

Research Article

Antigenotoxic effect of *Murraya koenigii* towards cyclophosphamide induced cytogenetic damage in mouse bone marrow cells

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ABSTRACT

The chemoprotective activity of *Murraya koenigii* methanolic extract has been studied using swiss albino mice bone marrow as an *in vivo* model. The methanolic extract (ME) effectively prevented cyclophosphamide (CP) induced chromosomal aberration. Animals were injected (i.p.) with 100mg/kg body weight of 50% methanolic extract (ME) of *M. koenigii* as a single dose & exposed to cyclophosphamide (50mg/kg) body weight ½ hr later. Bone marrow protection was studied by scoring aberrations in metaphase chromosomes. No drug toxicity was observed at this dose (100 mg/kg) b.wt. The effectiveness of methanolic extract (ME) of *M. koenigii* when administered gave a significant protection against CP alone group.

Keywords: *Murraya koenigii*, Micronuclei, Methanolic extracts

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. 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 chemo protection. Recent studies have indicated that some of the commonly used medicinal plants may be good sources of potent but nontoxic chemoprotectors⁴⁻⁷. But research on the radio protective property of plant products has not received the attention it deserves. *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, antidiabetic⁸, antifungal, antibacterial and used internally in dysentery and diarrhoea 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 investigated¹⁰ However, no study has been reported on its chemoprotective effect. The present investigation was undertaken to study the chemo protective property, if any of *M. koenigii*.

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 Shaukat Ali, Safia College, Bhopal.

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 filtrate was collected¹¹. This process of extraction was repeated for three times. The combined extract was filtered and concentrated under vacuum using SC110A Speed Vac[®] plus at 4°C. The extractive value of extract obtained was 14.94398% w/w.

Animals: Swiss albino mice of either sex weighing 30-40gm were obtained from Jawahar Lal Nehru Cancer Hospital and Research Centre. Animals were kept in cages and in environmentally controlled room. (23± 3°C, 12hr light & dark cycle) with free access to water. Animals were fed with standard diet pellets *labium*. Animals were randomly allocated to different experimental groups, three or four mice were used for each groups.

Experimental protocols were approved by institutional ethical committee of JNCH & RC, Bhopal, which follow guidelines of CPCSEA (Committee for the Purpose of Control & Supervision of Experiments on Animals) that complies with international norms of INSA. Mode of treatment: Chemotherapy – An intraperitoneal (i.p.) injection of 50mg./kg cyclophosphamide IP (Dabar New Delhi) was administered alone as well as with extract.

Number of micronuclei in PCE in the bone marrow of mice treated with *Murraya koenigii* extract (ME) chemotherapy (Cyclophosphamide).

Sr.No.	Treatment	Number of Micronuclei MNPCE			Total number of MNPCE
		MN1	MN2	MN3	
1.	Normal	11	-	-	11
2.	Vehicle alone (CMC)	13	-	-	13
3.	Methanolic Extract alone (<i>M. koenigii</i>)	24	-	-	24
4.	Cyclophosphamide alone	496	29	13	593
5.	Cyclophosphamide + ME (<i>M. koenigii</i>)	472	25	3	533

MNPCE / 1000 PCE in the bone marrow of mice treated with *Murraya koenigii* extract (ME) before chemotherapy (Cyclophosphamide).

Sr.No.	Treatment	Percentage MNPCE (%)
1.	Normal	2.3 ± 0.057735
2.	Vehicle alone (CMC)	1.21212 ± 1.15 E – 005
3.	Methanolic Extract alone (<i>M. koenigii</i>)	5.5 ± 0.11547
4.	Cyclophosphamide Alone	136.3833 ± 1.340502 ^{b, f}
5.	Cyclophosphamide + ME (<i>M. koenigii</i>)	97.26667 ± 1.281059 ^{c, e, x}

a, x, d = p < 0.05; b, y, e = p < 0.01 c, z, f = p < 0.001
a, b, c is compared to DDW (control); x, y, z is compared to CP alone ; d, e, f is compared to ME alone.

Number of micronuclei in NCE in the bone marrow of mice treated with *Murraya koenigii* extract (ME) before chemotherapy (Cyclophosphamide).

Sr.No.	Treatment	Number of Micronuclei MNNCE			Total number of MNNCE
		MN1	MN2	MN3	
1.	Normal	6	-	-	6
2.	Vehicle alone (CMC)	9	-	-	9
3.	Methanolic Extract alone (<i>M. koenigii</i>)	10	-	-	10
4.	Cyclophosphamide alone	114	22	11	191
5.	Cyclophosphamide + ME (<i>M. koenigii</i>)	88	16	4	132

Micro Nucleus Assay: Procedure- The mice were injected i.p. with 0.025% colchicines (Sigma, USA) and left for 2 hrs. to arrest the cells in metaphase. Then the animals were sacrificed by cervical dislocation, femur were dissected out and cleaned to remove adherent muscles. The mice femur marrow was flushed out with normal saline (0.84%). Cells smear was made. and the slides stained with May-Greunwald's-Geimsa and scored under fluorescent microscope. Polychromatic and normochromatic erythrocytes bearing micronuclei were scored. The Micronucleus assay, was done by the method of Schmid¹² with some modification in staining procedure¹³ Experimental protocol: Treatment schedule – Animals were divided into following seven groups of three to four animals each.

Group-I (Control) – Double distilled water
Group-II - (Vehicle alone) – Carboxy methyl cellulose (CMC)
Group-III - (Methanolic extract) – 50% Methanolic extract of *Murraya koenigii* (dose 100mg/kg)
Group-IV – Cyclophosphamide alone (50mg/kg)
Group-V - Cyclophosphamide alone plus methanolic extract of *Murraya koenigii* (dose- 100mg/kg)

STATISTICAL ANALYSIS

The data were analyzed by students test. Comparison between different groups were done by One Way ANOVA (Kypplot) using Graph PAD Instant software (USA), and the histograms for chromosomal aberrati

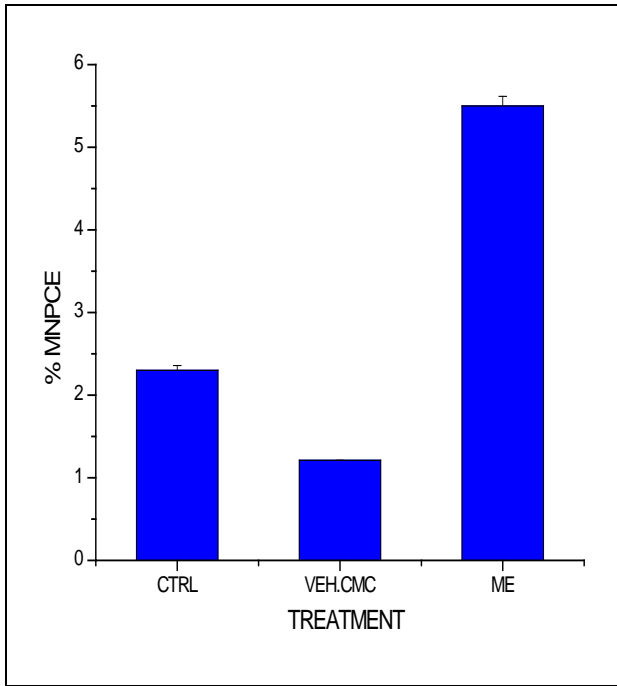


Figure .1. Effect of *M. koenigii* extract on the cyclophosphamide induced MNPCE in mouse bone marrow at 24 hrs.

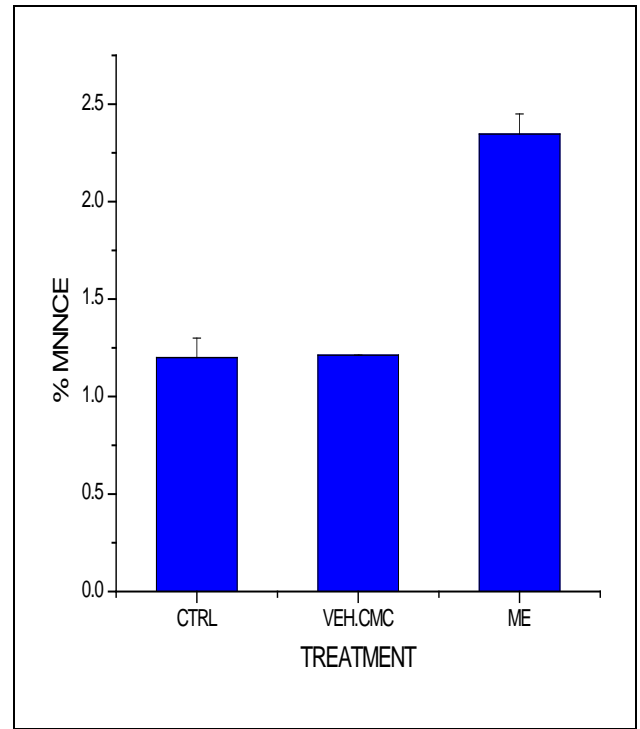


Figure.-2 Effect of *M. koenigii* extract on the cyclophosphamide induced MNNCE in mouse bone marrow at 24 hrs.

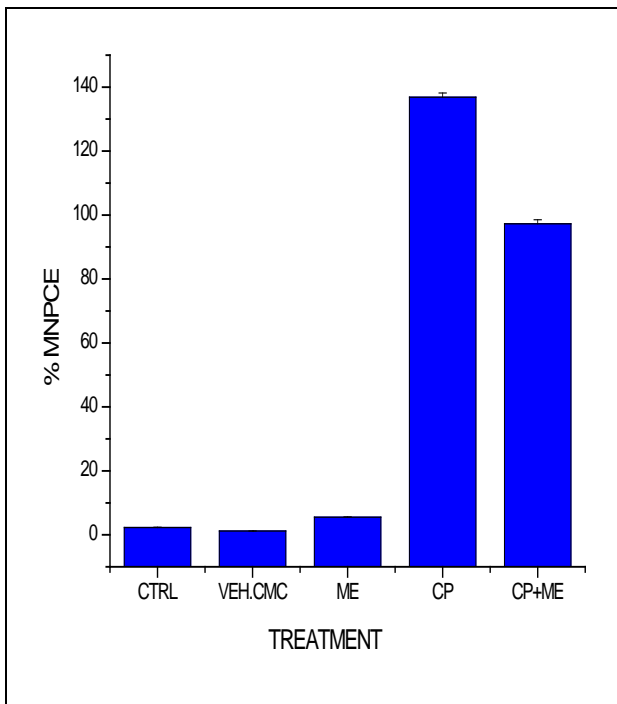


Figure - 3 Effect of *M. koenigii* extract on cyclophosphamide induced MNPCE in mouse bone marrow at 24 hrs.

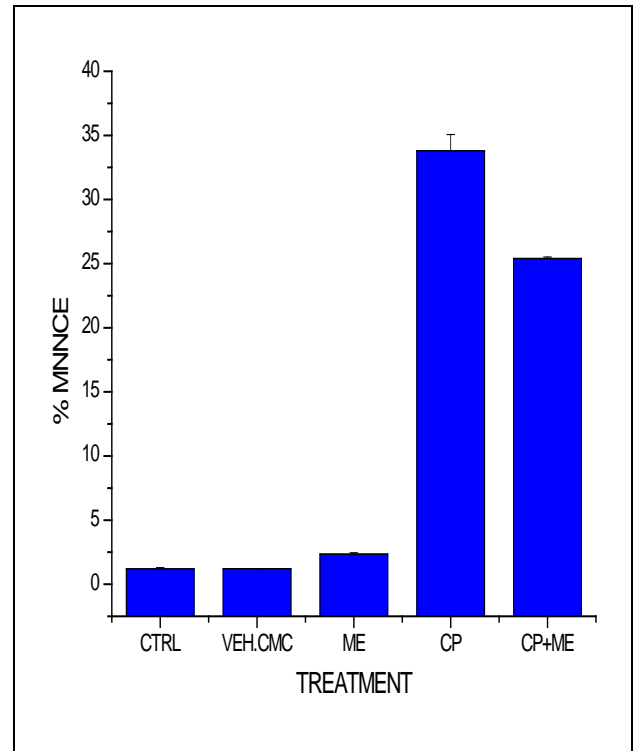


Figure-4 Effect of *M. koenigii* extract on cyclophosphamide induced MNNCE in mouse bone marrow at 24 hrs.

Ratio of PCE to NCE (P/N ratio) in the bone marrow of mice treated with *Murraya koenigii* extract (ME) before chemotherapy (Cyclophosphamide).

Sr.No.	Treatment	No. Cells per thousand Erythrocytes		Ratio of PCE to NCE (P/N ratio)
		PCE	NCE	
1.	Normal	478	500	0.956 ± 0.017474
2.	Vehicle alone (CMC)	508	497	1.02 ± 0.020817
3.	Methanolic Extract alone (<i>M.koenigii</i>)	491	509	0.964667 ± 0.018487
4.	Cyclophosphamide Alone	435	565	0.769 ± 0.005508 ^{b, e}
5.	Cyclophosphamide + ME (<i>M.koenigii</i>)	548	452	1.213333±0.018559 ^{b,d}

a, x, d = p < 0.05; b, y, e = p < 0.01 c, z, f = p < 0.001
a, b, c is compared to DDW (control); x, y, z is compared to CP alone ; d, e, f is compared to ME alone

MNNCE / 1000 NCE in the bone marrow of mice treated with *Murraya koenigii* extract (ME) before chemotherapy (Cyclophosphamide).

Sr.No.	Treatment	Percentage MNNCE (%)
1.	Normal	1.2 ± 0.1
2.	Vehicle alone (CMC)	1.21212 ± 1.15 E – 005
3.	Methanolic Extract alone (<i>M. koenigii</i>)	2.346667 ± 0.103333
4.	Cyclophosphamide Alone	33.79667 ± 1.271355 ^{b, e}
5.	Cyclophosphamide + ME (<i>M. koenigii</i>)	25.4 ± 0.1154701 ^{c, f, x}

a, x, d = p < 0.05; b, y, e = p < 0.01 c, z, f = p < 0.001
a, b, c is compared to DDW (control); x, y, z is compared to CP alone ; d, e, f is compared to ME alone.

on assay and micronuclei assay were drawn using Microcal Origin 6.0 software.

RESULT

The DDW control group showed a P/N ratio without a significant difference with the vehicle alone treated group as well with ME alone treated group. Chemotherapy brought about a significant increase in the frequency of MNPCE and MNNCE with a significance of P < 0.001 when compared to control, vehicle alone, ME alone group and a significant decrease in the P/N ratio. Treatment with methanolic extract of *M. koenigii* before Cyclophosphamide treatment produced a significant reduction in the number of MNPCE and MNNCE when compared to Cyclophosphamide alone with significant increase in the P/N ratio.

CP (50 mg/kg) increased the Percent polychromatic and normochromatic erythrocytes bearing micronuclei when compared to control, vehicle alone and ME alone groups. CP produced 136.38 ± 1.34 and 33.79 ± 1.271 resp. but methanolic extract of *M.koenigii* before Cyclophosphamide treatment produced a significant reduction in the percentage of MNPCE and MNNCE.

DISCUSSION

This study demonstrates a as chemo protective property of the *M.koenigii* leaf extract. The data clearly show that a single dose of 100 mg/kg of ME (*M.koenigii*) before CP (50mg/kg b.wt.) administered intraperitoneally can significantly decrease the cyclophosphamide induced chromosomal damage. Administration of the ME 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 ME treatment.

The chemoprotective 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 clastogenicity, evident in the reduced micronuclei in the bone marrow cells.

CONCLUSION

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 constituents, which



Erythrocytes with Micronuclei (MN)

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 in order to explore the full potential of this plant in human chemotherapeutic protection.

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