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ANTI-MUTAGENICEFFECT OF DALBERGIA LATIFOLIA ON SWISS ALBINO MICE

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ABSTRACT:

Aim of The Study: The present study was designed to evaluate the anti-mutagenic potential of Methanolic extract of Dalbergia latifolia, using micronucleus (MN) assay in mice bone marrow.

Materials and methods: The anti-mutagenic effect of Dalbergia latifolia was assessed using cyclophosphamide MN formation 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 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.

Results: Percentage Micronucleus Polychromatic erythrocytes (%MN PCE) formation significantly increased (P<0.001) after 24hrs. Of Cyclophosphamide treatment when compared to normal mice.

Conclusion: The study shows 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

Introduction

All chemicals that produce DNA damage leading to mutation or cancer are described as genotoxic1. 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 compounds2. 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 known3. 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 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].

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 district, Andhra Pradesh, identified and authenticated by Dr.K.Madhava chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, and Tirupati. The methanolic extract was obtained from Green Chem Herbal Extracts and Formulations, Bangalore, (Batch no: ETE/RD/01) from Dr. Rajendran.. All other chemicals used in the study are of AR grade.

5.3 CHEMICALS & DRUGS:

1. Cyclophosphamide (Endoxan, purchased from local market.)
2. Normal saline
3. Fetal Bovine serum (Himedia)
4. E.D.T.A disodium salt LR (Merc)
5. Anesthetic Ether I.P (TKM Pharma)
6. Glacial acetic acid LR (Merc)
7. Sodium hydrogen phosphate LR (Merc.)
8. Potassium Dihydrogen phosphate LR (Merc)
9. May-Grunewald stain (Himedia)
10. Giemsa stain (Himedia)
11. Sodium carbonate LR (Merc)
12. Glycerol LR (Merc)
13. Sodium hydroxide LR (Merc)
14. Methanol LR (Merc)
15. Potassium chloride LR (Merc)
16. Colchicine (Himedia)
17. Phosphate buffer saline (Himedia)

5.4 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

METHODS

DOSE SELECTION: Two doses of 100mg/kg & 200mg/kg body weight of methanolic extract of Dalbergia Latifolia root was selected as per the acute oral toxicity studies, performed below.

Preparation of phosphate buffer solution (pH=6.8)

Dissolved 2.366 gm. of Na2HPO4 in 250 ml of distilled water = Solution A

Dissolved 2.27 gm. of K2HPO4 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 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 tibia7.

Results

Phytochemical analysis of successive extract of bark of Dalbergia latifolia.

Table no. 1

Sl. 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	characteristic	Crystalline powder

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.

Table.3. Physical properties of Flavonoids:

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

Effect of methanolic extract Dalbergia latifolia (200,100, mg/kg; po; /day/7days) on Micronucleus formation in Bone Marrow cells:

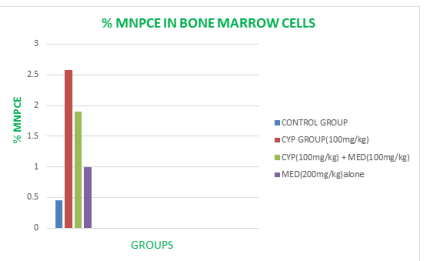
Percentage Micronucleus Polychromatic erythrocytes (%MN PCE) formation significantly increased (P<0.001) after 24hrs. Of Cyclophosphamide treatment when compared to normal mice. Administration methanolic extract Dalbergia latifolia (200,100 mg/kg; po/day/7days) to mice significantly decreased (P<0.001) Percentage Micronucleus Polychromatic erythrocytes (%MN PCE) formation levels observed after 24hrs when compared to Cyclophosphamide control group. Percentage Micronucleus Norm chromatic erythrocytes (%MN NCE) formation significantly increased (P<0.05) after 24h.of Cyclophosphamide treatment when compared to normal mice. Administration of methanolic extract Dalbergia latifolia (200,100 mg/kg: po/day/7days) to mice significantly decreased (P<0.05)

Percentage Micronucleus Norm chromatic erythrocytes (%MN NCE) formation levels observed after 24hrs when compared to Cyclophosphamide control groupEffect of methanolic extract Dalbergia latifolia (200,100, mg/kg; po; /day/7days) on Micronucleus formation in Bone Marrow cells:

SL. NO.	Groups	PCE	MN PCE	%MNPCE ±SEM	NCE	MN NC E	%MNN CE ±SEM	P/N RATIO
1	Vehicle Control	5500	28	0.46±0.05	5600	15	0.24±0.04	0.91±0.07
2	Cyclophosphamide (100mg/kg)	5675	162	2.58±0.05***	5560	54	*** 0.88±0.56	0.63±0.01***
3	D.latifolia 100mg/kg/7day+Cyp(100mg/kg)	5480	61	1.90±0.07 ###	5575	26	0.62±0.03##	0.76±0.01###
4	D.latifolia 200mg/kg/7days	5455	117	1.00±0.59 ###	5590	38	0.42±0.0###	0.85±0.01###

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

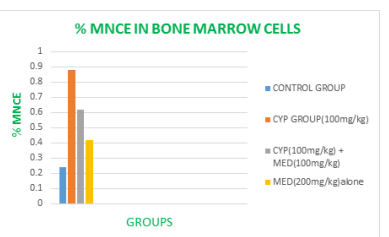
Effect of methanolic extract Dalbergia latifolia (200,1000, mg/kg; po; /day/7days) on Percentage Micronucleus Polychromatic erythrocytes (%MN PCE) formation Cyclophosphamide (100mg/kg; ip/day/single) in Bone Marrow cells:



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

###p<0.01 compared with cyclophosphamide group.

Effect of methanolic extracts Dalbergia latifolia (200,100, mg/kg; po; /day/7days) on p/n ratio Cyclophosphamide (100mg/kg; ip/day/single) in Bone Marrow cells.

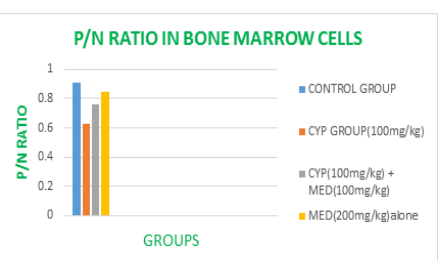


Values are expressed as mean ± SEM, (n=6) *** p<0.001 compared with normal control group.

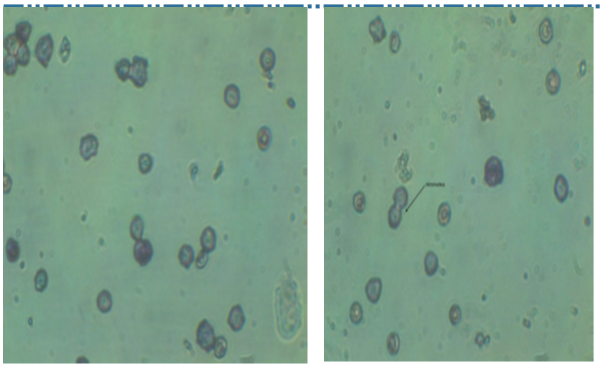
###p<0.01,##p<0.01 compared with cyclophosphamide groups.

Effect of methanolic extracts Dalbergia latifolia (200,100, mg/kg; po; /day/7days) on p/n ratio Cyclophosphamide (100mg/kg; ip/day/single) in Bone Marrow cells:

P/N Ratio in bone marrow cells



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



A) Normal Control **B) Micronucleus**

Conclusion

1. 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 and chromosomal aberration 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 and In vivo.

2. Hence we concluded that methanolic extract Dalbergia latifolia doesn't possess genotoxicity.

3. In conclusion, methanolic extract Dalbergia latifolia showed significant anti-mutagenicity in micronucleus in bone marrow cells of mice and also showed potent antimutagenic activity .

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