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Research Article

Evaluation of Mutagenic Effect (Antimutagenic) of Dalbergia Latifolia on Swiss Albino Mice

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ABSTRACT

The present study was designed to evaluate the anti-mutagenic potential of Methanolic extract of *Dalbergia latifolia*, using micronucleus (MN) and chromosomal aberration (CA) assay in mice bone marrow. The anti-mutagenic effect of *Dalbergia latifolia* was assessed using cyclophosphamide MN formation and CA in mice. The animals were pre-treated with the Methanolic extract of *Dalbergia latifolia* orally at two doses of 100, 200mg/kg body weight for seven days. In MN and CA test the two doses provided protection when given 24hrs prior to a single i.p administration of cyclophosphamide (100 mg/kg body weight). These results demonstrate that Methanolic extract of *Dalbergia latifolia* has got anti-mutagenic potential.

Keywords: Dalbergia latifolia, Methanolic extract, anti-mutagenic activity

INTRODUCTION

All chemicals that produce DNA damage leading to mutation or cancer are described as genotoxic[1]. Genotoxicity testing is an important part of preclinical safety assessment of any drug. It is designed to detect genetic damage such as gene mutations and chromosomal aberration, which may be reflected in tumorigenic or heritable mutation potential of the drug. As the mechanisms of micronucleus formation are related to those inducing chromosomal aberrations, both micronuclei and chromosomal aberrations can be accepted as assay systems to screen clastogenicity induced by test compounds[2]. 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. Micronucleus test and chromosomal aberration test are used for studying anti-mutagenic activity of a drug. One of the best ways to minimize the effect of mutagens and carcinogens is to identify the anti-clastogens /anti-mutagens substances which suppress or inhibit the process of mutagenesis by acting directly on the mechanism of cell and des-mutagens (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 anti-mutagenic and anti-carcinogenic food or drug additives[1].

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 des-mutagenesis: in which factors act directly on mutagens or inactivate them, the other is bio-anti-mutagenesis 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. Anti-mutagenesis 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[3]. 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[4]. 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

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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 tumor 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[5].

Table 1: Various tets performed

S. No.	Tests	Results
1	Tests for Steroids and Triterpenes	
	Salkowski test	+
	Libermann-Buchard test	+
	Kahleberg test	+
2	Tests for alkaloids	
	Mayer's reagent	-
	Dragandroff's reagent	-
	Hager's reagent	-
	Wagner's reagent	-
3	Tests for Saponins (Foam test)	-
4	Tests for phenolic compounds and Tannins	
	Ferric chloride test	+
	Gelatin test	+
	Lead acetate test	+
5	Tests for flavonoids	
	Sodium hydroxide test	+
	Ferric chloride test	+
	Shinoda's test	+
	ZINC-HCl reduction test	+
	Lead acetate test	+

Table.2. Percentage yield and physical characters of *Dalbergia latifolia* in different solvents.

Plant extract	Percentage yield	Colour	Odour	Nature
Methanol	3.73	Dark Brown	Character-istic	Non-Sticky

Table.3. Physical properties of Flavonoids

S. No.	Physical properties	Observation
1	State	Solid
2	Color	Dark brown
3	Odor	Characteristic
4	Nature	Non-Sticky

MATERIALS AND METHODS

Animals

Eight to ten weeks old Swiss albino mice having weight (25-30gm) were purchased from Central animal research facility NIMHANS Reg No.12/99 Bangalore. They were housed, five per poly propylene cage under standard laboratory conditions at room temperature (25° C± 2° C) with 12h light / dark cycle. The animals were provided with pellet chow and water ad libitum. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) of Karnataka College of pharmacy, Bangalore.

Plant material

The fresh root of *Dalbergia latifolia* was collected from Tirupati , Andhra Pradesh, identified and authenticated by Dr.K.Madhava chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, Tirupati. All other chemicals used in the study are of AR grade.

Chemicals & Drugs

- Cyclophosphamide (Endoxan, purchased from local market.)
- Normal saline
- Fetal Bovine serum (Himedia)
- E.D.T.A disodium salt LR (Merc)
- Anesthetic Ether I.P (TKM Pharma)
- Glacial acetic acid LR (Merc)
- Sodium hydrogen phosphate LR (Merc.)
- Potassium Dihydrogen phosphate LR (Merc)
- May-Grunewald stain (Himedia)
- Giemsa stain (Himedia)
- Sodium carbonate LR (Merc)
- Glycerol LR (Merc)
- Sodium hydroxide LR (Merc)
- Methanol LR (Merc)
- Potassium chloride LR (Merc)
- Colchicine (Himedia)
- Phosphate buffer saline (Himedia)

Instruments/ Equipments

1. Remi centrifuge
2. Digital pH meter (PHep, Hanna Instruments)
3. Coupling Jars
4. Microscopic Glass slides (Blue star)
5. Cover slips 22× 40 mm (Blue star)
6. Inverted microscope (Labomed, USA)
7. Micropipettes (Thermo Scientific)
8. RO water system (Millipore)
9. Reagent bottles
10. Pippetter tip

Preparation of Extracts

The leaves of Dalbergia latifolia was powdered (500g)

Table 4: Effect of methanolic extract of *Dalbergia latifolia (*200,100mg/kg: po/7days) on Chromosomal Aberration and Mitotic Index induced by Cyclophosphamide in mice bone marrow cells.

S. No.	Types of aberrations				Total number of aberrations	Mitotic index
	Rings	Exchanges	Breaks	minute		
Vehicle Control	1.00±0.25	0.66±0.33	0.16±0.17	0.66±0.33	2.33±0.42	4.33±0.7
Cyclophosphamide (100mg/kg)	4.00±0.57	2.16±0.30	4.66±0.71	4.50±0.61	16.17±0.83***	1.83±0.3*
D.latifolia 00mg/kg/7days+Cyp (100mg/kg)	1.00±0.44	0.83±0.30	1.66±0.55	0.83±0.30	4.33±0.55###	3.33±0.4ns
D.latifolia 200 mg/kg/7days alone	1.00±0.25	0.66±0.33	0.17±0.16	0.66±0.33	2.33±0.42###	4.66±0.5##

Values are expressed as mean ± SEM, (n=6) *** p<0.001, *p<0.05 compared with normal control group.
###p<0.01, ##p<0.01 compared with cyclophosphamide group.

and methanolic extract was prepared using soxhelt.

Extraction process. The methanolic extract was evaporated under reduced pressure using rotavapor evaporator. The yield of the extract was 3.73% g. A suspension was prepared using 2 % v/v tween 80 and administered orally.

Acute Oral Toxicity Studies (oppts870.1100)

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticides, and Toxic Substances) guidelines as follows[8,9].female albino rats of Wister strain (150-200g) were maintained under controlled standard animal house condition with access to food and water ad libitum.

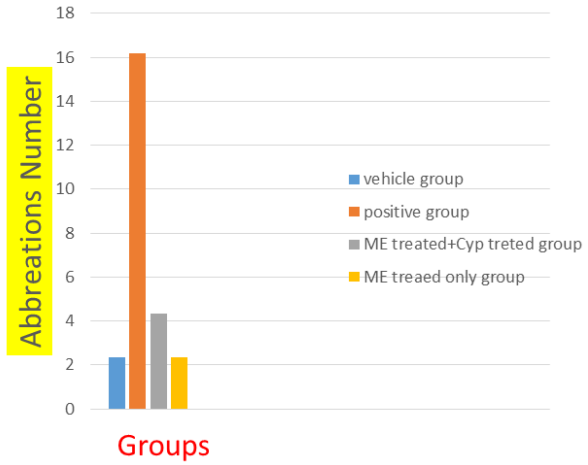

Figure 1: Aberration in bone marrow cells

METHODS

Dose Selection: Lethal dose 2000mg/kg selected and two doses of 100mg/kg & 200mg/kg body weight of methanolic extract of *Dalbergia Latifolia* leaves was selected as low dose and high dose as per the acute oral toxicity studies.The extract was subjected to the phytochemical test.

Preparation of phosphate buffer solution (pH=6.8)

Dissolved 2.366 gm. of Na_2HPO_4 in 250 ml of distilled water = Solution A

Dissolved 2.27 gm. of K_2HPO_4 in 250 ml of distilled water = Solution B

50 ml of solution A and 50 ml of solution B was taken and made up the volume to 1000 ml with distilled water

Preparation of suspending medium

5% bovine albumin solution was prepared by dissolving the required quantity of bovine albumin power in phosphate buffer (PH=7.2). The bovine albumin power is dissolve very carefully by adding the powder little by little to the solvent and mixed thoroughly, so as to avoid any coagulum. The final 5% albumin solution should be very clear and free from any protein lumps. Two drops of 1% sodium azide were added as a preservative.

Preparation of staining solution

May-Grunewald's stain was prepared by dissolving 0.2gm of the strain powder in 100 ml of methanol with slight heating and stirring. After it dissolved completely, it was filtered. Giemsa's stain was prepared by dissolving 1gm. of Giemsa's stain in 54 ml of glycerin. It was kept in a 60˚c oven for 2h. After cooling, 84 ml of methanol was added, stirred well and filtered.

Animals

Swiss albino mice of either sex 8-10 weeks old, weighing 25-30g were housed in plastic cages with paddy husk bedding. Animals were provided with food and water *ad libitum.*

Groups

Group 1: animals are treated with vehicle (n-6).

Group 2: animals are treated with cyclophosphamide (75-100mg/kg i.p)

Group 3: animals are treated with 100 mg/kg with methanolic extract for 7th day followed by Cyclophosphamide as challenging dose

Group 4: animals are treated with 200mg/kg with methanolic extract for 7day.

Procedure

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[6].

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

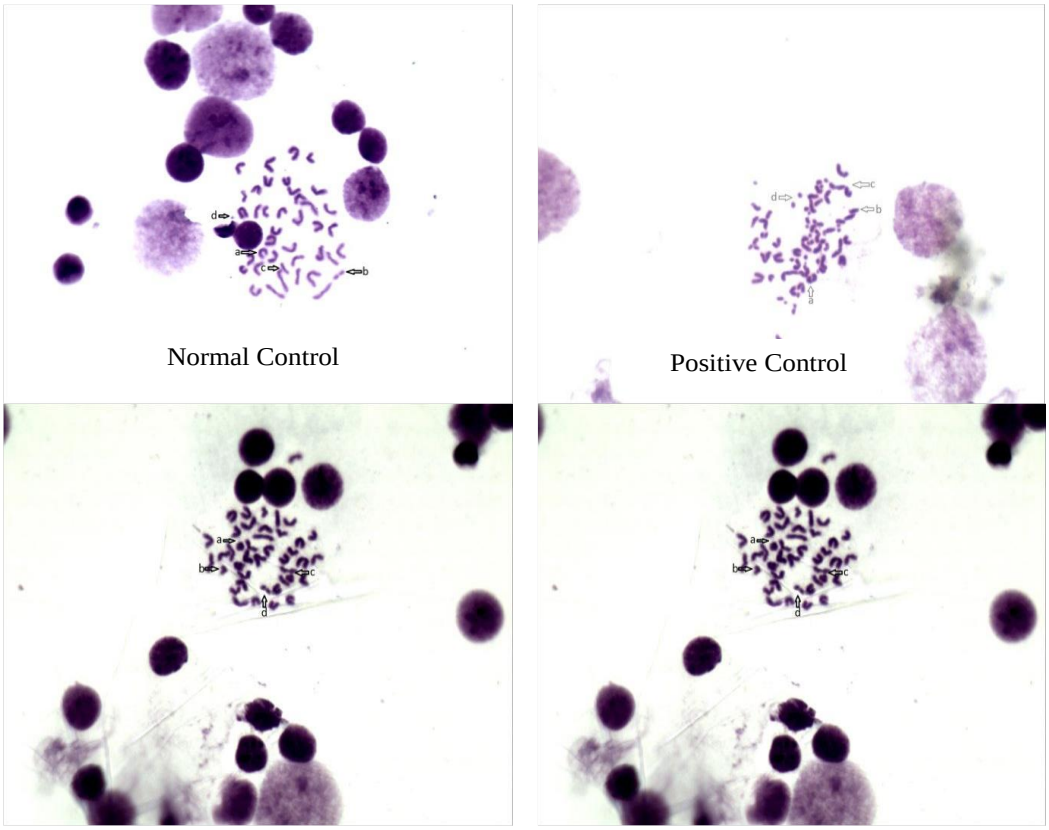

Figure 2: Effect of methanolic extract *Dalbergia latifolia* (100,200 mg/kg/po//7days) on Chromosomal Aberration in mice bone marrow cells.

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[7].

RESULTS

Phytochemical analysis of successive extract of bark of *Dalbergia latifolia.*

Physical examination of flavonoids

The isolated Flavonoids were subjected to physical examination and observation recorded in Table. The Flavonoids was a sticky solid mass with dark green colour. It odour is characteristic.

Chromosomal Aberration Test

Effect of methanolic extract Dalbergia latifolia (200, 100, mg/kg; po; /day/7days) on Chromosomal Aberrations and Mitotic Index induced by Cyclophosphamide (100mg/kg; ip/day/single dose) in mice bone marrow cells

Chromosomal aberration significantly increased (P<0.001) after 24 hrs. of Cyclophosphamide treatment when compared to normal mice. Administration of methanolic extract of *Dalbergia latifolia* (200,100, mg/kg; po/day/7days) to mice significantly decreased Chromosomal aberration levels observed after 24hrs when compared to Cyclophosphamide control group. Mitotic Index significantly decreased (P<0.001) after 24 hrs. Of Cyclophosphamide treatment when compared to normal mice. Administration of methanolic extract of *Dalbergia latifolia* (200,100, mg/kg; po/day/7days) mice significantly increased Mitotic Index levels observed after 24hrs when compared to Cyclophosphamide control group.

DISCUSSION

Mutagenicity is a broader term that refers to the ability to interact with DNA and/or the cellular apparatus that regulates the fidelity of the genome. It can be due to the exposure to various environmental, pharmaceutical pollutants, xenobiotic and some category of drugs produces unexpected and unidirectional changes in the genome. A chemical is considered to be mutagenic if it is capable of inducing heritable changes in the genotype of a cell as a consequence of alteration to, or loss of genes, chromosome or parts or chromosome. Many anticancer drugs, immunosuppressant's etc are the important categories used in the treatment of various human cancer invariably have cell toxicity and can also induce genotoxicity .one such example is cyclophosphamide. The

toxicity of cyclophosphamide is attributed to the generation of free radicals during its metabolism CYP is an effective anticancer drug that belongs to the class of nitrogen mustards. The cytotoxic effect of CYP is directly connected with free radical mediated metabolism. It is rapidly metabolized in the liver by cytochrome P-450 enzymes and generates active alkylating metabolites such as 4-hydroxycyclophosphamide, aldophosphamide, and acrolein, which interfere with cellular DNA synthesis in dividing cells and induce DNA single strand breaks[12] that may result in micronucleus formation and cell death[13,14].

CONCLUSION

From the present study, it was found that a significant decrease in mitotic index of cyclophosphamide treated animals, which can be due to the affected cell division in the bone marrow (Gonzalves et al., 2008). Methanolic extract of *Dalbergia latifolia* significantly inhibited the disturbances in the cell division of mouse bone marrow and therefore it showed anti-mutagenicity in micronucleus tests in bone marrow cells of mice. Mutation is one of the principle pathways that lead to cancer. The anti-mutagenic effects may be an important contributor in the use this compound as a potential anti-carcinogenic drug. Methanolic extract *Dalbergia latifolia* (ME) significantly inhibit the disturbances in the cell division by increasing mitotic index In vivo. Hence we concluded that methanolic extract *Dalbergia latifolia* doesn't possess genotoxicity. In conclusion, methanolic extract *Dalbergia latifolia* showed significant anti-mutagenicity in chromosomal abbreations in bone marrow cells of mice and also showed potent antimutagenic activity

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