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GENOTOXICITY: A REVIEW OF STUDIES ON SOME AYURVEDIC DRUGS

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ABSTRACT

Genotoxicity describes a deleterious action on a cell genetic material affecting its integrity. Ayurvedic medicines are generally based on herbal products and Ayurvedic practitioners usually make up their own medicines. They may use individual herbal extracts or a mixture of herbal extracts with vegetable, animal and mineral products and it is a basic principle of Ayurveda that anything can be used as a drug. This review article researches the ayurvedic drugs on genotoxicity is about it's therapeutic value. Then this review article reviews the various research studies on genotoxicity on ayurvedic drugs. This study will be very useful for future ayurvedic drug development and particularly for the life style diseases and disorders.

KEYWORDS: Genotoxicity, Ayurvedic drugs, lymphocyets, Heavy metals, comet assay and MNT.

1. INTRODUCTION

Genotoxic substances are capable of causing genetic mutation and of contributing to the development of tumors. Genotoxicants include the both-certain chemical compounds and certain types of radiations. Typical genotoxins like aromatic amines are believed to cause mutations because they are nuclieophillc and form strong covalent bonds with DNA resulting with formation of aromatic amine DNA adducts preventing accurate replication. Genotoxicity or genetic toxicology has evolved from the initial studies of gene mutability demonstrated first by Muller in 1927 using X-ray radiation, followed by Auerbach (1947). Genetic toxicology assesses the effects of chemical and physical agents on the hereditary material, DNA and on genetic processes of living cells (Prestone and Hoffmann, 2001). It also includes mutagenicity and carcinogenicity studies as well as studies on consequences of the DNA

damage and risk assessment (Li, 2000). There are many substances which cause or produce genotoxicity for example various chemicals insecticides, pesticides. Some drugs may also cause genotoxic effects like allopathic and ayurvedic drugs. Ayurvedic medicine has been used in India for thousands of years and is increasingly been used worldwide during the last few decades as evidenced by rapidly growing global and national markets of Ayurvedic drugs (Aneesh *et.al*, 2009). In India, around 25,000 effective plant-based formulations are used in traditional and folk medicine. More than 1.5 million practitioners are using the traditional medicinal system for health care in India (Kochhar *et. al*, 1981).

2. Genotoxicological studies on allopathic / homeopathic drugs

Anti-tumor activity of various drugs is based on the mechanism of action of these agents preventing the growth of the tumor cells. These cells unlike normal cells fail to respond to homeostatic control mechanism as a result their population expends due to continuous cell division (Schabel, 1975). The cell toxicity is due to the binding of drug at the alkyl groups directly to DNA bases that results in DNA damage in the term of single strand breaks. The genotoxicity and cytotoxicity of several drugs have already been evaluated by various scientists. Aly *et al*. (2003) studied genotoxicity and cytotoxicity of the anti-cancer gemcitabine and cisplatin separately and in combination *in vivo* on male mice bone marrow cells. Both gemcitabine (2, 2-diflorodeoxycytidine; dFdc) and cisplatin have significant anti-cancer activity against ovarian, head and neck, and non-small cell lung cancer. dFdc can be incorporated into DNA and RNA and inhibit DNA repair while cisplatine can form Pt- DNA abducts. Because of differences in mechanisms of action and toxicity profiles, combination of the two drugs has enormous clinical potential. These authors categorically stated that the combination of both is increasingly applied in clinical oncology. In CDDP (cis-diammine-dichloroplatinum) experiments doses of 6, 12, 24 & 36 mg kg^{-1} body weight where as for dFdc 40, 50, 60 and 80 mg kg^{-1}body weight were used. Three doses of drug combination i.e. (i) 4 mg kg^{-1} body wt. (CDDP) + 20 mg kg^{-1} body wt. (dFdc) (ii) 6 mg kg^{-1} body wt. (CDDP) + 20 mg kg^{-1} body wt. (dFdc) and (iii) 8 mg kg^{-1} body wt. (CDDP) + 20 mg kg^{-1} body wt. (dFdc) were used. Total chromosomal aberrations and sister chromatid exchange frequencies were increased after exposer to combined drugs as compared to exposure to each drug separately.

Blasiak *et al*. (2002) carried out *in-vitro* study on genotoxicity of anti-cancer drugs idarubicin and mitoxantrone in human lymphocytes. Idarubicin is an anthracycline analog with

presumed better anti-neoplastic activity and lesser toxicity. Using the alkaline comet assay, they formed that the drugs at 0.01-10 µM induced DNA damage in normal human lymphocytes. The effect induced by idarubicin was more pronounced than by mitoxantrone (P‹ 0.001). The cells treated with mitoxantrone at 1 µM were able to repair damage to their DNA within 30 minutes incubation where as the lymphocytes exposed to idarubicin needed 180 minutes.

Basu *et al*. (2001) studied mutagenic effect of Arsenic compounds in bacterial and animal cells. They found that Arsenic compounds are weak mutagens in these organisms. However, they are reported to produce clastogenic and anugenic effects and induce gene amplification, cellular transformation, DNA cross-links and DNA strand breaks in animal cells. Liver injury induced by chemicals and its recovery processes have been extensively studied to understand many cytotoxicological problems (Zimmerman, 1978; Plaa *et al*. 1991). Arsenic Album-200 can effectively combat chronic arsenic toxide in mice. Mice were injected subcutaneously with 0.0166 % arsenic trioxide at the rate of 1 ml/100g body weight, at an interval of 7 days until they were killed at 30 day, 60 day, 90 day or 120 day and were divided into three groups (i) one receiving a daily dose of Arsenicum Album-200 through oral administration, (ii) one receiving the same dose of diluted alcohol and (iii) another receiving neither drug, nor alcohol and compared with controls. The drug fed mice showed reduced toxicity at statistically significant levels in respect of all the parameters studied, thereby indicating protective potentials of the homeopathic drug against chronic arsenic poisoning (Banerjee *et al.* 2007).

Arsenic in ground water and it's accumulation in plants and animals have assumed a menacing proportion in a large part of West Bengal, India and adjoining areas of Bangladesh. Because of the tremendous magnitude of the problem, there seems to be no way to tackle the problem overnight. Efforts to provide arsenic free water to the millions of people living in these dreaded zones are being made, but are awfully inadequate. In quest for finding out an easy, safe and affordable means to combat this problem, a homeopathic drug, Arsenicum album-30, appears to yield promising results in mice (Mallick *et al.*, 2003).

Homeopathic medicines i.z. Avena sativa, Nux vomica, Arsenic album, Bryonia alba, Rhus toxicodendron, Arsenic album and Chamomilla were found to be effective in 10 C potency while Bryonia alba and Nux vomica in 200 C potency and Avena sativa in mother-tincture (Clinical Research Unit, Varanasi, 1994). The protective potentials of a potentized

homeopathic drug, Lycopodium-30 prepared from extract of spores of a plant, *Lycopodium clavatum* are used as a remedy for various liver ailments, which have been tested in mice chronically fed with p-dimethyl amino azo- benzene (p-DAB)- an initiator and phenobarbital- a promoter of hepatic cancer, by using some cytogenetic endpoints like chromosomal aberrations (CA), micronuclei (MN), mitotic index and sperm head abnormalities and toxicity biomarkers like acid and alkaline phosphatases, alanine and aspartate amino transferase and lipid peroxidation and reduced glutathione activities. The effect of chronic treatment of the carcinogens were assessed at different intervals of fixations and compared with the carcinogens and homeopathic remedy (Pathak *et al.*, 2006).

3. Genotoxicity induced by other agents

3.1 Heavy metals

Various inorganic (heavy metals) and organic compounds (pesticides), ayurvedic preparations are known to cause genotoxicity in various test systems. Several workers have studied the action of inorganic compounds such as arsenic, nickel and chromium, some plant essential oils and tobacco etc. in producing genotoxicity in different test system. Inorganic arsenic is considered the most potential human carcinogen, and humans are exposed to it from soil, water, air and food. In the process of arsenic metabolism, inorganic arsenic is methylated to monomethylarsonic acid finally to dimethylarsenic acid, followed by excretion through urine (Roy and Saha, 2000). Generally, the uses of these drugs have been discontinued because of high toxicity of arsenic compounds. Arsenic is used in the ayurvedic system of medicine to control hematological malignancies (Ireleaven *et. al*, 1993).

Chromium and nickel are naturally occurring elements present in several different forms in the environment. The general population is exposed to chromium by inhaling ambient air, ingesting food and drinking water containing chromium or by direct skin contact. Some studies were carried out on the genotoxicity exerted by nickel in the form of nickel chloride and chromium in the form of potassium dichromate, alone and in combination. Mitigating effect of curcumin was studies at two different exposure intervals (24 and 69 hours) in toxicant added human blood cultures. Nickel and chromium proved to be more toxic in 69 hours exposure than in 24 hours in induction of micronuclei (Rao *et. al*, 2008).

Lead toxicity has been reported with use of overdoses of lead containing Ayurvedic medicine, in the treatment of fatal infant encephalopathy, congenital paralysis and sensorineural deafness (Tait *et al.*, 2002). Moore and Adler (2000) reported 55 cases of heavy

metal intoxication associated with Ayurvedic HMPs in adults and children of the United States of America. They suggested that the quality of ayurvedic drugs and process of manufacturing and purification of metals are very important criteria which must be monitored because heavy metals in little amount are necessary for our health but beyond a particular limit it becomes toxic for our body.

Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes (Sandstead , 1994) and it plays a role in immune function (Solomons, 1998; Prasad, 1995), protein synthesis, wound healing (Heyneman, 1996), DNA synthesis, and cell division. Zinc also supports normal growth and development during pregnancy, childhood and adolescence (Simmer and Thompson, 1985; Fabris and Mocchegiani, 1995; Maret and Sandstead, 2006) and is required for proper sense of taste and smell (Prasad et *al*., 1997). A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system (Rink and Gabriel, 2000). Zinc toxicity can occur in both acute and chronic forms. Acute adverse effects of high zinc intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches. One case report cited severe nausea and vomiting within 30 minutes of ingesting 4 g of zinc gluconate (570 mg elemental zinc) (Lewis and Kokan, 1998).

Intakes of 150–450 mg of zinc per day have been associated with such chronic effects as low copper status, altered iron function, reduced immune function, and reduced levels of high-density lipoproteins (Hooper *et al*., 1980). Reductions in a copper-containing enzyme, a marker of copper status, have been reported with even moderately high zinc intakes of approximately 60 mg/day for up to 10 weeks (Institute of Medicine, Food and Nutrition Board, 2001). The doses of zinc used in the AREDS study (80 mg per day of zinc in the form of zinc oxide for 6.3 years, on average) have been associated with a significant increase in hospitalizations for genitourinary causes, raising the possibility that chronically high intakes of zinc adversely affect some aspects of urinary physiology (Johnson *et al*., 2007).

The comet assay has been widely accepted as a simple, sensitive, and rapid tool for assessing DNA damage and repair in individual eukaryotic as well as some prokaryotic cells, and has increasingly been applied in diverse fields ranging from genetic toxicology to human epidemiology. Many pesticides can be used with little risk to man and the environment. The cancer risk of pesticides has also been classified according to International Agency for Research on Cancer (IARC) and the US Environmental Protection Agencies (EPA) in 1989.

According to EAP, category B1 and B2 are awarded to agents probably carcinogenetic to humans which include pesticides such as aclifluorfen , alaculor, DDT and flopet etc. Category C is awarded to possibly carcinogenic agents which include cypermetherin and parathion among others. A positive incidence of cancer was observed among pesticides applicators exposed to chloropyriphos in an agricultural health study. The incidence of lung cancer was found to be significantly associated with both chlorophyriphos life time exposure days and chlorophyriphos intensity weighted exposure days. Individuals in the highest category of life time exposure days have a relative risk of lung cancer which was 2.18 times higher than those with no chlorophyriphos exposure (Lee *et al.,* 2004).

3.2 Ayurvedic preparations

The genetic toxicity of insecticide phosphamidon was evaluated by using the *in vitro* human lymphocyte test system and *in vivo* system using bone marrow of mice. The results indicate that the insecticide has potential to cause genetic damage (Patankar and Vaidya, 1980). The genotoxicity and cytotoxicity of several drugs have already been evaluated by various scientists. Various plant essential oils were able to induce chromosome aberrations and sister chromatid exchanges in human lymphocytes *in vitro*, and gene mutations in *Drosophila melanogaster* somatic cells *in vivo* (Lazutka *et. al*, 2001; Mierauskiene *et al.,* 2000). Jalani *et al*. (2006) carried out mutagenicity assessment of two herbal medicines, Urtan and Carmint in human leukocytes by single cell gel electrophoresis. Urtan and Carmint are examples of herbal medicines used in Iran for the treatment of hyperplasia, diuretic, urinary disfunction and antispasmodic action, carminative gastrointestinal disfunction respectively.

Sathya *et al.* (2009) described that Bhasmas, herbal preparations of ayurvedic origin, contain heavy metals in traces. Very little information is available on the pre-clinical toxicity or mutagenicity of these Bhasmas. Micronucleus assay and the comet assay were employed by them to study the endpoint of chromosomal damage and single / double – strand DNA breaks. The results revealed lack of induction of micronuclei or DNA damages as evidenced by the comet assay, despite the presence of traces of transformed toxic heavy metals. Besplug *et al*. (2004) used a new sensitive transgenic plant-based system to study genotoxicity and mutagenicity of atrazine present at minute concentrations in the liquid medium. This system gave us an opportunity to monitor the two main types of rearrangements, the frequency of homologous recombination and point mutations, which are indicators of the genotoxicity of atrazine. Atrazine present at low concentrations was found to be a strong inducer of

homologous recombination. On the other hand, it did not have a significant influence on the levels of A 3G and T 3G mutations. These results suggest that the transgenic plant-based biomonitoring system is a useful tool for analyzing the genotoxicity of water contaminated by atrazine. In the future this system can be used to study molecular mechanisms of genotoxicity and mutagenicity of atrazine and other triazine herbicides.

Kalantari *et al*. (2007) studied the genotoxic effects of herbal drops of garlic and pasipy using the micronucleus test. Maximum Tolerated Dose (MTD) was determined by a dose-response test. For each medicine three treatment groups were considered with doses of MTD, 1/2 MTD and 1/4 MTD, according to the CSGMT protocol (1995 Japan). Mitomicin C was used as a known genotoxic agent in positive control group. The peripheral blood samples before treatment (zero time samples) were considered as negative control. The appearance of a micronucleus was used as an index for genotoxic potential. The results obtained indicated that the herbal drops showed genotoxicity effect and it was dose-dependent as compared to the negative control group. The genotoxicity was significant ($p<0.05$) but the genotoxic effects of garlic and pasipy were "not significant" compared to the negative control group ($p>0.05$). Therefore, results if compared to the negative control group are significant and it is worth of consideration.

Dargan *et al*. (2008) studies the risk of heavy metal poisoning associated with the use of some Ayurvedic medicines. Many Ayurvedic medicines contain heavy metals, including lead, mercury and arsenic, and there have been numerous reports of clinically significant heavy metal poisoning related to their use. However, there have been a few studies that allow quantification of the incidence of this problem. There is limited regulation of these products in most areas of the world.

Ayurvedic medicinal products are used in the traditional Indian healing paradigm. According to the principles of Ayurvedic medicine, heavy metals are used in a detoxified state in these medicinal products because of their reputed therapeutic properties. However, the detoxification process is not followed strictly during manufacturing. It is possible for the resulting product to contain high levels of heavy metals. In addition to the JAMA study, other studies have found high levels of heavy metals in products sold in England and India. In Canada, Ayurvedic medicinal products are authorized for sale either as a natural health product bearing a Natural Product Number (NPN) or a drug product, bearing a Drug Identification Number (DIN). These eight digit numbers are preceded by NPN or DIN and

indicate that the product has been assessed by Health Canada for safety and effectiveness (Saper, 2004). The JAMA study reported high levels of heavy metals in the 14 Ayurvedic products presented in Table 3.1.

Table: 3.1 High levels of heavy metals in 14 Ayurvedic products Product

Products	Manufacturer	Indications on label
Bal Chamcha	Jalaram	problems associated with liver, digestion, teething, milk intolerance, irregular stools, regurgitation, bloating, parasites, colic, poor sleep, poor dentition*, and myalgias*
Bala Guti	Zandu	children's tonic
Bala Sogathi	Navjeevan	for healthy growth of children, teething, cough, cold, fever, diarrhea etc
Balaguti Kesaria	Kesari Ayurvedic Pharmacy	tonic tablets for babies with sudha and gold rickets, coryza*, cough griping, skin roughness, worms and dentition*
Gesari	Harinarayan Pharmacy	Indigestion, stomach problems, peptic pains*, uneasiness, bloating
Karela	Himalaya	Metabolism regulating
Maha Sudarshan Churna	Dabur	malaria, febrile conditions* varied etiology
Maha Sudarshan Churna	Zandu	Diaphoretic*, anti-malarial dyspepsia*, loss apatite
Mahalakshmi Vilas Ras with Gold	Baidyanath	weak lungs, cold, nasal problems, cough, runny nose, respiratory problems, blood deficiency, sinus HA, wound healing, asthma, palpitation, flu
Mahayograj Guggulu w/silver & Makardhwaj	Baidyanath	rheumatic pain, bile, pigmentation disorders, blood imfaction, eye problems, weakness
Navratna Rasa	Unjha Ayurvedic Pharmacy	general debility, rickets, calcium deficiency
Safi	Hamdard Pakistan	digestive systems, constipation, dermatitis
Shilajit	Syncom	tonic, bronchial skin piles, anemia, inflammation, excess fat, dyspepsia*, worms, constipation
Swarna Mahayograj Guggulu with Gold	Baidyanath	Pain, gas, rheumatism, CVA*, menstrual cycles, progesterone deficiency, strengthening arteries/veins, weak vital organs, mental disorders, fertility, menopause

* Dentition (dental problems); myalgias (muscle pain); coryza (runny eyes and/or nose); febrile conditions (fever); diaphoretic (perspiration); dyspepsia (stomach pain); and CVA (cerebrovascular accident).

Many workers have tested the mutagenicity of many substances by MNT. Maki- Paakkanen and Norppa (1987) tried induction of MN by vinyl acetate in mouse bone marrow cells and cultured human lymphocytes and found that the frequency of MN reach a peak at 0.5 and 1 mM and declined at 2mM because of toxic effect resulting in mitotic inhibition. Luomhara and Norppa (1994) worked on induction of MN in cultured human lymphocytes treated with

vinblastine before and after mitogen stimulation. Hydroquinone was found to be ineffective in the induction of the MNT in human peripheral blood lymphocytes by Doepker *et al.* (2000).

The degree of DNA migration measures the possible DNA strand breaks, alkali liable sites and incomplete excision repair sites and can be expressed as the olive tail moments- the percentage at DNA in the tail length. The micronucleus assay (MNT) is a widely used cytogenetic method to assay in vivo genotoxicity of various agents. Hayashi *et al.* (1998) Reported that MNT could be used to assess genotoxicity induced by anti-cancer drugs.

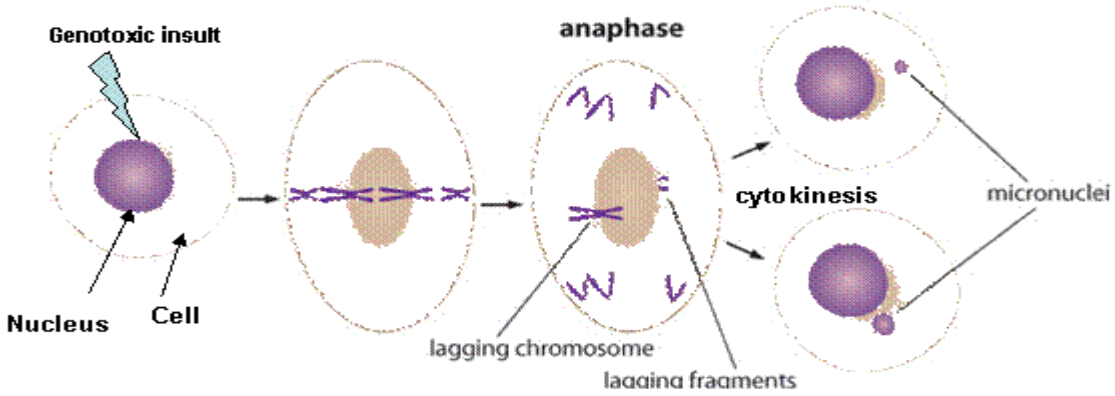

Fig. 1: Formation of Micronucleus

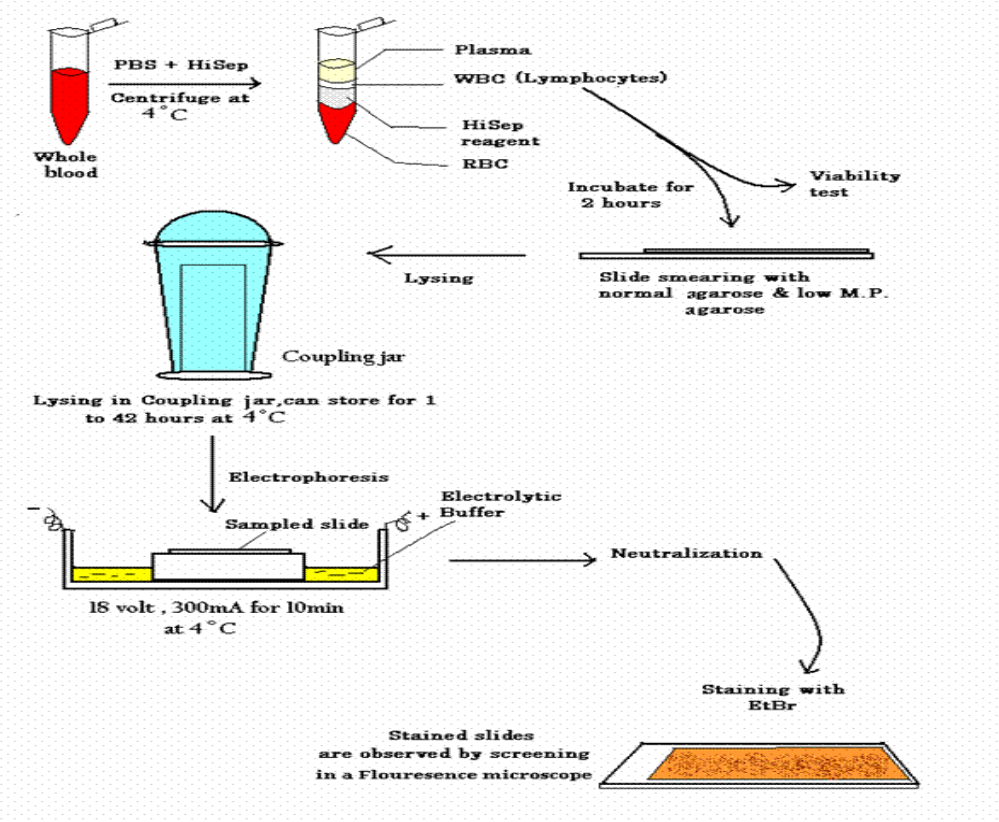

Fig. 2 Flow diagram showing sequential steps of comet assay

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