex formed by DNA polymerase d or e, the accessory replication proteins, the proliferating cell nuclear antigen (PCNA), RPA and the replication factor C [20].

d. Mismatch repair (MMR)

Mismatch repair (MMR) is a system that recognizes and repairs erroneous insertions, and mis-incorporation of bases. These can arise during DNA replication and MMR is a strand-specific repair. During DNA synthesis, the newly synthesized (daughter) strand may include incorrect bases. Examples of mismatch bases include base pairs like G/T or A/C. To repair these mismatched base pairs in the correct manner, it is very important to discriminate between the newly synthesized (mismatched) strand and the parental strand. The first step in MMR is recognition of the deformity caused by the mismatch. Thereafter, the template and the non-template strand are determined and the incorrect incorporated base is excised and replaced with the correct nucleotide. During the repair process not only the mismatched nucleotide is removed, but a few or up to thousands of bases of the newly synthesized DNA strand can be removed and replaced [21].

e. Chromosomal aberrations and repair

The other endpoint of genotoxicity, chromosomal aberrations, is caused by clastogenic chemicals. Chromosome aberrations can either be structural (clastogenic) or numerical (an eugenic). DNA damages like double-strand breaks (DSBs), threaten the integrity of chromosomes and viability of cells. Unrepaired or mis-repaired DSBs can lead to mutations, chromosome rearrangements, cell death and cancer [22,23,24,25]. Numerical chromosome aberrations (aneuploidy) can be either loss or gain of chromosomes per cell (like trisomy 21 in Down syndrome) and can be lethal or cause genetic diseases. Fortunately we also possess systems to repair DSBs, the last of the earlier mentioned repair systems. In mammalian cells, DSBs are mainly repaired by either homologous recombination repair (HRR) or non-homologous end-joining (NHEJ) repair, respectively [26, 27]. The main difference in HRR and NHEJ is the requirement of a homologous DNA sequence in HRR, which is therefore an error-free mechanism. In contrast, NHEJ, which does not use sequence homology is an error-prone mechanism[28, 29]. Another difference is their dependency of the cell cycle. HRR depending on the presence of an intact sister chromatid is more efficient during late S and G2 phase of the cell cycle, when sister chromatids are active in dividing cells. NHEJ not depending on a homologous DNA strand can repair DSBs in all cell cycle stages, G1, S and G2 phase[30-33].

It has been shown that HRR acts at the embryonic stage, where the embryonic cells were sensitive towards ionizing radiation, but its action in adults was not detected, unless NHEJ is disabled. It was concluded that the contribution of HRR and NHEJ can differ depending on mammalian developmental stage (i.e. cell type) and on the specific type of DNA damage.

f. Homologous recombination repair

Homologous recombination repair is an error-free repair system. The RAD52-group of proteins, including RAD50, RAD51, RAD52 and RAD54, and MRE11 play a major role in HRR. In the case of a DSB, the initial cellular response is there cognition of this break through the RAD50/MRE11/NBS1 complex. Subsequently, followed by nucleolytic processing of the broken ends of DNA into 3,-end single-stranded DNA. The single-stranded DNA is bound by RPA (replication protein A). After RPA is removed and replaced by RAD51, the RAD51 nucleoprotein filament mediates the search for a homologous duplex template DNA where after the complex of joint molecules between the broken DNA ends and the intact ds DNA repair template is formed. The Rad52, Rad54, Rad50 paralogues (such as Rad51B, Rad51C, Rad51D), Xrcc2, Xrcc3 andDmc1 are accessory to Rad51 at various stages of HRR. After polymerisation of nucleotides to restore degraded DNA strands and resolution of the recombination intermediates, the HRR is completed resulting in an error-free double-stranded DNA. The breast-cancer-susceptibility proteins BRCA1 and BRCA2 are involved in HRR as well, however, their role is not well understood [34, 35]. Loss of most HRR factors can lead to early or mid-embryonic lethality in mice [36]. This suggests that HRR plays an important role in development, presumably to repair spontaneously arising DNA damage which is in agreement with the findings that HRR and NHEJ can play different roles during the mammalian developmental stages [37].

g. Non-homologous end-joining repair

Non-homologous end-joining (NHEJ) is an error-prone repair mechanism. There are at least 3steps involved in NHEJ (Figure 5). The first step is the detection of the strand break and the end-binding mediated by DNA-PK consisting of the three subunits DNA-PKCS (DNA-dependent protein kinase catalytic subunit) and the KU80/KU70 heterodimer, which are involved in the formation of a molecular bridge that holds the broken DNA together. Hereafter, the NBS1/MRE1/RAD50complex is involved in the processing procedure that modifies non-matching and/or damaged DNA ends into incompatible and ligatable ends. Finally, in the ligation step, a complex consisting of DNA ligase IV and XRCC4 (X-ray-repair-cross-complementing defective repair in Chinese hamster mutant ligates the two DNA ends together forming an intact double-strand DNA molecule [38]. Recently, Cernunn os-XLF was discovered, which is also involved in NHEJ61. Cernunnos-XLF interacts and stimulates the DNA ligase IV-XRCC4 (LX) complex, which acts in the final ligation step in NHEJ [39].

IN VITRO TOXICOLOGY TESTING

The purpose of *in vitro* testing is to determine whether a substrate, product, or environmental factor induces genetic damage. One technique is cytogenetic assays using different mammalian cells. The types of aberrations detected in cells affected by a genotoxic substance are chromatid and chromosome gaps, chromosome breaks, chromatid deletions, fragmentation, translocation, complex rearrangements, and many more. The clastogenic or aneugenic effects from the genotoxic damage will cause an increase in frequency of structural or numerical aberrations of the genetic material. This is similar to the micronucleus test and chromosome aberration assay, which detect structural and numerical chromosomal aberrations in mammalian cells [40].

In a specific mammalian tissue, one can perform a mouse lymphoma TK+/- assay to test for changes in the genetic material. Gene mutations are commonly point mutations, altering only one base within the genetic sequence to alter the ensuing transcript and amino acid sequence; these point mutations include base substitutions, deletions, frame-shifts, and rearrangements. Also, chromosomes' integrity may be altered through chromosome loss and clastogenic lesions causing multiple gene and multilocus deletions. The specific type of damage is determined by the size of the colonies, distinguishing between genetic mutations (mutagens) and chromosomal aberrations (clastogens) [41].

Lastly, the SOS/umu assay test evaluates the ability of a substance to induce DNA damage; it is based on the alterations in the induction of the SOS response due to DNA damage. The benefits of this technique are that it is a fast and simple method and convenient for numerous substances. These techniques are performed on water and wastewater in the environment [42].

Chromosomal aberration [43]

The chromosome aberration test (CAT) and the micronucleus test (MNT) are most commonly used and well validated in vivo chromosome aberration tests. The CAT measures the occurrence of chromosome aberrations generally in bone marrow or peripheral blood cells. In the CAT the mitosis is arrested in the metaphase stage with a mitotic inhibitor colchicine. Metaphase preparations are examined for chromosome breaks and/or chromosomal rearrangements. The number of cells with chromosomal breaks is a measure for clastogenicity of chemicals for this test [44].

- Deletions: A portion of the chromosome is missing or deleted. Known disorders in humans include Wolf-Hirschhorn syndrome, which is caused by partial deletion of the short arm of chromosome 4; and Jacobsen syndrome, also called the terminal 11q deletion disorder.
- Duplications: A portion of the chromosome is duplicated, resulting in extra genetic material. Known human disorders include Charcot-Marie-Tooth disease type 1A which may be caused by duplication of the gene encoding peripheral myelin protein 22 (PMP22) on chromosome 17.
- Translocations: A portion of one chromosome is transferred to another chromosome. There are two main types of translocations:

- Reciprocal translocation: Segments from two different chromosomes have been exchanged.

- Robertsonian translocation: An entire chromosome has attached to another at the centromere - in humans these only occur with chromosomes 13, 14, 15, 21 and 22.

- Inversions: A portion of the chromosome has broken off, turned upside down and reattached, therefore the genetic material is inverted.
- Insertions: A portion of one chromosome has been deleted from its normal place and inserted into another chromosome.
- Rings: A portion of a chromosome has broken off and formed a circle or ring. This can happen with or without loss of genetic material.
- Iso chromosome: Formed by the mirror image copy of a chromosome segment including the centromere.

Animals were sacrificed by cervical dislocation after 24h of administration of the clastogen. 90 min. prior to death, each animal was injected with 0.04% colchicine in a dose of 4 mg/kg i.p for mitotic arrest. Colchicine solution was prepared in distilled water. Animals were cut open and femur and tibia from both the legs were quickly removed and muscle mass cleaned away from the bones. For collection of bone marrow, the upper end of femur was cut open, till a small opening was visible. A 22 gauge needle was inserted to ensure that the upper end was

open. About 0.5 ml of 0.56% (or 0.075 M) hypotonic potassium chloride solution was taken in a syringe and the needle was inserted at the lower epiphyseal end. The bone marrow was flushed into a clean cavity block. If the marrow collected was solid, it was dispersed by repeated aspiration and flushing with the help of the syringe. Similarly tibial marrow was also collected. Altogether 2 ml of hypotonic potassium chloride solution was used to collect the marrow from both femur and tibia.

A fine marrow suspension was made by gently mixing the contents with 3 ml of hypotonic potassium chloride solution in the cavity block. Suspension was transferred to a clean graduated centrifuge tube with the help of a hypodermic syringe and allowed to stand for 20 min [45].

A. Fixation

- After 20 min., the cells were centrifuged at 800 rpm for 4 min.
- Supernatant was removed by gentle aspiration until a small volume (0.1-0.2 ml) remained above the pellet.
- The pellet was resuspended in the remaining volume on a cyclo (vortex) mixer.
- The cells were then agitated on the cyclo-mixer with drop wise addition of 0.5 ml of freshly prepared cold fixative (methanol: gl. Acetic acid, 3:1).
- The resulting suspension was allowed to stand at room temperature for 15-20 min.
- Then it was centrifuged at 800 rpm for 4 min.
- Supernatant was gently aspirated leaving a small volume over the pellet.
- The cells were resuspended in the remaining volume.
- About 2 ml of fresh, cold fixative was added drop wise with agitation.
- Steps 6-9 were repeated thrice with the last-but-one fixation lasting at least 1h under refrigeration.
- Fixative was changed for the last time just prior to the preparation of the slides.
- The cell suspension was divided into two parts- one part stored (in about 2ml of fixative) tightly capped at 4˚c in a refrigerator; whereas the other part was used for immediate preparation of slides.

B. Slide Preparations:

For overnight stored cells, fixative was changed two more times by repeating steps 6-9. Slides used for smearing were free from any scratches, greasy or particulate matter. Two separate drops were dropped from a height of 30-40 cm on to the chilled slides kept in a refrigerator for at least 1hr before use. The slides were quickly dried by passing twice or thrice over a low intensity flame. It is important that all fluid dries up quickly and completely to stick the cell firmly to the glass surface. Adequate numbers of slides were prepared depending upon the frequency of scorable metaphase cells, with a minimum of two slides per animals. Slides were then stained with Giemsa's stain diluted with phosphate buffer PH=6.8 (1 ml of 5% Giemsa solution in 40 ml of buffer for 5-10 minutes). Quality of staining was checked on test slides before all slides were stained. After staining, the slides were washed rapidly in 3 or 4 changes of buffered water so that differentiation could take place. The lower surface of each slide was wiped with acetone and the slides were then air-dried.

C. Observation

Slides were scanned under higher magnification (100 X oil immersion) for examination of each individual metaphase. At least 100 well-spread metaphase cells were screened per animal. Only those cells that were well spread, where nuclei were free of cytoplasm and chromosomes did not overlap were selected of aberrations. Mitotic index (MI) was determined from the ratio of dividing to non-dividing cells. The MI was determined by counting the number of metaphases in 1000 cells per animal.

Micronucleus Assay [46]

Micronucleus is also the name given to the small nucleus that forms whenever a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei during cell division. In newly formed red blood cells in humans, these are known as Howell-Jolly bodies. In normal people and many other mammals, which do not have nuclei in their red blood cells, the micronuclei are removed rapidly by the spleen. Hence high frequencies of micronuclei in human peripheral blood indicate a ruptured or absent spleen. In mice, these are not removed, which is the basis for the in vivo Micronucleus test.

We have to inject cyclophosphamide (100mg/kg), 24 hours before starting the experiment.

The experimental animals were sacrificed by cervical dislocation. Animals were cut open and femur and tibia were removed. For collection of marrow, the upper end of the femur was cut open, till a small opening was visible. A needle was inserted to ensure that the upper end was open. About 0.5 ml of the suspending medium was taken in a syringe and the needle was inserted at the lower epiphyseal end. The marrow was flushed into a clean cavity block. If the marrow collected was solid, it was dispersed by repeated aspiration and flushing with the help of the syringe. Similarly tibial marrow was also collected. Altogether 2.0 ml of the suspending medium was sufficient to collect the marrow from femur and tibia. A fine marrow suspension was then transferred to a centrifuge tube and centrifuged at 1000 rpm for 8 min. The supernatant was drawn off and marrow films made by smearing a small drop on a clean glass. Then the slides were air-dried.

Staining procedure

The smears were fixed in absolute methanol for 10 min. (The methanol should be absolutely pure, so as to avoid artifacts in the slides). The slides were kept in coupling jars containing May Grunwald's stain freshly diluted with equal volume of phosphate buffer (PH=6.8) for 15 min. The slides were transferred to Giemsa, freshly diluted with phosphate buffer (1:6) and kept for 10 min. washed the slides rapidly in 3 or 4 changes of buffered water. The slides were then allowed to stand undisturbed in buffered water for 5 min., so that differentiation could take place. Slides were air-dried.

Scoring

The slides were scanned under oil immersion (100X) in Labomed – Model Digi 2 microscope (90V-260V) for the presence of MN in PCE and NCE was determined by counting a total of about 2000 erythrocytes per animal. A total of 500 erythrocytes were counted for PCE NCE ratio.

Statistical Analysis

The statistical significance of the results was tested using and one-way ANOVA.

IN VIVO TESTING

The purpose for *in vivo* testing is to determine the potential of DNA damage that can affect chromosomal structure or disturb the mitotic apparatus that changes chromosome number; the factors that could influence the genotoxicity are ADME and DNA repair. It can also detect genotoxic agents missed in *in vitro* tests. The positive result of induced chromosomal damage is an increase in frequency of micronucleated PCEs. A micronucleus is a small structure separate from the nucleus containing nuclear DNA arisen from DNA fragments or whole chromosomes that were not incorporated in the daughter cell during mitosis. Causes for this structure are mitotic loss of acentric chromosomal fragments (clastogenicity), mechanical problems from chromosomal breakage and exchange, mitotic loss of chromosomes (aneugenicity), and apoptosis. The micronucleus test *in vivo* is similar to the *in vitro* one because it tests for structural and numerical chromosomal aberrations in mammalian cells, especially in rats' blood cells [47].

Prevention of genotoxicity

Genotoxic effects such as deletions, breaks and/or rearrangements can lead to cancer if the damage does not immediately lead to cell death. Regions sensitive to breakage, called fragile sites, may result from genotoxic agents (such as pesticides). Some chemicals have the ability to induce fragile sites in regions of the chromosome where oncogenes are present which could lead to carcinogenic effects. In keeping with this finding, occupational exposure to some mixtures of pesticides are positively correlated with increased genotoxic damage in the exposed individuals [48]. DNA damage is not uniform in its severity across populations because Individuals vary in their ability to activate or detoxify genotoxic substances, which leads to variability in the incidence of cancer among individuals. The difference in ability to detoxify certain compounds is due to individuals' inherited polymorphisms of genes involved in the metabolism of the chemical. Differences may also be attributed to individual variation in efficiency of DNA repair mechanisms [49].

The metabolism of some chemicals results in the production of reactive oxygen species which is a possible mechanism of genotoxicity. This is seen in the metabolism of arsenic which produces hydroxyl radicals, which are known to cause genotoxic effects [50]. Similarly, ROS have been implicated in genotoxicity caused by particles and fibres. Genotoxicity of non-fibrous and fibrous particles is characterized by high production of ROS from inflammatory cells [51].

Flavonoids have been reported to possess a wide range of biochemical and pharmacological activities, both potentially detrimental and protective. One of the effects of flavonoids is the ability to modulate the xenobiotic metabolism. Various studies have indicated that a potential basis for protection is interference with enzymes such as cytochrome p450 which plays an important role in metabolic activation of wide range of carcinogens[51].

Drugs presently being used as anti mutagenic agents are busulfan, carmustine, etoposide etc.

Plant-derived polyphenolics and other chemicals with antioxidant properties have been reported to inhibit the expression of genotoxic activity by pro-oxidant chemicals. *In vitro* and *in vivo* studies with ionizing radiation suggest that hydroquinone (HQ) may have similar protective effects. The protective effect of HQ may be due to enzyme induction or a direct antioxidant effect of HQ against oxidants commonly present in the diet [52].

Ellagic acid peracetate (EAPA), which unlike ellagic acid (EA) has demonstrated time-dependent inhibition of liver microsomes catalysed AFB_1-epoxidation as measured by AFB_1 binding to DNA. EAPA was more potent than EA in preventing bone marrow and lung cells from AFB_1-induced genotoxicity. EAPA was acted upon by microsomal acetoxy drug: protein transacetylase (TAase) leading to modulation of the catalytic activity of certain functional proteins (cytochrome P450, NADPH cytochrome c reductase and glutathione S-transferase), possibly by way of protein acetylation[53].

Non-flavonoid compounds such as simple phenolics (C6), phenolic acids (C6-C1), cinnamic acid and related compounds (C6-C3) also showed anti mutagenic effects[55].

CONCLUSION

A genotoxic agent is a drug or a chemical which causes changes or aberrations or mutations in the DNA structure and may lead to cancer. They act by changing the chromosomal structures, forming rings, breaks, joins etc. These can be identified by the chromosomal aberration

test. Any drug which prevents the genotoxic effect of clastogenic agents are said to be anti- clastogenic or anti-mutagenic agent.

DNA repair mechanisms, metabolism of harmful chemical clastogens and use of anticancer drugs are the major treatments for genotoxicity. The drugs which are used for treatment of genotoxicity and also act as anti cancer agents are alkylating agents, intercalating agents and enzyme inhibitors.

Plant extracts like flavonoids, ellagic acid etc, are found to possess pharmacological activity and hence being used as anti mutagenic agents.

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