REVIEW: Protective Potential of Herbal Plants and their Constituents on the Clastogenicity Produced by Anticancer Treatments.

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Abstract
Cancer is a worldwide disease according to WHO cancer is the second largest occurring disease after heart diseases. Treatments in cancerous patients were very harmful to our body and long term treatment may cause the serious side effect on patients. Hepatotoxicity, nephrotoxicity, cardiotoxicity, clastogenicity etc. Clastogenotoxicity may initiate the mutation and causes detrimental effect on the patient health. It is very serious topic today's life. Several reports published show's that natural or herbal treatment will capable to reduce the severe affect of anticancer drugs. Our review on those selected plants that having protective affects against genotoxicity produced in cancerous patients.

Keyword : Genotoxicity, Cancer, Mutation, Clastogenotoxicity, Herbal.

Introduction: Cancer is the worldwide serve health disease both in developing as well as non developed countries. In 2008, 12.7 million people were diagnosed with cancer across the world and 7.6 million people died from cancer (1). Predictable treatment of cancer includes interventions such as psychosocial support, surgery, radiotherapy and chemotherapy (2). Due to rising the speed of mortality associated with cancer and adverse or toxic side effects of cancer chemotherapy and radiation therapy, it very necessary to discovery of protective shealth against anticancer agents toxicity that derived from nature, especially plants, is currently under investigation.herbal medicament are very famous from the history in many toxicity so we collected all those plant list that contains such special anti genotoxicity properties that we search already. There is wide classification of anticancer drugs and many reported drugs that effect on the genetic material as side effect.

- Cyclophosphamide
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- **Emblica myrobalan, Emblica officinalis Gaertn.** (Amla) extract and its major active component ascorbic acid on the in vivo clastogenicity of two chemicals namely Benzo(a)pyrene (a well-known carcinogen) and Cyclophosphamide (an anticancer drug) in mice. Extract help to recovery the reduced glutathione contents as well as the activities of glutathione-S-transferase, glutathione reductase and glutathione peroxidise.

- **Ficus benghalensis** herbal plant used in Ayurveda as a traditional medicinal. In the present investigations, the preventive effect of *Ficus benghalensis* bark extract was evaluated against cyclophosphamide induced of chromosomal Abbreviation and micronucleus formation in the rat bone marrow cells. The single oral administration of *Ficus benghalensis* bark extract at the dose of 250, 500 and 800 mg/kg body weight, 24 hours prior the administration of cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations and Chromosomal Abbreviation and protective potential against anticancer drug in dose dependent manner in bone marrow cells of rat as compared to cyclophosphamide group.

- **Solanum lycopersicum** fruit extract has been protective potential against cyclophosphamide (CP)-induced chromosomal aberrations in the bone marrow cells of the mice. Single i.p. administration of *Solanum lycopersicum* fruit extract at different test doses, namely 500, 1000 mg/kg body weight have provided protection, when given 24 hr prior to the single i.p. administration of cyclophosphamide (50 mg/kg body weight). It’s seems to have a preventive potential against CP-induced chromosomal aberrations in the bone marrow cells of the mice.

- **Crocus sativus** (Saffron) obtained from dried stigmas belonging to the family Iridaceae. Over the decades, it has been used in traditional medicine for various diseases. The anticlastogenic effects of saffron have been reported in the present study. The constituents of saffron include safranal, crocin and crocetin. Safranal a monoterpenic aldehyde which is an essential oil of saffron showed antioxidant activity. Crocin and crocetin which are the carotenoids of saffron possess inhibitory effect on free radical chain reactions, because most carotenoids are lipid-soluble and might act as highly-efficient free radical scavengers. Saffron in experiments with Swiss albino mice significantly inhibited the genotoxicity of cyclophosphamide, which is shown by decrease comet tail length, tail moment and percent DNA in the tail.

- **Caryocar brasiliense** (Caryocaraceae) pulp, popularly known in Brazil as pequi, against clastogenicity induced by cyclophosphamide was evaluated using mouse bone marrow cell micronuclei test, Chinese hamster ovary cell (CHO-K1) chromosome aberration test ,based on the oxidative damage to 2-deoxy-D-ribose (2-DR) induced by hydroxyl radicals (OH) generated by the reaction between ascorbic acid and (Fe III)-EDTA. In mouse bone marrow cells the extract showed a protective effect against micronuclei induced by cyclophosphamide but did not hinder with polychromatic bone marrow erythrocyte proliferation, except when the mice had been treated with the highest dose of cyclophosphamide. When pretreated by adding 0.01, 0.05 or 0.1 mL of extract per mL of cell culture medium 24 or 48 h before cyclophosphamide there was a protective effect against chromosome breaks and a significant decrease in the mitotic index. The extract also had a protective effect against oxidative hydroxyl radical damage. This study suggests that *C. brasiliense* pulp aqueous extract has anticlastogenic potential, possibly due to its antioxidative properties.
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- **Plumbago zeylanica**, commonly known as white leadwort, found in the plains of Bengal and southern India, was tested for its possible *in vivo* protective effect against cyclophosphamide-induced genotoxicity. Pretreatment with the alcoholic root extract of *Plumbago zeylanica* (250 and 500 mg/kg orally for 5 days) significantly reduced the frequency of micronucleated polychromatic erythrocytes (MnPCEs), increased the PCE/NCE (normochromatic erythrocyte) ratio in the bone marrow. Both doses of *Plumbago zeylanica* were effective in exerting a protective effect against cyclophosphamide-induced genotoxicity. (Sivakumar, 2006)

- **Buchanania lanzan** To explain the consequence of ethanolic extract of bark extract against cyclophosphamide induced genotoxicity. Pretreatment with *B. lanzan* 250, 500 and 1000 mg/kg, p.o., daily for 7 days significantly reduced the chromosomal damage with concomitant changes in antioxidants and detoxification systems. (9)

- **Caralluma tuberculata** extract used for cytotoxicity in the bone marrow cells induced by standard drug cyclophosphamide. In the extract-treated animals there was a significant and dose-dependent increase in the DNA content of the liver, with a negligible effect on the protein content. Combined treatment with *C. tuberculata* and cyclophosphamide showed that *C. tuberculata* diminished the effect of cyclophosphamide on DNA levels; however, RNA levels were further suppressed, resulting in increased cytotoxicity. Pretreatment with *C. tuberculata* extract significantly reduced the clastogenicity of cyclophosphamide. These results indicated the involvement of different phytoconstituents acting by different routes. (10).

- **Pothomorphe umbellata** root extract and its isolated active principle, the 4-nerolidylcatechol (4-NC) used to estimate the mutagenicity and antimutagenicity, in bone marrow cells of mice using the micronucleus test. Swiss male mice were orally treated for 4 days with extract (200, 100 or 50 mg/kg/day) or 4-NC (50, 25 or 12.5 mg/kg/day) previous exposed with a single dose (200 mg/kg) of cyclophosphamide, 24 h after the end of the treatment. The results demonstrated that the PUE and 4-NC did not have any mutagenic effect on bone marrow cells; quite the opposite, there was a protective effect against genotoxicity induced by cyclophosphamide. (11).

- **Caseariasylvestris** leaves used to investigated the genotoxic effects of a *C. sylvestris* crude ethanolic extract on Hepatoma Tissue Culture (HTC cells) of *Rattus norvegicus* and Chinese hamster V79 cells in culture, using the comet assay. For the genotoxic evaluation the cells were treated with three different concentrations (0.5, 1 and 2 mg/ml) of extract prepared from a 25 mg/ml aqueous solution. The positive control was cyclophosphamide for HTC cells and methyl methanesulfonate for V79 cells. The results showed that the extract of *C. sylvestris* presented no genotoxic effects and not modified effect inducing DNA damage by alkylating agents cyclophosphamide and methyl methanesulfonate in HTC and V 79 cells respectively. (12)

- **Aloysia triphylla** a perennial, bushy plant originally from South America has long been used in traditional medicine. Its aqueous extract contains considerable amounts of polyphenolic compounds, namely flavonoids and phenolic acids. This study was undertaken to investigate the chemoprotective effects of cedron leaves infusion against the genetic damage induced by acrylamide (AA) by using the alkaline version of the comet assay technique. The results suggest that the infusion could exerts an in vivo chemo protective action, probably due to its scavenging potency towards free radicals (13).

- **Cisplatin**

*Terminalia. chebula* Annona crassiflora *P. maderaspatensis Hemidesmus indicus*
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Solanum paniculatum    Aloysia triphylla        Rubia cordifolia    Coccoloba mollis  
Bixa orellana    Vitis vinifera

- **Terminalia chebulia** used to evaluated the genotoxicity activity in rat bone marrow cells induced by cisplatin. The fruit of *Terminalia chebula* was extracted with hydro alcoholic solvent methanol: water mixture. Phytochemical like tannins, saponins, and flavonoids was identified in the extract. The aberration scored for extract induced by cisplatin treated group was 30.99±1.376% which was found to be significantly less as compared to cisplatin treated group. Thus the results suggested that the extract contains some active principles which might be contain significant non-genotoxic activity.(14).

- **Annona crassiflora** (Araticum) widely used in humans as therapeutic medicine to treat several diseases such as diarrhea, rheumatism and syphilis. Acetogenins contents cytotoxic, antitumogenic, and antiparasitic properties. In this study, mutagenic, antimutagenic and cytotoxic effects of *araticum* leaves ethanolic extract were evaluated by micronucleus test. For all doses, micronucleated polychromatic erythrocytes (MNPCES) frequency was evaluated at 24, 48 and 72 hours after treatment. The frequency of MNPCES was evaluated 36 hours after exposure. Cytotoxicity was evaluated by the polychromatic and normochromatic erythrocytes ratio (PCE/NCE). For our study we concluded that extract contain anti clastogenotoxicity.(15)

- **Phyllanthus maderaspatensis**. Linn (PME) was studied on cisplatin-induced nephro- and genotoxicity in male Swiss albino mice. The treatment of mice with different doses of PME (400 and 600 mg/kg body weight) for 7 days before the administration of a single i.p. dose of cisplatin (5 mg/kg) exhibited significant chemoprotective activity. Renal dysfunction was evaluated biochemically by measuring the concentration of blood urea nitrogen (BUN) and serum creatinine and histologically by light microscopy. Genotoxicity was evaluated by the bone marrow micronucleus assay. A single dose of cisplatin significantly elevated the levels of blood urea nitrogen, serum creatinine, and the kidney to body weight ratio, but pretreatment with PME (600 mg kg⁻¹day⁻¹) for 7 days significantly attenuated the cisplatin-induced nephrotoxicity. The frequency of micronucleated polychromatic erythrocytes (MNPCES) in the bone marrow was determined at 24 h after the administration of cisplatin. After administration of cisplatin, the frequency of MNPCES distinctly increased. In mice treated with PME before cisplatin application, there was a decrease in the number of MNPCES when compared with mice injected with only cisplatin. Ethanol extract of PME thus has a marked free radical scavenging effect indicating its antioxidative property. The results suggest that the ethanol extract of PME has a protective effect against cisplatin-induced nephro- and genotoxicity through its antioxidant property.(16)
• *Hemidesmus indicus* aqueous extract of roots was investigated for its *in vivo* antigenotoxic effect against cisplatin-induced cytogenetic damage. Swiss albino mice were administered with various doses of the extract either singly (50, 100 and 200 mg/kg body weight) or as split doses (10, 20 and 40 mg/kg bw/day) for five consecutive days by orally. As endpoints, chromosome aberrations, micronuclei in polychromatic erythrocytes, mitotic index and PCE/NCE ratio were estimated. The extract protected the bone marrow cells from cisplatin-induced genotoxicity in an inverse dose-dependent manner. However, the extract was cytotoxic at all doses. The presence of saponins, tannins, phenols, terpenoids, flavonoids and coumarins in the crude extract could explain these effects.(17)

• *Solanum paniculatum* is a plant species widespread throughout tropical America, especially in the Brazilian Savanna region. It is used in Brazil for culinary purposes and in folk medicine to treat liver and gastric dysfunctions, as well as hangovers. Because of the wide use of this plant as a therapeutic resource and food, the present study aimed at evaluating the mutagenic and cytotoxic effects of *S. paniculatum* ethanolic leaf and fruit extracts using the mouse bone marrow micronucleus test. Our results indicate that neither *S. paniculatum* ethanolic leaf extract nor its ethanolic fruit extract exhibit mutagenic effect in mice bone marrow; however, at higher doses, both extracts presented cytotoxic activity.(18).

• *Aloysia triphylla* (cedron) the cisplatin-induced genetic damage in mouse bone marrow cells protected by cedron-leaf infusion. (I) untreated, (II) negative control, (III) treated with cedron-leaf infusion (5%), (IV) treated with cisplatin (6 mg/kg b.w.), (V) pretreated with infusion and treated with cisplatin and (VI) positive control (cyclophosphamide, 20 mg/kg b.w.). Based on the tail moment values found, four types of comets were distinguished. The results suggest that infusion could exert its in vivo antigenotoxic action by enhancing the antioxidant status of bone marrow cells. The found could be attributed to its scavenging potency towards free radicals.(19).

• *Rubia cordifolia* evaluated the Genotoxicity produced by cisplatin at a dose of 12 mg/kg body weight after administered intraperitoneally to Swiss albino mice. Another set of animals was given hydro-alcoholic extract of *Rubia cordifolia* at different doses along with cisplatin treatment. The antioxidant levels, serum creatinine, serum urea etc. were analyzed. The extract could significantly decrease the cisplatin induced nephrotoxicity as inferred from the tissue antioxidant status in the drug administered animals. Remarkable change was observed in serum creatinine and urea levels. Lipid peroxidation in the kidney and liver tissues was also considerably reduced in *Rubia cordifolia* extract treated animals (20).

• *Coccoloba mollis* (Polygonaceae) is a medicinal plant popularly used in cases of memory loss, stress, insomnia, anemia, impaired vision, and sexual impotence, but the scientific literature, to date, lacks studies on the biological effects of this species, particularly with regard to cytotoxicity and induction of DNA damage. The aim of the present study was to assess ethanolic extracts of the roots and leaves of *C. mollis* for genotoxicity. For these evaluations comet assay, micronucleus test. Both extracts induced DNA damage at a concentration of 20 g/mL in the comet assay, but no genotoxicity was detected with any of the treatments carried out in the micronucleus test (21).

• *Bixa orellana* contains bixin in the main carotenoid found in annatto seeds. The antioxidant properties of this compound are associated with its ability to scavenge free radicals, which may reduce damage and protect tissues against toxicity caused by anticancer drugs such as cisplatin. In this study, the genotoxicity and antigenotoxicity of bixin on cisplatin-induced toxicity in PC12 cells was assessed. Cytotoxicity was evaluated using the MTT assay, mutagenicity, genotoxicity, and protective effect of bixin were evaluated using the micronucleus test and comet assay. PC12 cells were treated with bixin (0.05, 0.08, and 0.10 g/mL), cisplatin (0.1 g/mL) or a combination of both bixin and cisplatin. Bixin was neither cytotoxic nor genotoxic compared to the controls. In the combined treatment bixin significantly reduced the percentage of DNA in tail and the frequency of micronuclei induced by cisplatin. This result suggests that bixin can function as a protective agent, reducing cisplatin-induced DNA damage in PC12 cells, and it is possible that this protection could also extend to neuronal cells. Further studies are being conducted to better understand the mechanisms involved in the activity of this protective agent prior to using it therapeutically.(22)

• *Vitis vinifera* (Grape) seed extract. It contains the most beneficial groups of plant flavonoids, proanthocyanidins oligomers. Grape seed extracts contain 92 to 95% proanthocyanidins oligomers. These
flavonoids are potent antioxidants and exert many health-promoting effects. Their effects include the ability to increase intracellular vitamin C levels, decrease capillary permeability and fragility, scavenge oxidants and free radicals. The activity of proanthocyanidins oligomers is approximately fifty times greater than that of vitamin C and vitamin E, in term of antioxidant action. Grape seed extract prevents oxidative injury by modulating the expression of antioxidant enzyme systems. The oxidative DNA damage in the brain regions of aged rats was modulated by grape seed extract administration. Grape seed extract enhanced the antioxidant status and decreased the incidence of free radical-induced lipid peroxidation in the central nervous system of aged rats. (23).

- **Doxorubicin**

  - **Baccharis dracunculifolia** (Asteraceae), have been used as an antipyretic, stomachic and health tonic in Brazil. The present study was to investigate the potential mutagenic effect of *B. dracunculifolia* ethyl acetate extract (Bd-EAE) and its influence on the mutagenicity induced by the chemotherapeutic agent doxorubicin (DXR) using the rat bone marrow and peripheral blood micronucleus test. Wistar rats were divided into 10 treatment groups. Five groups received DXR (90 mg/kg body weight, b.w., intraperitoneally) to induce mutagenicity and three of these groups received a single oral dose of Bd-EAE at a concentration of 6, 12 or 24 mg/kg b.w. prior to DXR administration. The results showed that Bd-EAE itself was not mutagenic, in the rat micronucleus assay. In animals treated with Bd-EAE and DXR, the number of MNPCEs was significantly decreased compared to animals receiving DXR alone. The putative antioxidant activity or the interference of one or more of the active compounds of Bd-EAE with mutagenic metabolic pathways may explain its effect on DXR mutagenicity. (24).

  - **Panax ginseng** is one of the most widely prescribed herbal medicines for the treatment of cancer, diabetes, chronic inflammation, and neurodegenerative and cardiovascular diseases. Since the use of alternative medicines in combination with conventional therapy may increase the risk of unwanted interactions, we investigated the possible genotoxicity of a water-soluble form of the dry root of *P. ginseng* (2.5, 5.0 or 10.0 mg/mL) and its ability to protect against the genotoxicity of doxorubicin (DOX; 0.125 mg/mL) by using the *Drosophila melanogaster* wing somatic mutation and recombination test (SMART) with standard and high-bioactivation crosses of flies. (25).

  - **Proanthocyanidins** can protect against doxorubicin-induced mutagenicity in mice. Pretreatment of mice with proanthocyanidins (100 mg/kg/day, orally) for 7 days and simultaneously with doxorubicin (12 mg/kg, i.p.) for another day, significantly reduced the frequency of bone marrow DNA strand breaks and micronucleated polychromatic erythrocytes compared to doxorubicin-treated mice alone. Furthermore, proanthocyanidins caused a reduction in bone marrow suppression induced by doxorubicin treatment. In male germline, orally administration of proanthocyanidins (100 mg/kg/day, orally) for 7 consecutive days before and 7 consecutive days after treatment with doxorubicin (12 mg/kg, i.p.). Conclusively, this study provides for the first time that proanthocyanidins have a protective role in the abatement of doxorubicin-induced mutagenesis and cell proliferation changes in germinial cells of mice that reside, at least in part, in their radical scavenger activity. Therefore, proanthocyanidins can be a promising chemopreventive agent to avert secondary malignancy and abnormal reproductive outcomes risks in cancer patients receiving doxorubicin-involved treatment. (26)
Vitamin A (VA) on the induction of chromosomal aberrations (CA) in rat bone marrow cells and to investigate its modulating effect on chromosomal damage induced by doxorubicin (DXR). Wistar rats were treated with VA (7.5, 15 and 30 mg/kg body wt) once a day for 2 days by gavage before injecting DXR (90 mg/kg body wt). Rats in the control group were treated with corresponding doses of water and olive oil. Animals treated with the medium dose of VA (15 mg/kg body wt) plus single dose of DXR presented a statistically significant reduction in total number of CA and in number of abnormal metaphases (P < 0.05). However, when compared with control and DXR groups, the low and high VA doses (7.5 and 30 mg/kg body wt) were found to be less efficient than the medium dose VA (15 mg/kg body wt) in terms of parameters analyzed. Furthermore, the high dose of VA group (30 mg/kg body wt) was found to be clastogenic (P < 0.05). This study concludes that the protective effect of VA against chromosome damage is dose dependent.

Radiation

Spirulina platensis Zataria multiflora Olea europaea Syzygium cumini

Spirulina platensis extract using the micronucleus test in polychromatic erythrocytes of bone marrow of mice for evaluated the radioprotective effect done in chemotherapy. In this system the extract caused a significant reduction of the micronucleus frequencies induced by gamma-radiation.(28).

Zataria multiflora radioprotective effect of hydroalcoholic extract was investigated against genotoxicity induced by γ irradiation in human lymphocytes. Peripheral blood samples were collected from human volunteers and incubated with Z. multiflora extract at different concentrations (5, 10, and 50 μg/mL) for 1 hour. At each dose point, the whole blood was exposed in vitro to 150 cGy of cobalt-60 γ irradiation, and then the lymphocytes were cultured with mitogenic stimulation to determine number of the micronuclei in cytokinesis-blocked binucleated cells. The treatment of lymphocytes with extract showed a significant decrease in the incidence of micronuclei binucleated cells, compared with similarly irradiated lymphocytes without extract against γ irradiation.(29).

Olea europaea leaves extract contain polyphenolic having radioprotective effects, the flavonoids diosmin and rutin, which are widely used as pharmaceuticals; and the sulfur-containing compounds dimethylsulfoxide (DMSO) and 6-n-propyl-2-thiouracil (PTU) were determined by using the micronucleus test for anticlastogenic activity, evaluating the reduction of the frequency of micronucleated polychromatic erythrocytes (MnPCEs) in bone marrow of mouse before and after X-ray irradiation. Olea europaea is the only substance that showed a significant anticlastogenic activity both before and after X-ray irradiation treatments.(30).

Syzygium cumini Linn. or Eugenia cumini (SC; black plum, Jamun, family Myrtaceae) was studied on the alteration in the radiation-induced micronuclei formation in the cultured human peripheral blood lymphocytes. Treatment of lymphocytes to various concentrations of SC resulted in a dose dependent increase in the micronuclei-induction, especially after 25–100 μg/ml extract. The exposure of human lymphocytes to various concentrations of SC extract before 3 Gy γ-irradiation resulted in a significant decline in the micronuclei-induction at all the drug doses when compared with the non-drug treated irradiated cultures. A nadir in MBNCE frequency was observed for 12.5 μg/ml drug concentration, where the MBNCE frequency was approximately fourfold lower than that of the non-drug treated irradiated cultures. Therefore, this dose
may be considered as an optimum dose for radiation protection. Our study demonstrates that the leaf extract of *S. cumini*, a plant traditionally used to treat diabetic disorders protects against the radiation-induced DNA damage.

- **Ocimum sanctum** leaves show radioprotective effect on chromosome aberrations. Adult Swiss mice were whole-body exposed to 1–6 Gy of gamma radiation with/without pretreatment with 10 mg/kg b.wt. of extract intraperitoneally for 5 consecutive days. Radiation was given 30 min after the last injection. Metaphase plates were prepared from femur marrow on days 1, 2, 7 and 14 post-treatment and the frequency of aberrant cells and individual aberrations were scored. Extract alone did not have any significant effect on the chromosomes. Maximum percent of aberrant cells was observed at 24 h in all the exposed groups. These results show that extract affords in vivo protection against radiation-induced cytogenetic damage. Free radical scavenging is a likely mechanism of OE protection.

References


