

Evaluation of antimutagenic potential of *Lagenaria siceraria*, *Desmodium gangeticum* and *Leucas aspera*

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ABSTRACT

Objective: The aim of the current study was to evaluate antimutagenic (antitumor) activity of methanolic extract of *Lagenaria siceraria* fruit, *Desmodium gangeticum* and *Leucas aspera*. **Method:** The group distribution for animal was, negative control, only extract treated, positive control (cyclophosphamide treated), and two treatment control group for each plant extract. Mutagenicity was induced by administering cyclophosphamide (20-25 mg/kg, i.p) 24 hrs prior to sacrifice animals. **Result:** The preliminary phytochemical tests showed presence of alkaloids, polyphenols, flavanoids and tannins in alcoholic extract. Its antioxidant and cytoprotective activity of the extracts has already been reported. On the basis of that we have decided to evaluate its antimutagenic activity *in vivo* in mice using bone marrow chromosomal aberration assay. Methanolic extract of selected plants had shown significant reduction in percentage of cells with aberration in chromosomal aberration assay. The results obtained by these experiments, strongly suggesting that *Lagenaria siceraria* fruit extract having potent antimutagenic property than *desmodium gangeticum* and *Leucas aspera*. The overall antimutagenic activity of above three extract were in the order

of *Lagenaria siceraria* > *Desmodium gangeticum* > *Leucas aspera*. The observed antimutagenic activity of selected plant against Cyclophosphamide might be associated with its antioxidant constituents such as poly-phenolic compound, flavanoids and other micronutrients. **Conclusion:** Therefore, from the present study, it can be concluded that methanolic extract of selected indigenous plant extract possesses antimutagenic property.

Key words: Antimutagenic activity, *Lagenaria siceraria*, *Desmodium gangeticum*, *Leucas aspera*, Chromosomal aberration assay.

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INTRODUCTION

Herbal medicines are more popular for the treatment of many diseases due to popular belief that green medicine is safe, easily available and with lesser side effects. Sometimes crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects.¹

Reactive oxygen species (ROS) damage cellular DNA and play an important role in process like mutagenesis, carcinogenesis and aging.² Antioxidants identified for antimutagenic and anticarcinogenic properties. Routine consumption of naturally occurring antioxidants has capacity to protect organisms and cells from oxidation.³ Indigenous plants also provide a rich source for antioxidants that are known to play very important role to protect against various disease.⁴

Few studies validating the medicinal uses of *Lagenaria siceraria* fruits, *desmodium gangeticum* and *leucas aspera* have been reported. Various extracts of *Lagenaria siceraria* fruits, *desmodium gangeticum* and *leucas aspera* were found to have anti-inflammatory, analgesic, hepatoprotective, antihyperlipidemic, diuretic and antibacterial activities.⁵⁻⁷

Keeping in mind the great medicinal value and high content of polyphenols, flavanoids; present investigation was planned to study the antigenotoxic effect of methanolic extract of *Lagenaria siceraria* fruits, *desmodium gangeticum* (aerial part) and *leucas aspera* (aerial part).

MATERIALS AND METHOD

Experimental animals: Adult Swiss albino mice, weighing 30 ± 5 gm were used in the study. Animals were exposed to natural day and night cycles, with ideal laboratory conditions in terms of ambient temperature and humidity. Temperature during the time of carrying out the experiment was between $24 \pm 2^\circ$ and humidity 50-60%. Animals were fed *ad*

libitum with Amrut brand mice feed supplied by Pranav Agro Industries and RO water. The experiment was carried out after obtaining the permission from Institutional Animal Ethics Committee (Approval number: IAEC/2012/02) and care of animals was taken as per the CPCSEA guidelines.

Plant material: The herbal material was collected from different sources. All herbs were identified and authenticated by botanist. All herbs were sun-dried and powdered using grinder. The powder of *Desmodium gangeticum*, *Leucas aspera* and *Lagenaria siceraria* were subjected to extraction. *Desmodium gangeticum* and *Leucas aspera* were extracted with methanol using Soxhlet apparatus by continuous hot extraction. Fruit powder of *Lagenaria siceraria* was macerated with 100% methanol for 18 hrs. After each extraction, the solvent was recovered using distillation assembly, and the extracts were concentrated under reduced pressure. The final yield of extracts were calculated and stored in air-tied container for experiment.

Chemicals: Cyclophosphamide (CP) was procured from Getwell pharmaceutical, Gurgaon, Haryana (Batch No 3GCYOZ). Colchicine (Batch No T8371720) was obtained from Sisco Research Laboratory, Mumbai. Methanol, acetic acid, and potassium chloride were obtained from Chemdyes Corporation, Vadodara, Gujarat, India.

Experimental design: The animals were randomized into 11 groups consisting of 5 animals in each group for evaluating influence of *Lagenaria siceraria* methanolic extract (LSME), *Desmodium gangeticum* methanolic extract (DGME) and *Leucas aspera* methanolic extract (LAME) on chromosomal aberration assay. Group I served as normal control receiving water and normal food. Group II, III, IV served as extract control and treated with 200 mg dose of extract for fourteen consecutive days.

Table 1: Posology

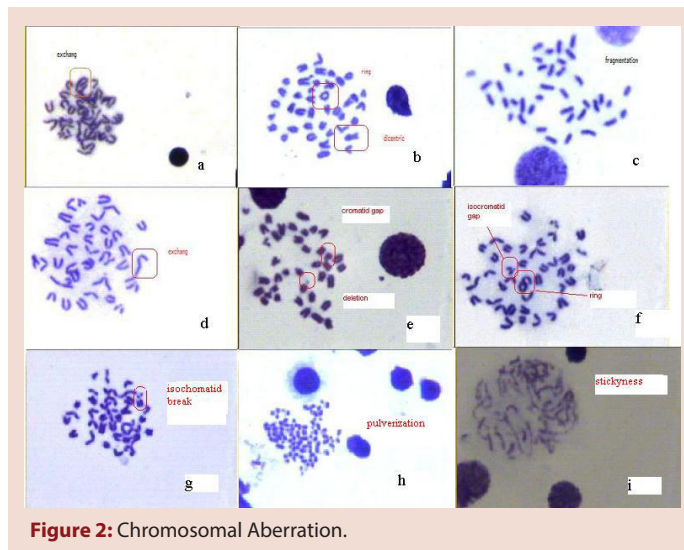
Group	Drug	Dose	Duration	Parameters
I	Water	0.4 ml	2 weeks	
II, III, IV	LS-2, DG-2, LA-2 respectively	200 mg/kg	2 weeks	Chromosomal aberrations and mitotic index
V	Cyclophosphamide (CP)	20 mg/kg	24 hrs	
VI, VII, VIII	LS-1 + CP, DG-1 + CP, LA-1 + CP	100 mg/kg	2 weeks	
IX, X, XI	LS-2 + CP, DG-2 + CP, LA-2 + CP	200 mg/kg	2 weeks	

Table 2: Frequencies of chromosomal aberration in bone marrow erythrocytes. Values are expressed as mean ± SEM. n=5

Group	Drug	Dose	Total No. of cells analyzed	Total No. of aberrations		
				LSME	DGME	LAME
I	Water	0.4 ml	500	3.60 ± 0.92	3.60 ± 0.92	3.60 ± 0.92
II, III, IV	LS-2, DG-2, LA-2 respectively	200 mg/kg	500	0.80 ± 0.37	1.80 ± 0.37	3.00 ± 1.22
V	Cyclophosphamide (CP)	20 mg/kg	500	64.40 ± 5.30	64.40 ± 5.30	64.40 ± 5.30
VI, VII, VIII	LS-1 + CP, DG-1 + CP, LA-1 + CP	100 mg/kg	500	41.40 ± 1.50	45.80 ± 4.75	67.40 ± 4.52
IX, X, XI	LS-2 + CP, DG-2 + CP, LA-2 + CP	200 mg/kg	500	16.60 ± 1.53	42.20 ± 4.94	50.20 ± 1.31

Table 3: Mitotic Index of bone marrow cells of Control, LS-2, CP (20 mg/kg), LS-1 + CP, LS - 2 + CP. Values are expressed as Mean ± SEM, n=5, ###P<0.001 vs Normal Control, *P<0.001 vs CP, *P<0.05 vs CP, ns = not significant**

Group	Dose	Total No. of cells counted per animal	Mitotic Index (%) (Mean±SEM)
Control	Water	500	7.36 ± 0.17
LS - 2	200 mg/kg	500	7.44 ± 0.14 ^{ns}
CP	20 mg/kg	500	4.40 ± 0.37 ^{###}
LS - 1 + CP	100 mg/kg	500	5.04 ± 0.24 [*]
LS - 2 + CP	200 mg/kg	500	6.00 ± 0.22 ^{***}



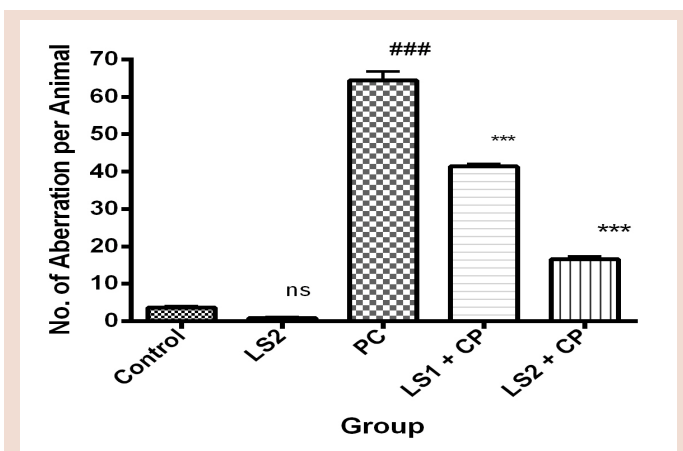


Figure 3: Data Showing comparison of chromosomal aberration in control, LS - 2, CP, LS - 1 + CP, LS - 2 + CP groups. † n=5, ###P<0.001 vs Control. ***P<0.001 vs CP ns = not significant CP = Cyclophosphamide treated group LS - 1 = *Lagenaria siceraria* treated (100 mg/kg) LS-2 = *Lagenaria siceraria* treated (200 mg/kg).

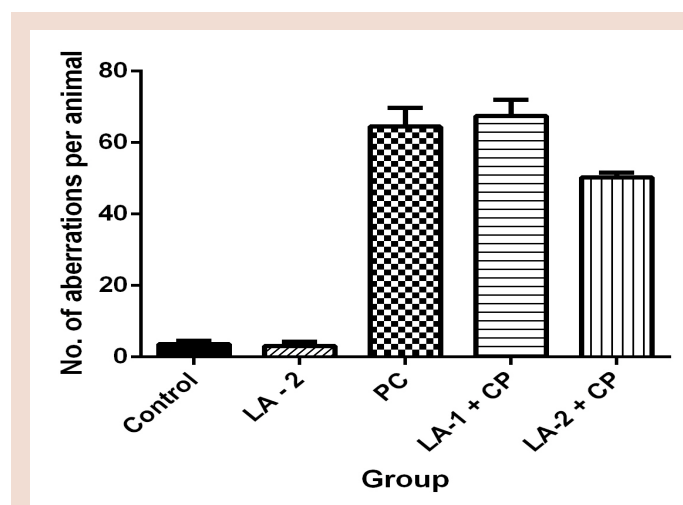


Figure 5: Data Showing comparison of chromosomal aberration in control, LA - 2, CP, LA - 1 + CP, LA - 2 + CP groups. † n=5, ###P<0.001 vs Control. *P<0.05 vs CP, ns = not significant CP = Cyclophosphamide treated group LA-1=*Leucas aspera* treated (100 mg/kg) LA-2=*Leucas aspera* treated (200 mg/kg).

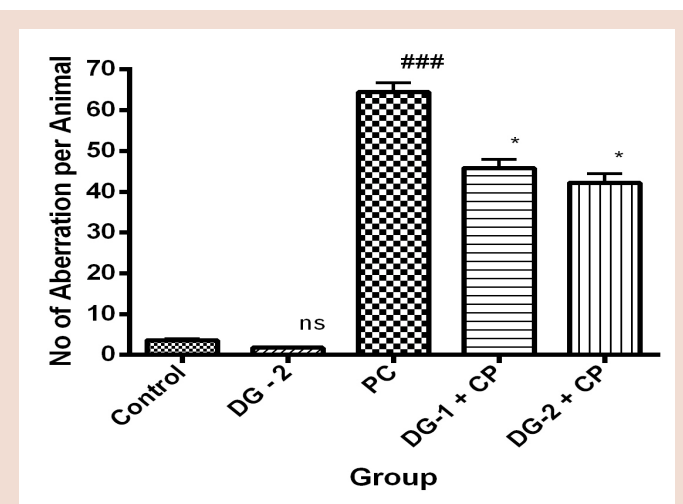


Figure 4: Data Showing comparison of chromosomal aberration in control, DG - 2, CP, DG - 1 + CP, DG - 2 + CP groups. † n=5, ###P<0.001 vs Control. *P<0.05 vs CP, ns = not significant CP = Cyclophosphamide treated group DG-1 = *Desmodium gangeticum* treated (100 mg/kg) DG-2=*Desmodium gangeticum* treated (200 mg/kg).

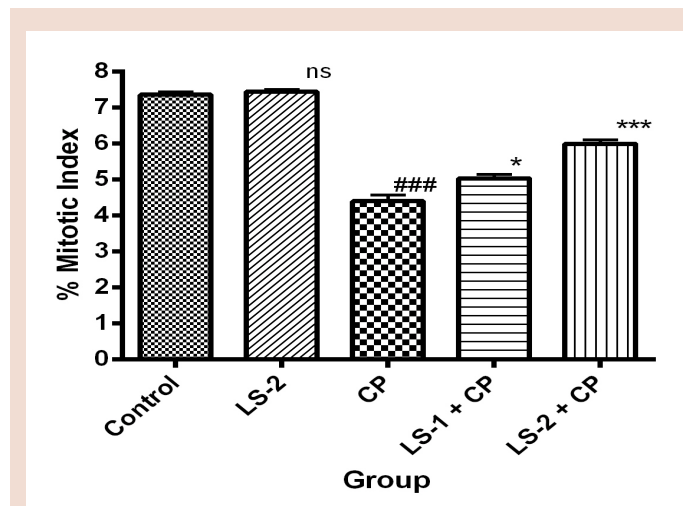


Figure 6: Data Showing comparison of % Mitotic Index in control, LS - 2, CP, LS - 1 + CP, LS - 2 + CP groups † n=5, ###P<0.001 vs Control. ***P<0.001 vs CP, *P<0.05 vs CP, ns = not significant CP = Cyclophosphamide treated group LS-1=*Lagenaria siceraria* treated (100 mg/kg) LS-2=*Lagenaria siceraria* treated (200 mg/kg).

Group V served as positive control and treated with CP single dose 20 mg/kg intra-peritoneally 24 hour prior to termination. Group VI, VII, VIII were treated with 100 mg dose of test drugs and Group IX, X XI were treated with 200 mg dose of test drug LSME, DGME and LAME respectively for 14 consecutive days and sacrificed on 15th day giving CP single dose 20 mg/kg intra-peritoneally 24 hour prior to termination. (Table 1).

Chromosomal aberration assay

Animals were injected colchicine intra-peritoneally at the dose of 4 mg/kg body weight, on the 15th day in order to arrest dividing cells in metaphase and sacrificed by cervical dislocation, 90 min after the colchicine treatment. Bone marrow cells from both femurs were extracted, subjected to hypotonic shock treatment (KCL 0.075M), for about 30 min, at room temperature and then centrifuged at 1000 rpm for 10 min. the cells

were fixed 5 times using freshly prepared methanol-acetic acid (3:1). The cells were spread on clean glass slides that were dried on hot plate at 40°C. One more drop of fixative was added on slides to see more reliable pictures of chromosomes and then the slides were air dried at room temperature and finally stained with 5% dilution of Giemsa reagent in phosphate buffer (pH 6.8) for 15 min. the chromosomes of 100 cells in metaphase abnormalities (Figure 1, 2) were analyzed per group with a 100X oil immersion objective, using a Trinocular Microscope. Metaphases with chromosomes and chromatid breaks, gaps, rings, stickiness, dicentric, centric fusion and deletion (if any) were recorded.

Mitotic index (MI)

Stages of mitotic index among 500 cells are calculated per animal. MI is calculated by using the formula.⁸

$$MI = (A/A+B) 100$$

A= no. of dividing cells (metaphases+ anaphases)

B= no. of non dividing cells.

RESULTS

Chromosomal aberration assay

Evaluation of chromosomal aberration was conducted at two dose levels. In this assay chromatid gap and break, chromosomal gap and break, exchange, deletion, fragmentation, pulverization, stickiness, ring, and dicentric chromosomes were taken as a parameter to score the total number of cells with aberration. Total number of cells with aberration was calculated in negative control, only extract treated, Positive control, and test drug treated group.

For all three plant extract common negative control and positive control group was kept. Negative control group was given water and positive control group was treated with cyclophosphamide (CP) shows statistically significant result ($***P<0.001$).

With reference to results obtained through the experiment total number of aberrations was seen in negative control is 3.60 ± 0.92 . There is no significant increased frequency of aberration was found in the group treated with *Lagenaria siceraria* (200 mg/kg , 0.80 ± 0.37), *Desmodium gangeticum* (200 mg/kg , 1.80 ± 0.37) and *Leucas aspera* (400 mg/kg , 3.00 ± 1.22^{ns}) when compared with negative control group (Table 2). Thus, *Lagenaria siceraria*, *Desmodium gangeticum* and *Leucas aspera* exhibited no signs of genotoxicity in chromosomes of mice bone marrow cells.

The results indicates that there is significant ($p<0.001$) increase in frequency of chromosomal aberration in positive control group ($64.40 \pm 5.30^{***}$), compare to the results of negative control group.

Effects of the oral prophylactic treatment with 100, 200 mg/kg/day of *Lagenaria siceraria*, *Desmodium gangeticum* and *Leucas aspera* methanolic extract on the total no of aberration in the bone marrow of normal and mice exposed to cyclophosphamide, are described in Table 2. Further results indicates that two weeks treatment with *Lagenaria siceraria*, *Desmodium gangeticum* shows great reduction in the percentage of aberration compare to positive control group (Figure 3, Figure 4 respectively) which is statistically significant ($*p<0.001$, extremely significant, $*p<0.05$, significant respectively).

Results indicates that two weeks treatment with *Leucas aspera* 200 mg per kg shows reduction in the total no. of aberration compare to positive control group ($p<0.05$, significant) while *Leucas aspera* 100 mg per kg shows not significant reduction in chromosomal aberration (Figure 5).

Mitotic Index

The data summarized in Table 3 revealed that control group receiving water shows the value of mitotic index 7.36 ± 0.17 . The positive control group receiving cyclophosphamide for 24 hours prior to sacrifice the animal, shows the percentage of mitotic index 4.40 ± 0.37 ($p<0.001$). Decrease in percentage of mitotic index in CP treated group shows that there is decrease in cell proliferation rate of bone marrow cells of mice. There was significant ($p<0.001$) increase in value of mitotic index was seen with the *Lagenaria siceraria* treatment as compare to positive control group. Group treated with *Lagenaria siceraria* alone did not shown any significant differences compare to negative control group (Figure 6).

DISCUSSION

The results obtained in this study indicate that *Lagenaria siceraria* fruit extract and *Desmodium gangeticum* arial part extract pretreatment significantly reduce the frequency of structural chromosomal aberrations

induced by CP in bone marrow cells (Figure 3, 4). While pretreatment with *Leucas aspera* reduce the frequency of structural chromosomal aberrations in higher dose selected dose (Figure 5).

The relationship between cell cycle progression and increase and decrease in cell proliferation is examined by indentifying the mitotic index. Decrease in percentage of mitotic index in CP treated group shows that there was decrease in cell proliferation in bone marrow cells of mice, while pretreatment with *Lagenaria siceraria* shows a significant increase in mitotic activity of cells. The improvement in mitotic activity of bone marrow cells of animals pre-treated with *Lagenaria siceraria* may focus attention on the beneficial effect of *Lagenaria siceraria* in cancer chemotherapy.

CONCLUSION

The antigenotoxic effect of methanolic extract of *Lagenaria siceraria* fruit, *Desmodium gangeticum* and *Leucas aspera* arial part was seen against the mutation induced by cyclophosphamide.

As per literature survey antioxidant and cytoprotective activity activity of *Lagenaria siceraria* fruit, *Desmodium gangeticum* and *Leucas aspera* has been proven. A reason for mutagenic effect is the genotoxicity of reactive oxygen species (ROS) and antioxidants in such cases can act as stabilizers of homeostasis. So there is an increasing interest in the protective biochemical function of natural antioxidants contained in medicinal plants, which prevent oxidative damage caused by oxygen-free radical species. Keeping in mind number of previous investigation about antioxidant and cytoprotective nature of selected indigenous plant extract it can be anticipated that antimutagenic activity observed in the present study may be via antioxidant mechanism.

Therefore, from the present study, it can be concluded that selected indigenous plant methanolic extract possesses antimutagenic property. The observed antimutagenic activity of selected indigenous plant extract-against cyclophosphamide might be associated with active compounds and its antioxidant constituents such as poly-phenolic compound, flavanoids and other micronutrients. The significant antimutagenic activity showed by *Lagenaria siceraria*, *Desmodium gangeticum* and *Leucas aspera* provide a scientific validation for the traditional use of these plants. Further investigations will be needed to evaluate the same activity of selected plant extract on other test system as well as to characterize the active compounds in detail.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

None.

ABBREVIATION USED

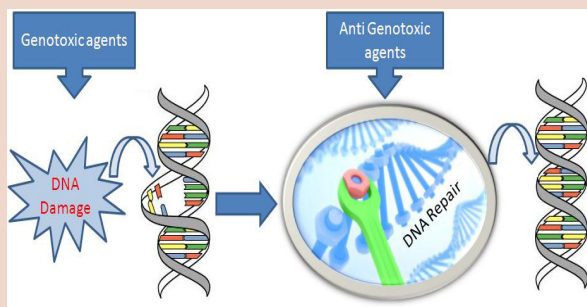
CP: Cyclophosphamide; LSME: *Lagenaria siceraria* methanolic extract; DGME: *Desmodium gangeticum* methanolic extract; LAME: *Leucas aspera* methanolic extract.

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PICTORIAL ABSTRACT



SUMMARY

- Antimutagenic activities of some indigenous plants were observed using Chromosomal aberration assay. The selected plant *Lagenaria siceraria*, *Desmodium gangeticum* and *Leucas aspera* shows significant antimutagenic activity.

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