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Original Article

RENOPROTECTIVE ACTIVITY OF *HIPPOPAHE* LEAF EXTRACT IN TOTAL BODY ^{60}CO-GAMMA-IRRADIATED MICE: AN OXIDATIVE AND HISTOPATHOLOGY STUDY

MANU SAINI[1, 2], MADHU BALA[1*], HUMAIRA FAROOQI[2], MZ ABDIN[2], JAGDISH PRASAD[1]

[1]Department of Radiation Biology, Institute of Nuclear Medicine and Allied Sciences, Brig. S. K. Mazumdar Marg, Delhi-110054, India.
[2]Department of Biotechnology, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India. Email: bala44@gmail.com

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ABSTRACT

Objective: This study investigated the time dependent effects of radioprotective dose of *Hippophae rhamnoides* leaf extract (coded as SBL-1) on kidney injuries in ^{60}Co-gamma-irradiated (10Gy) mice and also studied the underlying mechanisms.

Methods: Swiss albino Strain 'A' male mice (9 weeks old) were divided into different Groups: Group I (sterile water only); Group II (whole body ^{60}Co-gamma-irradiated, 10Gy); Group III (administered SBL-1, 30mg/kg body weight, intraperitoneally); Group IV (administered SBL-1, 30mg/kg body weight, intraperitoneally and after 30min whole body ^{60}Co-gamma-irradiated, 10Gy). Animals were sacrificed; kidneys were excised and processed for biochemical assays as well as tissue histology on day 1, 2, 3, 5, 7, and 15.

Results: Irradiation caused significant reduction in total thiol (T-SH) ($p<0.05$), in activity of catalase, superoxide dismutase (SOD) ($p<0.01$), glutathione-s-transferase (GST) ($p<0.05$) and increase in activity of alkaline phosphatase (ALP) and lipid per-oxidation (LPx) ($p<0.05$); increase in glomeruli shrinkage, nuclear degeneration, tubular dilations, widening of tubular lumen and collapsing of cellular architecture from day 2 till day 7. By day 12 all animals died. In Group IV animals, significant ($p<0.05$) increase in T-SH was from day 2-5, decrease in catalase and SOD activity on day 3 only and in GST at day 2 only. Mild changes in the tissues histology were observed till day 7; by day 10 no changes were observed in comparison to Group I. The early changes in Group III animals were self-reversible by day 5.

Conclusion: SBL-1 was non-toxic and countered radiation induced oxidative stress and tissue histological changes in kidney.

Keywords: *Hippophae rhamnoides*, Radiation, Kidney injury, Lipid per-oxidation.

INTRODUCTION

Total body exposure to low linear energy transfer (LET) ionizing radiation such as X-rays and gamma (γ) rays produce a dose dependent flux of free radicals and reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), hydrogen radicals ($H^{\bullet}$), oxide radicals ($O^{\bullet}$) and superoxide radicals ($O_2^{\bullet-}$) by a cascade of events, ultimately producing a state of oxidative stress leading to multiple inter- and intra-cellular damages [1, 2]. Under normal physiological conditions, a number of antioxidant defense mechanisms (via non-enzymatic radical scavengers as well as by anti-oxidant enzymes) play important role in maintaining the intra-cellular state of redox homeostasis, either directly by detoxifying ROS or indirectly by regulating their levels. Among the antioxidant enzymes, superoxide dismutase (SOD), catalase and glutathione-s-transferase (GST), are some of the important enzymes providing protection from oxidative injuries. SOD detoxifies ROS by catalyzing the dismutation of $O_2^{\bullet-}$ into H_2O_2 which is then converted to O_2 and H_2O in presence of catalase. Presence of both SOD and catalase is important to minimize the deleterious effects of superoxide radicals. GSH functions as a catalyst for disulfide exchange reactions and also contributes in H_2O_2 detoxification [3]. GSTs have an important role in conjugating GSH to the products of endogenous lipid per-oxidation and inactivating organic hydroperoxides, thus protecting the cell from the deleterious effects of oxidative stress [4]. The increased use of ionizing radiation in industry, warfare as well as in diagnostics and therapeutics has increased the threat of radiation accidents and therefore, the injuries to the mankind. Radiation injuries after total body irradiation are dependent on the exposure dose as well on the organ sensitivity. Kidneys are vital organs and serve important physiological functions such as excretion of waste products from the body and maintaining the balance of body fluids. During radiotherapy (of total body or upper abdominal) kidneys become important dose-limiting organs [5]. Although, the markers of renal toxicity after radiation exposure are not well understood, yet, GSTs are among the best characterized enzymes for renal injury [6]. More recent studies have reported increase in lipid per-oxidation and decrease in levels of antioxidant enzymes within 24h in kidneys of mice, which were given total body exposure to sub-lethal and lethal doses of gamma radiation [7, 8]. It was also reported that the kidneys could be protected from the early radiation induced oxidative stress up to 24h if the experimental mice were pretreated with gallic acid or with extract from *Acorus calamus*. However, the classic 30 day survival assay to determine the total body radiation protection potential, revealed that gallic acid rendered only 20% and *Acorus calamus* rendered only 5% radiation protection in total body irradiated (10Gy) mice [8, 9]. Development of medical interventions for protection from radiation injuries is a highly challenging field. Even after decades of research, no radioprotective drug has been approved for human use, till date. Furthermore, no radioprotective agent has been reported which is renoprotective as well as renders high survival benefit at lethal doses of low LET ionizing radiations.

In our laboratory, plants growing at high altitude regions of Indian Himalayas were explored for their radioprotective efficacy. Significantly high radioprotective potential of a standardized extract prepared from leaves of *Hippophae rhamnoides* (coded as SBL-1) was reported earlier [10]. *Hippophae rhamnoides* L., (common name Seabuckthorn; Family: Elaeagnaceae) is a plant growing naturally as well as cultivated in North-West Himalayas at 7000-15,000 feet. It is known for anti-oxidant and medicinal properties [11, 12]. It was demonstrated that one time treatment with SBL-1 at 30mg/kg body weight (b.w.), 30min prior to ^{60}Co-gamma-radiation (10Gy), rendered more than 90% survival i.e. >90% mice population survived till day 30 and beyond, while all the non-SBL-1 treated, ^{60}Co-gamma-irradiated (10Gy) mice died within 12 days (0% survival) after irradiation [10]. For development of an effective radiation countermeasure, it is

important to investigate the radioprotective potential of the candidate drug on different organs. The present study was undertaken to investigate the effects of SBL-1 (at radioprotective dose) on the early as well as late kidney injuries in the experimental mice, which were total body irradiated with lethal dose of ^{60}Co-gamma-radiation; and also to investigate the mechanisms of action of SBL-1.

MATERIALS AND METHODS

Chemicals

Nitroblue tetrazolium (NBT), Glutathione reduced (GSH), Glutathione oxidized (GSSG), Nicotinamide adenine dinucleotide phosphate (NADP), 1-chloro-2,4-dinitrobenzene (CDNB), NADPH, 1,2-dithio-bis-nitrobenzoic acid (DTNB) and Folin's reagent were purchased from Sisco Research laboratories Ltd., Mumbai, India. Sodium carbonate and trichloroacetic acid (TCA) was purchased from Himedia, India. *para* Nitrophenylphosphate (PNPP) was purchased from Calbiochem, India. Thiobarbituric acid (TBA), Aluminum chloride, Bovine serum albumin (BSA) and EDTA were purchased from Sigma Aldrich., St. Louis, MO, USA. Toulene, Ethyl acetate, Formic acid and HPTLC plates; RP-18 F254S (20x20 cm) were purchased from E. Merck, Mumbai, India. Reference standards Gallic acid ethyl ester, purity 98% w/w, and Rutin, purity 97% were purchased from Acros Organics, Fischer scientific; and Quercetin, purity 98% was purchased from Fluka Biochemika.

Plant extract preparation and standardization

Fresh green leaves of *Hippophae rhamnoides L.* (family *Elaegnaceae*) common name Seabuckthorn, (identified and confirmed by ethno-botanist; records preserved at museum, Defence Institute of High Altitude Research (DIHAR), Leh, India, specimen No SBTL-2006) were collected from a specific natural habitat of North-west Himalayas. The leaves were shade dried, powdered, soaked in distilled water and supernatant was lyophilized. The dried powder (yield 0.125g/g) was coded as SBL-1 and was standardized with reference to gallic acid ethyl ester, quercetin dihydrate and rutin trihydrate using high performance thin layer chromatography (HPTLC) method [13]. Briefly, the separation of each compound was carried out on silica gel 60 F_{254} pre-coated TLC aluminum plates, while allowing linear ascending (9cm) development at room temperature, in twin trough glass chamber saturated with suitably designed mobile phase. In SBL-1, total phenolic content (expressed as gallic acid equivalent) was determined by Folin-Ciocalteau method [14]. Total flavonoid content (expressed as quercetin equivalent) was determined by Aluminium chloride method [15]. Total tannin content (expressed as tannic acid equivalent) was determined by protein precipitation method [16]. The total thiol (T-SH) content was measured as per the method of Sedlak and Lindsay [17].

Animals

Adult Swiss albino Strain 'A' male mice (8-10 weeks old, weighing 28±2 g), from an inbred colony maintained at Institutional Animal Experiment Facility were used. The animals were maintained under controlled environment at 26±2^{0}C and were offered food and tap water *ad libitum*. The animals were issued after the approval from Institution Animal Ethics Committee and experiments were conducted in accordance with CPSEA guidelines.

Experimental procedure

The animals were divided into four groups. Group I-untreated control (administered sterile water (vehicle) only intraperitoneally), Group II-whole body ^{60}Co-γ-irradiated (10Gy), Group III-administered SBL-1 alone and Group IV-administered SBL-1, 30 min prior to whole (total) body ^{60}Co-γ irradiation (10Gy). Group I had 3 animals only while Group II, III and IV had 18 animals per Group. Each experiment was repeated two times. SBL-1 was dissolved in water and administered intraperitoneally (i.p) at 30mg/kg b.w. For whole body irradiation, each mouse was placed in a separate wire mesh container and given one time exposure to deliver 10Gy radiation dose using ^{60}Co-gamma-ray source (GC-220, Atomic Energy of Canada Ltd., Canada, dose rate of 0.31 rad/sec).

Tissue extraction

The animals were anesthetized and sacrificed by cervical dislocation. Three animals were sacrificed from each Group (II, III and IV) on day 1, day 2, day 5 and day 7. All animals of Group I were sacrificed on day 1. The number of animals surviving in Group II after day 10 was too small and was not sufficient to collect statistically significant data. Therefore, the data from Group II is presented only up to day 7.

On day 15, three animals of Group III and Group IV were sacrificed. From all the animals the kidneys were removed immediately after sacrifice and weighed. One kidney of each animal was fixed in 10% neutral buffered formalin for 48h for histopathological study; the contralateral kidney of the same animal was homogenized in ice-cold phosphate buffer saline (10%, w/v) in a homogenizer (Scientific systems) at 4^{0}C. The homogenate was centrifuged at 10,000 rpm for 20 min at 4^{0}C, and the resultant supernatant was used for subsequent biochemical analyses. For histology studies the animals sacrificed on day 2, 5, 7, 10 and 15 were used.

Biochemical parameters

To determine the lipid per-oxidation (LPx) malondialdehyde (MDA) formation per unit protein was measured by method of Ohkawa *et al* [18]. The absorbance was measured at 535 nm. Alkaline phophatase activity (ALP) was measured by Bessey Lowry-Block method [19] in terms of p-nitrophenol (PNP) formation. The changes in absorbance were measured at 405 nm. Total thiol (T-SH) content (mM/mg) was determined using the method of Sedlak and Lindsay [17]. The absorbance was recorded at 412 nm. Catalase activity was measured by the method of Claiborne [21]. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated and expressed as mmol H_2O_2 consumed/min/mg protein. SOD activity was measured by the method of Niskimi *et al* [22] and was expressed in terms of % inhibition of NBT reduction. The intensity of chromagen was measured at 560 nm. GST activity was assayed by the method of Habig *et al* [20] using CDNB as substrate. The changes in the absorbance were recorded at 343 nm and enzymes activity was expressed in terms of mmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6×10^3/M/cm.

Histopathological studies

The kidneys were processed as per standard procedure of dehydration and rehydration followed by paraffin embedding. The paraffin embedded kidneys were then sectioned (5 µm thickness) using the microtome and stained with hematoxylin and eosin (H & E), which were then examined under light microscopy (100X and 400X).

Statistical analysis

The results are presented as mean+ standard deviation (SD) of six animals for each time point. Statistical analyses of the results were performed using ANOVA (analysis of variance) followed by posthoc T-test.

RESULTS

Characterization of SBL-1 extract

The SBL-1 (per gram) contained 0.23+0.002g polyphenols equivalent to gallic acid; 0.093+0.008g flavonoids equivalent to quercetin; 0.32+0.006g tannins equivalent to tannic acid; and 0.827M total thiols. The HPTLC chromatogram of 3 marker compounds gallic acid ethyl ester, quercetin dihydrate and rutin trihydrate is presented in Fig.1. The content of gallic acid ethyl ester, quercetin dihydrate and rutin trihydrate were 12.09, 4.7 and 8.7mg/g respectively.

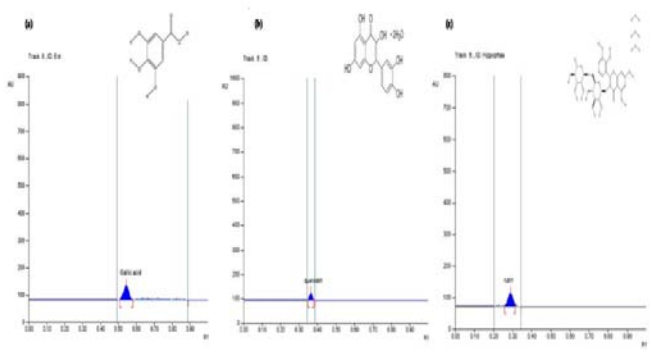

Fig. 1: (a) HPTLC chromatogram of gallic acid ethyl ester, (b) chromatogram of quercetin dihydrate, (c) chromatogram of rutin trihydrate as detected in SBL-1

Alterations in antioxidant status

In comparison to Group I animals, the Group II animals showed a significant increase ($p<0.05$) in LPx from day 1 till day 7. In Group III animals the values were not significantly different than Group I on all study days. In Group IV animals significant increase ($p<0.05$) was observed in LPx till day 3 only and thereafter the values were not different than the Group I animals (Fig 2a). The levels of ALP in Group II showed significant increase ($p<0.05$) in comparison to Group I animals from day 1 till day 7. In Group III the ALP levels were comparable to Group I animals. In Group IV animals ALP level was significantly high ($p<0.05$) in comparison to Group I on day 3 only while the levels were comparable to Group I animals at all other days (Fig 2b). In comparison to Group I animals, the levels of T-SH in Group II animals decreased significantly ($p<0.05$) from day 1 till day 5 but were not significantly different on day 7. In Group III animals the T-SH levels showed significant ($p<0.05$) increase at day 1, 2, 3, 5 and 15. In Group IV animals the levels of T-SH increased significantly ($p<0.05$) at day 2, 3 and 15 while on all other days the values were not different than Group I animals (Fig 2c).

In comparison to Group I animals, the activity of catalase was significantly ($p<0.01$) decreased in Group II animals on all days. In Group III animals the catalase activity increased significantly at day 1 only, thereafter the values were not different than Group I animals. In Group IV animals the activity of catalase was significantly ($p<0.05$) decreased only at day 3, thereafter, the activity was not different in comparison to the Group I animals (Fig 2d).

The activity of SOD was significantly ($p<0.01$) decreased in Group II animals from day 1 till day 7 when compared with Group I animals. In Group III animals the SOD activity differed significantly ($p<0.05$) at day 1 only. In Group IV animals SOD activity decreased significantly ($p<0.05$) at day 3 only and thereafter, the values were not different in comparison to the Group I animals (Fig 2e).

The activity of GST was significantly ($p<0.05$) decreased in Group II animals from day 1 till day 7 when compared to Group I animals. In Group III animals the GST activity increased significantly at day 5, 7 and 15 in comparison to Group I animals. In Group IV animals the GST activity significantly ($p<0.05$) decreased only at day 2 and thereafter the values were not significantly different in comparison to the Group I (Fig 2f).

Histopathology

The typical view of histological examination of the kidney sections (for day 2, 5 and 7) as observed in this study is presented in Fig 3. A summary of changes observed on day 2, 5, 7, 10 and 15 is presented in Table 1, on the scale of 0-5; where 0 represents no change in comparison to Group I animals and 5 represents maximum change in comparison to Group I animals of this study.

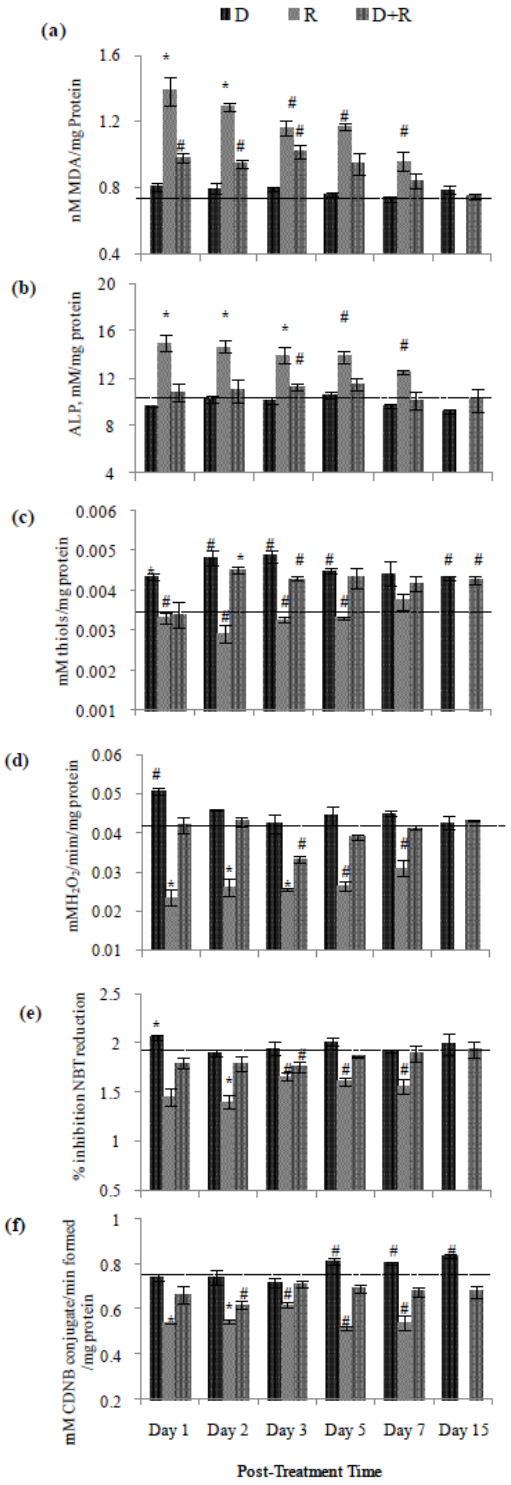

Fig. 2: (a) Malondialdehyde (MDA), (b) alkaline phospahatse (ALP), (c) total thiol levels and (d) catalase activity, (e) SOD activity and (f) GST activity recorded in kidneys of mice treated with SBL-1 alone (D), Radiation (R) and SBL-1+ radiation (D+R). The data was collected on different days after one time treatment. Dashed line represents values obtained from untreated animals. Each value represents mean+SD of six animals. The data presented significant difference with respect to untreated control at p<0.05 (#) and at p<0.01 (*).

The transverse sections of kidney of Group I animals showed normal histoarchitectural pattern of the Bowman's capsule as well as the distal and proximal convoluted tubules. In comparison to Group I the kidney sections from Group II animals showed multiple histological alterations. The interstitial hemorrhages were observed at day 5 and 7. The glomerulus shrinkage showed an increasing trend from day 2 till day 7. The renal tubules showed nuclear degeneration, dilations, widened lumen, enucleated cells and substantially increased inter-tubular spacing from day 2 till day 7. Large numbers of apoptotic nuclei were observed at day 7. In Group III animals a slight shrinkage in glomerulus and some enucleated cells in tubules were observed only at day 10. These changes were not observed on any other day. In Group IV animals, in comparison to Group I animal's mild interstitial hemorrhages were observed at day 7 only, which were not observed on any other day. Small amount of glomerular shrinkage was observed at day 2, 7 and 10 but not on day 15. Nuclear degeneration in few cells was observed at day 2, 5, 7 and 15. Slight widening of lumen though was observed at day 2, 5, 10 and 15, but in comparison to Group II animals the extent of widening was much smaller. The small increase in inter-tubular spacing observed in Group IV, animals as compared to Group I animals, at day 2 and 5 but was completely recovered at day 7 and 10.

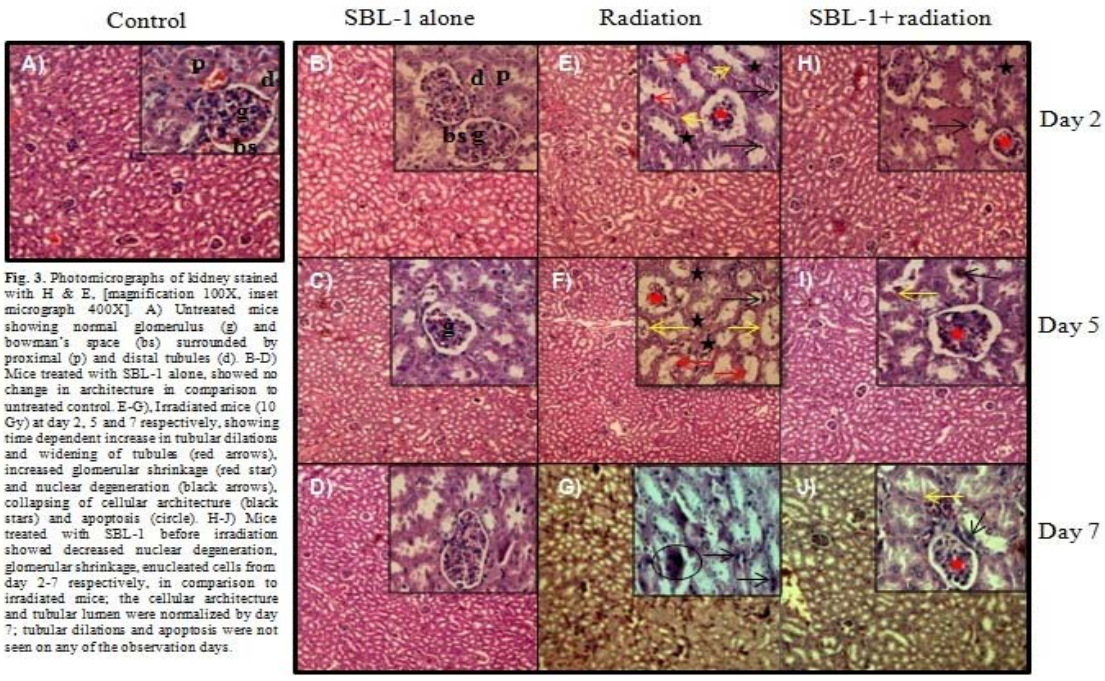

Fig. 3. Photomicrographs of kidney stained with H & E, [magnification 100X, inset micrograph 400X]. A) Untreated mice showing normal glomerulus (g) and bowman's space (bs) surrounded by proximal (p) and distal tubules (d). B-D) Mice treated with SBL-1 alone, showed no change in architecture in comparison to untreated control. E-G), Irradiated mice (10 Gy) at day 2, 5 and 7 respectively, showing time dependent increase in tubular dilations and widening of tubules (red arrows), increased glomerular shrinkage (red star) and nuclear degeneration (black arrows), collapsing of cellular architecture (black stars) and apoptosis (circle). H-J) Mice treated with SBL-1 before irradiation showed decreased nuclear degeneration, glomerular shrinkage, enucleated cells from day 2-7 respectively, in comparison to irradiated mice; the cellular architecture and tubular lumen were normalized by day 7; tubular dilations and apoptosis were not seen on any of the observation days.

Table1: Time dependent kidney damage score. ↔ denotes no change in kidney architecture in comparison to untreated control mice. ↑ denotes increased trend in comparison to untreated control.

		Day 2		Day 5		Day 7		Day 10		Day 15	
		Grade	Trend	Grade	Trend	Grade	Trend	Grade	Trend	Grade	Trend
Interstitial	D	0	↔	0	↔	0	↔	0	↔	0	↔
Hemorrhages	R	0	↔	2	↑	4	↑				
	DR	0	↔	0	↔	1	↑	0	↔	0	↔
Glomerular	D	0	↔	0	↔	0	↔	1	↑	0	↔
Shrinkage	R	2	↑	3	↑	4	↑				
	DR	1	↑	0	↔	1	↑	1	↑	0	↔
Nuclear	D	0	↔	0	↔	1	↑	0	↔	1	↑
Degeneration	R	3	↑	4	↑	5	↑				
	DR	1	↑	1	↑	1	↑	0	↔	1	↑
Tubular Dilations	D	0	↔	0	↔	0	↔	0	↔	0	↔
	R	3	↑	4	↑	5	↑				
	DR	0	↔	0	↔	0	↔	0	↔	0	↔
Widened Tubular	D	0	↔	0	↔	0	↔	0	↔	1	↑
lumen	R	4	↑	4	↑	5	↑		↔		↑
	DR	1	↑	1	↑	0	↔	1	↑	1	↑
Enucleated	D	0	↔	0	↔	0	↔	1	↑	0	↔
Cells	R	3	↑	4	↑	5	↑				
	DR	1	↑	1	↑	1	↑	0	↔	0	↔
Apoptosis	D	0	↔	0	↔	0	↔	0	↔	0	↔
	R	0	↔	0	↔	5	↑				
	DR	0	↔	0	↔	0	↔	0	↔	0	↔
Collapsing	D	0	↔	1	↑	0	↔	0	↔	0	↔
	R	3	↑	4	↑	5	↑				
	DR	1	↑	1	↑	0	↔	0	↔	0	↔

DISCUSSION

Whole body exposure to lethal doses of low LET ionizing radiation causes dysfunctioning or failure of multiple organs. Increase in cellular oxidative stress due to overproduction of ROS is considered to be responsible for majority of this damage [23]. The kidney is an important and radiosensitive organs. Radiation nephropathy occurs after abdominal or total body irradiation [24]. Events leading to kidney damage after total body exposure to lethal doses of radiation are not well investigated. Body's innate mechanism has many enzymes and non-protein compounds that protect from the damaging effects of free radicals and ROS produced during normal metabolism and also due to external stimuli. The antioxidant enzymes such as SOD, catalase and GST work together with non-enzymatic antioxidants such as T-SH to neutralize free radicals and counter the oxidative cellular damage. A reduction in the activity of these enzymes is likely to adversely affect the resistance of cellular lipids, proteins and DNA to oxidative damage [25]. Lipid per-oxidation, an indicator of oxidative damage in the kidney [26], involves oxidative degradation of polyunsaturated fatty acids (PUFA). Lipid per-oxidation in biological membranes causes alterations in the membrane structure and function resulting in decreased membrane fluidity as well as inactivation of many membranes bound enzymes [27].

In this study significant increase in lipid per-oxidation, ALP levels and decrease in T-SH content in the irradiated animals (Group II, Fig 2) was in line with the earlier studies [8]. The elevation in lipid per-oxidation in Group II (Fig 2a) was due to high ROS and free radicals generated after irradiation. The increased ALP levels in Group II animals (Fig 2b) indicated the increased phosphorylation or tissue damage caused by ROS and free radicals. SOD dismutates $O_2^{\bullet-}$ and catalase detoxifies the resultant H_2O_2. Decrease in SOD and catalase in irradiated animals (Fig 2d, e) suggested that detoxification of $O_2^{\bullet-}$ and H_2O_2 was adversely effected. GSTs are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to the thiol Group of GSH, producing less toxic forms [28]. The significant decrease of GST activity (Fig 2f) after total body irradiation indicated insufficient detoxification in kidney. Decreased T-SH also suggested the insufficient detoxification of free radicals in Group II animals (Fig 2c). The observed major histological alterations in Group II animals (Fig 3, Table 1, interstitial hemorrhages, glomerular shrinkage, nuclear degeneration, tubular dilations and apoptotic nuclei) indicated the cumulative effects of damage to lipids, protein and DNA resulting in death of animals of Group II by day 12.

In SBL-1 pretreated and lethally irradiated animals (Group IV) the restoration of normal levels of T-SH and GST activity from day 2 onwards (Fig 2c, f); catalase and SOD activity from day 3 onwards (Fig 2d, e), together with lipid per-oxidation and ALP levels from day 5 onwards, demonstrated the protective effect of SBL-1. Only mild histological alterations observed in SBL-1 pretreated lethally irradiated animals in comparison to Group I animals till day 10 (Fig 3, Table 1) also demonstrated the kidney tissue protective action of SBL-1. The SBL-1 contained phenolics, flavanoides and tannins. The quantity of gallic acid ethyl ester, quercetin dihydrate and rutin was in the ratio of 12091.15, 4691.66 and 8684.72 (µg/g of dried leaf extract) [13]. Gallic acid has strong antioxidant properties [29]. The phenolic compounds possess strong antiradical properties and the number of hydroxy groups connected with the aromatic ring, in ortho or para-position relative to each other, enhances antioxidative and antiradical activity of phenolic acids [30]. The flavanoids such as rutin and quercetin also possesses antioxidant properties [31]. The number of hydroxy groups and the presence of a 2, 3-double bond and orthodiphenolic structure enhance the antiradical and anti-oxidative activity of flavonoids [30].

Rutin also acts as anti-inflammatory agent [32]. The SBL-1 is also rich in tannins and thiols. It was reported that the polyphenolic nature of tannic acid (relatively hydrophobic "core" and hydrophilic "shell") is responsible for its antioxidant action [33]. Thiols act as intracellular antioxidants by scavenging free radicals as well as by influencing the enzymatic reactions. It is proposed that the action of all these constituents in SBL-1 was responsible for countering the radiation induced changes in SOD, catalase and GST activity in Group IV animals resulting in the renoprotective action.

The renoprotection by SBL-1 also contributed to survival of more than 90% animals beyond day 30 as observed earlier [10]. No change in lipid per-oxidation and ALP throughout the study in Group III animals suggested the non-toxic nature of SBL-1. *Hippophae* leaves were reported to be non-toxic in SD rats [34]. Although significant increase in SOD and catalase activity was observed in Group III animals on day 1, the effects were completely reversed by day 2 and the activity of SOD and catalase was not different than the Group I animals from day 2 onwards (Fig 2d, e). The increased GST activity from day 5 onwards in Group III animals was probably to balance the increased T-SH levels in kidney of animals treated with SBL-1 (Group III).

Development of medical countermeasures to protect living organisms from damaging effects of ionizing radiations is an important and challenging field. Such drugs have utility not only in situations such as nuclear warfare or nuclear accidents but could also be investigated for their utility as adjuvant to radiotherapy to protect the normal tissues. Since no chemical radioprotective drug has been approved till date due to the severe toxicity, the plant extracts demonstrating radio-protective effects are gaining more acceptability due to their non-toxic nature.

In conclusion the results of present study demonstrated that the damage to kidney caused by whole body exposure to lethal dose of gamma radiation can be countered by administration of SBL-1 prior to irradiation.

CONFLICT OF INTEREST

We have nothing to disclose.

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