ISSN: 0974-6943

Research Article

Radioprotective activity of *Murraya koenigii* (L.) on cellular antioxidants in *Swiss albino mice*

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Received on:18-09-2008; Accepted on :16-01-2009

ABSTRACT

The radioprotective activity of methanolic extract of *Murraya koenigii* (L.) leaves was studied in mouse liver. Adult Swiss albino mice were injected i.p. with 100 mg/kg of the *Murraya koenigii* (L.) extract (MKE) for 5 consecutive days and exposed to 4Gy gamma radiation 30 min after last injection. The extract itself increased the GSH and enzymes significantly above levels, whereas radiation significantly reduced all values. Pretreatment with the extract could check the radiation induced depletion of GSH and all the enzymes and maintain their levels within or above control range. Pretreatment with the extract also reduces lipid peroxidation rate induced by radiation. The result demonstrates that *Murraya koenigii* (L.) leaves posses good antioxidant activity in vitro and is able to protect against radiation induced depletion in cellular antioxidants.

Key words: Radioprotection, MKE, GSH, Lipid peroxidation

INTRODUCTION

In biological systems, the normal processes of oxidation produce highly reactive free radicals. Exposure of mammalian systems to radiations induces damaging effects leading to cell death and an increased risk of diseases particularly cancer [1]. These free radicals generated as a result of radiation or oxidative stress can readily react with and damage other molecules, including DNA in cell nuclei or mitochondria resulting in the occurrence of diseases [2, 3, 4]. Antioxidants are molecules that can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged [5]. Consequently, there is a growing interest in developing new radioprotectants in preventive medicines as well as adjuvant therapy. Most of the effective radioprotectants such as WR-2721 developed so far are synthetic and are reported to be toxic [6]. Thus, there is a need to develop radioprotectants from natural sources especially from edible or medicinal plants/ herbs as these are regarded as non-toxic even at higher concentrations. The importance of usage of ethnomedicines is increasing nowadays as they have less or no side effects, low cost and are, often easily accessible to the common people. The present study was primarily aimed to this end, wherein the radioprotecting property of *Murraya koenigii* (L.) commonly known as Meethi neem in Hindi of *Rutaceae* family was studied.

Murraya koenigii (L.) [7] is an aromatic, deciduous shrub or a small tree up to 6 m. in height found throughout India up to an altitude of 1,500 m. commonly in forests often as gregarious under growths. It is cultivated for its aromatic leaves. The leaves of *Murraya koenigii* yield a number of carbazole alkaloids namely murrayanine [8], mahanimbine [9],girinimbine [10], murrayacine [11], isomurrayazoline [12], mahanine, koenine, koenigine, koenidine [13], koenimbine[14] and murrayazoline [15]. Many medicinal properties such as antioxidant [16], anticarcinogenic [17], antibacterial , antifungal [18], hypoglycaemic [19] and anti-lipid peroxidative [20] have been attributed to this plant. The leaves are traditionally used as antiemetic, antidiarrhoeal, and blood purifier. They are considered as tonic, stomachic, and carminative. The oil is applied externally to bruises and eruptions. The leaves are used extensively as a flavouring agent in curries and chutneys [21, 22]. The present study was carried out to investigate the effect of *Murraya koenigii* extract MKE on cellular antioxidants in mice exposed to gamma radiation.

MATERIALS AND METHODS

Chemicals-TBA, Deoxyribose, Ascorbic acid, DETAPAC, BHT, GSSG, Glutathione reduced, DTNB and NADPH were obtained from Hi-media, India. NaCl, DMSO and Tris were

ISSN: 0974-6943

purchased from Rankem, India.Na_2HPO_4, NaH_2PO_4, KH_2PO_4, EDTA and $NaHCO_3$ were obtained from Qualigens, India. NaOH and KCl were from Ranbaxy, India. TCA and H_2O_2 were purchased from Merck limited, India. MDA and Glutathione reductase were from Sigma Aldrich, USA. Cumene hydroperoxide was obtained from Acros, Belgium. NaN_3 was obtained from Sulab reagents, India. $FeCl_3$ was purchased from S.D. fine-chem. ltd., India.

Plant extract-The leaves of *Murraya koenigii* were collected locally from Bhopal (M.P.) during the months of August-September, 2005.The shade dried leaves were powdered and extracted with 50% methanol by cold maceration method. The extract was concentrated to get solid residue and the percentage yield was calculated on the basis of fresh and dry weight. The yield of MKE was approximately (16% w/w).

Animal model-Adult Swiss albino strain mice were procured after obtaining clearance from the Jawaharlal Nehru Cancer Hospital Ethical Committee. The mouse colony was maintained in the animal house of the Research Department, Jawaharlal Nehru Cancer Hospital, Bhopal. The mice were housed in polypropylene cages containing sterile paddy husk (procured locally) as bedding and maintained under controlled conditions of temperature (23±2°C), humidity (50±5%) and light (12:12h of light: dark respectively). The animals were fed commercial mice feed and acidified water ad libitum. Mice of either sex, 6-8 weeks old and weighing 22±2g, were selected from the above colony for the experiments.

Irradiation-The animals were whole body irradiated exposed to gamma rays from a ^{60} Co Gammatron teletherapy unit (Theratron, Canada) at a dose rate of 1 Gy/min (4 Gy in 4 min). Dosimetry was done using a beam therapy dosimeter (Dose 1 Sweden Scanditronix and Unidose PTW, Germany), used routinely to calculate the dose. During irradiation, mice were kept in a well-ventilated Perspex box (20 cm x 20 cm x 4.5 cm). The SSD (source surface distance) was adjusted to 100cm.The 30 cm x 30 cm of field size was taken.

Methodology

***In-vitro* antioxidant activity**—Inhibition of Fenton reaction generated OH radicals was showed using this method. The reaction mixture consisted of 100µl deoxyribose, 50µl ferric chloride, 50µl EDTA, 100µl H_2O_2 in 550 µl phosphate buffer saline. Different concentrations of the extract (5-400µg) or phosphate buffer saline, in case of blank, was added to reaction mixture to make a final volume of 1 ml. The reaction mixture was then incubated for 1 h at room temperature. The mixture was then incubated for 20 min in a boiling water bath with 0.5 ml of 3% of TCA and 0.5 ml of 1% of TBA, cooled and centrifuged. The absorbance was measured at 532 nm in an UV spectrophotometer. The test tube with PBS was considered as blank and DMSO was used as positive control [23].

***In-vivo* study of cellular antioxidants**-

The animals were divided into 4 groups of 12 each. The animals of Group I were injected intraperitoneally (i.p.) with double distilled water (DDW) for 5 consecutive days. Group II was injected i.p. with 100mg/kg of MKE for 5 consecutive days. Animals of Group III were injected i.p. with DDW for 5 consecutive days and sham irradiated 30 min after last injection. Group IV was injected i.p. with 100mg/kg of MKE for 5 consecutive days and sham irradiated 30 min after last injection.

Measurement of Biochemical parameters

Three animals of each group were killed by cervical dislocation at 30 min, 1 h, 2 h, 4 h of intervals to estimate the various enzyme levels. The liver was perfused with 0.9% NaCl and dissected out. The weight of liver was taken and 10% tissue homogenate was prepared with saline – EDTA or Sucrose solution (0.25M) or 10% Trichloroacetic acid or Tris HCl (pH 7). The remaining sample was centrifuged at 10,000 rpm for 10 min. at 4°C. The supernatant was used for the estimation of Glutathione, Glutathione reductase, Glutathione peroxidase, Glutathione transferase and lipid peroxidation [24, 25, 26].

Estimation of GSH (Glutathione)

Total GSH was measured by the enzyme recycling method of Tietze with modification. Homogenization of accurately weighed approximate 50 mg of the liver tissue was done with 5 ml of 10% TCA. Homogenate was centrifuged at 10000 rpm for 10 min at 4°C, the supernatant was diluted 10 fold in phosphate buffer and kept on ice. A 300µl aliquot of this sample was combined with 450µl phosphate buffer, 100µl GSSG reductase and 50µl DTNB. After incubating for 1 min 100µl NADPH was added and the absorbance was monitored for 2 min at 412nm. The change in absorbance / min was determined and this value was converted to µM of GSH in comparison to known standard. A Standard Curve is obtained by using different concentration of reduced glutathione (0-50 µM) and the standard sample was processed like that of test sample.

Estimation of Glutathione peroxidase

A 10% tissue homogenate was prepared using 0.25 M sucrose. The reaction mixture of the test consisted of 2.58 ml of phosphate buffer (0.05 M) containing EDTA, 0.01 ml of NaN_3 (1.125M), 0.1 ml of NADPH (0.0084 M), 0.01 ml of

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GSH reductase and 0.1 ml of GSH (0.15 M).To the above mixture, 0.1 ml of the test sample was added and incubated at room temperature for 5 min. The reaction was started by the addition of 0.1 ml of cumene hydroperoxide (0.0022M).The absorbance at 340 nm was recorded for 5 min.

Estimation of Glutathione Reductase

A 10% tissue homogenate was prepared using saline – EDTA. The reaction mixture consisted of 2.6 ml of phosphate buffer, 0.1 ml of EDTA (15 mM) and 0.1 ml of GSSG (65.3 mM).To the above mixture 0.1 ml of sample was added. The mixture was preincubated in the thermostat cell compartment of the spectrophotometer at 37 ^{0}C for 5 min.The reaction was started by the addition of 0.05 ml of NADPH (9.6mM) and absorbance was monitored at 340 nm for 5 min.

Estimation of Glutathione –S- Transferase

0.1ml of CDNB (30mM), 0.6 ml of sample and 2.2 ml of KH_2PO_4 was added. The reaction mixture was preincubated in the thermostat cell compartment of the spectrophotometer at 37 ^{0}C for 5 min.The reaction was initiated by the addition of 0.1 ml of GSH (30 mM).The absorbance was recorded for 5 min at 340 nm.

Lipid peroxidation assay

A 10 % tissue homogenate was prepared in tris-HCl. The homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was collected. The reaction mixture consisted of 0.1 ml of KCl (150 mM), 0.1 ml of ascorbic acid (0.3 mM) and 0.1 ml of tris – HCl buffer (0.2 M).To the mixture was added 0.1 ml of test sample and incubated at 37 ^{o}C for 1 h. To the above were added 1 ml of 20 % TCA, 2ml of 0.67 % TBA, 0.1 ml of DETAPAC (70 µM) and 0.1 ml of BHT (3.5mM) and incubated in a boiling water bath for 15 min and cooled to room temperature and centrifuged for 5 min at 2000 rpm. Absorbance was measured at 540 nm. A Standard Curve was obtained using different concentration of MDA.

Statistical evaluation of the data was done by Student't' test (Graph PAD In stat software, Kyplot). A value of $p < 0.05$ was considered to be significant.

Results

***In-vitro* antioxidant activity-** It has shown the property to inhibit the formation of TBARS. The MKE inhibited the formation of TBARS at concentrations above 25µM till 300µM, with no further increase at higher concentrations. The MKE showed a significantly ($p < 0.0001$) inhibitory effect on TBARS. [Fig-1]

***In-vivo* study on cellular antioxidants-**

Glutathione- The normal level of glutathione in control animals ranged from 50.06 ± 0.05 – 53.3 ± 0.05 µM/mg tissue during the experiment. The group treated with drug extract resulted in significant ($p<0.0001$) elevation in GSH compared to control group. The level gradually decreased with time but maintained the level near control range. Pretreatment with the MKE resulted in significant ($p<0.0001$) elevation in GSH compared to RT alone at 30 min to 4 h interval. [Fig-2]

Glutathione peroxidase- The GSH-Px activity in control liver ranged from 2 .3 ±0.05 – 2 .6 ± 0.05 µ moles/min/mg protein. Leaf extract alone treated groups showed an elevated GSH – Px activity significantly ($p=0.0081$) compared to control group. The normal values were restored by 24 h. Exposure to radiation markedly decreased the GSH –Px activity. Pretreatment with the MKE resulted in significant ($p=0.0073$) elevation in G-Px activity compared to RT group alone. [Table-1]

Glutathione –S- Transferase - The GT activity in control liver ranged from 1.1 ± 0.1 – 1.4 ± 0.05 µ moles / min/ mg/ protein. Injection of the extract alone marginally increased ($p=0.0808$) the level of the above enzyme compared to control group. Irradiation results in decrease in GT activity. Pretreatment with the MKE resulted in significant ($p=0.0031$) elevation in GT activity compared to RT alone. [Table-1]

Glutathione reductase- The GR activity in control animals ranged from 0.20 ± 0.006 – 0.3 ± 0.05 µ moles/ min/ mg protein. An i.p. injection of the extract increased the GR activity significantly ($p=0.0004$) compared to control group and returned to normal values at 24 h. Irradiation markedly decreased in GR activity from 30 min to 4h post- irradiation. Pretreatment with MKE resulted in significant ($p=0.0009$) elevation in GR activity compared to RT alone. [Table-1]

Antilipid peroxidative effect- Irradiation resulted in a gradual increase in the level of lipid peroxidation from 30 min to 1 h after exposure and then increased steeply to reach a maximum at 4 h It remained significantly ($p=0.0009$) higher than the control level. Extract alone had marginally decreased the level of lipid peroxidation in liver compared to control. Pretreatment with MKE significantly ($p=0.0004$) reduced the radiation induced increase in the level of lipid peroxidation compared to the group that received the radiation alone. [Fig-3]

Discussion

The deleterious biological sequences of both ionizing and non ionizing radiations especially with respect to causing mutation and carcinogenesis are well documented. The interaction of these radiations results in the radiolysis of water in biological

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Table.1. Effect of 50 % methanolic extract of *Murraya koenigii* on radiation induced glutathione peroxidase, glutathione reductase and glutathione transferase activity in irradiated mice liver

Treatment (GPx-µM/min/mg/protein)	30 min	1 h	2 h	4h
DDW	2.3±0.05	2.5±0.12	2.5±0.05	2.6±0.06
Murraya koenigii extract	2.3±0.05 [c]	2.6±0.05 [b]	2.62±0.1	2.7±0.12
DDW + RT (4 Gy)	2.4±0.1	2.3±0.02	2.0±0.12	1.8±0.05
Murraya koenigii extract + RT(4Gy)	2.45±0.11	2.5±0.11 [2]	2.3±0.05 [2]	2.24±0.08 [3]
Treatment (GR-µM/min/mg/protein)				
DDW	0.3±0.05	0.25±0.05	0.2±0.006	0.25±0.05
Murraya koenigii extract	0.3±0.005	0.4±0.02 [b]	0.4±0.01 [a]	0.5±0.0066[b]
DDW + RT (4 Gy)	0.65±0.02	0.5±0.003	0.4±0.008	0.15±0.02
Murraya koenigii extract + RT(4Gy)	1.1±0.04 [1]	0.9±0.04 [1]	0.7±0.01 [1]	0.6±0.01 [1]
Treatment (GT-µM/min/mg/protein)				
DDW	1.4±0.05	1.2±0.05	1.3±0.05	1.1±0.1
Murraya koenigii extract	1.5±0.05	1.5±0.11 [b]	1.3±0.05	1.4±0.11
DDW + RT (4Gy)	1.75±0.02	1.04±0.02	1.02±0.01	1.0±0.006
Murraya koenigii extract +RT(4 Gy)	1.9±0.05 [2]	1.6±0.11 [3]	1.4±0.05 [3]	1.2±0.11

[a] : p <0.0001 [b] : p<0.1 [c] : p<0.01 compared to DDW. , [1] : p <0.0001 [2] : p <0.1 [3] : p<0.01 compared to RT alone

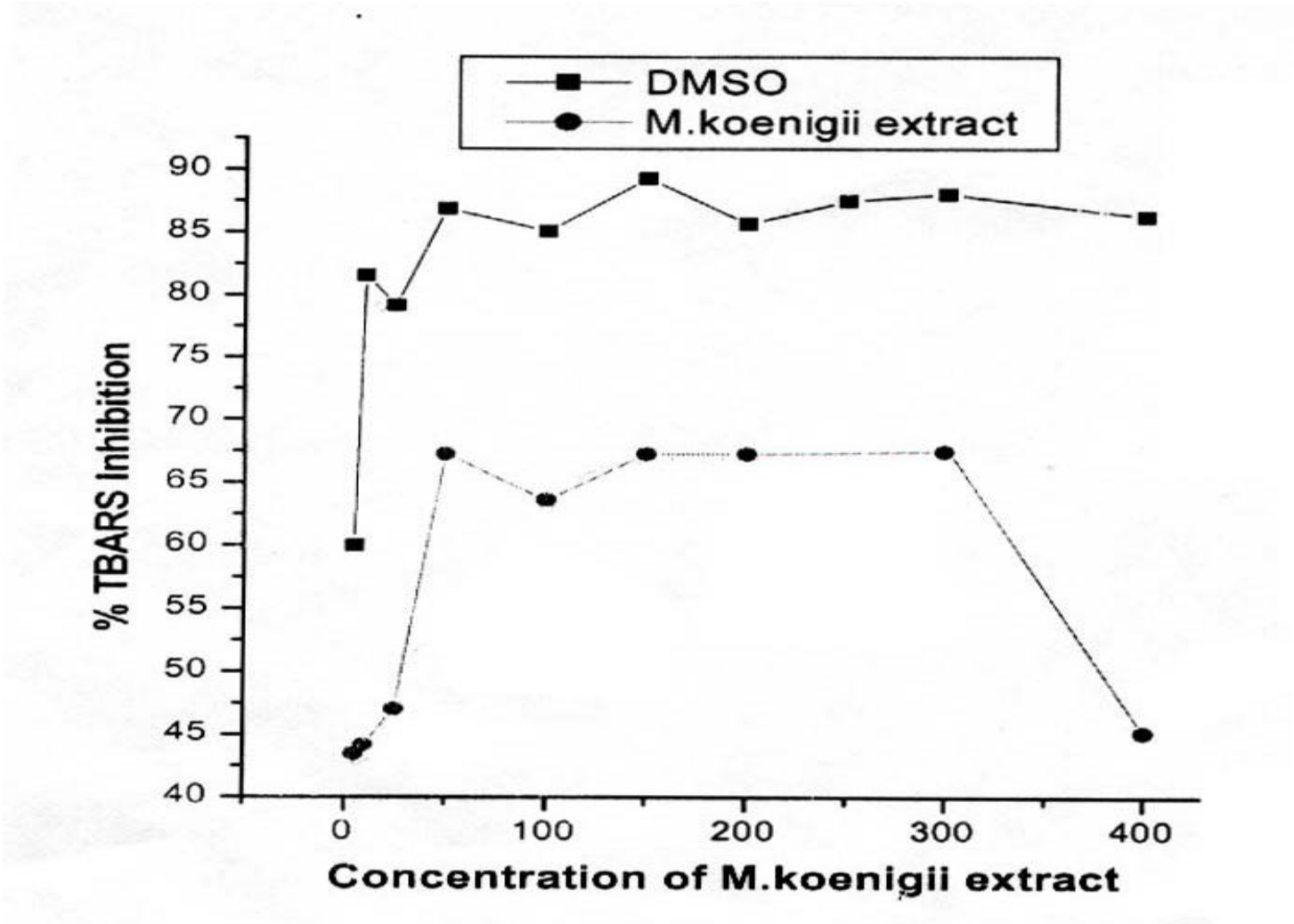

Fig.1.*In- vitro* antioxidant activity of 50 % methanolic extract of *Murraya koenigii*

ISSN: 0974-6943

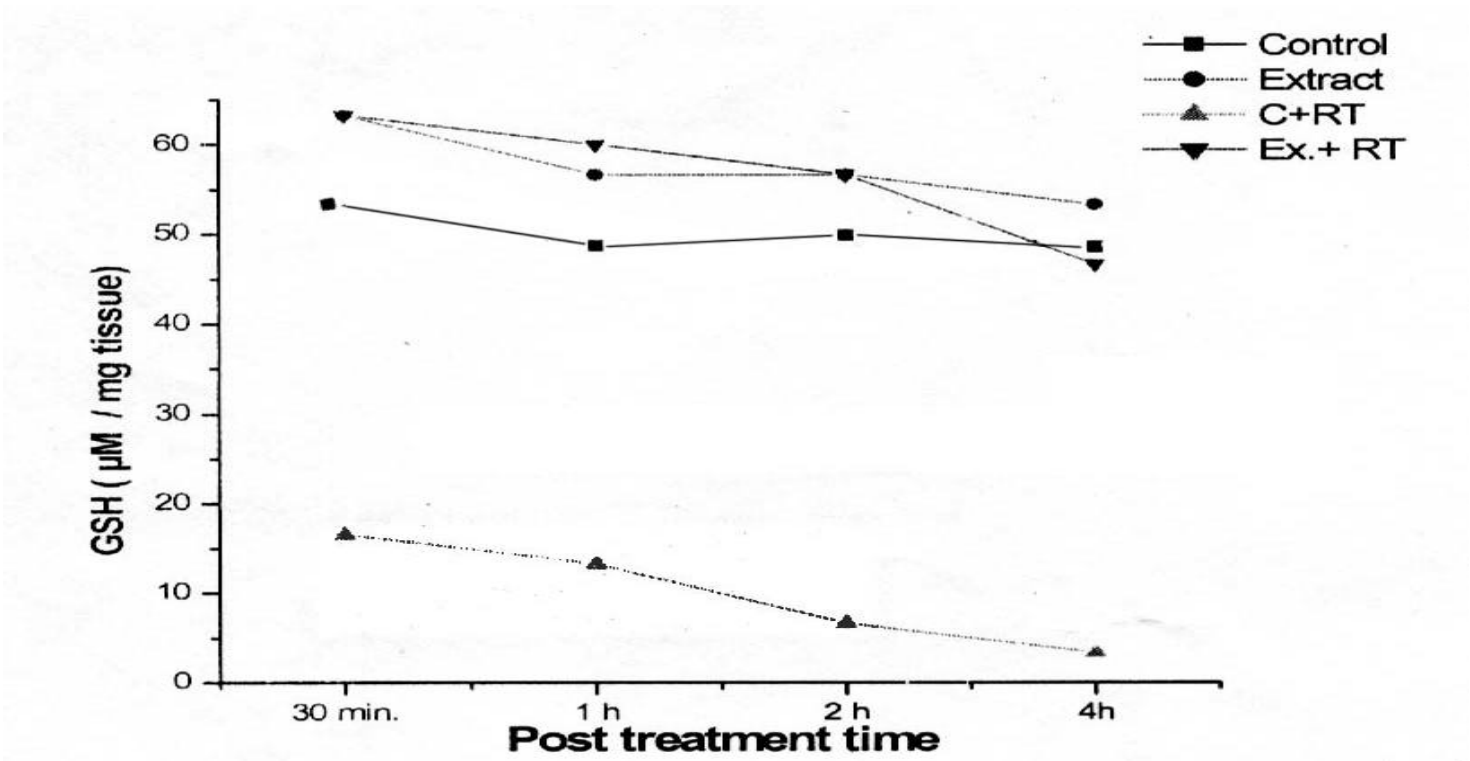

Fig.2. Effect of 50% methanolic extract of *Murraya koenigii* on radiation induced glutathione changes in mice liver

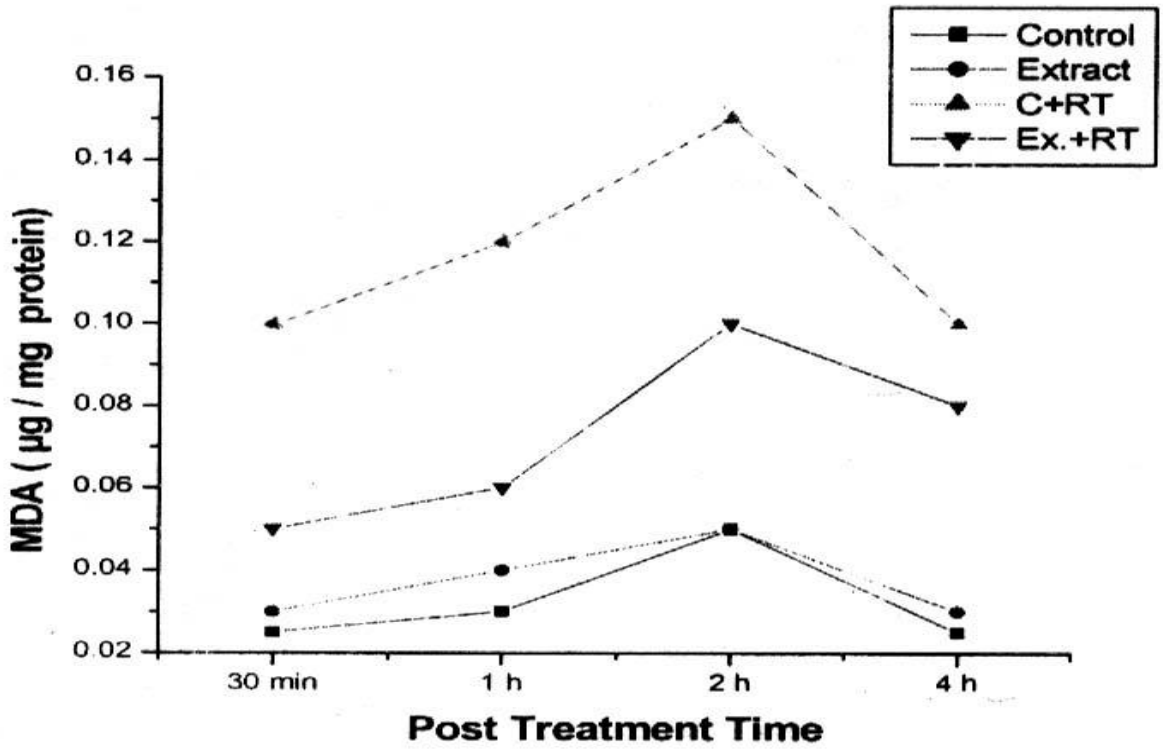

Fig.3.Effect of 50 % methanolic extract of *Murraya koenigii* on radiation induced rate of lipid peroxidation

ISSN: 0974-6943

system resulting in the generation of OH*, H* and superoxide radicals. These reactive species are known to cause degradation of important macromolecules including DNA and membranes. Thus, the high level of unsaturated lipids are most susceptible to oxidative damage, resulting in the disruption of cellular integrity, inactivation of cellular components and lead to cellular cytotoxicity. The DNA molecules are also prone to radiation induced lesions due to presence of various reactive sites (base and sugar) in them.

In the present study the MKE has shown good *in vitro* free radical scavenging activity. The Fenton reaction was used to generate hydroxyl radicals in a test system and the free radical scavenging activity was determined by the degradation of deoxyribose. Fe^{+++} - ascorbate – EDTA – H_2O_2 system produces hydroxyl radicals which react with deoxyribose and set off a series of reactions that result in the generation of Thiobarbituric acid reactive substance (TBARS). The measurement of TBARS thus gives an index of free radical activity [27]. The drug extract showed a significantly inhibitory effect on TBARS.

Many antioxidant protectors are thought to act through the inherent defense system by stimulating the cellular antioxidants.GSH is an important factor in this defense system. The main mechanism by GSH protection are radical scavenging , restoration of damaged molecules by hydrogen donation, reduction of peroxides and maintenance of protein thiols in reduced state. GSH has an important role in determining the inherent radiosensitivity of cells. Radiation interacts with biological molecules and produces toxic free radicals leading to damage of DNA and altered membrane potentials [28]. In addition, protein synthesis and repair systems are also impaired. It is demonstrated that GSH, together with GSH-Px, is involved in a cellular defense system against peroxidation. It appears that the highly elevated glutathione level at the time of exposure enabled the cells to cope with free radical stress of irradiation. The increase in GSH content may be due to an exchange reaction between the protector and protein bound GSH and that of protein-bound GSH might play a role in the protection of cells against radiation [29].

In the *in-vivo* study, whole body irradiation resulted in significant depletion in GSH level and reduced activity of GR and GSH-Px. But in the irradiated mice the normal synthesis/ repair was disrupted due to damage to DNA and membranes. This could be explaining continuous depletion of GSH and the related enzymes even at 24 h post-irradiation. Pretreatment with extract of MKE checked this depletion and maintained the GSH level above control level and also restored the activity of GR and GSH-Px [30]. It appears that the highly elevated glutathione level at the time of exposure enabled the cells to cope with free radical stress of irradiation. The present study demonstrates that the MKE cause a reduction in radiation induced lipid peroxidation in the liver of mice. Lipid peroxidation can be initiated by hydrogen abstraction from lipid molecules by lipid radiolytic products, including hydroxyl and hydroperoxyl radicals. Melondialdehyde (MDA) formed from the breakdown of poly-unsaturated fatty acids, serves as convenient index of determining the extent of the peroxidation reaction. Peroxidation of lipid generates MDA, which reacts with TBA to give a red species TBARS [31], which can be measured. It was observed that the drug extract treatment prevents the interaction of free radicals with membrane lipids.

Acknowledgement

The author wish to thank Dr. P. Uma Devi, Prof. and Head, Dept. of Research, Jawahar Lal Nehru Cancer Hospital, Bhopal for providing constant support, invaluable suggestions and patience throughout the course of the present investigation.

REFERENCES

1.Pierce D A, Preston D L. Radiation related cancer risks at low doses among atomic bomb survivors. Radiat Res 2000; 154: 178-186.

2.Devasagayam T P A, Tilak J C, Boloor K K. Free radicals and antioxidants in human health. Curr Stat Fut Prosp JAPI 2004; 52: 794-804.

3. Cerutti A A.Oxidant stress and carcinogenesis. Eur J Clin Inves 1991; 21:1-11.

4. Ames B. Micronutrients prevent cancer and delay aging. Toxicology 1998;
102: 5-18.

5. Halliwell B. Antioxidant defense mechanisms the end . Free Radical Research.1998; 31: 261-72.

6. Rades D, Fehlauer F, Bajrovic A, Mahlmann B , Richter E, Alberti W Serious adverse effecs of amifostine during radiotherapy in head and neck cancer patients. Radiother Oncol 2004; 70: 261-264.

7. Medicinal Plants of India. Indian council of medicinal research,Cambridge printing works, New Delhi, 1987: p. 289-295.

8.Chakraborty D P, Barman B K, Bose P K. On the constitution of murrayanine, a carbazole derivative isolated from *Murraya koenigii*. Spreng Tetrahedron 1965; 21: 681.

9.Narasimhan N S. Structures of mahanimbin and koenimbin. Tetrahedron Lett No. 1968; 53: 5501.

10. Chakraborty D P, Barman B K, Bose P K. On the structure of girinimbine, a pyranocarbazole derivative isolated from *Murraya koenigii*. Spreng Sci Cult 1964; 30: 445.

11. Chakraborty D P, Das K C, Chowdhary B K. Structure of murrayacine. J Org Chem 1971; 36: 725.

12. Bhattacharyya L, Roy S, Chakraborty D P . Structure of the Carbazole alkaloid iso-murrayazoline from *Murraya koenigii*. Phytochemistry 1982; 21:2432.

13. Narasimhan N S, Paradkar M V, Kelkar S L. Alkaloids of *Murraya koenigii*,Structures of mahanine, koenine, koenigine and koenidine. Indian J Chem 1970 a,b; 8: 473

14. Kureel S P, Kapil R S, Popli S.P. Synthesis of koenine, koenimbine girinimbine. Chem Ind No. 1970 a; 39:1262.

15. Bordner J, Chakraborty D P, Chowdhary B K , Ganguli S N , Das K C, Weinstein B. The crystal structure of murrayazoline. Experientia 1972; 28: 1406.

16. Abraham A. Anti-oxidant effects of curry leaf, *Murraya koenigii* and mustard seeds, *Brassica juncea* in rats fed with high fat diet.

Indian Journal of Experimental Biology 1997; 35:148-150.

17. Khan B A, Abraham A. *Murraya koenigii and Brassica juncea* – alterations on lipid profile in 1-2 dimethyl hydrazine induced colon carcinogenesis. Investigational New Drugs 1996; 14: 365-369.

18. Goutam M P, Purohit R M. Antimicrobial activity of essential oil of leavesof *M.koenigii*. Indian J Pharm 1974; 36 : 11.

19. Narayana K, Sastry K N V. Hypoglycaemic effect of *Murraya koenigii* in normal and alloxan diabetic dogs. Mysore J Agric Sci 1975; 9: 132.

20. Khan B A, Abraham A. Role of *Murraya koenigii* (curry leaf) and *Brassica juncea* (mustard) in lipid peroxidation. Indian Journal of Physiology and Pharmacology 1996; 40: 155.

21. Gopalan C, Rama Sastri B V, Balasubramanian S C. Nutritive value of Indian foods, National Institute of Nutrition, Hyderabad. Indian Council of Medical Research, New Delhi, 1984 : p. 66, 117.

22. The wealth of India. Council of Scientific and Industrial Research,New Delhi, 6: L-M, 1998 : p. 446-448.

23. Uma Devi P , Ganasoundari A. Radioprotective effect of leaf extract of Indian medicinal Plant *Ocimum sanctum.* Indian J Exp Biology 1998; 33: 205-208.

24. Uma Devi P, Ganasoundari A. Modulation of glutathione and antioxidant enzymes by *Ocimum sanctum* and its role in protection against radiation injury. Indian J Exp Biol 1998; 37: 262-268.

25.Uma Devi P, Ganasoundari A , Vrinda B. Radiation protection by the *Ocimum sanctum* flavonoids orientin and vicenin: Mechanisms of action. Radiat Res 2000; 154: 455-460.

26. Kazuo Nakagawa. Decreased glutathione S- transferase activity in mice livers by acute treatment with lead,independent of alteration in glutathione content Toxicology letters 1991; 56: 13-17.

27. Halliwell B, Gutteridge J M C. For mation of TBARS from deoxyribose in presence of iron salts. FEBS Lett 1981; 128: 347-352.

28.Barker J L , Motague E D, Peters L J. Cancer 1980; 45:625-629.

29. Rotruck J T, Pope A L, Ganther H E, Swanson A B, Hafeman DG, Hoekstra W G. Selenium: Biochemical role of a component of glutathione peroxidase. Science 1973; 179: 588.

30. Habig W B, Pabst M J, Jakoby W B. Glutathione-S-transferases: first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130.

31. Niehaus W G, Samuelson S. Formation of Malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968; 6: 126.

Source of support: Nil, Conflict of interest: None Declared