Original Paper

Anticlastogenic activity of *Plumbago indica* using Mouse Bone Marrow - Micronucleus Test

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ABSTRACT

Certain human cancers can be prevented by identification of mutagenic agents in the environment and protecting humans from exposure to such agents. The purpose of this study is to evaluate the anticlastogenic activity of Plumbago indica in mouse bone marrow cells. The known clastogen used was mitomycin C at 1.5mg/kg body weight. The Plumbago indica extract is treated with mitomycin C at three different dose levels viz., 0.5, 1 & 2mg/kg at varying time periods. The pretreatment was carried out at 3, 6, & 12 hours, and the animals were sacrificed after 24 hours of the last administered injection. The percent inhibition of micronucleated polychromatic erythrocytes (MNPCEs) was found to be more than 50% at all time points of pretreatment. Plumbago indica was found to be effective at 12 hours before treatment of mitomycin C (~80%). The inhibition of micronuclei in MNPCEs induced by mitomycin C was not effective at simultaneous and post treatment of Plumbago indica.

Key Words: *plumbagin, anticlastogenicity, mitomycin C*

Introduction

Regular or substantial consumption of certain plant materials has been associated with a lowered cancer risk at various sites.[1,2] These plant materials and their tissue extracts have been demonstrated to contain a variety of antimutagenic substances.[3] In recent years there has been an increasing awareness that certain naturally occurring substances in such plants and other sources have protective effects against environmental mutagens/carcinogens and endogenous mutagens.[4] *Plumbago indica* is a subscandent shrub known locally as "Raktachitrak," that grows wild in peninsular India and West Bengal. The root of this plant contains an acrid, crystalline principle called plumbagin. Plumbagin is a gastric stimulant and appetite stimulator. The tincture of the root is used in indigenous medicine for the treatment of secondary syphilis, leprosy, and also for the relief of dyspepsia, piles, and flatulence. It is also said to be a good remedy for controlling postpartum hemorrhage. Further, both the root and the root-bark are used in making caustic pastes used in rubefacient applications (external).

The *in vivo* bone marrow micronucleus assay allows an effective assessment of both chromosomal damage and chromosomal loss induced by chemicals, because it is simpler and faster than chromosomal analysis. The root extract of *Plumbago indica* has been reported to inhibit the mutagenicity in Ames test.[5,6] The antimutagenic effect of *Plumbago indica* has been documented under *in vitro* conditions, but the action in animal system needs more research. Therefore, it was decided to study the anticlastogenic potency of the root extract of *Plumbago indica* in bone marrow cells of mice against the mutagen mitomycin C using the *in vivo* micronucleus test.

Materials and Methods

Animals: Male Swiss albino mice aged 7-10 weeks weighing 22-30g were taken from the animal house of Jai Research Foundation for the conduct of the study. They were housed in groups of five in polypropylene cages bedded with paddy husk. The animals were maintained at 22 ± 2°C with 50-55% relative humidity and a

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12h light/dark cycle. Food and water were given ad libitum.

Chemicals: Mitomycin C was used as known mutagen (Biochem Laboratories). Plumbagin (Sigma, CAS No.481-42-5) was used as the test compound. The other chemicals used were foetal calf serum (PAA Laboratories), dimethyl sulfoxide (Qualigens), and giemsa and maygrunwald stains (Himedia Laboratories).

Anticlastogenicity: The dose levels of 0.5, 1 & 2 mg/kg b.wt. of *Plumbago indica* was treated with known mutagen mitomycin C (1.5 mg/kg b.wt.). *Plumbago indica* was dissolved in dimethyl sulfoxide. The test compound was injected intraperitoneally to all groups of mice. *Plumbago indica* was injected 12h, 6h & 3h before mitomycin C injection, simultaneously with mitomycin C, and 3h after mitomycin C injection. A separate group for vehicle control was included along with a positive control group (mitomycin C). All pre, simultaneous, and post *Plumbago indica* treated animals were sacrificed after 24h of last treatment.

Treatment schedule: The *Plumbago indica* was treated with mitomycin C at three different dose levels at varying time periods. The extract was treated 3h, 6h & 12h before the injection of Mitomycin C (pretreatment). In the second case, the extract was treated along with mitomycin C (simultaneous treatment). In the third case, the extract was treated 3h after the mitomycin C injection (posttreatment). A total of 17 groups were assigned for treatment with each group comprising 5 male mice.

Bone marrow micronuclei preparation and scoring: Bone marrow micronuclei slides were prepared by using the standard method.[7] The slides were actually coded before analysis. The polychromatic erythrocytes (PCEs), normochromatic erythrocytes (NCEs) and micronucleated polychromatic erythrocytes (MNPCEs) were scored. Approximately 2000 PCEs and corresponding NCEs were scored from each animal to assess the micronuclei frequency, and to estimate P/E ratio. The data were statistically analysed using Graphpad Prism software.

Results and Discussion

The results of *in vivo* micronucleus test using bone marrow sampling after 24 hours showed significant effects at 12hours pretreatment. *Plumbago indica* when assessed by pretreatment at 3 hours, 6 hours, and 12 hours before mitomycin C injection showed promising anticlastogenic nature. The animals treated 3 hours be fore mitomycin C injection at dose levels of 0.5, 1, and 2 mg/kg b. wt. did not show significant bone marrow toxicity, i.e., P/E ratio. Moreover, the number of

Table 1 Effect of *Plumbago indica* pretreated at varying time points against Mitomycin C

Treatment	Dose (mg/kg)	%MNPCE	%Total MN	P/E Ratio
	Pretreatment before 3 h of MMC treatment			
DMSO	0.0	0.35	0.21	0.439
MMC	1.5	1.48	0.76	0.416
MMC+PLM	0.5	0.75	0.38	0.502
MMC+PLM	1.0	0.68	0.34	0.497
MMC+PLM	2.0	0.62	0.32	0.508
	Pretreatment before 6h of MMC treatment			
MMC+PLM	0.5	0.55	0.32	0.489
MMC+PLM	1.0	0.47	0.27	0.500
MMC+PLM	2.0	0.39	0.21	0.491
	Pretreatment before 12h of MMC treatment			
MMC+PLM	0.5	0.31	0.16	0.491
MMC+PLM	1.0	0.30	0.16	0.496
MMC+PLM	2.0	0.24	0.13	0.504

MMC - Mitomycin C
PLM - Plumbago indica
MNPCE - Micronucleated Polychromatic Erythrocytes
DMSO - Dimethyl sulfoxide

micronucleated polychromatic erythrocytes is reduced in a dose dependent manner. The dose level of 2mg/kg b. wt. of *Plumbago indica* showed 58.11% inhibition of micronucleated polychromatic erythrocytes induced by mitomycin C. The other dose levels 1 and 0.5mg/kg showed 54.06 and 49.33%, respectively. Another three groups of animals treated 6 hours before the mitomycin C injection also showed reduced micronucleated polychromatic erythrocytes at all the dose levels. The percent inhibitions of micronucleated polychromatic erythrocytes in these groups were comparatively more than the 3 hours pretreated animals. The lowest dose of 0.5 mg/kg *Plumbago indica* inhibited more (62.84%) than the highest dose 2 mg/kg in 3 hours pretreated animals (49.33%). 73.65% of micronucleated polychromatic erythrocytes were inhibited by 2 mg/kg of *Plumbago indica*. The results with 3 hours and 6 hours pretreatment revealed the effectiveness of *Plumbago indica* with 6 hours. The third parameter of pretreatment 12 hours before mitomycin C showed a significant inhibition of micronuclei than the 3 and 6 hours treatment. A maximum of 83.79% inhibition was observed at 2 mg/kg of *Plumbago indica*. The dose level of 0.5 and 1mg/kg exhibited 79.06% and 79.73%, respectively (**Table 1**).

Plumbago indica was also treated simultaneously with mitomycin C. The results did not show any reduction in the number of micronucleated polychromatic erythrocytes. The dose level of 0.5 mg/kg was seen slightly influencing the number of micronucleated polychromatic erythrocytes, but the count was in the normal range of mitomycin C. The simultaneous treatment was not showing any effect of *Plumbago indica*. Another group of animals were treated with *Plumbago indica* 3 hours after the mitomycin C injection. The results here, again were like simultaneous exposure showing almost negative effect on induced micronucleated polychromatic erythrocytes. The highest dose showed 3.32% inhibition (**Table 2**).

During the full duration of the study, the animals did not show any abnormal symptoms, and there was no mortality observed. The animals were sacrificed after 24 hours. The results from **Tables 1** and **2** clearly indicate that *Plumbago indica* is effective when administered before the injection of mitomycin C. Even though the pretreatment was significant in reducing the induced MNPCEs, it is found to be more effective at 12 hours before the administration of mitomycin C. Since the anticlastogenic effect was not seen at simultaneous and posttreated *Plumbago indica* it is assumed that the mechanism of action of *Plumbago indica* is not intracellular. *Plumbago indica* appears capable of eliminating the mutagen before it actually enters the cell as evidenced by pretreatment observation. As the anticlastogenic effect of *Plumbago indica* was observed only at pretreatment (before mutagen addition), the possible mechanism of action should be extracellular, which is generally classified as a desmutagen.

Table 2 Effect of *Plumbago indica* simultaneously and posttreated against Mitomycin C

Treatment	Dose (mg/kg)	%MNPCE	%Total MN	P/E Ratio
	Simultaneous treatment with MMC			
Vehicle	0.0	0.35	0.21	0.439
MMC	1.5	1.48	0.76	0.416
MMC+PLM	0.5	1.54	0.78	0.390
MMC+PLM	1.0	1.38	0.70	0.410
MMC+PLM	2.0	1.47	0.74	0.360
	Posttreatment after 3h of MMC treatment			
MMC+PLM	0.5	1.58	0.79	0.348
MMC+PLM	1.0	1.50	0.77	0.455
MMC+PLM	2.0	1.43	0.73	0.437

MMC - Mitomycin C
PLM - Plumbago indica
MNPCE - Micronucleated Polychromatic Erythrocytes

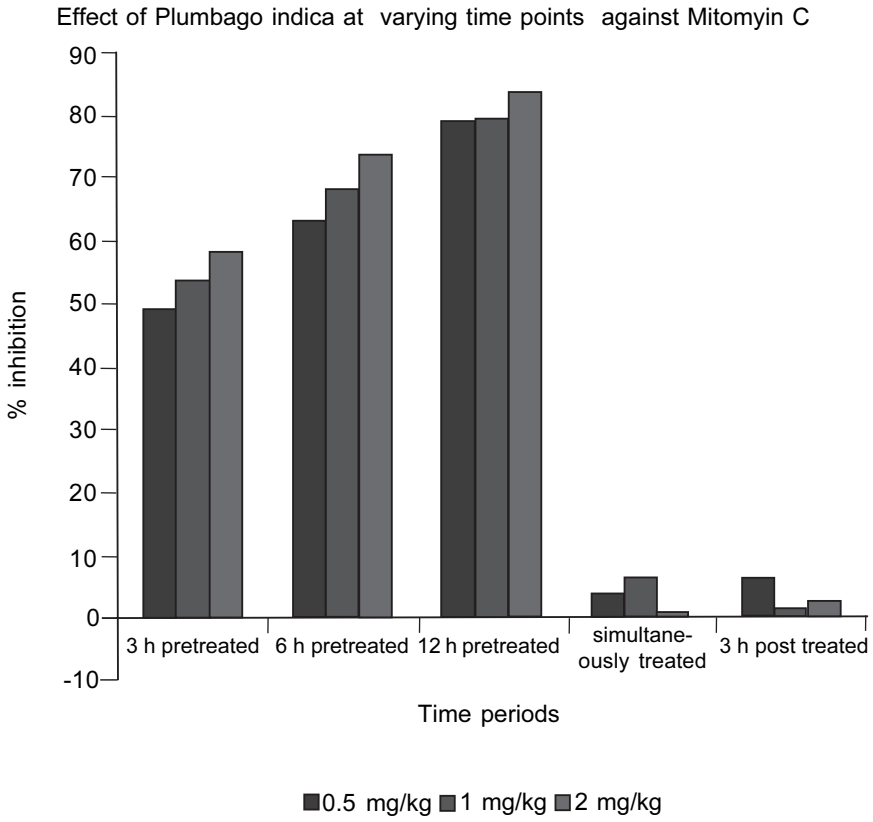

Fig 1

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