Indian Journal of Experimental Biology
Vol.50, March 2012 pp. 209-215

Amelioration of ionizing radiation induced lipid peroxidation in mouse liver by *Moringa oleifera* Lam. leaf extract

Mahuya Sinha[a], Dipesh Kr Das[a], Sanjukta Datta[b], Santinath Ghosh[b] & Sanjit Dey[a]*

[a]Department of Human Physiology and [b]Department of Chemical Technology, University Colleges of Science, Technology and Agriculture, 92 APC Road, Kolkata 700 009, India

Received 15 April 2011; revised 9 December 2011

Protective effect of *Moringa oleifera* leaf extract (MoLE) against radiation-induced lipid peroxidation has been investigated. Swiss albino mice, selected from an inbred colony, were administered with MoLE (300 mg/kg body wt) for 15 days before exposing to a single dose of 5 Gy ^{60}Co-gamma radiation. After treatments, animals were necropsied at different post irradiation intervals (days 1, 7 and 15) and hepatic lipid peroxidation and reduced glutathione (GSH) contents were estimated to observe the relative changes due to irradiation and its possible amelioration by MoLE. It was observed that, MoLE treatment restored GSH in liver and prevented radiation induced augmentation in hepatic lipid peroxidation. Phytochemical analysis showed that MoLE possess various phytochemicals such as ascorbic acid, phenolics (catechin, epicatechin, ferulic acid, ellagic acid, myricetin) etc., which may play the key role in prevention of hepatic lipid peroxidation by scavenging radiation induced free radicals.

Keywords: Gamma radiation, Lipid peroxidation, Liver, Reactive oxygen species

Among the environmental stresses, ionizing radiation is a crucial risk factor for human being. Ionizing radiation initiates the decomposition reaction of water, producing a variety of reactive oxygen species (ROS) such as hydroxyl radicals ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), superoxide anion radicals ($O_2^{\cdot -}$) etc[1]. These reactive species react with the molecules of cell membranes and causes a chain reaction called lipid peroxidation (LPO).

Among all free radicals $OH^{\cdot}$ is considered to be rapid initiators of the lipid peroxidation process, due to removal of hydrogen atoms from unsaturated fatty acids[2]. $OH^{\cdot}$ reacts with polyunsaturated fatty acids produces a lipid radical ($L^{\cdot}$), which in turn reacts with molecular oxygen to form a lipid peroxyl radical ($LOO^{\cdot}$). The $LOO^{\cdot}$ can abstract hydrogen from a neighboring fatty acid to produce a lipid hydroperoxide ($LOOH^{\cdot}$) and a secondary lipid radical[3]. The $LOOH^{\cdot}$ formed may suffer reductive cleavage by reduced metals, such as Fe^{++}, producing lipid alkoxyl radical ($LO^{\cdot}$). Both alkoxyl and peroxyl radicals stimulate the chain reaction of lipid peroxidation by abstracting additional hydrogen atoms. This chain reaction is the reason for making the process of lipid peroxidation highly dangerous. This destructive process alters integrity, fluidity, permeability and functions of biomembranes; damages membrane-bound enzymes[4]; modifies low density lipoprotein (LDL) to proatherogenic and proinflammatory forms and generates potential toxic products[5]. LPO products have been shown to be mutagenic and carcinogenic[6]. Therefore it is crucial to search for a protective agent which may prevent radiation induced lipid peroxidation.

In recent times, there has been an increased interest on plant research in exploring their advantageous aspects owing to their natural origin, cost effectiveness and less side effects. One such plant, *Moringa oleifera* Lam. (Sy. *Moringa pterygosperma* Gaertn, Family: Moringaceae) is referred as "Miracle tree" in tropics and sub-tropics.

The leaf of *Moringa oleifera* has a wide range of beneficial effects which was predicted in Indian system of medicine (Ayurveda and Unani). *Moringa oleifera* leaves have been reported to possess immunomodulatory[7] and wound healing[8] properties. It was also reported to prevent gastric ulceration[9]. There are reports that the leaf extracts prepared using either by methanol or by ethanol inhibit microsomal lipid peroxidation[10]. *Moringa* also showed radio

*Correspondent author
Telephone: +91-9830211512
Fax: +91-33-2351-9755
E-mail: sanjitdey2003@yahoo.com

protective potential as the leaf extract protects bone marrow chromosomes against radiation induced damage[11]. Therefore, it has been hypothesized that leaf extract of *Moringa oleifera* may inhibit the radiation induced lipid peroxidation.

Liver is the most metabolically active organ and it reflects any systemic derangement upon ionizing radiation. After radiation exposure liver is affected significantly[12,13]. It has been reported that extract from *Moringa oleifera* is able to protect the liver against acetaminophen[14], carbon tetrachloride induced damage[15]. However, the effect of *Moringa* leaf against radiation induced alteration in liver has not been reported.

Hence, in the present investigation an attempt has been made to study the protective effect of *Moringa oleifera* leaf extract in radiation induced oxidative insult. The protective effect was evaluated in terms of lipid peroxidation using liver as an experimental model. The study also aimed at identifying phytochemicals present in *Moringa oleifera* leaf extract which may be responsible for the prevention of *in-vivo* lipid peroxidation.

Materials and Methods

Chemicals—Thiobarbituric acid (TBA), trichloroacetic acid (TCA) was purchased from E Merck Co. (Darmstadt, Germany). 5, 5'-dithio-bis (2-nitro benzoic acid) (DTNB), HPLC standards of catechin, epicatechin, ferulic acid, ellagic acid, myricetin were purchased from Sigma (St Louis, MO, USA). All other chemicals used were of analytical grade.

Preparation of plant extract—Leaves of *Moringa oleifera* were collected from a specific tree in September and authenticated from Botanical Survey of India, Howrah, India (Voucher no. CNH/I-I/10)/2009/Tech.II/352). Leaves were air-dried, powdered and extracted with 50% ethanol. The extract was filtered and the filtrate was vacuum evaporated to completely remove the solvent and a powdered form was obtained. This powder form was dissolved in double-distilled water (10 mg/ml) with the help of a cyclomixer just before oral administration. This aqueous ethanolic *Moringa oleifera* leaf extract (10 mg/ml) has been referred as MoLE.

Hydroxyl radical scavenging activity—Hydroxyl radical scavenging activity was determined according to the method described by Singh *et al.*[16] MoLE (1 ml) in ethyl alcohol was taken in test tubes. Iron-EDTA (1 ml) solution (0.1% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of EDTA (0.018%) and 1 ml of dimethyl sulfoxide (DMSO) (0.85% v/v in 0.1 *M* phosphate buffer, *p*H 7.4) were added to these tubes and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90ºC for 15 min. The reaction was terminated by the addition of 1 ml of ice cold TCA (17.5% w/v). 3 ml of nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min and absorbance taken at 412 nm against reagent blank. Vitamin E was used as a standard.

% Hydroxyl radical scavenging activity=
(1-Absorbance of sample/Absorbance of blank) × 100.

Phytochemical characterization—Total antioxidant capacity of the extract was measured using standard Kit (Biovision, California). The assay was performed as per the detailed instructions of the manufacturer. The ascorbic acid content of MoLE was quantitatively determined according to the 2, 6-dichlorophenolindophenol dye method[17]. Total polyphenol content of the extract was quantified according to the method of Taga *et al.*[18] with modifications. MoLE was mixed with 2% Na_2CO_3 and allowed to stand at room temperature for 2 min. After the incubation 50% Folin-Ciocalteu's phenol reagent was added and the reaction tube was allowed to stand for another 30 min at room temperature prior to read the absorbance at 720 nm. Chromatographic separation of polyphenols were done by a Thermo Hypersil (Runcorn, UK) ODS (4.6×125 mm, 3µm) column with a C-18 guard column (Bandapak C18, 4.6×10 mm, 10µm). Both columns were placed in a column oven set at 35ºC. The HPLC system consisted of a Merck Hitachi (Hitachi, Tokyo, Japan) Lachrom Pump L-7100, Lachrom auto sampler L-7200, diode array detector (DAD) L-7450 and interface D-7000. Gradient elution was employed with a mobile phase consisting of 50 m*M* H_3PO_4, *p*H 2.5 (solution A) and acetonitrile (solution B) as follows: isocratic elution 95% A/ 5% B, 0-5 min; linear gradient from 95% A/ 5% B to 50% A/ 50% B, 5-55 min; isocratic elution 50% A/ 50% B, 5-65 min; linear gradient from 50% A/ 50% B to 95% A/ 5% B, 65-67 min; post time 6 min before next injection. The flow rate of the

mobile phase was 0.7 ml/min and the injection volumes were 10 µl of the standards and leaf extract. HPLC coupled with UV-Visible DAD (Diode array detector) was employed to separate, identify and estimate phenolic compounds in the aqueous ethanolic extract of *Moringa* leaf. These individual phenolic compounds have been identified in the MoLE according to their retention times and spectral characteristics of their peaks against those standards[19].

Experimental animals—Swiss albino male mice (*Mus musculus*), 6-8 weeks old with body weight of 24±2 gm were obtained from an inbred colony (Bengal chemical and Pharmaceuticals Ltd., Kolkata, India). Mice were maintained according to the guidelines set by Institutional Animal Ethical Committee (IAEC, India), maintained under the controlled conditions of temperature (23±2°C), humidity (50±5%) and a 12-h light-dark cycle. Animals were given standard mice feed (procured from Hindustan Lever Ltd., Mumbai) and water *ad libitum.*

Irradiation—Mice were irradiated using ^{60}Co gamma chamber (Board of Radiation and Isotope Technology, Mumbai) at Saha Institute of Nuclear Physics, Kolkata, India. Unanaesthetized animals were restrained in well-ventilated perspex boxes. They were exposed whole body to gamma radiation, at a dose-rate of 1 Gy/min and a source-to-surface distance of 77.5 cm.

Determinations of optimum dose of MoLE—Mice were divided into 5 groups of 10 animals each and MoLE (75, 100, 150, 300, 450 mg/kg body weight) was orally administered for 15 days. One hour after the last administration, animals were exposed to whole body 5 Gy gamma radiation. All treated animals were observed for 30 days for any signs of radiation sickness. The optimum dose, 300 mg/kg body weight, derived from this dose selection experiment has been used in this study.

Experimental design—Mice selected from an inbred colony were divided into 4 groups.

Group I: (Control) Mice given distilled water through oral gavages once in a day for 15 consecutive days.

Group II: Mice were given distilled water orally equivalent to MoLE for 15 days and 1 h after the final administration animals were exposed whole body to 5 Gy gamma radiations.

Group III: Mice were orally supplemented MoLE (300 mg/kg body wt/day) for 15 consecutive days.

Group IV: Mice were administered orally MoLE (300 mg/kg body wt/day) for 15 consecutive days once daily and after 1 hour of last dose; they were exposed whole body to 5 Gy gamma radiations.

Following varied treatments, mice were necropsied at 1, 7, 15 days post irradiations and livers were collected. Liver homogenate (10%) was prepared in a glass-Teflon homogenizer using phosphate buffer saline (*p*H 7.4) for biochemical estimation.

Estimation of lipid peroxidation in liver—Lipid peroxidation was estimated in terms of Thiobarbituric acid reactive substance (TBARS). TBARS in the homogenate was estimated using standard protocol[20]. Briefly, the homogenate was incubated with 15% trichloroacetic acid, 0.375% thiobarbituric acid and 5 N HCl at 95°C for 15 min, the mixture was cooled, centrifuged and the absorbance of the supernatant measured at 535 nm against appropriate blank. The amount of lipid peroxidation was determined using $\varepsilon=1.56\times10^5/M/\text{cm}$ and expressed as n mol of TBARS/g tissue.

Determination of Reduced glutathione content—Reduced Glutathione was determined according to the method described by Moron *et al.*[21] Liver homogenates were treated with 0.1 ml of 25% TCA and the resulting precipitate was pelleted by centrifugation. Free endogenous sulfydryl was assayed in a total 3 ml volume by adding 2 ml of 0.5 m*M* 5, 5 dithio-bis 2- nitrobenzoic acid (DTNB) prepared in 0.2 *M* phosphate buffer (*p*H 8) to 1 ml of the supernatant. The GSH reacts with DTNB to form a yellow-colored complex. The absorbance was read at 412 nm. Total protein content was estimated by the standard method of Lowry *et al.*[22]

Statistical analysis—Values are given as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) with Tukey's post hoc test was done for various groups of animals and $P<0.05$ was considered significant.

Results

Hydroxyl radical scavenging assay—EC_{50} (µg/ml), the concentrations to scavenge 50% of hydroxyl radical were found to be 20.24±1.5 µg/ml and 32.5±1.9 µg/ml for MoLE and standard (Vitamin E), respectively. The extract inhibition value was found to be lesser than standard having better hydroxyl radical scavenging activity than vitamin E.

Characterization of MoLE—Total antioxidant capacity was performed to measure the amount of a range of antioxidant molecules and the total antioxidant capacity of MoLE was 1.39 mole of trolox equivalent. Among total antioxidants, it was revealed that 100 g of dry leaf contains 0.0368 g ascorbic acid and 5 g total polyphenol. Different types of polyphenols were identified and estimated by HPLC profile of the extract is shown in Fig. 1. No interfering peaks were noted for the samples and the peaks were identified as catechin (peak 1), epicatechin (peak 2), ferulic acid (peak 3), ellagic acid (peak 4), myricetin (peak 5). Moreover, epicatechin (23.12%) and ferulic acid (53.26%) comprised the major peaks (Fig. 1).

Determination of optimum dose—Irradiated animals exhibited signs of radiation sickness within 4-5 days with the first death occurring on 9th day post 5 Gy irradiation. All irradiated mice showed radiation sickness such as lethargy, irritability, ruffling of hair and also showed 40% mortality within 30 days. Leaf extract control did not confer any toxic effects during the study period. Leaf extract treatment prior to irradiation caused reduced levels of radiation sickness compared to the irradiated animals. Treatment of mice with 75 mg/kg body weight prior to irradiation increased 20% protection in comparison to radiation control and first death occurred on 20th day. 100 mg/kg body weight and 150 mg/kg body weight leaf extract treatment before irradiation delayed the onset of radiation induced death and survivability were 90% after 30 days. 300 mg/kg body weight and 450 mg/kg body weight leaf extract treated plus irradiated animals achieved maximal survival of 100% (after 30 days). Thus, 300 mg/kg body weight was determined as the optimum dose (Fig. 2).

Inhibition of up regulation of LPO level in liver by MoLE—In irradiated group (Group II), lipid peroxidation level on day 1 was 5.38±0.48 n mol of TBARS/g tissue, day 7 was 6.24±0.23 n mol of TBARS/g tissue and on day15 the level was 6.88±0.16 n mol of TBARS/g tissue. The LPO levels in Group II were increased significantly (P<0.05) compared to control group at all autopsy intervals. In leaf extract treated group (Group III), lipid peroxidation significantly decreased compared to control on day 1 (2.64±0.19 n mol of TBARS/g tissue) and on day 15 (2.56±0.24 n mol of TBARS/g tissue). In Group IV i.e. MoLE treated as well as irradiated group, the lipid peroxidation on day 1 was 2.8±0.24 n mol of TBARS/g tissue, day 7 was 3.12±0.19 n mol of TBARS/g tissue and day 15 was 3.36±0.34 n mol of TBARS/g tissue. MoLE pretreatment prior to irradiation caused significant (P<0.05) decrease in the LPO levels on day 7 and day 15 as compared to irradiated groups (Fig. 3).

Inhibition of radiation-induced changes of hepatic reduced glutathione (GSH) by MoLE—In irradiated group, the GSH content showed significant (P<0.05) decrease at day 7 (590.37±41.98 n mol/mg protein),

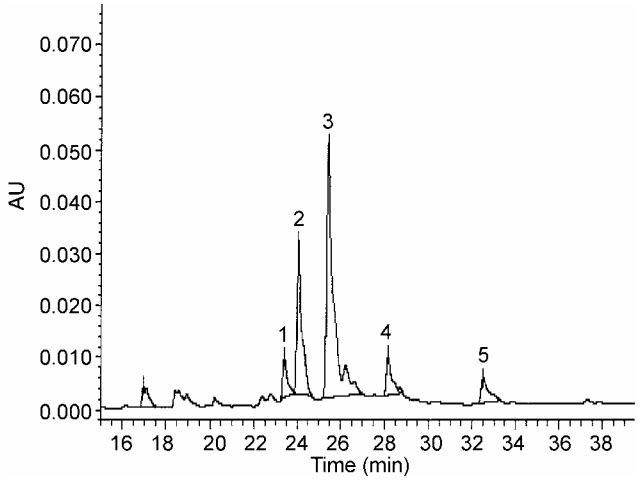

Fig. 1—HPLC-DAD chromatograms of flavonoids and phenolic acid mixture from *Moringa* leaf extract were recorded at 280 nm for catechin (Peak 1), epicatechin (Peak 2) and 340 nm for ferulic acid (Peak 3), ellagic acid (Peak 4) and myricetin (Peak 5).

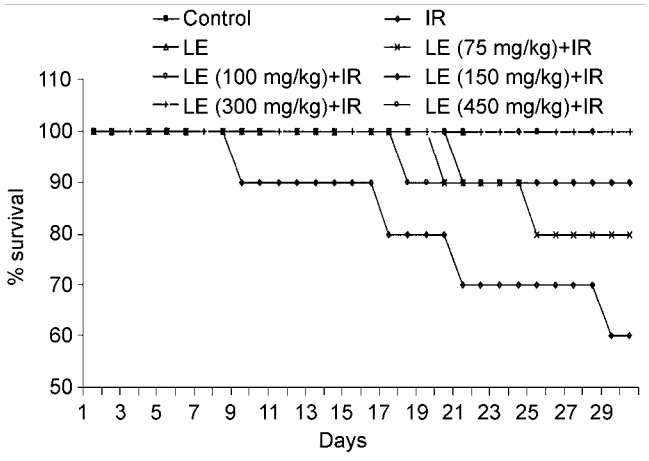

Fig. 2—Survival curve for the determination of optimum dose of *Moringa oleifera* leaf extract. 30 days survivability study was performed using Swiss albino mice (N=10) with or without *Moringa* leaf extract treatment at 5 Gy of gamma irradiation.

day 15 (617.62±59.68 n mol/mg protein) compared to control mice. In leaf extract treated group (Group III), GSH significantly increased compared to control on day 7 (954.25±29.8 n mol/mg protein) and on day 15 (1015.5±29.34 n mol/mg protein). In Group IV the GSH levels were 826±36.36 n mol/mg protein, 885.62±23.98 n mol/mg protein and 911±34.79 n mol/mg protein at day 1, 7, 15 respectively. Notably, in MoLE pretreated irradiated mice, a significant (P<0.05) increase in GSH content was observed at all intervals when compared with irradiated mice (Fig. 4).

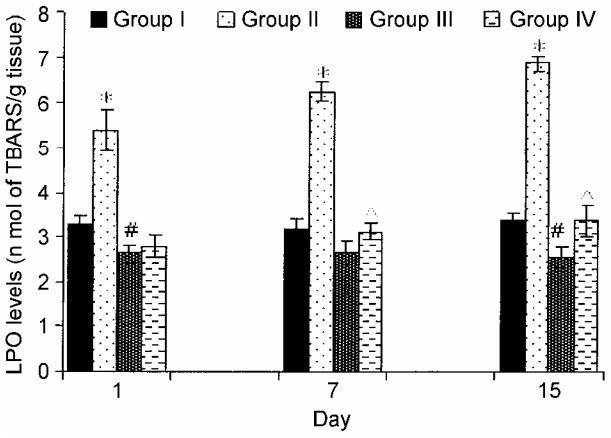

Fig. 3—Changes in Lipid peroxidation level (n mol of TBARS/g tissue) in liver of Swiss albino mice in different groups. [Values are Mean ± SEM of 8 animals. Significance level: P<0.05. *Irradiated versus control; #MoLE treated versus control; ^MoLE+radiation versus Irradiated].

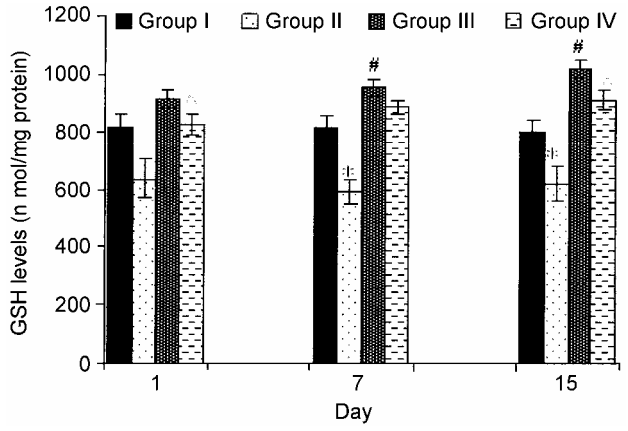

Fig. 4—Reduced glutathione (GSH) levels (n mol/ mg protein) in the liver of Swiss albino mice in different groups. [Values are Mean ± SEM of 8 animals. Significance level: P<0.05. *Irradiated versus control; #MoLE treated versus control; ^MoLE+radiation versus Irradiated].

Discussion

The present investigation deals with the radioprotective mechanism of *Moringa oleifera* leaf extract against radiation induced lipid peroxidation, as the measurement of lipid peroxidation is a convenient method to monitor oxidative cell damage. In this study it was observed that 5 Gy gamma radiation caused augmentation in LPO during the entire test period. The basic effect of radiation on the cellular membrane is believed to be peroxidation of membrane lipids. Gamma radiation produces reactive oxygen species (ROS)[1] which causes LPO. Lipid peroxidation, within the membrane has a destructive effect on the functional state of cell. The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative agents at the cost of antioxidants. Thus, in the present study, inhibition of LPO in biomembranes has been caused by antioxidants present in *Moringa oleifera*.

It was also observed that, radiation caused depletion in GSH levels in entire test period. Under normal conditions, the inherent defense system like glutathione protects against oxidative damage. GSH is a versatile protector and executes its radio protective function through free radical scavenging, restoration of the damaged molecules by hydrogen donation or by reduction of peroxides and maintenance of thiols in the reduced state[23]. The decrement of GSH could be due to an enhanced utilization of the antioxidant system during detoxification of the free radicals generated by radiation. This depletion of glutathione further enhanced the lipid peroxidation.

The present study clearly demonstrated that MoLE protected liver from radiation induced lipid peroxidation in entire study period. The result also showed that *Moringa* leaf extract quenched the hydroxyl radical which is the key mediator of lipid peroxidation. Moreover we showed that *Moringa* leaves possess variety of phytochemicals such as ascorbic acid, phenolics etc. HPLC data of phenolics revealed that MoLE contains a range of important antioxidant molecules such as catechin, epicatechin, ferulic acid, ellagic acid and myricetin. Ascorbic acid is considered to be the one of the most important antioxidant[24] and also acts to protect membranes against peroxidation by enhancing the activity of α-tocopherol, the chief lipid-soluble and chain-breaking antioxidant. The flavonoids, typical

phenolic compounds are powerful chain-breaking antioxidants[25]. It was reported that LPO can be inhibited by flavonoids, possibly through their activity as strong superoxide radical scavengers[26] and singlet oxygen quenchers. It is concluded that the above phytochemicals and other free radical scavengers present in total antioxidant pool of the leaf extract may neutralize the effect of radiation induced free radicals, thus preventing lipid peroxidation in mice. Some of these phytochemicals individually conferred protection when analyzed with same biochemical tests (data not shown). In this study, the GSH levels maintained after irradiation when the mice were pretreated with the leaf extract. This is because of its less utilization in the system as the phytoantioxidants defend against radiation induced free radicals. The restored GSH also plays an important role in inhibiting lipid peroxidation. Therefore the protection by *Moringa oleifera* has been attributed to phytoantioxidants present in MoLE.

Rao *et al.* showed similar preventive potential of methanolic extract of *Moringa* leaf after whole body irradiation[11]. Previously it was reported that water extract of *Moringa oleifera* leaf also possess radioprotective potential (data not shown). Several reports of the hepatoprotective potential of this leaf have been also documented[14,15,27]. Recently we have reported that *Moringa oleifera* leaf extract prevents radiation induced oxidative stress in mice[28].

In conclusion, *Moringa oleifera* leaf may be considered as a promising radiopreventive agent especially for the nuclear workers or for defense personals assuming possibility of nuclear exposure. The present study showed the capability of a vegetable phytoextract against the whole body radiation generated systemic stress in the mammalian model. The leaf extract efficiently prevented the lipid peroxidation and restored the GSH levels, two immediate markers of radiation stress. This popular vegetable may also be used as supplement for the patients who undergo total body irradiation (TBI) during different clinical maneuvers such as bone marrow transplantation, to prevent higher levels of lipid peroxidation. However, this needs further investigation before clinical trials.

Acknowledgement

A Junior Research Fellowship (JRF) from UGC-DAE Consortium for Scientific Research, Kolkata, India to MS is gratefully acknowledged. DKD obtained the fellowship from Centre for Research in Nanoscience and Nanotechnolofy, University of Calcutta. The research was partially supported by UGC-DAE, Consortium for Scientific Research, Kolkata Centre. Thanks are due to Dr. M. Nandi of SINP, Kolkata for technical supports.

References

1 Sadani G R & Nadkarni G D, Changes in lipid peroxide levels and the activity of reactive oxygen scavenging systems in thyroid tissue after exposure to radioactive iodine in rats, *Thyroid*, 7 (1997) 937.

2 Rollet-Labelle E, Grange M J, Elbim C, Marquetty C, Gougerot-Pocidalo M A & Pasquier C, Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis, *Free Radical Biol Med*, 24 (1998) 563.

3 Catala A, An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay, *Int J Biochem Cell Biol*, 38 (2006) 1482.

4 Halliwell B & Gutteridge J M C, Production of hydroxyl radicals in living systems In: *Free Radicals Biology and Medicine*, (Clarendon Press, Oxford), 1989, 31.

5 Greenberg M E, Li X M, Gugiu B G, Gu X, Qjn J, Salomon R G & Hazen S L, The lipid Whisker model of the structure of oxidized cell membranes, *J Biol Chem*, 283 (2008) 2385.

6 West J D & Marnett L J, Endogenous reactive intermediates as modulators of cell signaling and cell death, *Chem Res Toxicol*, 19 (2006) 173.

7 Gupta A, Gautam M K, Singh R K, Kumar M V, Rao C V, Goel R K & Anupurba S, Immunomodulatory effect of *Moringa oleifera* Lam extract on cyclophosphamide induced toxicity in mice, *Indian J Exp Biol*, 48 (2010) 1157.

8 Rathi B S, Bodhankar S L & Baheti A M, Evaluation of aqueous leaves extract of *Moringa oleifera* Linn for wound healing in albino rats, *Indian J Exp Biol*, 44 (2006) 898.

9 Debnath S & Guha D, Role of *Moringa oleifera* on enterochromaffin cell count and serotonin content of experimental ulcer model, *Indian J Exp Biol*, 45 (2007) 726.

10 Siddhuraju P & Becker K, Antioxidant Properties of various Solvent Extracts of Total Phenolic Contituents from Three Different Agroclimatic Origins of Drumstick Tree (*Moringa oleifera* Lam.), *J Agr Food Chem*, 51 (2003) 2144.

11 Rao A V, Devi P U & Kamath R, In vivo radioprotective effect of *Moringa oleifera* leaves, *Indian J Exp Biol*, 39 (2001) 858.

12 Kumar R, Singh P K, Arora R, Sharma A, Prasad J, Sagar R, Singh S & Sharma R K, Radioprotection by *Podophyllum hexandrum* in the liver of mice: A mechanistic approach, *Environ Toxicol and Pharmacol* 20 (2005) 326.

13 Bhatia A L & Jain M, *Spinacia oleracea* L. protects against gamma radiations: a study on glutathione and lipid peroxidation in mouse liver, *Phytomedicine* 11 (2004) 607.

14 Fakurazi S, Hairuszah I & Nanthini U, *Moringa oleifera* Lam prevents acetaminophen induced liver injury through restoration of glutathione level, *Food Chem Toxicol*, 46 (2008) 2611.

15 Selvakumar D & Natarajan P, Hepato-Protective activity of *Moringa oleifera* Lam in carbon tetrachloride induced Hepato Toxicity in Albino Rats, *Phcog Mag*, 4 (2008) 97.

16 Singh R P, Murthy C K N & Jayaprakash G K, Studies on the antioxidant activity of promgranate (*Pumica granatum*) pod and seed extracts using in vitro models, *J Agr Food Chem*, 50 (2002) 81.
17 Jones E & Hughes R E, Foliar ascorbic acid in some angiosperms, *Phytochemistry*, 22 (1983) 2493.
18 Taga M S, Miller E E & Pratt D E, Chia seeds as a source of natural lipid antioxidants, *J Am Oil Chem Soc*, 61 (1984) 928.
19 Mattila P, Astola J & Kumpulainen J, Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections, *J Agric Food Chem*, 48 (2000) 5834.
20 Beige A J & Aust S D, Microsomal lipid-peroxidation, *Meth Enzymol*, 52 (1978) 302.
21 Moron M S, Depierre J W & Mannervik B, Levels of glutathione, glutathione reductase and glutathione S- transferase activities in rat lungs and liver, *Biochim Biophys Acta*, 582 (1979) 67.
22 Lowry O H, Rosebrough N J, Farr A L & Randall R J, Protein Measurement with the folin phenol reagent, *J Biol Chem*, 193 (1951) 265.
23 Bump E A & Brown J M, Role of glutathione in the radiation response of mammalian cells in vitro and in vivo, *Pharmac Ther*, 47 (1990) 117.
24 Moser U & Bendich A, Vitamin C. In: *Machlin, L.J. (Ed.), Handbook of Vitamins, 2nd Edition*, (Marcel Dekker, New York), 1991, 195.
25 Torel J, Cillard J & Cillard P, Antioxidant activity of flavonoids and reactivity with peroxl radical, *Phytochemistry*, 25 (1986) 383.
26 Baumann J, Wurm J & Von BF, Prostaglandin synthetase inhibition by flavonoids and phenolic compounds in relation to their $O2^{\cdot-}$ scavenging properties, *Arch Pharm*, 313 (1980) 330.
27 Pari L & Kumar N A, Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats, *J Med Food*, 5 (2002) 171.
28 Sinha M, Das D K, Bhattacharjee S, Majumdar S & Dey S, Leaf extract of *Moringa oleifera* prevents ionizing radiation-induced oxidative stress in mice, *J Med Food,* 18 (2011) 1167.