

Effect of *Ailanthus Excelsa* Against Cyclophosphamide Induced Genotoxicity

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Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. These changes may involve a single gene or gene segment, a block of genes or chromosomes. The term clastogen is used for agents giving rise to structural chromosome aberrations. A clastogen can cause breaks in chromosomes that result in the loss or rearrangements of chromosome segments (1). In vitro and in vivo tests that measure chromosomal aberrations in metaphase cells can detect a wide spectrum of changes in chromosomal integrity. The assays that detect either chromosomal aberrations or micronuclei are appropriate for detecting clastogens (2).

In somatic cells, Cyclophosphamide produces gene mutations, chromosome aberrations, micronuclei and sister chromatid exchanges in a variety of cultured cells in the presence of metabolic activation as well as sister chromatid exchanges without metabolic activation. It can also produce chromosome damage and micronuclei in rats, mice and Chinese hamsters (3). The present study designed to investigate the effect of *Ailanthus excelsa* on chromosome in bone marrow cells of swiss albino mice. Swiss albino male mice (20-25 g) were separately group housed in ambient room temperature ($25 \pm 2^\circ \text{C}$) and relative humidity ($50 \pm 5\%$), maintained at 12:12 hr dark-light cycle. Food and water were available ad libitum. All procedures employed in the present study were approved by Institutional Animal Ethics Committee and carried out under strict compliance with Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests, Government of India. Animals were acclimatized to the experimental conditions for a period of one week before actual experimentation. CP(Cyclophosphamide) and colchicine were purchased from Sigma-Aldrich Co., St. Louis, MO, US. Fresh *Ailanthus excelsa* leaf was obtained from Bhopal and it get authenticated by Saifia college,

Bhopal. The leaves were rinsed with water and dry under shade. Material of known weight was Soxhleted using hydroalcoholic solvents. N-butanol soluble fraction was considered for saponins isolation. N-butanol fraction was further treated with chilled diethyl ether, which resulted in formation of saponin precipitate which were further treated with diethyl ether again after separation and then they were dissolved in ethanol and solvent was evaporated in low rate for getting crystallized saponins which were further confirmed by froth test. These components were considered as crude saponins for further assay.

Acute toxicity study was carried out using Swiss albino mice ($25 \pm 2 \text{ gm}$) according to the OECD 423 guide line. Four groups of mice were assigned to a different treatment. Two test groups were treated with different dose of saponin reach extract of leaves of *Ailanthus excelsa* 24 hours before treatment of Cyclophosphamide (50 mg/kg bd. wt). Positive control group treated with only Cyclophosphamide (50 mg/kg bd. wt), Colchicines (4 mg/kg b.wt) was administered intraperitoneally 1 hours before the harvest of the cells. Animals were sacrificed by cervical dislocation and femur bone was excised. Bone marrow was aspirated by flushing with normal saline in the centrifuge tube and Flush the suspension in the tube properly to get good cell suspension. Centrifuged for 10 min at 1000 rpm. Supernatant discarded and Pellet was treated with pre-warmed (37°C) KCl on cyclomixer. Left the above suspension in a water bath (37°C) for 30 min. Again Centrifuged and supernatant discarded. Pellet was treated with freshly prepared cornoy's fixative (methanol: acetic acid = 3:1) on cyclomixer. Once again Centrifuged and supernatant discarded above step of treatment with Cornoy's fixative was repeated 3 times to get debris free white pellet. Cornoy's fixative (quantity sufficient) added to pellet and got a good cell suspension. Slides were made with Air Drop Method. The

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Received: 17.06.2012, Accepted: 14.06.2014

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Slides were stained with 5% Giemsa's solution for 15 min and slides rinsed in distilled water blotted. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1000 × (100×10) for each groups. Different types of chromosomal aberrations such as chromatid breaks/gaps, centromeric association and chromatid fragmentation were scored and expressed as % chromosomal aberrations (4). All results were analyzed by One way analysis of variance (ANOVA) and post-hoc analysis was performed with Bonferroni's test. Value of P<0.05 was considered to be statistically significant in all the cases. Acute toxicity studies (OECD - 423guideline) of *Ailanthus excelsa* revealed that there was no toxic up to dose of 2000 mg/kg nor any significant variation in behavior of animal was observed. CP (50 mg/kg i.p) single dose So, for present experimental studies the 1/10th and 1/5th dose of *Ailanthus excelsa* was selected i.e. 100mg/kg and 200mg/kg. As depicted in Table 1, CP (50 mg/kg i.p) single dose administration significantly increased in total aberrant cells (%) as compared to control group. Further, comparison by Bonferroni's test showed that *A. excelsa* leaves, crude saponin extract (100 and 200,mg/kg) significantly and dose dependently increase incidence of % aberrant cells. The observations suggest that saponin` reach extract of leaves of *Ailanthus excelsa* has mutagenic activity

REFERENCES

1. EFSA Scientific Committee; Draft Scientific Opinion on Genotoxicity Testing Strategies applicable in food and feed safety assesment. European Food Safety Authority (2011). Available online: www.efsa.europa.eu.
2. Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use, international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, 2008, S2(R1).
3. Suchitra P, Suresh J, Archana T. Therapeutic use of cyclophosphamide and its cytotoxic action : A challenge for researchers. *J Pharmacy Res* 2011;4(8):2755-7.
4. Agrawal RC. In: Induction of chromosomal aberrations by propoxur in bone marrow cell. *Biomedical and Environmental sciences*.pp 292-295.

Table 1. Chromosomal aberration test in bone marrow swiss albino mice. Separate groups of mice were administered with (1) Saline (p.o) (2) Saline (p.o) + CP (50 mg/kg, i.p) (3) *A. excelsa* (100 mg/kg, p.o) + CP (50 mg/kg, i.p) (7) *A. excelsa* (200 mg/kg, p.o) + CP (50 mg/kg, i.p). CP (50 mg/kg i.p) was administered after 1 hr. last dose of *A. excelsa* treatment. Thereafter, 24 the animal was sacrificed and test was performed. All the value expressed as mean ± SD (n=6).

	Break	Fragmentation	Deletion	Polyploidy	Pulverized	Ring	Total Aberration
Group 1	3.83 ±1.72	2.83 ± 1.47	3.67± 1.63	0.00	0.00	0.00	4.83 ± 1.72
Group 2	31.33±3.01	24.17 ± 2.56	17.33 ± 3.27	6.67 ± 1.97	6.17 ± 1.47	5.83 ± 1.60	42.33 ± 3.50
Group 3	34.17±2.04	26.17±2.32	19.83±2.32	7.17±1.72	6.83±1.47	7.33±1.63	44.17±2.56
Group 4	41.33±2.58	31.33±3.33	27.67±2.16	10.67±1.21	11.33±2.16	11.50±2.26	49.83±2.32