Global Veterinaria 10 (2): 128-133, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.10.2.65189

Protective Effect of *Murraya koenigii* (Curry Leaf) Leaves Extract Against Genotoxicity Induced by Cyclophosphamide in Mouse Bone Marrow Cells

[1]Aditya Mishra, [2]Vishhal Gupta, [3]Hemant Nagar and [1]Vishal Shrivastav

[1]Department of Pharmacology,
Millennium College of Pharmacy, Bhopal, (M.P.) India
[2]Faculty of Pharmaceutical Sciences,
Millennium College of Pharmacy, Bhopal (M.P.), India
[3]Faculty of Pharmacy,
Sapience Bio Analytical Research Laboratory, Bhopal (M.P.), India

Abstract: This study was undertaken to investigate the effects of *Murraya koenigii* (curry leaf) Leaves extract on Cyclophosphamide induced genotoxicity in mice bone marrow cells using the micronucleus and chromosomal aberration test. Mice were orally (gavages) pre-treated with solutions of Aqueous and Ethanolic extract of *Murraya koenigii* which was prepared at two different doses (100 and 200 mg/kg b.w.) of both the extracts for seven consecutive days. Mice were injected intraperitoneally on the seventh day with Cyclophosphamide (50 mg/kg b.w.) and killed after 24 h for the evaluation of micro nucleated polychromatic erythrocytes (MnPCEs) and the ratio of PCE/NCE (polychromatic erythrocyte/normochromatic erythrocyte). Higher doses (200 mg/kg b.w.) of extract significantly reduced MnPCEs induced by Cyclophosphamide ($P < 0.01$). In Chromosomal aberrations test, various types of Chromosomal aberrations were counted. Higher doses of both extract significantly reduced the aberrations ($P < 0.01$). On the basis of our result it may be concluded that the leaves of *Murraya koenigii* have the antimutagenic potential that may prevent the mutagenic effect of various cytotoxic drugs.

Key words: Genotoxicity · Micronucleus · Cyclophosphamide · Chromosomal Aberrations

INTRODUCTION

Genotoxicity is a property possessed by some substances that makes them harmful to the genetic information contained in organisms. While there are many different factors that can affect DNA, RNA and other genetic materials, the property of genotoxicity only applies to those substances that actually cause harm to the genetic information. A substance that has the property of genotoxicity is known as a genotoxin. Micronuclei are cytoplasmic chromatin-containing bodies that appears in the cell like a small satellite nucleus around the cell nucleus, due to chromosome fragments or entire chromosomes that are not incorporated in the main nucleus after cell division. The presence of micronuclei (MN) in cells is considered as a biomarker of damage to the DNA. The micronucleus test is an *in vivo* and *in vitro* short-time screening cytogenetic test is a widely used method for assessing genotoxicity of chemicals in organisms. Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. Besides that it is a well known carcinogen which biotransformed principally in the liver to active alkylating metabolites by a mixed function microsomal oxidase system. These metabolites interfere with the growth of susceptible rapidly proliferating malignant cells. The mechanism of action is thought to involve cross-linking of tumor cell DNA [1-3].

The Indian plant *Murraya koenigii* (L.) spreng commonly known as curry leaf, belong to family Rutacea is native of India and found everywhere in India, it commonly occurs in foothills of Himalaya region andhra Pradesh, Assam, Kerela, Tamilnadu and Maharashtra. *Murraya koenigii* leaf is a common spice in daily used

Corresponding Author: Aditya Mishra, Department of Pharmacology, Millennium College of Pharmacy, Bhopal, (M.P.) India, Plot No. 3, Bagdilkusha, 462023, Bhopal (M.P.), India. Tel: +09827230580.

food in India and other countries. Leaf contains various phytoconstituents [4, 5] such as carbazole alkaloids and phenolic compounds in rich amount both are responsible for antioxidant [6, 9] and many other activities of drug. Antioxidants are used in prevention of various diseases such as skin disease, cancer etc. Volatile oil is used as a fixative for soap, perfume. The leaves, bark and root of the plant are used in the indigenous medicine as a tonic, stimulant, carminative and stomachic. It also has the anticancer [10], anti inflammatory [11], anti-ulcer, antidiarrhoeal [12], hypoglycaemic [13, 15], antinociceptive [16], anticholinesterase [17], anthelmintic activity [18] and antiamnesic activity [19]. In the present investigation antimutagenic effect of aqueous and ethanolic leaf extract were investigated on Cyclophosphamide induced genotoxicity in mice bone marrow cells.

MATERIALS AND METHODS

Animals: Animal care and handling as per CPCSEA guideline. The experiment was carried out on Swiss albino mice of 4 months, of both sexes, weighing between 25 to 30 gm. They were provided from Sapience Bio-analytical Research Lab, Bhopal (M.P.), India. The animals were acclimatized to the standard laboratory conditions in well cross ventilated animal house at temperature 25±2°C, relative humidity 44-56% and light and dark cycles of 12-12 hours respectively for 1 week before and during the experiments. The animals were fed with standard diet and water *ad libitum*.

The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no.1413/PO/A/11/CPCSEA).

Chemicals: Cyclophosphamide (CP) was purchased from united local market (brand name of Uniphos-500) and Cholchicine from Himedia laboratories Pvt.Ltd. CAS No. 64-86-8. All the other chemicals were used are highest purity and analytical grade.

Collection of Plant Material: Leaves of *Murraya koenigii* were collected from indrapuri area in month of August. Leaf were separated and made completely clean, dust free.

Authentification of Plant: Plant was identified and authentified by Prof. Zea ull Khan (Prof. of Botany), Department of Botany, Safia College, Bhopal (M.P.) and a specimen voucher no. 132/ Bot/Safia/2012 was issued.

Extraction Process of Murraya Koenigii Leaf: The collected, cleaned powder of leaf of *Murraya koenigii* were used for the extraction process. The powder of leaf (200 g) material were evenly packed in the soxhlet apparatus and extracted with two solvents Ethanol and Aqueous by hot continuous extraction process for about 26 h separately. The extracts were filtered while hot through whatmann filter paper to remove any impurities if present. The extracts were concentrated by vacuum distillation to reduce the volume 1/10. The concentrated extracts were transferred to 100 ml beaker and to removing solvent were evaporated on the water bath. Then they were collected and placed in desiccator to remove the excessive moisture. The dried extracts were packed and labelled in air tight container for the further studies such as a phytochemical screening and pharmacological activities [20].

Experimental Design: The experiment was carried out on white mice (Swiss strain) of 4 months, of both sexes, weighing between 25 to 30 gm. In the experiment, a total no of 36 mice were used. The mice were divided into 6 groups comprising of 6 mice in each group as follows (these Experimental design were followed in both screeninig models).

Group 1: Normal control rats received 1 ml/100 gm b.w. using an intragastric tube for 7 days.

Group 2: Positive control, received a single dose of Cyclophosphamide (CP, 50 mg/kg b.w., i.p.) in saline intraperitoneally.

Group 3: Treated with a single i.p. dose of CP, 1 h after the last dose of ethanolic extract of *Murraya koenigii* (100 mg/kg per day for 7 days) in distilled water by intragastric tube.

Group 4: Treated with a single i.p. dose of CP, 1 h after the last dose of ethanolic extract of *Murraya koenigii* (200 mg/kg b.w. per day for 7 days) in distilled water by intragastric tube.

Group 5: Treated with a single i.p. dose of CP, 1 h after the last dose of aqueous extract of *Murraya koenigii* (100 mg/kg b.w. per day for 7 days) in distilled water by intragastric tube.

Group 6: Treated with a single i.p. dose of CP, 1 h after the last dose of aqueous extract of *Murraya koenigii* (200 mg/kg b.w. per day for 7 days) in distilled water by intragastric tube.

Micronucleus Assay: The micronucleus test was carried out according to Schmid [21] to evaluate chromosomal damage in experimental animals. Mice were sacrificed by cervical dislocation 24 h after Cyclophosphamide (CP) treatment. Mice were dissected and both of femur bone was excised. Bone marrow was aspirated by flushing with normal saline in the centrifuge tube containing HBSS solution. After centrifugation for 10 min at 1000 rpm, Supernatant was discarded. The cells were dispersed by gentle pipeting and collected by centrifuge at 1500 rpm for 10 min at 4°C. Cell pellet was resuspended and a small drop of the viscous suspension was putted on the end of a slide and spreaded by pulling the material behind a polished cover glass held at an angle of 45 degree. The preparation was then dried and fixed for 2-5 min smears were prepared. The slides were coded to avoid observed bias. Smears were stained for 5 min in may-Gruenwald solution and stain for 10 min in Giemsa respectively. Then slides rinsed in distilled water, clean back side with filter paper then dry on the slide warmer. For each experimental point, six mice were used and a total of 6000 PCEs were scored per each experimental point to determine the percentage of MnPCEs and PCE /NCE ratio [22].

Chromosomal Aberration Test: Mice were sacrificed by cervical dislocation. Cyclophosphamide was injected to 24 hr and Colchicine was injected 2 hrs before sacrificed. Mice were dissected and both of femur bones were excised. Bone marrow was aspirated by flushing with normal saline in the centrifuge tube. The suspension was flushed in the tube properly to get good cell suspension and centrifuged for 10 min at 1000 rpm. Supernatant was discarded and the Pellet was treated with pre-warmed (37°C) KCl (0.56%) on cyclomixer. Left above suspension in a water bath (37°C) for 20 min. Pellet was treated with freshly prepared Conroy's fixative (Methanol: Glacial Acetic Acid, 3:1) on cyclomixer. Cornoy's fixative was repeated 3 times to get debris free white pellet. To pellet Cornoy's fixative (quantity sufficient) was added to get a good cell suspension. Slides were made with Air Drop Method and stained with (Giemsa's -3 min, Methanol-3 min and DDW- 1 Dip respectively) and observed under microscope in 40×10 x and than in 100×10x magnifications. A total of 100 well spread metaphase plates were scored for chromosomal aberrations such as gaps, chromatid breaks, polyploidy centromic association and fragmentation were counted and data of scoring was expressed as percentage chromosomal aberrations. Statically significance was calculated using students-t test at $p<0.05$.

Statistical Analysis: All the values are expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and followed by Turkey multiple comparison tests by employing statistical software, GraphPad InStat 3. Differences between groups were considered significant at $P < 0.05$ levels.

RESULTS AND DISCUSSION

Among the alkylating agents used for the treatment of wide range of cancers, CP is one of the widely used drugs. Acrolein and phosphoramide are the active compounds of CP. These active compounds of the CP slow down the growth of cancerous cells by interfering with the actions of DNA within those cells [23, 24]. The mutagenicity of CP in particular is related to formation of the ultimate cytotoxic metabolite phosphoramide mustard through the intermediate agent's hydroxycyclophosphamide and deschloroethylcyclophosphamide, which is capable of inducing DNA crosslink's and strand lesions. It has been tested extensively for its genotoxic effects both *in vitro* and *in vivo* in different test systems giving consistently positive results [25, 26]. Several lines of studies have demonstrated that the CP and many other chemotherapeutic agents cause gene mutations, CA and rearrangements and aneuploidy in somatic cells as well as an increased frequency of secondary treatment-related tumours in human cancer survivors [27, 28]. The results presented here actually indicate that aqueous and alcoholic extracts from *murraya koenigii* exhibit Antimutagenic activity against the *in vivo* DNA damaging effect of the indirectly acting alkylating agent CP. Numbers of micronuclei were found decrease with higher the doses (200mg/kg) of *Murraya koenigii* extract (Aq.- 1.85±0.17, Alcoholic- 1.68±0.18) (Table 1 and Fig.1). On the other hand, there was a very less decrease of micronuclei in the group of lower doses of *Murraya koenigii* extract (Aq. - 2.72±0.21, Alcoholic- 2.58±0.22). It was noteworthy that different doses of *Murraya*

Table 1: Effect of *Murraya koenigii* extract on micronucleus formation in mouse bone marrow cells.

Groups	MNPCE±S.E.M.	PCE/NCE±S.E.M. RATIO
I	0.341±0.18	0.43±0.03
II	7.47±0.27a***	0.22 ±0.07
III	2.72±0.21a*,b**	0.25±0.09
IV	1.85±0.17b**	0.36±0.03
V	2.58±0.22a*,b**	0.27±0.01
VI	1.68±0.18b**,	0.34±0.04

The data obtained were analyzed by one-way ANOVA followed Turkey multiple comparison test. Each value represents the mean±S.E.M., n= 6. *P < 0.05, ** P < 0.01, P < 0.001. (PCE/NCE denotes polychromatid erythrocytes/ nonchromatid erythrocytes).

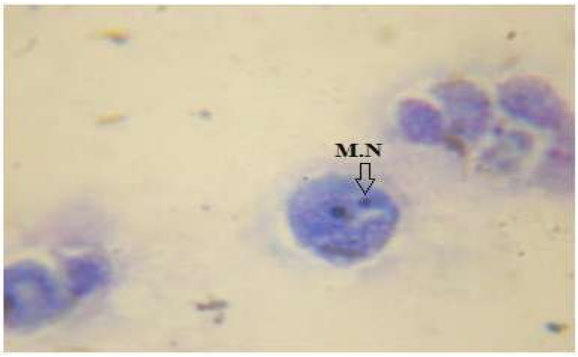

Fig. 1: The photograph shows Micronucleus

Table 2: Effect of *Murraya koenigii* extract on chromosomal aberration in mouse bone marrow cells

Group	Mean±S.E.M. (C. Ab.)	Mean C.B.	Mean C.F.	Mean C.G.	Mean R.F.	Mean C.A.	% Protection
I	18.6±2.18	11	6	1.6	Nil	Nil	-
II	48.3±4.28a**	18	12	10.3	4	4	-
III	38.6±2.26 a**,b*	15.3	13.2	6.1	2	1	25.19
IV	36.8±2.46						
a**, b*	17.3	11.2	4.3	3	1	31.25	
V	40.5±1.27						
a**, b*	18.3	16	3.2	1	2	19.25	
VI	35.2±2.47						
a**, b*	16.1	16	3.1	Nil	Nil	37.21	

The data obtained were analyzed by one-way ANOVA followed Turkey multiple comparison test. Each value represents the mean±S.E.M., n= 6. *P < 0.05, ** P< 0.01.

C.Ab. - Chromosomal Aberration, C.B. - Chrometid Break

C.F. - Chrometid Fragment, C.G. - Chrometid Gap

R.F. - Ring formation, C.A. - Centromeric Association

koenigii extract used in this experiment were note cytotoxic for PCE/NCE ratio as compared to positive control group.

In case of chromosomal aberration test (Table.2), we report the protective effect of *Murraya koenigii* extract against Cyclophosphamide induced chromosomal aberrations in mouse bone marrow cells. The two test doses of aqueous and alcoholic extract. Provided protection when given 24 hour prior to the single i.p. administration of Cyclophosphamide (50 mg/kg). Thus, tested *Murraya koenigii* extract seems to have a preventive potential against Cyclophosphamide induced chromosomal aberrations in Swiss mouse bone marrow cells at the doses tested.

CONCLUSION

On the basis of our result, we may conclude that higher doses (200mg/kg) of *Murraya koenigii* extract have shown significant protection against Cyclophosphamide-induced micronucleus formation and chromosomal aberrations. It may be concluded that antimutagenic effects of *Murraya koenigii* due to carbazole alkaloids, tannins and flavonoids present in the extract. This finding, therefore, confirmed health benefits of *Murraya koenigii* and ingredient of food and as a medicinal plant to reduce mutagenicity.

ACKNOWLEDGEMENT

This research was self financed and supported by Sapience Bio Analytical Research Lab. Bhopal M.P. India. We would like to express our heartiest thanks to our parents, without their help and kind support we would have been unable to complete this work.

REFERENCES

1. Abeer, H., A.E. Rahim and N.A. Hafiz, 2009. Investigation on the protective effect of grape seed and linseed oils against Cyclophosphamide induced genotoxicity in mice. Global Veterinaria, 3: 377-382.

2. Hosseinimehr, S.J., M. Azadbakht and A.J. Abadi, 2008. Protective effect of hawthorn extract against genotoxicity induced by cyclophosphamide in mouse bone marrow cells. Environmental Toxicology and Pharmacology, 25: 51-56.
3. Asita, A.O., M.E. Dingann and S. Magama, 2008. Lack of modulatory effect of asparagus, tomato and grape juice on cyclophosphamide-induced genotoxicity in mice. African Journal of Biotechnology, 7: 3383-3388.
4. Palanisamy, A. and S. Pillai, 2007. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic β-cells in experimental diabetes in rats. Chemico-Biological Interactions, 165: 155-165.
5. Chowdhury, J.U., N.I. Bhuiyan and M. Yusuf, 2008. Chemical composition of the leaf essential oils of Murraya koenigii (L.) Spreng and Murraya paniculata Jack. Bangladesh J. Pharmacol., 3: 59-63.
6. Reisch, J., O. Goj, A. Wickramasinghe and H.M.T.B. Herath, 1992. Carbazole alkaloids from seeds of Murraya koenigii. Phytochemistry, 31: 2877-2879.
7. Russel, S., M. Ramsewak, G. Nair, M. Strasburg and L. David, 1999. DeWitt, Biologically Active Carbazole Alkaloids from Murraya koenigii. J. Agric. Food Chem., 47: 444-447.
8. Das, A.K., V. Rajkumar and D.K. Dwivedi, 2011. Antioxidant effect of curry leaf (*Murraya koenigii*) powder on quality of ground and cooked goat meat. International Food Research Journal, 18: 563-569.
9. Yukari, T., K. Hiroe, Nordin, J. Agric, J. Lajis and N. Nobuji, 2011. Antioxidative Activity of Carbazoles from Murraya koenigii Leaves. Food Chem., 49: 5589-5594.
10. Yih Kok, S., L.Y. Mooi, K. Ahmad and M.A. Sukari, 2012. Anti-tumour promoting activity and antioxidant properties of girinimbine isolated from the stem bark of Murraya koenigii. Molecule, 17: 4651-4660.
11. Muthumani, P., S. Venkatraman, K.V. Ramseshu, R. Meera, P. Devi and B. Kameswari, 2009. Pharmacological studies of anticancer, anti inflammatory activities of *Murraya koenigii* (Linn) Spreng in experimental animals. J. Pharm. Sci. & Res., 1: 137-141.
12. Sharma, P., G. Vidyasagar, A. Bhandari, S. Singh, U. Bhadoriya, S. Ghule, *et al*., 2012. A pharmacological evaluation of antidiarrhoeal activity of leaves extract of Murraya koenigii in experimentally induced diarrhoea in rats. Asian Pacific Journal of Tropical Disease, pp: 230-233.
13. Yadav, S., V. Vats, Y. Dhunnoo and J.K. Grover, 2002. Hypoglycemic and Antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. Journal of Ethnopharmacology, 82: 111-116.
14. Kesari, A.N., S. Kesari, S.K. Singh, R.K. Gupta and G. Watal, 2007. Studies on the glycemic and lipidemic effect of *Murraya koenigii* in experimental animals. Journal of Ethnopharmacology, 112: 305-311.
15. Kesari, A.N., R.K. Gupta and G. Watal, 2005. Hypoglycemic effects of *Murraya koenigii* on normal and alloxan-diabetic rabbits. Journal of Ethnopharmacology, pp: 247-251.
16. Patil, R.A., P.M. Langade, P.B. Dighade and Y.A. Hiray, 2012. Antinociceptive activity of acute and chronic administration of Murraya koenigii L. leaves in experimental animal models. Indian J. Pharmacol., 44: 15-19.
17. Tembhurne, S.V. and D.M. Sakarkar, 2011. Antiamnesic effect of petroleum ether extract of Murraya Koenigii (Linn) leaves involving possible Anticholinesterase and cholesterol lowering mechanism. Asian Journal of Pharmaceutical and Clinical Research, 4: 1.
18. Sharma, U.S., U.K. Sharma, A. Singh, N. Sutar and P.J. Singh, 2010. *In vitro* anthelmintic activity of *Murraya Koenigii* Linn leaves extracts. International Journal of Pharma and Bio Sciences, 1: 3.
19. Tembhurne, S.V. and D.M. Sakarkar, 2010. Beneficial Effects of Ethanolic Extract of Murraya Koenigii (Linn) Leaves in Cognitive Deficit Aged Mice Involving Possible Anticholinesterase and Cholesterol Lowering Mechanism. International Journal of Pharm Tech Research, 2: 181-188.
20. Handa, S.S., S. Khanuja, G. Longo and D.D. Rakesh, 2008. Extraction Technologies for Medicinal and Aromatic Plants. International centre for science Earth, Environmental and Marine Sciences and Technologies International Centre for Science and High Technology. Italy, pp: 21-66.
21. Schmid, W., 1975. The micronucleus test. Mutat. Res., 31: 9-15.
22. Pandey, S. and R.C. Agrawal, 2010. Clastogenic analysis of *bauhinia variegata* bark extract using micronucleus assay in mouse bone marrow cells. American-Eurasian Journal of Toxicological Sciences, 2: 112-114.
23. Hales, B.E., 1982. Comparison of the mutagenicity and teratogenicity of cyclophosphamide and its active metabolites, 4-hydroxycyclophosphamide, phosphoramide mustard and acrolein. Cancer Res., 42: 3016-3021.

24. Haubitz, M., 2007. Acute and long-term toxicity of Cyclophosphamide. Tx Med., 19: 26-31.
25. Das, S. and R.C. Agrawal, 2011. Evaluation of Antimutagenic potential of ripe aegle maemelos fruit extracts using micronucleus and chromosomal abrerration assy in mouse bone marrow. Irjp, 2: 187-191.
26. Mathur, D. and R.C. Agrawal, 2011. Evaluation of *in vivo* antimutagenic potential of fruits extracts of Withania coagulans. Der Pharma Chemica, 3: 373-376.
27. Kruawan, K. and K. Kangsadalampai, 2006. Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs. Thai J. Pharm. Sci., 30: 28-35.
28. Kumpati, P., K. Sundramoorthy, T.S. Sathiyavedu and R. Rabandi, 2004. Interactive effects of saffron with garlic and curcumin against cyclophosphamide induced genotoxicity in mice. Asia Pac. J. Clin Nutr, 13: 292-294.