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Protective Role of *Murraya Koenigii* Leaf Extract on Adriamycin Induced Micronuclei in Mice Bone Marrow Erythrocytes

K. Rudrama Devi*, Minny Jael .P and Kusumlatha. C

Human Genetics and Toxicology Lab, Dept of Zoology, Osmania University, Hyderabad-7,India.

Corres. Author: rudramadevi_k@yahoo.com

Abstract: The present study was under taken to observe the chemoprotective effect of *Murraya Koenigii* leaves methanolic extract against adriamycin induced cytogenetic damage in bone marrow erythrocytes of mice. Two experiments were conducted. In the first experiment animals were fed with 120,240,480mg/kg body weight of *Murraya Koenigii* extracts for 7 day orally. *Murraya Koenigii Leaf* extracts (MKL) treatment has not showed any mutagenicity indicated that the extracts are non mutagenic. In the second experiment, four groups of animals were maintained. When *Murraya Koenigii* leaf extract pretreated and concurrent administration of adreamycin 16mg/kg single dose intraperitonially reduced the frequency of micronuclei in polychromatic erythrocytes when compared with adriamycin induced significant increase of micronuclei was observed. The P/N ratio was also reached to normalicy in pretreated MKL extract group of animals. Thus the results clearly indicate administration of MKL extract gave a significant protection in adriamycin genotoxicity and it is positive cytotoxic modulator of chemotherapeutic strategy.

Key Words: Murraya Koenigii Leaf Extract, adriamycin, Micronuclei, Protection.

Introduction

Antitumor agents are used for common therapy against many of human cancer. However as with many drugs that have mammalian toxicity as a target, physiological side effects can occur and genotoxic effect rise to secondary tumors [1]. The anthracyclin antibiotic adriamycin (doxorubin) is one of most effective chemotherapeutic agents against a wide variety of cancers. The tumor that respond better breast and esophageal, carcinomas, asteosarcoma, soft tissue sarcomas, hodgkins and non-hodgkin lymphoma. Because of its beneficial effects it is used as gastric cancer, bile duct pancreatic and endometrial carcinomas [2].

Doxorubin induces mutations and chromosomal aberrations in normal and tumor cells [3]. It has be proposed the capacity of doxorubin to inhibit DNA synthesis as a result of mode of action. Deoxyrubin has a high affinity of cell nuclei and about 60% of total intracellular of deoxryrubin is found in cell nucleus. It binds to DNA polymerase and inhibits nucleic acid synthesis, responsible for formation of protein – linked DNA double strand breaks[4]. Further cellular enzymes are capable of converting doxyrubin into free radical metabolites. For treatment of many types of

cancer, Adriamycin is used in chemotherapy, it is important to reduce its toxicity to normal cells a goal can be achieved by concurrent administration of free radical scavenging agents such as antioxidants [5].Further the consumption of frutits and vegetables can minimize to some extent the occurrence of some cancers [6].

Murraya Koenigii family – rutaceae. (English; curry leaf tree, hindi:metha tree neem, Sanskrit nahnib) has been used in Indian recipe preparation since several centuries . It posses antioxidant properties. Further this plant had shown to relieve the pain in kidney, diarrhea, anti diabetic antifungal anti bacterial [7-12]. Animals administered with dimethyl hydrazine hydrochloride has been studied. So far no studies are reported about its chemopreventive effects in animals. Hence in the present investigation a study was undertaken to study the chemoprotective activity against cisplatin induced micronuclei in bone marrow erythrocytes of mice.

Materials and Methods

Chemicals

Adriamycin kindly provided by Director, MNJ Institute of oncology and mytomycin from biochem pharma limited. The chemicals used in the study are purchased from Ranboxy Laboratories Hyderabad.

Animals

Six to eight weeks old male mice (*Mus Musculus*) of swiss albino mice weighing about 25-27 gms procured from National Institute of Nutrition, Hyderabad, were used in this study. The mice were housed in poly propylene cages in a well ventilated room and were provided with standard pellet diet(M/S Lipton India limited) and water adlabitum.

Collection of plant

The fresh leaves of *Murraya Koenigi* were collected from the local market and identified by Professor M. Pratiba Devi of Botany Department fresh leaves were washed under tap water and shade dried and powdered. 50% of methanolic extract of the powder (500gms) was prepared with the help of cold maceration, at room temperature for about 20 hrs shaking frequently. The extracts were filtered and concentrated with vacuum rotovapour at 4^{o}c. The value of extract obtained was86.321% w/w on dry basis.

Dosage schedule

Two experiments were conducted. In the first experiment four groups were maintained to study whether the plant extract is toxic or not in bone marrow cells. Hence the group I received control saline where as group II, group III & group IV were orally administered with doses of 120mg /kg/bw, 240mg/kg and 480mg/kg/wt of MKL extract for seven days.

In the experimental groups the groupI as only control vehicle group II animals treated with 480 mg/kg/bw methanolic extract of MKL for 7 days. Group III is Adriamycin 16mg/kg single dose intraperitonially. Group IV *Murraya Koenigi* leaf treated (480mg/kg /bW) pre treated Adriamycin + 16mg/kg intraperitonially one day prior to last treatment.

Micronucleus test

All the animals were killed after twenty four of last treatment and bone marrow preparations were made. The control and experiment groups were killed by cervical dislocation femur bones were dissected out and cells were flushed with total bovine serum into tubes. Smears were fixed with methanol and stained with Giemsa. The slides were screened for the presence of micronuclei in polychromatic erythrocytes of bone marrow cells in control and experimental group of animals. A total of 2000 polychromatic erythrocytes were examined for each animal under 100 x magnifications [13]. Student paired t test was used to detect statistical significance among the different groups. 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 cells. The scoring was done separately for each animal and it was observed that there was no significant difference between individual animals of same group. The ratio of polychromatic to norm chromatic erythrocytes was used to estimate the effect on the proliferative activity of bone marrow cells

Results and Discussion

The micronucleus test is an effective method for the genotoxicity of environmental mutagens and carcinogens. Since micronuclei (MN) are formed during cell division due to lagging of acentric chromosomes, chromatid fragments are entire chromosome, that are not included in the main daughter nuclei during metaphase, anaphase cell division can produced micronuclei [14,15]. The most frequently used genotoxicity test in mammals is the micronucleus test which provides simple and rapid indirect measure of structural and numerical aberrations [16] and it can performed only in dividing

cells. A micronucleus is literally a small nucleus. The cell organelle contains the genetic material of fragmented DNA. During cell division the genetic material replicates divides between two daughter cells that are produced. If this process is disrupted, the chromosomes are broken or damaged by chemicals then the distribution of genetic material between the two daughter nuclei during cell division may be affected or formed new nuclei may be micronucleus clearly observed under microscope.

Adriamycin is a potent antitumor agents used for the treatment of many cancer. It is demonstrated that this drug has the potential for initiating genetic events in non-tumor cells in human and in animal systems. The results showed that adriamycin induced micronuclei in polychromatic erythrocytes male and female mice. The results are in agreement with other reports of Adriamycin cytotoxicity [17,18,19]. The biochemical mechanism of adriamycin causes cytotoxicity is unclear. However when it intercates with DNA generates free radicals. Two pathway of mechanisms has been proposed. Two different pathways of free radical formation of adriamycin have been described. First is formation of semiquinone free radical the semi quinine can be transferred to a C7 radical that can also mediate cellular damage. The reduction of doxorubicin by 2 electrons generates a secondary alcohol metabolite doxorubicinol. The second pathway doxorubicin free radicals come from an enzymatic mechanism that involves reactions with iron. For example Fe3+reacts with doxorubicin in a redox reaction after which the iron atom accepts an electron and a Fe2+ deoxyribicin free radical complex is produced. This iron doxorubicin complex can reduce oxygen to hydrogen peroxide and other active species [20-21].

Table: 1 Frequency of micronuclei in bone marrow erythrocytes of mice treated with MLE extract.

Dose Groups	MN in poly chromatic cells	MN in Normochromatic cells	Micronuclei in total cells	P/N ratio
Control	26/12080 (0.21)	12/13106 (0.09)	38/25180 (0.15)	0.94
120mg/kg	28/12610* (0.22)	15/13160 (0.11)	43/25820 (0.19)	0.93
240 mg/kg	30/12240* (0.24)	18/13110 (0.13)	48/25550 (0.18)	0.92
480mg/kg	30/12306* (0.24)	16/12660 (0.01)	46/25366 (0.18)	0.97

*P>0.05

Table: 2 Protective effects of Micronucleus leaf extract on Adriamycin induced micronuclei in bone marrow erythrocytes of mice.

Dose Groups	MN in polychromatic cells	MN in normochromatic cells	MN in total cells	P/N ratio	Protection (%)
GI .Control Saline	20/12110 (0.16)	10/12110 (0.08)	30/24220 (0.01)	1.00	-
GII. MLE 480mg.kg	26/12008 (0.21)	12/13006 (0.09)	38/25014 (0.15)	0.92	-
Group III Adriamycin 16mg/kg	292/12240***a** (2.40)	32/23100 (0.13)	324/35340 (0.91)	0.52	-
Group IV MLE 480mg/kg+16mg/kg	102/12400***b** (0.82)	60/14800 (0.35)	162/29200 (0.55)	0.83	34.16

*P<0.01

a- Denotes significance compared with the control group.

b- Denotes significance compared with ADR treated mice.

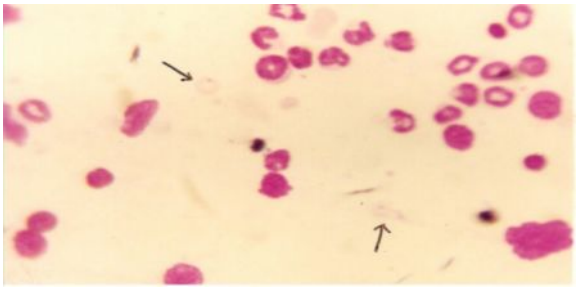

Fig. 1 The presence of micronucleus in adrimycin treated animals

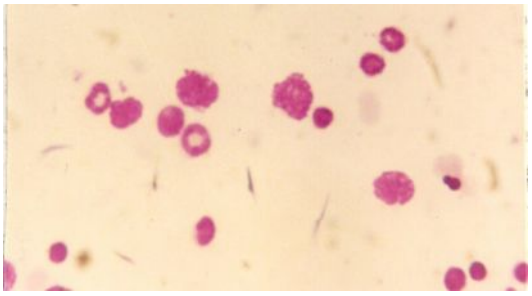

Fig. 2 The absence of micronucleus in MKL extract treated animals

The animals were treated with methanol extract of *Murraya Koenigii* of three doses showed a increase at all dose levels in polychromatic erythrocytes of mice. However the differences in the frequency of micronuclei between control and treated groups were insignificant (P>0.05) (table 1). The P/N ratio is not changed and the values were observed equal to the control values.

There was significant increase in the frequency of micronuclei from in control(0.18%)to Adriamycin treated groups(2.40%)(Fig.1). Whereas the pre treatment with the methanolic extract of *Murraya Koenigii* results showed a reduction in the induction of micronuclei when compared with Adriamycin alone(table 2). The P/N ratio was decreased in Adriamycin treated animals but concurrent administration of *Murraya Koenigii* leaf extract (MKL) brings the values to lower range (0.82%). This indicates the chemoprotective nature of the *Murraya Koenigii* leaves. It is used as flavours to curry and sambar preparation especially by south Indias and it is available economically cheaper. The difference in the frequency of micronuclei between the group III & Group IV showed statistically significant (P<0.01). Thus the data indicate MKL extract supplementation reduced the cytotoxicity induced by Adriamycin(fig. 2).

The *in vivo* micronucleus test is one of the best methods to screen the clastogenetic effects of chemicals and drugs [13]. Using this procedure the mutagenecity of various alkylating agents, pesticides and drugs in swiss albino male mice has been reported [22,23,24]. The results of the present study clearly demonstrates as chemoprotective property of *Murraya Koenigii* leaf extract. The column chromatography led to the isolation of 3 compounds SU-I, II, III from petroleum ether and chloroform extract. Thus compounds were found to have anti inflammatory property in rank order of (SU II 60% SU III 58.72% SU I 57.36%) [25]. Further these leaf extract improved the learning of aged mice in hypoxic condition at 300kg/mg and 500mg/kg of extract when pretreated for 15 days [26]. Further *Murraya Koenigii* stem bark extract in ether (SU-II) showed anti cancer activity. Whereas dry plant powder showed a compound showed significant antidiabetic activity in strepto$_3$tocin induced rats. The present results are comparable with earlier studies that *Murraya Koenigii* can significantly decrease the chromosomal damage caused by cyclophosmide. The acetone extract of the bark of Murraya Koenigii exhibited significant reduction in the ccl_4 induced hepatotoxicity as it reduced the SGOT, SGPT alkaline phosphates and total billirubin[27,28,29]. In literature there are no reports on antitumogenic and antimutagenic effects of MKL extract in mice. This is the first report indicating the protective role of MKL extract against adriamycin induced cytogenetic damage in bone marrow cells of mice.

It is well known that consumption of fruits and vegetables is associated and are known to prevent chromosomal and DNA damage in animals [30,31]. Usually antimutagens acting in rodents are active in human too[32]. Our results have a practical decline of genotoxic effects of cisplatin in cancer patients some health care workers as nurse and pharmaceutical plant workers handle this drug which may alternate the higher risks for development of secondary malignancy and for abnormal reproductive outcomes due to its antioxidant activity of Murraya Koenigie leaf extract.

Conclusion

From the above studies it is concluded that was potential candidate ***Murraya Koenigii* Leaf Extract** as protective agent against adriamycin induced genotoxic effect in somatic cells of mice. The combined treatment of adriamycin and MKLE extract holds a promise as a safe and effective chemotherapeutic strategy.

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