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CYTOPROTECTIVE ROLE OF *HELICTERES ISORA* IN CHROMOSOMAL ABERRATIONS AND MICRONUCLEUS FREQUENCIES IN RAT BONE MARROW CELLS AGAINST CISPLATIN INDUCED GENOTOXICITY

Sunny Sharma[1]*, Seema Bhargava[2] and Anil Mehta[3]

[1,2,3]Department of Biotechnology, Faculty of Science and Technology, Mewar University, Gangrar, Chittorghar, Rajasthan India.

***Corresponding Author: Sunny Sharma**

Department of Biotechnology, Faculty of Science and Technology, Mewar University, Gangrar, Chittorghar, Rajasthan India.

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ABSTRACT

In the present investigations, the preventive effect of *Helicteres isora* fruit extract was evaluated against cisplatin induced chromosomal abbreviations and micronucleus frequencies in the rat bone marrow cells. The single PO administration of *Helicteres isora* methanolic extract at the dose of 200 and 400mg/kg body weight, 24 hours prior the administration of cisplatin (at the dose of 6 mg/kg) significantly prevented the micronucleus formations and chromosomal abbreviations in a dose dependent manner in bone marrow cells of rat as compared to cisplatin group. The highest number of frequencies was observed in rat treated with only cisplatin and least micronucleus frequencies and chromosomal aberrations were observed in 400mg/kg dose group of *Helicopters isora* (methanolic fruit extract) extract. The animals treated with different doses of *Helicteres isora* extract, i.e. 200 and 400 mg/kg showed a significant reduction ($p<0.05$) in the frequencies of micronucleus as well as chromosomal aberrations as compared to cisplatin group. The frequencies decrease with an increase in the dose of extract. There was a significant dose-effect relationship between chromosomal aberrations, micronucleus frequencies and dose of *Helicteres isora* methanolic fruit extract. It seems to have a preventive potential against Cisplatin-induced mutagenic effect in rat bone marrow cells.

KEYWORDS: *Helicteres isora*, Mutagenicity, Micronucleus, Bone marrow, Cisplatin, chromosomal abbreviation.

INTRODUCTION

Cisplatin (*cis*-diamminedichloroplatinum II) is broadly used as an antineoplastic agent for the handling of numerous solid tumors, including cancers of the lung, ovary, bladder, testis, head and neck, cervix and endometrium.[1] Cisplatin show excellent anticancer action, but clinical use of cisplatin is frequently restricted by its adverse side effects, such as hepatotoxicity and severe nephrotoxicity.[2,3] Although cisplatin is a highly effective chemotherapeutic agent for many types of cancer, one concern regarding its use is that its genome destabilizing effect may cause somatic mutations in tumor cells that potentially result in resistance to other drugs or unfavorably alter other characteristics of the malignancy.[4] In somatic cells the mutations not only involved in the carcinogenesis process, but also have role in the pathogenesis of other chronic degenerative diseases, such as heart diseases and atherosclerosis, which are the main reasons of death in the human population.[5] One of the most desire ways to decrease the effect of mutagens and carcinogens is to recognize the anticlastogens, antimutagens and desmutagens in our diets and increasing their use. Nature has granted us with many medicinal plants. There is a necessity to discover them for practice as antimutagenic and anticarcinogenic food or drug additives.[6] *Helicteres isora* Linn belongs to the family Sterculiaceae. It is commonly found in India from Jammu eastwards to Nepal, Bihar, Central, Western and Southern India, West Bengal and the Andaman islands. Due to screw like look of its fruit it is commonly known as Marodphali, Enthani, Marorphali, *etc*. It is a medium size tree attaining a maximum high of 5m.[7] It is shown in Literature that *Helicteres isora* plant shows antioxidant, anti-inflammatory, antipyretic and antispasmodic activities.[8-9] The stem barks and root of *Helicteres isora* are considered to be astringent, expectorant, demulcent and anti-galactagogue and are beneficial in colic, scabies, empyema, gastropathy, diabetes, diarrhea and dysentery.[10] The fruits are demulcent, constipating, astringent, acrid, vulnerary, refrigerant, stomachic, vermifuge, haemostatic and urinary astringent. They are valuable in vitiated situations of pitta ophthalmitis, colic, flatulence,

diarrhea, dysentery, wounds, ulcers, hemorrhages, epistaxis and diabetes.[11]

MATERIAL AND METHODS

Helicteres isora fruits were collected from central India The specimen was identified and confirmed by Dr. Zia ul Hassan HOD, Department of Botany Saifia Science collage, Bhopal. India. (Authentication No 407/Bot/Saif/16)

Extraction

The fruits of *Helicteres isora* were shade dried at room temperature, grinded into fine powered was exhaustively defatted with petroleum ether (60-80°C) and successively extracted with methanol (MeOH) using the soxhlet apparatus for 6 hours.[12]

Animals

Wistar rats (150-200 g) were provided by animal house PBRI, Bhopal, India. The animals were housed in standard conditions of temperature (25±2^{0}C) & 12 hr light-dark cycle. The rats were fed with commercial diet and water *ad Libitum*. The animal experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC), Pinnacle Biomedical Research Institute (PBRI), Bhopal, (M.P) as per CPCSEA guidelines (1824/PO/ERe/S/15/CPCSEA).

Micronucleus Assay

In micronucleus assay, 24 male Wistar rats at age 6-7-wk. old weighing 150-200 g were divided into 4 groups (6 rats per group), the extract at the volume doses level such as 200, 400 mg/kg body weight was injected 24 hours before the treatment of cisplatin, to 12 animals. The positive control group received single i. p. injection of 6 mg/kg cisplatin in 0.9% saline. The animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as described by Schmid (1975) and modified by Aron *et al.* (1980). After staining with May-Gruenwald and Giemsa, a total 1000 cells were scored at the magnification of x1000 (100 x 10x) for each group. The data are expressed as the average number of micronucleated cells/thousand polychromatied erythrocytes cells (PCE) cells/animals (±SE) for a group of six animals.[13.14] The results were compared with the vehicle treated and positive control group using Student's't' test with significance determined at $p<0.05$.

Chromosomal aberrations assay

For chromosomal aberrations 24 Wistar rats were divided into 4 groups for each assay the extract at the volume doses level such as 200 and 400 mg/kg body weight was injected 24 hours before the treatment of cisplatin, to 12 animals. The positive control group received single i. p. injection of 6 mg/kg cisplatin in 0.9% saline and a single treatment of colchicine 5 mg/kg. The animals were sacrificed by cervical dislocation, animal dissected and femur bone was excised. Bone marrow was aspirated by flushing with normal saline in the centrifuge tube. Flush the suspension in the tube properly to get good cell suspension. Centrifuged for 10 min at 1000 rpm. Supernatant discarded. Pellet was treated with pre-warmed (37ºC) KCl on cyclomixer. Left above suspension in a water bath (37ºC) for 20 min. Centrifuged and supernatant discarded. Pellet was treated with freshly prepared Cornoy's fixative on cyclomixer. Centrifuged and supernatant discarded. Above step of treatment with Cornoy's fixative was repeated 3 times to get debris free white pellet. To pellet added Cornoy's fixative (quantity sufficient) to get a good cell suspension. Slides were made with Air Drop Method. Stained (Giemsa's -3 min, Methanol-3 min and DDW- 1 Dip) and observed under microscope in 40X10 xs and then in 100X10x magnifications. No. of cells having aberration and the particular aberrations were scored (Total 100 cells were counted).[15]

RESULTS

Cisplatin treatment group showed a significant increase in the number of chromosomal aberrations and micronucleus formation when compared to vehicle groups. Detailed information related to the micronucleus frequencies and chromosomal aberrations was shown in table 1 and table 2. The highest number of frequencies was observed in rat treated with only cisplatin and least micronucleus frequencies and chromosomal aberrations were observed in 400mg/kg dose group of extract. The animals treated with different doses of *Helicteres Isora* extract, i.e. 200 mg/kg and 400 mg/kg showed a significant reduction ($p<0.05$) in the frequencies of micronucleus as well as chromosomal aberrations as compared to cisplatin group.

The frequencies decrease with an increase in the dose of *Helicteres Isora* extract. There was a significant dose-effect relationship between chromosomal aberrations, micronucleus frequencies and dose of *Helicteres Isora* extract. There was a statically significant difference between cisplatin group and the different dose groups. 200 mg/kg and 400 mg/kg dose group has significant less chromosomal aberrations and micronucleus frequencies when compare to the cisplatin treated group.

Table 1: Frequencies of micro-nucleated polychromatic erythrocytes MNPCEs and PCE/ NCE ratio in rat bone marrow after oral administration of 200 mg/kg and 400 mg/kg of *Helicteres isora* methanolic extract.

Group	MNPCE ± S.E.M		PCE/NCE± S.E.M
Vehicle	3.50±0.548		2.90±0.217
Cisplatin	31.00±4.195		1.23±0.495
200mg/kg	21.17±5.193*		1.98±0.340*
400mg/kg	11.33±2.006*		2.27±0.263*

MNPCEs = Micronucleated Polychromatic Erythrocytes PCE = Polychromatic Erythrocyte. All data presented in Mean ± SD (n=6), * - P<0.05 as compared to cisplatin treated group.

Table 2. Frequencies of chromosomal abbreviation in rat bone marrow after oral administration of 200 mg/kg and 400mg/kg of *Helicteres isora* extract.

Group	Fragment	Break	Ring	Deletion	Dicentric	Polypoidy	%total aberration
Vehicle	2.17±1.329	1±1.095	1±1.095	3.67±0.816	2.33±0.816	0±0.000	4.33±0.816
Cisplatin	27±4.858	24.67±3.502	13.33±2.066	31.33±1.633	4.33±1.506	4.00±1.265	46.33±4.803
200 mg/kg	25.33±4.320*	19.67±5.854*	10.67±3.266*	24.00±5.367*	2.00±1.265*	0.00±0.000*	21.00±3.521*
400mg/kg	21.00±4.858*	15.00±4.858*	6.33±2.658*	20.67±3.502*	2.33±0.816*	0.00±0.00*	17.33±1.633*

Above mention results shows protective nature of Helicteres isora at 200mg/kg and 400 mg/kg against cisplatin in rat bone marrow. All data presented in Mean ± SD (n=6), * - P<0.05 as compared to cisplatin treated group.

DISCUSSION

Cisplatin is a cytotoxic drug used against several types of cancers including Lung, Head and Neck and Testicular cancer, etc. The anticancer drug cisplatin is one of the most active cytotoxic agents in the treatment of cancer, and its positive action is dose dependent. However, its use is limited primarily by its nephrotoxicity.[16] In addition, other fewer toxic effects, such as hepatotoxicity, ototoxicity and neuropathy, can occur and harmfully affect patients given high doses of cisplatin.[17] Cisplatin brings mitochondrial dysfunctions, particularly inhibition of the electron transfer system, resulting in higher production of superoxide anions, hydrogen peroxide and hydroxyl radicals.[18] The micronucleus test and chromosomal aberration tests from the rat bone marrow cells are the most suitable genotoxicity tests. In the present study the cytoprotective activity of *Helicteres isora was* evaluated by measuring their inhibitory effect on cisplatin induced mutagenesis. It is indicated in the results, cisplatin induced chromosomal damage in mouse bone marrow cells. These fragmented chromosomes were condensed to form micronuclei which are not comprised in the main nucleus.[19] *Helicteres isora* decreased the cisplatin induced formation of micronuclei in MNPCEs and PCE/NCE, which may be due to the inhibition of cisplatin induced chromosomal damage. In the chromosomal aberration test, there was a significant, time dependent rise in the total no. of chromosomal aberrations, of cisplatin treated animals, when compared with normal vehicle animals. *Helicteres isora* significantly inhibits the cisplatin induced chromosomal aberrations and micronucleus formation, which may be due to inhibition of cisplatin induced toxicity. *Helicteres isora* possess cytoprotective activity against cisplatin induced toxicity.

CONCLUSION

The present cytoprotective study indicated that a Methanolic extract of *Helicteres isora* fruit may have good cytoprotective activity against cisplatin induced toxicity. There was a significant dose-effect relationship between chromosomal aberrations, micronucleus frequencies and dose of *Helicteres isora* methanolic fruit extract. It seems to have a preventive potential against Cisplatin-induced toxic effect in rat bone marrow cells. This finding, therefore, confirmed the health benefits of *Helicteres isora* fruit as a medicinal plant to reduce toxicity.

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