



RESEARCH ARTICLE

EVALUATION OF *ARGEMONE MEXICANA* FRUITS EXTRACT USING MICRONUCLEUS ASSAY IN MOUSE BONE MARROW CELLS

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Herbs have always been used as a common source of medicines. *Argemone mexicana* is a vital herbal plant used in Ayurveda as a traditional medicinal system of India. In the present investigation, the preventive effect of *Argemone mexicana* fruits extract was evaluated against cyclophosphamide-induced micronucleus formation in the mouse bone marrow cells. The single *i.p.* administration of *Argemone mexicana* fruits extract at the dose of 50, 100 and 150 mg/kg body weight, 24 h prior the administration of cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations in a dose dependent manner in bone marrow cells of mice as compared to cyclophosphamide group. Therefore, plant fruits extract seems to have a preventive potential against CP-induced micronucleus formation in swiss mouse bone marrow cells.

Key words: *Argemone mexicana*, Mutagenicity, Micronucleus, Bone Marrow, Cyclophosphamide.

INTRODUCTION

Literature is enriched with several reports indicating cytotoxic potential of natural and synthetic compounds from diverse sources *viz.* sponges, plants and microorganisms (Devienne *et al* 2002; Gordaliza, 2010; Dahiya and Gautam, 2011) Micronuclei are cytoplasmic chromatin-containing bodies that appear in the cell like a small satellite nucleus around the cell nucleus, due to chromosome fragments or entire chromosomes that are not incorporated in main nucleus after cell division. The presence of micronuclei (MN) in cells is considered as a biomarker of damage to the DNA. The micronucleus test is an *in vivo* and *in vitro* short time screening cytogenetic test which is a widely used method for assessing genotoxicity of chemicals in organism (Heddle, 1973; Schmid, 1975; Meier *et al* 1999). *Argemone mexicana* Linn is known as Satyanashi which is medium

size tree belonging to family *Papaveraceae* and is a strong branched prickly annual, 60-90 cm in height with yellow latex and simple, sessile and spiny leaves. Flowers are large, bright yellow, terminal on the short leafy branches, fruits are prickly capsules, oblong-ovoid, opening by 4-6 valves, seeds are numerous (Dwivedi *et al* 2008). The seeds contain 22-36% of pale yellow non-edible oil, called *Argemone oil* or *Katkar oil*, which contains the toxic alkaloids sanguinarine and dihydrosanguinarine. The plant contains alkaloids such as berberine, protopine, sarguarine, optisine, chelerytherine etc. The seed oil contains myristic, palmitic, oleic, linoleic acids etc. (Mukherjee and Namhata, 1990). According to Ayurveda, the plant is diuretic and purgative which destroys worms. It cures leprosy, skin-diseases, inflammations and bilious fevers (Satpathy and Panda, 1992). The present

investigation is aimed at studying the anti-mutagenicity activity of *Argemone mexicana* Linn in order to justify the traditional claims endowed upon this herbal drug as a rasayana.

MATERIALS AND METHODS

Plant material

The fresh fruits of *Argemone mexicana* Linn were collected from Idgah Hills, Bhopal (**Figure 1**) and authenticated by Dr. Pramod Patil (Botanist) at Govt. M.L.B. Girls (Autonomous) college, Bhopal (MP) and Sheet no. of Herbarium is 903.



Fig. 1. Fresh fruits of *Argemone mexicana* Linn.

Preparation of extract

The fruits of *A. mexicana* were washed with double-distilled water, dried in shade and powdered by grinder. The powder was treated with petroleum ether up to 3 h for defating. 50 g of powder was taken in separating funnel and 50% methanol was added to it followed by gentle mixing. After every 24 h, extract was collected in a beaker till the solvent appears colorless. Cycle was repeated 3 times. Then, extract was dried into powder by water bath at 55°C and hot air oven at 45°C. Total weight of extract powder was measured and 16% yield of extract was obtained (Alade and Irobi, 1993).

Animals

The study was conducted on the random bred, 4-5 weeks old and 25±2 g body weight swiss albino mice of both sexes. These were maintained under controlled conditions of temperature (25±2°C), light (12 light: 12 dark). They were housed in good laboratory condition and were given standard mouse pellet diet and water *ad libitum*. Animals were housed in polypropylene plastic cages. The study protocol is approved by Institutional Animal Ethical Committee having number 2004/ec/2010.

Micronucleus assay

The swiss albino were taken and divided into 5 groups, each group containing six animals.

Group I was control, no treatment given to this group. Group II was treated with cyclophosphamide 50 mg/kg *i.p.* body weight (Agrawal and Kumar, 1998). Group III, Group IV and Group V were treated with cyclophosphamide 50 mg/kg *i.p.* along with *Argemone mexicana* extract 50 mg/kg, 100 mg/kg and 150 mg/kg *i.p.* body weight, respectively. After intraperitoneal administration of cyclophosphamide, 50 mg/kg body weight animals were sacrificed by cervical dislocation and bone marrow cells were harvested. From freshly killed animals, bone marrow were 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 normochromatic 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 student 't' test.

RESULTS AND DISCUSSION

In Group I (Control), no MNPCE were found, it means Group I did not possess mutagenic effect. In Group II (cyclophosphamide 50 mg/kg *i.p.* body weight), maximum number of MNPCE were found which means cyclophosphamide exhibited maximum mutagenic effect as compared to control group (**Figure 2**). In Group III (AME 50 mg/kg *i.p.* body weight + cyclophosphamide 50 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 and Group V also decreases the

MNPCE and increase the antimutagenic effect (Table 1). It means *Argemone mexicana* hydro-methanolic fruits extract at the dose of 50 mg/kg, 100 mg/kg and 150 mg/kg drug showed decrease in the Micronucleus formation when given for 24 h before the cyclophosphamide at the dose of 50 mg/kg. The effect was observed in dose dependent manner (Pandey and Agrawal, 2010). The phytochemical study indicates the presence of flavonoid which is responsible for antimutagenic activity (Brown, 1980).

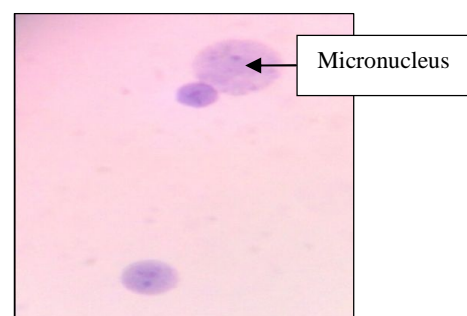


Fig. 2. Micronucleus in mice bone marrow cell

Table 1. Effect of *Argemone mexicana* fruits extract on micronucleus formation in mouse bone marrow cell.

S. No.	Treatment	MNPCE±S.E.M.	PCE/NCE±S.E.M. ratio
1.	Control	0.00	0.479±0.105
2.	Cyclophosphamide 50 mg/kg	2.666±0.093	0.733±0.044
3.	50 mg/kg AME ^a + cyclophosphamide 50 mg/kg alone	1.666±0.025	0.679±0.088
4.	100 mg/kg AME + cyclophosphamide 50 mg/kg	0.833±0.167	0.658±0.102
5.	150 mg/kg AME + cyclophosphamide 50 mg/kg	0.333 ^b ±0.609	0.577±0.056

Data represented as Mean±S.E.M. (one way ANOVA and Student 't' test)

^aAME-*Argemone mexicana* extract; ^b Showed significant activity by Student 't' test $p < 0.05$

CONCLUSION

Argemone mexicana is a vital herbal drug in Ayurveda. The beneficial antimutagenic effects of *Argemone mexicana* fruits extract may be due to either individual or combined effect of its constituent. The mechanism underlying the antimutagenic action of *Argemone mexicana*

fruits extract is not clear. All these data point to the possibility of developing an extract of *Argemone mexicana* Linn as a novel, potential agent in area of cancer chemotherapy. However, further studies are required to elucidate exact mechanism of action of *Argemone mexicana* extract.

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