



EVALUATION OF PLUMBAGO ZEYLANICA L. PLANT EXTRACT USING MICRONUCLEUS ASSAY IN MOUSE BONE MARROW CELLS

Botany

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ABSTRACT

Herbal plants have always been used as a common source of medicines. *Plumbago zeylanica* is the most common plant used in Indian traditional system of medicine. In the present investigation, the preventive effect of *Plumbago zeylanica* plant extract was evaluated against cisplatin-induced micronucleus formation in the mouse bone marrow cells. The single i.p. administration of *Plumbago zeylanica* extract at the dose of 200 and 400 mg/kg body weight, 24 h prior the administration of cisplatin (at the dose of 6 mg/kg) have significantly prevented the micronucleus formations in a dose dependent manner in bone marrow cells of mice as compared to cisplatin group. Our result indicate that plant extract seems to have preventive potential against cisplatin-induced micronucleus formation in swiss mouse bone marrow cells.

KEYWORDS

Plumbago zeylanica, Mutagenicity, Micronucleus, Bone Marrow, Cisplatin.

Introduction

P. zeylanica is the most common plant used in Indian traditional system of medicine. A native of South Asia, the species is distributed throughout most of the tropics and subtropics; growing in deciduous woodland, savannas and scrub lands from sea level up to 2000 m altitude. 1-3 the root is used as laxative, expectorant, astringent, abortifacient and in dysentery. Tincture of root bark is used as antiperiodic. The leaves are caustic and used in treatment of scabies. *Plumbago* are chemically characterized by the presence of naphthoquinones, flavonoids, terpenoids and steroids, many of them being responsible for several biodynamic activities. Popular name of *Plumbago zeylanica* is lead wort. This plant is also known by several names in different parts of the world. In India its common name is "Chitrak".

Collection and identification of plant material

The plant materials were purchased from the local market Bhopal (MP) India and were authenticated by Dr. Zia Ul Hasan (Prof. and Head) Department of Botany, Safia College of Science, Bhopal. The plant materials were shade dried, reduced to coarse powder and stored in airtight container till further use.



Experimental design for micronucleus method

Swiss albino mice were randomly divided into four groups and each group contain six animals and treated as per protocol with slight modification.

Micronucleus assay

The swiss albino were taken and divided into 4 groups, each group containing six animals. Group I was control, no treatment given to this group. Group II was treated with cisplatin 6 mg/kg i.p. bodyweight. Group III, Group IV was treated with cisplatin 6 mg/kg i.p. along with *Plumbago zeylanica* extract 200 mg/kg, 400 mg/kg and i.p. body weight, respectively. After intraperitoneal administration of cisplatin 6mg/kg body weight animals were sacrificed by cervical dislocation and bone marrow cells were harvested. From freshly killed animals, bone marrow was removed from muscle by use of gauze and fingers. Bone marrow cell was aspirated by flushing with Hank's Balanced Salt Solution (HBSS) with help of a syringe. The tube was centrifuged at 1000 rpm for 5 min. The supernatant is removed and the cells in the sediment are carefully mixed by aspiration. The cycle was repeated three times and a small drop of the viscous suspension was put on the end of a slide and spread by pulling the material behind a polished

cover glass held at an angle of 45°. The preparation was then dried on slide warmer and fixed for 2-5 min. Slides were dipped in methanol for 10 min for the fixation of cells. Then, staining was carried out in ordinary vertical staining jars. Staining was done first for 5 min in May-Grunewald solution and then, for 10 min in Giemsa. Slides were rinsed in distilled water, blotted, cleaned with filter paper on back side of followed by drying on slide warmer. Erythrocytes cells were scored for micronuclei under the microscope. At least 1000 cells per animals were scored for the incidence of micronuclei. The ratio of polychromatic erythrocytes (PCEs) to nonchromatic erythrocytes (NCEs) was determined for each animal by counting a total 1000 erythrocytes (Aaron *et al* 1989). Then statistical analysis was done by one way ANOVA and Bonferroni test.

Group-I: Vehicle control

Group-II: Cisplatin 6 mg/kg b.w., i.p.

GROUP-III: *Plumbago zeylanica* 200 mg/kg., b.w.p.o.

GROUP-IV: *Plumbago zeylanica* 400 mg/kg., b.w.p.o.

RESULTS AND DISCUSSION

In Group I (Control), no MNPCE (micronucleated polychromatic erythrocytes) were found, it means Group I did not possess mutagenic effect. In Group II (cisplatin 6mg/kg i.p. body weight), maximum number of MNPCE were found which means cisplatin exhibited maximum mutagenic effect as compared to control group. In Group III (Pz200mg/kg i.p. body weight + cisplatin 6 mg/kg i.p. body weight), as compared to Group II, lowest number of MNPCE were found which means Group III possesses antimutagenic effect and Group IV decreases the MNPCE and increase the antimutagenic effect (TABLE:1)

Table1: Effect of *Plumbago zeylanica* extract on micronucleus formation in mouse bone marrow cell.

S.N O	GROUP	PCE mean±sd	NCEmeam±sd	PCE/NCEmean±sd
1.	Vehicle	2.33±0.942	1.50±0.957	1.13±0.610
2.	Cisplatin (6 mg/kg)	55.50±13.586	14.50±4.958	4.02±0.906
3.	Ext P. zeylanica (200 mg/kg)	33.67±3.091	13.67±0.745	2.42±0.195
4.	Ext P. zeylanica (400 mg/kg)	16.00±2.039	9.00±1.732	1.78±0.402

Data are expressed in mean±sd, Data were analysed by one way ANNOVA followed by Bonferroni test. Number of animals in each group n=6, All groups are compared with cisplatin control. *p<0.05, NS non significant.

CONCLUSION

The beneficial antimutagenic effects of *Plumbago zeylanica* extract may be due to either individual or combined effect of its constituent. The mechanism underlying the antimutagenic action of *Plumbago*

zeylanica extract is seen in higher dose. Hence it can be concluded that *Plumbago zeylanica* extract did not exhibit any genotoxicity. The extract was found to be neither mutagenic nor carcinogenic and hence can be used safely

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