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IN VIVO PROTECTIVE EFFECTS OF *MURRAYA KOENIGII* LEAF EXTRACT AGAINST CYCLOPHOSPHAMIDE INDUCED MICRONUCLEI IN BONE MARROW CELLS OF MICE

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ABSTRACT

In the present study the genetic protective nature of *Murraya koenigii* leaf extract were carried out in Cyclophosphamide CP) induced micronuclei in polychromatic erythrocytes of mice. Two experiments were conducted in Swiss mice. In one experiment the animal were administered with various doses of curry leaves as 100mg/kg, 200mg/kg and 400mg/kg to assess the anti-mutagenic nature of leaf extract in another experiment the priming the animals with two doses of curry leaf extract and intraperitoneal injection of CP 50mg/kg to experimental animal. all animals were sacrificed after24 hours and slides prepared and screened for the presence of micronuclei in bone marrow cells of mice. when given curry leaf extract the percentage of micronuclei were lower no significant incidence was observed however when primed with curry leaf extract in CP induced genetic damage there was a reduction was noted indicating protective nature of leaf extract. Hence the data clearly indicate use of curry leaf extract is essential in chemotherapy regimen.

KEYWORDS: *Murraya koenigii* leaf extract, Cyclophosphamide, micronuclei, mice.

INTRODUCTION

A major problem associated with cancer chemotherapy is the severe side effects resulting from normal tissue damage. Consequently, agents which protect normal tissues against chemotherapy can increase the patient tolerance to chemotherapy.[1] Several chemicals have been found to provide good chemical protection in experimental animals, but their clinical utility is limited by the drug toxicity on repeated administration1. The only drug approved for

clinical use in cancer therapy patients is amifostine, a synthetic phosphorothioate compound, which also produces side effects of its own, like nausea, vomiting and hypotension.[2,3] Moreover amifostine is very expensive. Therefore, there is a need to find nontoxic and inexpensive drugs for clinical chemoprotection.[4-7] Recent studies have indicated that some of the commonly used medicinal plants may be good sources of potent but nontoxic chemo protective effects. *Murraya koenigii* (family–rutaceae, Eng- curry leaf tree, Hindi- metha neem, Sanskrit – mahanimb) has been an ingredient of Indian diet since several centuries. Its constituents have been shown to possess antioxidant properties, antidiabetic[8], antifungal, antibacterial and used internally in dysentery and diarrhea and also for checking vomiting. The juice of the plant is taken to relieve pain associated with kidney.[9] The anti-oxidant potential of curry leaves in rats treated with chemical carcinogen, dimethyl hydrazine hydrochloride has been investigated.[10] Hence studies were carried out to evaluate the anti-mutagenic potential of curry leaf extract in Cyclophosphamide induced micronuclei in bone marrow cells of mice using micronucleus test.

MATERIALS AND METHODS

Preparation of Methanolic Extract: Collection of plants – The fresh leaves of *Murraya koenigii* were collected from the region of Madhya Pradesh (Bhopal) in the month of February and were identified by Botanist Professor prof pratiba devi. Fresh leaves were washed under tap water and shade dried and powdered. 50% methanolic extract of the powder (100gm) was prepared with the help of cold maceration. And was allowed to stand at room temperature for about 18 hrs. after shaking frequently for 6 hrs. the filterate was collected.[11] This process of extraction was repeated for three times. The combined extract was filtered and concentrated under vacuum using SC110A Speed Vac plus at 40C. The extractive value of extract obtained was14.94398%w/w.

ANIMAL MAINTENCE

The study was conducted on random 6-7 weeks old and 24- 28 gm body weight male Swiss albino mice. They were maintained under controlled conditions of temperature and lightTable-1 and the photographs of micronuclei are shown (light: dark, 12 hrs: 12 hrs).

They were provided standard mice feed and water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC, Ref. No. 2157/225/2006).

MICRONUCLUES TEST: For micronucleus test, three doses of 100, 200 and 400mg/kg body weight was administered. MKL extract were dissolved in double distilled water and administered to mouse 24 hours prior to CP administration. The animals were scarified 6 hrs after the last administration, bone marrow preparations were made by an air drying technique and stained with May Grunewald and Giemsa stains according to the method described by Schimid.[12] For each animal 2000 polychromatic erythrocytes (RBC) and corresponding normochromatic RBC were scored for the presence of micronuclei the appearance of micronuclei in polychromatic erythrocytes was used as an indicator of genetic damage. The ratio of polychromatic to normochromatic RBC was utilized to estimate the effect on the proliferative activity of bone marrow. The data obtained from these studies were analyzed using t-test.

RESULS

The results on the frequency of micronuclei in control was 0.25% and the values were 0.30%, 0.35% and 0.32% after the administration of, 100and200mg/kg 400 *Murraya koenigii* leaf extract respectively (Table-1). Hence, the results clearly indicate the non-mutagenic nature of MKL extract in bone marrow cells of mice. In Cyclophosphamide treated group, there was a significant increase in the percentage of micronuclei (1.50) in bone marrow cells of mice when compared to control –II value (0.20) (Table-II). However the frequency of micronuclei is decreased to 0.85%, 0.75% and 0.95% after the co-administration of 100 and 200mg/kg 400mg/kg of MKL extract. The P/N ratio in bone marrow cells showed a decrease when compared with control values. The differences in the frequency of micronuclei in control and lead treated group were found to be significant ($P< 0.05$), Table-II. The frequency of inhibition was as 24.63, 36.23and 11.59 at primed of 100, 200 mg/kg treated groups however at primed 400mg/kg groups the percentage of inhibition observed was insignificant, thus the data clearly indicate the use of two doses i.e. 100 and 200mg/kg groups only.

Table 1: Frequencies of micronuclei in bone marrow erythrocytes of mice administered with various doses of *Murraya koenigii* leaf extract.

Groups Mg/kg	Micronuclei in polychromatic cells (P)	Micronuclei in normochromatic cells	Micronuclei in total P+N cells	P/N ratio
Control	20/8000 (0.25)	10/8200(0.12)	30/16200(0.18)	0.97
100MKL	24/8000(0.30)	16/9600(0.16)	40/17600(0.24)	0.83
200MKL	28/8000(0.35)	18/9800(0.18)	42/17800(0.25)	0.81
400MKL	26/8000(0.32)	12/8400(0.14)	38/16400(0.23)	0.95

The values in the parenthesis are percentages.

The $P>0.05$ level, hence the difference is considered to be statistically insignificant.

Table 2: Frequency of micronuclei in bone marrow erythrcytes of mice treated with Cyclophosphamide primed with *Murraya koenigii* leaf extract.

Group	Dose/ treatment	Micronuclei in polychromeatic cells (P)	Micronuclei in normochromatic cells (N)	Micronuclei in total P+N cells	P/N ratio	% inhibition
1	control	16/8000(0.20)	10/8010(0.13)	26/16020(0.16)	0.98	
2	50CP	85/8000(1.50)	44/8800(0.50)	129/16800(0.76)	0.90	
3	100 Mk+50CP	68/8000(0.85)	20/8906(0.22)	88/16906(0.52)	0.89	24.63
4	200Mk+50CP	60/8000(0.75)	24/9100(0.26)	84/17100(0.49)	0.87	36.23
5	400Mk+50CP	76/8000(0.95)	20/8282(0.24)	96/16282(0.58)	0.96	11.59

The values in parentheses are percentage

**denotes statistically significant as compared to control group at $P < 0.01$ level

*denotes statistically significant as compared to group II at $P < 0.05$ level

DISCUSSION

The invivo micronucleus test is one of best methods to screen the clastogenic effects of chemicals and drugs. using this procedure the mutagenicity of various alkylating agent drugs.[13-16] was also established the CP at 50 mg/kg showed significant increase in percentage of micronuclei in bone marrow cells of mice. Hence present results are comparable with that of Asita *et al.*[17] who investigated the intraperitoneal injection of mice with a single dose of 40 mg/kg body weight of Cyclophosphamide induced a significant increase in the frequency of MNPCE, 24 hr after injection, when compared with animals that received water treatment. The present results are comparable to Santos Renato *et al.*,[18] who reported that Cyclophosphamide at 135mg/kg dose induced a significant increase in the frequency of micronuclei in polychromatic erythrocytes of male mice M.koenigii leaf extract. The data clearly show that a two doses of 100 mg/kg and 200mg/kg of ME (M.koenigii) before CP (50mg/kg b.wt.) administered intraperitoneally can significantly decrease the cyclophosphamide induced micronuclei in bone marrow cells of mice damage. Administration of the MKL further enhanced the bone marrow protection, as indicated by the significant reduction in polychromatic and normochromatic erythrocytes bearing micronuclei at 24 hr. after CP (administered intraperitoneally) compared to MKL treatment. The chemo protective effect of several natural products has been associated with their antioxidant property. Earlier studies from other laboratories have shown that M.koenigii possesses antioxidant activities. This may have a role in the protective effect of ME against and CP clastogenecity, evident in the reduced micronuclei in the bone marrow cells.[19] The present results can be comparable with earlier study of Rudramadevi *et al.*, MKL showed protection

at all doses tested in Adriamycin induced micronuclei in bone marrow cells of mice. Several reports showed chemo protective nature of MKL cancer cell lines.[20] M.koenigii possesses potential secondary metabolites that could be developed as anti-cancer agents. In one study, the cytotoxic activity was evaluated for three extracts: hexane, ethylacetate, andmethanol of M. koenigii leaves against the HeLa cell line. The extracts were reported as being potently cytotoxic in nature in HeLa cancer cells. These results established the potential of M. koenigii as an anticancer agent in vitro.[21] Additional evidence for the anticancer activity of M. koenigii has been obtained from rodent cancer cell lines, as well as different in vivo cancer models.[22-25, 26, 27] In an early study, histopathological evidence showed that M. koenigii extract treatment generated a decline in neoplasms in the colon.[28] The anticancer activity of mahanine and isomahanine in human oral squamous cell carcinoma CLS-354 has also been reported.[29] Natural antioxidants from plant sources have been considered a promising therapy for the prevention and treatment of these diseases, especially neuro degenerative disorders, cardiovascular diseases, cancer, and other conditions. Various natural bioactive compounds, such as mahanine, mahanimbine, isolongifolene, koenimbine, Isomahanine, koenoline and –methyl murrayamine, are present in M.koenigii and exhibit remarkable antioxidant properties.[29] The results can be comparable with our work the protective effect of ascorbic acid, garlic extract and *Solanum lycopersicum* fruit extract against chemicals induced cytotoxicity in bone marrow cells of mice.[30,31]

CONCLUSIONS

Thus, the present study demonstrates that nontoxic doses of an extract of the leaves of M. koenigii protect bone marrow chromosomes exposed to cyclophosphamide. Murraya leaves have been reported to contain the antioxidants like Vit. A. and other constituent may be responsible for the chemoprotective properties of the extract. As Murraya leaves are used as flavors to the preparation and as a spice in different curries and is freely available in India, it is worthwhile to conduct detailed studies order to explorer the full potential of this plant in human cancer chemotherapy stragy for public health interest further studies using this leafy extract with using different protocols are in pgress.

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