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

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ABSTRACT

The present investigation was carried out to evaluate the protective effects of *Murayya koenigii* leaf extract against cyclophosphamide induced genotoxicity in bone marrow cells of mice. The administration of *Murraya koenigii* leaf extract (MKL) extract at various doses i.e. 100, 200and 400mg /kg. When treated individually did not induce chromosomal aberrations in somatic cells of mice in 24hrs. A single Intraperitoneal administration of 50mg/kg of cyclophosphamide induced significant increase in the percentage of CAS insomatic cells of mice. However after co administration of three doses of MKL extract there was a dose dependent decrease in the % of CAS was observed. When animals were administered with MKL 100, 200 and 400mg/kg/bw orally for seven days prioer to Cyclophasrimide (50mg/kg/bw) was given intraperitoneally as a single dose. For each experimental group control, animals were maintained. 24hrs after the administration of the last dose, the animals were sacrificed and air dried metaphase preparations were made and processed for identification of chromosomal aberrations in somatic cells of mice. An increase in the percentage of Chromosomal aberrations was observed after 50mg/kg of Cyclophosphamide in treatment. But when animals primed with MKL, there was a decrease in the frequency of chromosomal aberrations. Thus the results clearly indicated the protective role of MKL on Cyclophosphamide induced genotoxic damage in somatic cells of mice.

KEYWORDS

Cyclophosphamide, *Murraya koenigii* leaf extract, Chromosomal aberrations and Somatic cells.

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INTRODUCTON

There are plenty of antineoplastic medicines are in common use to combat more than a few kinds of malignancies. These anticancer drugs such as cycloxan Cyclophosphamide, Cytoxan, Tamoxifen, Cisplatin, Paclitxel and Gemcitabine cyclophosphane have been studied their structure

exclusively mutagenic nature and revealed that their chemical structure for the source for cytotoxicity and genotoxicity. These drugs have expressing clastogenic outcomes in a number of test systems. Potential genetic damage due to drugs and other chemicals is well recognized. Extensive studies have been carried out on mutagenicity of various drugs in microorganisms, insects, and mammals and in exposed population[1-3]. Cyclophosphamide (CP) is a nitrogen mustard alkylating agent from the oxazophorines group. It is used to treat Hodgkin's disease, lymphomas, leukemia, Wegener's granulomatosis, severe rheumatoid arthritis, and lupus erythematosus[4-6]. It is also used in combination with other drugs to treat breast cancer, leukemia, and ovarian cancer. In spite of CP therapeutic importance, a wide range of adverse effects were recorded. Sweetman[7] reported many side effects; including hemorrhagic cystitis, alopecia and hyperpigmentation of skin may develop after high or prolonged dosages and can be life-threatening.

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], anti-fungal, antibacterial and used internally in dysentery and diarrhea and also for checking vomiting. The MKL extract can be used to treat different renal ailments[9]. The anti-oxidant potential of curry leaves in rats treated with chemical carcinogen, dimethyl hydrazine hydrochloride has been investigated[10]. Several reports have shown the curry leaf extract anti diabetic[11] anti-inflammatory neuroprotective[12-14] effects. However, no study has been reported on its chemo protective effect. The present investigation was undertaken to study the protective property, of curry leaf extract in CP induced chromosomal damage in somatic cells of mice.

MATERIAL AND METHODS

Animals

The study was conducted after taking the approval of Institutional Ethical Committee on twenty adult male Swiss albino mice 30 to 50 days old and weighing around to 30 to 40g were maintained in plastic cages under controlled lighting conditions (12:12 light and dark cycle) relative humidity (50±5%) and temperature (37±2ºC) fed with mice feed and were given ad libitium access to water. The experimental animals were administered with Cyclophosphamide and primed with various doses of MKL extract.

Dosage schedule

In the present study two experiments were conducted. The animals were fed orally with cyclophosphamide and MKL extract and categorized in to following groups Group I: controls Group II: MKL extract 100mg/kg Group III: MKL extract 200mg/kg Group IV: MKL extract 400mg.

For priming experiment 5 groups were treated as follows.

Group I: Controls

Group II: Cyclophosphamide 50mg/kg

Group III: MKL extract 100mg/kg + Cyclophosphamide 50mg/kg

Group IV: MKL extract 200mg/kg + Cyclophosphamide 50mg/kg

Group V: MKL extract 400mg/kg + Cyclophosphamide 50mg/k

Analysis chromosomal aberrations in somatic cells of mice

The animals were killed two days after administration of the last dose. The bone marrow was flushed into clean glass Petri dishes with hypertonic solution (0.56% KCl) were used to get a homogeneous cell suspension. It was then collected in clean centrifuge tubes and incubated at 37ºC for 45 minutes. Four slides for each were prepared from control and experimental animals. The staining was done within 24 h of preparation according to the method Preston *et al*[15]. The slides were screened for 50 well spread metaphases per animal for the presence of various types of chromosomal aberrations like gaps breaks, fragment, chromatid separations and polyploids in control and treated group of animals. The differences in the frequencies of chromosomal aberrations between control and treated groups were analyzed using Chi-square test. For calculating mitotic index (MI) a minimum of 1000 cells were counted for each animal results selected for *Murraya koenigii* extract were 100 200 and 400mg/kg body weight at 24hr time intervals. The mutagenic effects of the extract were studied on

somatic cells of mice for different time intervals. The results were recorded (Table No.1).

RESULTS AND DISCUSSION

At 24hrs the percentage of chromosomal aberrations for 100, 200 and 400 mg/kg body weights of fruit extract in the treated groups recorded were 3.33, 3.66 and 3.00% respectively when compared with that of controls 2.0% (Table No.1). The differences in the frequencies of chromosomal aberrations between controls and MKL treated mice for 24 were analyzed by X2 test and the results were found to be insignificant (P>0.05, Table No.1).

At 24 hrs of the study the controls have shown 2.4% of the chromosomal abnormalities when 50mg/kg body weight of cyclophosphamide was recorded (Table No.2). The uprimed mice with cyclophosphamide have shown the chromosomal aberrations were 17.33% respectively. The highest dose has shown maximum abnormal metaphases. Priming with 200mg/kg body weight of MKL, the effect has decreased. There was decrease observed and the aberrations were 11.33% respectively. Similarly with 200mg/kg body weight the recorded values were 13.33.6% and with 600mg/kg body weight there was a decrease for 50mg/kg body weight of cyclophosphamide with 15.33% respectively at 24 hrs (Table No.2). The differences in the frequencies of the chromosomal aberrations were analyzed by X2 test and the results observed were found to be significant (P<0.01, Table No.2).

Discussion

The actively proliferating cells from bone marrow provide maximum information on the effect of any test compound. The transition from proerythroblast to erythrocytes takes about seven cell division cycles. Each cell cycle takes 10-11 hrs and the terminal mitosis completed in about 10hrs before the transition of orthochromatid erythroblast to polychromatic erythrocytes. In view of the above to see the long and short term effect of test compound on cells, the sampling time ranged was from 6-72 hrs has taken in present observation. There are different type of chromosomal aberrations observed in present analysis. These aberration are classified into structural, numerical and other abnormalities. Structural aberration includes gaps, breaks, fragments, terminal deletion and centric fusion these end points serve as indicators for assessing the mutagenic effect of test substance the CP at 50mg/kg showed significant increase in percentage of micronuclei in bone marrow cells of mice. Rudramadevi and keasavrao[16] reported a signicant increase in the frequency of chromosomal abberrations 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 40mg/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 two doses of 100mg/kg and 200mg/kg of MKL (*M. koenigii*) before CP (50mg/kg b.wt.) administered intraperitoneally can significantly decrease the cyclophosphamide induced chromosomal aberrations 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. 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[19]. Several reports showed chemo protective nature of MKL cancer celllines[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, and methanol 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[22-27]. The medicinal properties of M. koenigii have been accredited to sever a chemical constituents of different carbazole alkaloids and other important metabolites, like terpenoids, flavonoids, phenolics, carbohydrates, carotenoids, vitamins, and nicotinic acid from different parts of the M. koenigii plant.orted[28]. 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[29,30]. The results of present study it indicate protective nature of MKL in cyclophosphamide induced chromosomal aberration in animals. Such protective nature of other plant extracts such as *Phylthanthus emblica, Garlic extract, Solanum lycopersiun, Curcumin extract* against anti-cancer drug has been reported in our lab[31-33].

Table No.1: Frequency of Chromosomal aberrations recorded in somatic cells of mice with various doses of *Murraya koenigii* extract

S.No	Treatment	Normal metaphases scores	Abnormal metaphases scores
1	Control	294(98.00)	6 (2.00)
2	100mg/kg MKL	290(96.60)	10 (3.33)
3	200mg/kg MKL	289(96.44)	11 (3.66)
4	400mg/kg MKL	291(97.00)	9 (3.00)

The values in parentheses are percentages

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

Table No.2: Frequency of Chromosomal aberrations recorded in somatic cells of mice treated with Cyclophosphamide and primed with *Murraya koenigii* extract

S.No	Dose	Normal metaphases	Abnormal metaphases	Inhibition
1	Control	292(96.33)	8(2.66)	
2	Cp 50mg/kg	248(83.66)	52(17.33)	
3	100mg/kg MKL + 50mg/kg CP	266(89.34)	34(11.33)*	40.10*
4	200mg/kg MKL + 50mg/kg CP	260(88.66)	40(13.33)*	31.81*
5	400mg/kg MKL + 50mg/kg CP	254(86.77)	46(15.33)	15.22

The values is parentheses are percentages

The *p<0.05 level, hence the difference is considered to be statistically significant

CONCLUSION

From the above studies, it is concluded that *Murrayya koenigii* leaf extract was a potential candidate as protective agent to cyclophosphamide induced genotoxic effect in somatic cells of mice. The combined treatment of cyclophosphamide and GBE holds a promise as a safe and effective chemotherapeutic strategy.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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