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Evaluation of Salicin Isolated from *Salix subserrata* as a Radioprotector against Gamma Irradiation Induced Ultrastructural and Electrophoretic Changes in Spleen Tissue in Rats

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Abstract

The aim of this study was to investigate the radioprotective effect of salicin against irradiation effect on spleen tissue in male rats. Lipid peroxidation product (MDA) level was measured as thiobarbituric acid reactive substance. Ultrastructural examination was carried out in spleen tissue by scanning electron microscope (SEM). The polyacrylamide gel electrophoresis for native protein, lipoprotein and zymogram were carried out in spleen homogenate. As expected, salicin resisted the irradiation effect and declined the MDA level in spleen homogenate of all treated groups. The alterations which were occurred as a result of irradiation in the spleen tissue could not be detected microscopically but they were detected electrophoertically at levels of protein and isozymes. Salicin prevented the qualitative mutagenic effect of irradiation on the electrophoretic protein pattern in the irradiated salicin simultaneous treated group (SI = 0.73). It showed the highest protective effect against qualitative mutagenic irradiation effect in catalase pattern in irradiated salicin pre-treated group (SI = 0.80). It could not prevent the abnormalities occurred qualitatively and quantitatively as a result of irradiation in peroxidase pattern in all irradiated salicin treated groups. While the esterase pattern showed the same electrophoretic pattern in the all irradiated salicin treated groups. The results suggested the radioprotective ability of salicin against gamma irradiation effect on various ultrastructural and electrophoretic patterns in spleen tissue of male rats.

1 Introduction

Gamma rays are a packet of pure electromagnetic rays which are photons of high frequency and high energy and hence short wave length[1]. They can penetrate into living tissues or cells and result in transduction of radiation energy to biological materials. The absorbed energy of ionizing radiation can break chemical bonds and cause ionization of different molecules including water and different biological essential macromolecules as DNA[2], membrane lipids and proteins[3].

It has been reported that whole-body gamma irradiation induces oxidative stress. The most important consequences of oxidative stress are lipid peroxidation, protein oxidation and depletion of antioxidants[4,5]. It was found that irradiation decreases tissue concentrations of natural nonenzymatic antioxidants[6,7] and causes induction of lipid peroxidation as evidenced by increased malondialdhyde (MDA)[8].

Spleen plays an important role in immune functions by proliferating lymphocytes. The integrity of the immune system depends upon the normal functioning of the lymphoid organs so that the alterations in the homeostasis of spleen tissues will affect immune responses[9]. It was demonstrated that spleen was the most biosensitive organs to low doses of irradiation in rats[10]. Irradiation caused alterations in spleen tissue and it caused induction of DNA affecting the radiosensitive gene in the spleen of rats[11,12]. Ezz,[13] showed that

spleen taken to study the ameliorative effect of radioprotector against irradiation induced oxidative stress and immune responses in rats.

Proteins are the most complex compounds and at the same time the most characteristic of living matter. They are present in all viable cells; they are the compounds which, as nucleoproteins, are essential for cell division and, as enzymes and hormones, control many chemical reactions in the metabolism of cells. Thus, the separation and characterization of the individual proteins facilitate the study of the chemical nature and physiological function of each protein[14]. They are major targets for oxidative damage due to their abundance and rapid rates of reaction with a wide range of radicals and excited state species[15]. Changes in the protein patterns of the tissues may reflect specialization and adaptation in the organisms. It is worthy to note that each protein is considered as reflect to the activity of specific gene through the production of enzyme, which act as catalyst to produce the demanded protein; this type of produced protein is responsible for a specific biological character[16]. The radiation-induced alteration of the protein structure was observed by measuring the changes in the molecular properties of the proteins[17]. Recently, it was found that irradiation showed significant increase in protein carbonyls by 73%[18].

Antioxidants enzymes as catalase (CAT) and peroxidase (GPx) are important in the elimination of free radicals[19,20]. They are involved to counteract the toxicity of ROS[21]. These enzymes are the first line of defense against oxidative injury. Superoxide dismutase is the primary step of the defense mechanism in the antioxidant system against oxidative stress by catalyzing the dismutation of 2 superoxide radicals (O^{2-}) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2)[22]. H_2O_2 can synthesize a highly reactive OH, is neutralized by the combined action of CAT and GPx in all vertebrates[23,24]. These enzymes act in coordination and the cells may be pushed to oxidative stress state if any change occurs in the levels of enzymes[21].

Irradiation can exert a significant inflammatory response in cells. So, it is essential to develop methods to target the radiosensitive organs and / or to protect the normal tissues. Antioxidants eliminate the free radicals and neutralize reactive oxygen species (ROS) before they can do their damage. However, much remains unknown about mechanisms of radio-protection. Development of protective agents presented new solutions for recovery of undesired tissue damage induced by irradiation[25,26].

The discovery of radioprotectors for the first time seemed to be very promising and has attracted the interest of a number of radiobiologists. Although synthetic radioprotectors such as the aminothiols have yielded the highest protective factors; typically they are more toxic than naturally occurring protectors[27].

Salicin ($C_{13}H_{18}O_7$) is a natural product extracted from several species of *Salix* (willow) and *Populus* (poplar), and was also found in *Gaultheria procumbens* (wintergreen) and in *Betula lenta* (sweet birch), the volatile oils of which consist almost entirely of methyl salicylate[28]. Salicin is considered as natural aspirin. It is very possible to be digested without side effects in the stomach and kidneys, while acetylsalicylic acid is known to upset the stomach and in some cases damage kidneys. Scientists believe that this is because salicin is converted to acetylsalicylic acid after the stomach has absorbed it[29]. It is a pro-drug that is gradually transported to the lower part of the intestine, hydrolysed to saligenin by intestinal bacteria, and converted to salicylic acid after absorption. It thus produces an antipyretic action without causing gastric injury[30].

It belonged to the phenolic compounds which are believed to work synergistically to promote healthy conditions through a variety of different mechanisms, such as enhancing antioxidant activity, impacting cellular processes associated with apoptosis, platelet aggregation, blood vessel dilation, and enzyme activities associated with carcinogen activation and detoxification[31,32].

The present main objective is to optimize salicin as a radioprotector against effect of gamma irradiation on the spleen tissue in the hope that this compound may be further explored as novel antioxidative radioprotector.

2 Materials and Methods

2.1 Salicin isolation

Salicin was extracted and isolated from fresh young leaves of willow trees *(Salix subserrata, Salix safsaf)* according to method suggested by Mabry *et al*.[33] and purified according to method described by Kur'yanov *et al*.[34] then identified qualitatively by advanced chromatographic techniques.

2.2 Acute toxicity test

The safety of salicin orally was evaluated by determination it's LD_{50}. Forty eight adult female albino mice weighing 20-25 g was used to study acute toxicity. It was divided into 6 groups each of 8 mice. The groups were treated orally with rising doses of 500, 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of aqueous solution of salicin solution. Mortality was recorded 24 hrs post treatment. The LD_{50} was calculated according to the equation suggested by Paget and Barnes[35].

2.3 Animals

Seven groups of male rats weighing between 150-200 gm per one obtained from the animal house laboratory of national research centre. Ten rats in each group. All the animals were kept under normal environmental and nutritional conditions. The animal groups

were divided into Rats were non-irradiated and non-treated with salicin representing Control group; Rats were non-irradiated but treated with the safe dose of salicin (was about 150 mg / Kg) taking in the consideration weight of each rat representing Salicin treated group; Rats were irradiated at the dose 7 Gy and non-treated with salicin representing Irradiated group; Rats were treated with salicin for 15 days followed by irradiation at the 15th day representing Irradiated salicin pre-treated group; Rats were treated with salicin for 15 days followed by irradiation at the 15th day then the treatment was continued daily for another 15 days representing Irradiated salicin prepost-treated group; Rats were irradiated and treated with salicin at the same time of irradiation and continue daily for 15 days representing Irradiated salicin simultaneous treated group; and Rats were irradiated at the same gamma dose then left without treatment for 15 days. At the 15th day, the rats were treated with salicin for another 15 days representing Irradiated salicin post-treated group.

2.4 Irradiation

Whole body of the animals was exposed to an acute single dose of 7 Gy delivered at a dose rate of 1.167 Rad/Sec. using cobalt-60 (Co^{60}) from the biological irradiator gamma cell source belonging to Middle Eastern Regional Radioisotopes Center for Arab Countries, Dokki, Cairo, Egypt.

2.5 Lipid peroxidation measurement

Lipid peroxidation level was measured as thiobarbituric acid reactive substance in spleen homogenate according to method of Ohkawa *et al*.[36].

2.6 Scanning Microscopic examination (SEM).

This examination was carried out in piece of spleen tissue using SEM. The tissue was preserved in gluteraldhyde purchased from Gpr Chemicals Co. It was prepared according to the method suggested by Tánaka[37] who reported that the specimen showed be passed through series of the dehydration steps by placing it in ethyl alcohol then incubated for 15 min. then coated with the golden atoms to be ready for the electron microscopic examination.

2.7 Electrophoretic protein and lipoprotein patterns

Total protein was determined in spleen homogenate according to Bradford[38]. The sample was mixed with the sample buffer. The protein concentration in each well must be about 70 µg protein. Proteins were separated through polyacrylamide gel electrophoresis (PAGE). Electrode and gel buffer and polyacrylamide stock were prepared according Laemmli[39]. After electrophoretic separation, the gel was gently removed from the apparatus and put into a staining solution of coomasie brilliant blue for native protein pattern[40] and staining solution of sudan black B (SBB) for lipoprotein pattern[41].

2.8 Isozyme

Native protein gel was stained for peroxidase pattern using certain stain prepared according to the method suggested by Rescigno *et al*.[42], for catalase pattern according to method described by Siciliano and Shaw[43] and for esterase pattern according to method of Baker and Manwell[44].

2.9 Data analysis

The polyacrylamide gel plate was photographed, scanned and then analyzed using Phoretix 1D pro software (Version 12.3). The similarity index (S.I.) compares patterns within, as well as, between irradiated and non-irradtated samples. The similarity values were converted into genetic distance (GD) according the method suggested by Nei and Li[45].

3 Statistical Analysis

All the grouped data were statistically evaluated with SPSS/16.00 software. The results were expressed as mean ± SE of studied groups using the analysis of variance test (one-way ANOVA) followed by student's t-test. P values of less than 0.05 were considered to indicate statistical significance. The means of irradiated groups and the salicin treated groups were individually compared with those of control group. The irradiated group was compared with irradiated salicin treated groups.

4 Results

4.1 Lipid peroxidation

As compared to control, irradiation caused significant ($P < 0.05$) elevation in the MDA level in spleen tissue. Salicin administration showed the ameliorative effect against irradiation by reducing MDA level in all irradiated salicin treated rats. From the data compiled in Table 1, it was found that salicin showed the most suitable antagonistic effect against irradiation on spleen of irradiated salicin pre-treated group as compared to irradiated group.

4.2 Spleen ultrastructure

The ultrastructural observations in spleen tissue of control rats revealed normal tissue surface (Fig. 1a). Salicin administration showed normal appearance, no ultrastructural changes and no deviation from control (Fig. 1b). Irradiation caused no obvious abnormalities on the spleen surface indicating to inability of this radiation dose to cause any differences observed microscopically on surface of the spleen tissue (Fig. 1c). In the irradiated salicin pre-treated group, there was superfacial lesions with cellular loses (Fig. 1d).

Spleen tissue showed smooth appearance in irradiated salicin simultaneous treated group with presence of blood aggregates on

the tissue surface (black arrow) (Fig. 1e). In the irradiated salicin prepost-treated group, it was found that there was surface erosion (red arrow) with presence of blood aggregates (black arrow) (Fig. 1f).The irradiated salicin post-treated group displayed deep cracking with widening the gap between the cells (Fig. 1g).

Table 1: Effect of irradiation, salicin and their combination in various treatment modes on level of lipid peroxidation in spleen tissue of male rats

Group / Organ	Control	Sal.	Irr.	Irradiated salicin treated groups			
				Pre-treated	Simultaneous	Prepost-treated	Post-treated
Spleen	137.49	150.40	1050.08[a]	231.60[a,b]	344.75[a,b]	443.94[a,b]	462.54[a,b]
(nmol/g)	± 4.23	± 6.18	± 10.55	± 6.46	± 9.82	± 6.66	± 8.96

A : Different from control at P < 0.05, b : Different from the irradiated group at P < 0.05, Note: Sal. ; salicin, Irr. : Irradiated

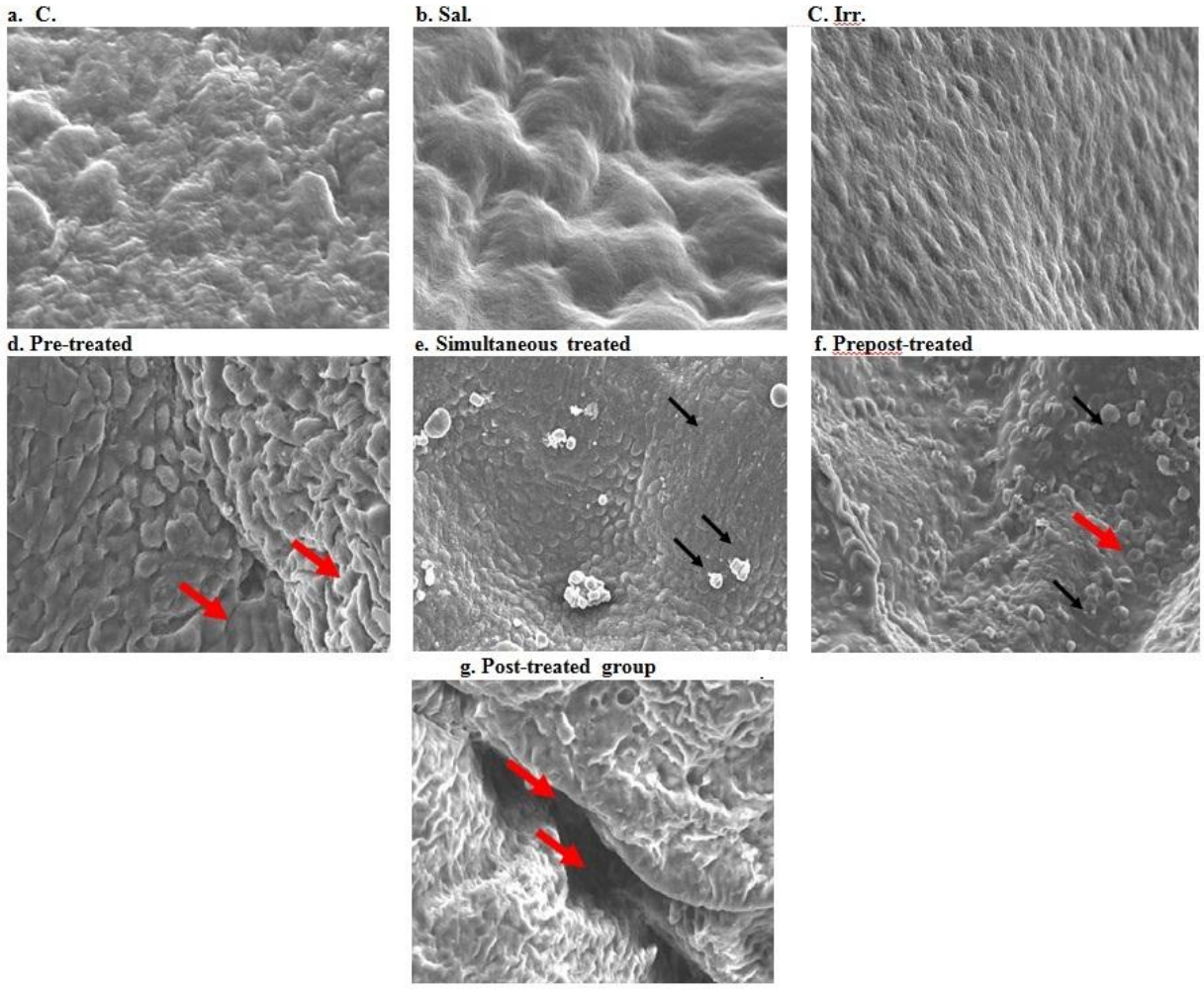

Fig. 1: The scanning electromicrograph showing effect of gamma irradiation on spleen tissue and the protective effect of the salicin when compared to the control and the salicin treated male rats

4.3 Electrophoretic protein pattern

As shown in Table 2 and illustrated in Fig. 2, there were no common bands in all groups but there was one characteristic band appeared

in irradiated salicin post-treated group with R_f 0.32 (Mwt 55.32 KDa and B% 7.13). Irradiation caused severe qualitative mutation represented by disappearance of 2 bands with deviation of the 3rd, 4th, 5th and 6th bands to be appeared with R_{fs} 0.21, 0.48, 0.58 and 0.64 (Mwts 121.29, 25.82, 18.82 and 16.84 KDa) respectively. Salicin showed the highest antagonistic effect against the qualitative mutagenic effect of irradiation in the irradiated salicin simultaneous treated group. It could not prevent the quantitative mutation which was represented by increasing B% of the 5th normal band (R_f 0.63, Mwt 17.16 KDa and B % 55.42).

From the SI values, it was found that the lowest SI value (SI = 0.15) was recorded with irradiated salicin post-treated group and the highest SI value (SI = 0.67) was recorded with irradiated salicin pre-treated and simultaneous treated groups. As compared to SI value of the irradiated group (SI = 0.20), salicin prevented the irradiation effect in all groups except irradiated salicin post-treated group.

Table 2: Data of the electrophoretic protein pattern in spleen tissue of control, irradiated and irradiated salicin treated groups at different therapeutic modes in male rats

Control			Salicin			Irradiated			Irradiated salicin treated											
									Pre-treated			Simultaneous			Prepost-treated			Post-treated		
Rf.	Mwt	B. %	Rf.	Mwt	B. %	Rf.	Mwt	B. %	Rf.	Mwt	B. %	Rf.	Mwt	B. %	Rf.	Mwt	B. %	Rf.	Mwt	B. %
0.11	199.20	5.92	0.11	195.70	6.85	0.21	121.29	13.40	0.12	189.63	17.29	0.16	159.48	10.25	0.06	234.17	18.25	0.06	233.01	2.66
0.18	140.64	10.79	0.16	159.48	5.89	0.48	25.82	9.18	0.24	99.73	25.69	0.24	101.81	8.93	0.23	103.92	31.56	0.14	175.09	8.84
0.25	93.60	6.82	0.21	120.18	19.91	0.58	18.82	12.29	0.62	17.27	57.03	0.51	22.94	16.53	0.61	17.65	50.19	0.25	90.61	7.99
0.52	22.45	14.30	0.49	24.39	21.40	0.64	16.84	65.13	—	—	—	0.58	18.89	8.87	—	—	—	0.32	55.32	7.13
0.62	17.31	13.66	0.58	18.56	6.73	—	—	—	—	—	—	0.63	17.16	55.42	—	—	—	0.48	25.67	9.44
0.66	16.47	48.50	0.66	16.56	39.23	—	—	—	—	—	—	—	—	—	—	—	—	0.53	21.31	10.03
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.62	17.48	53.92
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

R_f : Rate of Flow, Mwt. : Molecular Weight, B. % : Band Percent

Note : Arrangement of the bands at each lane is not correlated with the other bands in the other lanes

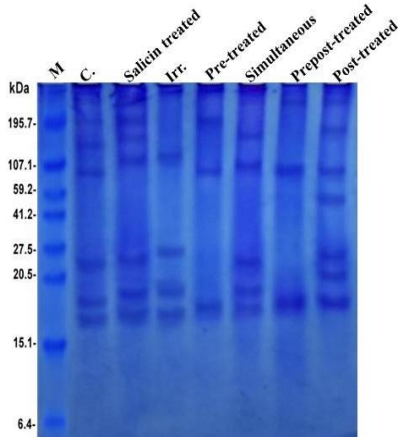

Fig. 2: Electrophoretic pattern showing effect of salicin against the irradiation effect on protein pattern in spleen tissue of male rats

4.4 Electrophoretic lipoprotein pattern

Lipoprotein pattern showed thatthere were 2 common bands appeared in all groups with R_{fs} 0.04 and 0.97 (B % 23.77 and 35.31) (Table 3 and illustrated in Fig. 3).

Irradiation caused alterations represented by disappearance of 2 normal bands with deviation of normal band to be appeared with R_f 0.13 (B % 28.51). There was no quantitative mutation.Salicin could not prevent the irradiation effect represented by deviation of 2 normal bands to be appeared with R_f 0.13 and 0.51 (B % 20.30 and 12.42) in the irradiated salicin pre-treated group, by deviation of one normal band to be appeared with R_f 0.11 (B % 14.35) with appearance of one abnormal band with R_f 0.33 (B % 11.64) in the irradiated salicin simultaneous treated group and represented qualitatively by disappearance of one normal bands with deviation of the 2nd normal band to be appeared with R_f 0.11 (B % 20.81) and quantitatively by increasing B % of the normal band appeared with R_f 0.98 (B % 59.39) in the irradiated salicin post-treated group. While in the irradiated salicin prepost-treated group, salicin could not prevent the alterations represented qualitatively by disappearance of 2 normal bands and quantitatively by increasing B % of the normal bands appeared with Rfs 0.04 and 0.99 (B % 37.94 and 62.06).

From the SI values, salicin decreased the irradiation effect on the band number and arrangement in the irradiated salicin pre-treated (SI = 0.60) and simultaneous treated groups (SI = 0.73).

4.5 Electrophoretic esterase pattern

As shown in Table 4 and illustrated in Fig. 4, there were 3 common bands appeared in all the groups with R_{fs} 0.18, 0.36 and 0.80 (B % 21.52, 49.21 and 21.37). Irradiation caused no quantitative mutation but it caused qualitative alterations represented by disappearance of

the 3rd normal type of the enzyme pattern without appearance of abnormal bands. As compared to control, irradiation showed the same electrophoretic esterase pattern in the irradiated and all irradiated salicin treated groups. The SI values were equal in all irradiated salicin treated groups (SI = 0.86). There was complete similarity between these groups. The highest SI value (SI = 1) was recorded with the salicin treated group.

Table 3: Data of the electrophoretic lipoprotein pattern in spleen tissue of control, irradiated and irradiated salicin treated groups in male rats

Control		Salicin		Irradiated		Irradiated salicin treated							
						Pre-treated		Simultaneous		Prepost-treated		Post-treated	
Rf.	B. %	Rf.	B. %	Rf.	B. %	Rf.	B. %	Rf.	B. %	Rf.	B. %	Rf.	B. %
0.04	23.77	0.04	15.83	0.04	38.58	0.04	29.92	0.04	20.97	0.04	37.94	0.04	19.80
0.07	6.06	0.07	8.47	0.13	28.51	0.08	7.74	0.08	5.00	0.99	62.06	0.11	20.81
0.17	17.83	0.13	21.00	0.98	32.91	0.13	20.30	0.11	14.35	—	—	0.98	59.39
0.49	17.03	0.38	19.50	—	—	0.51	12.42	0.33	11.64	—	—	—	—
0.97	35.31	0.52	18.67	—	—	0.97	29.61	0.49	11.07	—	—	—	—
—	—	0.98	16.51	—	—	—	—	0.99	36.97	—	—	—	—

R_f.: Rate of Flow, B% : Band Percent

Table 4: Data of the electrophoretic esterase pattern in spleen tissue of control, irradiated and irradiated salicin treated groups in male rats

Control		Salicin		Irradiated		Irradiated salicin treated							
						Pre-treated		Simultaneous		Prepost-treated		Post-treated	
Rf.	B. %	Rf.	B. %	Rf.	B. %	Rf.	B. %	Rf.	B. %	Rf.	B. %	Rf.	B. %
0.18	21.52	0.20	19.28	0.19	29.73	0.19	28.62	0.20	13.37	0.19	25.17	0.19	25.05
0.36	49.21	0.36	38.18	0.35	43.08	0.36	44.06	0.35	52.73	0.34	44.29	0.35	50.75
0.66	7.91	0.66	18.08	0.81	27.20	0.82	27.32	0.81	33.90	0.82	30.54	0.80	24.21
0.80	21.37	0.81	24.46	—	—	—	—	—	—	—	—	—	—

R_f.: Rate of Flow, B.% : Band Percent

Note: Arrangement of the bands at each lane is not correlated with the other bands in the other lanes

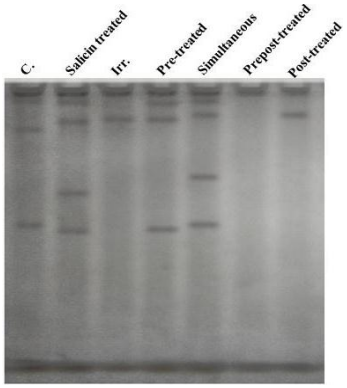

Fig. 3: Electrophoretic pattern showing effect of salicin against the irradiation effect on lipoprotein pattern in spleentissue of male rats

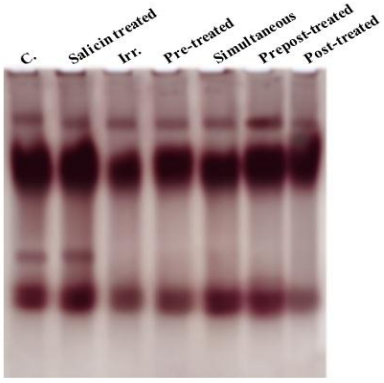

Fig. 4: Electrophoretic pattern showing effect of salicin against the irradiation effect on esterase pattern in spleen tissue of male rats

4.6 Electrophoretic catalase pattern

The electrophoretic catalase pattern showed that there were no common bands appeared in all groups (Table 5 and illustrated in Fig. 5).

Irradiation caused qualitative alteration represented by disappearance of one normal type with appearance of one abnormal band with R_f 0.28 (B % 69.42). Salicin administration showed the highest protective effect against qualitative mutagenic effect of irradiation in the irradiated salicin pre-treated group. It could not prevent the irradiation effect which was represented qualitatively by appearance 2 abnormal bands with R_{fs} 0.28 and 0.59 (B % 19.45 and 29.30) in the irradiated salicin simultaneous treated group, with R_{fs} 0.16 and 0.27 (B % 34.62 and 25.57) in the irradiated salicin prepost-treated group and R_{fs} 0.20 and 0.57 (B % 32.77 and 26.19) in the irradiated salicin post-treated group. It could not prevent the quantitative mutation which was represented by decreasing the B % of the 1st type of the catalase enzyme in all irradiated salicin treated groups.

The SI values showed that the lowest SI value (SI = 0.33) was observed with irradiated salicin simultaneous group and the highest value (SI = 0.8) observed with irradiated salicin pre-treated group. In the irradiated salicin prepost-treated group, it was observed that all the bands were not matched with all bands of the other groups. Salicin treatment minimized the irradiation effect the irradiated salicin pre-treated (SI = 0.80) and post-treated group.

Table 5: Data of the electrophoretic Catalase pattern in spleen tissue of control, irradiated and irradiated salicin treated groups in male rats

Control		Salicin		Irradiated		Irradiated salicin treated							
						Pre-treated		Simultaneous		Prepost-treated		Post-treated	
Rf.	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**
0.06	73.44	0.06	22.25	0.06	73.44	0.06	22.25	0.06	73.44	0.06	22.25	0.06	73.44
0.69	26.56	0.16	37.46	0.69	26.56	0.16	37.46	0.69	26.56	0.16	37.46	0.69	26.56
—	—	0.55	14.60	—	—	0.55	14.60	—	—	0.55	14.60	—	—
—	—	0.62	11.22	—	—	0.62	11.22	—	—	0.62	11.22	—	—
—	—	0.68	14.47	—	—	0.68	14.47	—	—	0.68	14.47	—	—

R_f.: Rate of Flow, B.% : Band Percent.

Note: Arrangement of the bands at each lane is not correlated with the other bands in the other lanes

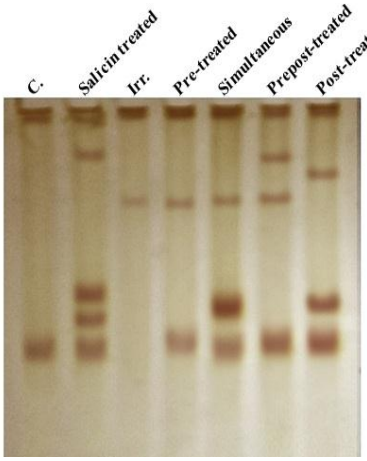

Fig. 5: Electrophoretic pattern showing effect of salicin against the irradiation effect on catalase pattern in spleen tissue of male rats

4.7 Electrophoretic peroxidase pattern

All the data were recorded in Table 6 and illustrated in Fig. 6. There were no common bands in all groups. Irradiation caused qualitative alterations represented by disappearance of the 1st and 6th types with appearance of one abnormal band with R_f 0.27 (B % 30.98) and deviation of the 2nd, 3rd and 5th types to be appeared with R_{fs} 0.33, 0.40 and 0.62 (B % values 8.14, 9.02 and 18.89). Salicin administration could not prevent the abnormalities occurred qualitatively and represented by disappearance of normal bands, appearance of abnormal bands and deviation of some normal bands to be appeared with different data. It could not prevent the alterations which were represented quantitatively in all irradiated salicin treated groups by increasing B % of some normal bands.

The SI values showed that the lowest SI value (SI = 0.15) was observed in irradiated group and the highest value (SI = 0.43) noticed in the salicin treated group. In the irradiated salicin pre-treated group, it was observed that all the bands were not matched with all bands of the other groups.

5 Discussions

Spleen of the male rats was selected to be under study due to sensitivity of the male rats to irradiation damages more than female rats. This was in agreement with Ezz, (2011)[13] who showed that spleen taken to study the ameliorative effect of radioprotector against irradiation induced OS and immune responses in male rats.

During results of the present study, the MDA level elevated significantly as a result of radiation exposure in spleen tissue. This was in accordance with the results obtained by Dixit *et al*. (2012)[46] who reported that irradiation at the doses 2, 6 and 10 Gy enhanced the MDA level. This may refer to increasing in intracellular ROS concentration which leads subsequently to oxidative stress[47] and decrease in activity of antioxidant enzymes with possible damage of cellular membranes[48]. Kergonou *et al*. (1981)[49] showed that the MDA level increased after radiation exposure due to radiosensitivity of spleen and also because the MDA is released from tissues in plasma and trapped from plasma in kidney and spleen.

Table 6: Data of the electrophoretic peroxidase pattern in spleen tissue of control, irradiated and irradiated salicin treated groups in male rats

Control		Salicin		Irradiated		Irradiated salicin treated							
						Pre-treated		Simultaneous		Prepost-treated		Post-treated	
Rf.	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**	**Rf.**	**B. %**
0.19	26.24	0.18	19.78	0.19	26.24	0.18	19.78	0.19	26.24	0.18	19.78	0.19	26.24
0.36	10.31	0.33	17.59	0.36	10.31	0.33	17.59	0.36	10.31	0.33	17.59	0.36	10.31
0.42	10.01	0.42	8.03	0.42	10.01	0.42	8.03	0.42	10.01	0.42	8.03	0.42	10.01
0.52	22.99	0.52	15.50	0.52	22.99	0.52	15.50	0.52	22.99	0.52	15.50	0.52	22.99
0.65	10.32	0.62	8.90	0.65	10.32	0.62	8.90	0.65	10.32	0.62	8.90	0.65	10.32
0.71	10.56	0.69	10.03	0.71	10.56	0.69	10.03	0.71	10.56	0.69	10.03	0.71	10.56
0.78	9.57	0.80	20.17	0.78	9.57	0.80	20.17	0.78	9.57	0.80	20.17	0.78	9.57

R_f.: Rate of Flow, B.% : Band Percent

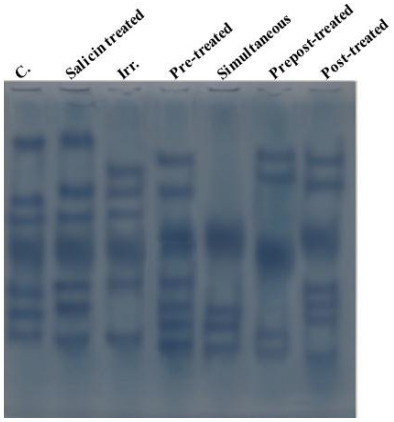

Fig. 6: Electrophoretic pattern showing effect of salicin against the irradiation effect on peroxidase in spleen tissue of male rats

Although it is well known that irradiation induced cellular injury due to the harmful effects of the free radicals which play a key role in irradiation induced apoptosis[50], it caused no obvious alterations detected microscopically during the current study.

During the current study experiment, it was showed severe alterations detected electrophoretically at level of protein and isozymes although irradiation showed no morphological alterations on the spleen surface. This might refer to the irradiation effect which caused DNA strand breaks due to increased production of ROS which attack DNA in splenocytes[51] and / or the effect on radiosensitive gene in spleen tissue[12].

Ramanathan and Misra (1979)[52] reported that irradiation induced changes in lipid metabolism in spleen of rats. The fatty acid composition of spleen was profoundly altered 24 hrs after irradiation**.** Free radicals implicated in OS reactions, which can damage cells and tissues and cause disorders in the immune system[53].

As reported by Hamzaa *et al.* (2012)[54], it was found that the phenolic compounds have ameliorating effects against oxidative damage induced by gamma-irradiation through inhibition of lipid peroxidation, improvement of lipid profile and enhancement of the antioxidant activity.

Salicin hydrolyzes in the gastrointestinal tract to give D-glucose and salicyl alcohol. Upon absorption, salicyl alcohol is oxidized into

salicylic acid[55]. Thus in the current study, the effect of salicin was attributed to its hydrolysable form salicylic acid.

The effect of salicylic acid was compatible with an antioxidant profile: it inhibited lipid peroxidation and increasedglutathione synthesis, but did not modify the activitiesof glutathione-related enzymes[56]. The effect of the salicylic acid on lipid peroxidation may be explainable by the ability of salicylic acid to absorb hydroxyl ions[57] and thus impede a main step in the process of membrane lipid peroxidation. Salicylic acid might spare glutathione stores by avoiding factors that stimulate glutathione depletion. Two observations support this notion: the percentage of oxidized glutathione was reduced, and the activities of enzymes associated with maintaining glutathione levels were not modified substantially[56]. Salicylic acid showed a direct effect on the glutathione system. This effect may be related with the ability of both to react with hydroxyl radicals[57,58].

On the other hand, Rebouch and Seim (1998)[59] and Ibrahim *et al*. (2007)[60] recorded that salicin might induce elevation in activities of the AOs as glutathione peroxidase in these tissues. It might act by improving the turnover of fatty acids peroxidated by the free oxygen radicals during normal metabolism. It might be added to category of the natural products as olive oil, Nigella sativa oil and pomegranate extract which play vital role in male fertility[61].

The present results showed that irradiation caused alterations in all electrophoretic patterns in spleen tissue. This was in agreement with results reported by many previous studies which suggested that irradiation produces ROS that damage proteins, lipids and nucleic acid[62].

The current experiment showed that irradiation decreased the ordered structure of proteins. This was in agreement Moon and Song, (2001)[17] who suggested that radiation caused initial fragmentation of polypeptide chains and, as result, subsequent aggregation and degeneration of proteins by scavenging ROS produced by irradiation. The difference in the protein fractions separated electrophoretically after radiation exposure was in agreement with Pleshakova *et al*., (1998)[63] who reported that irradiation caused a rise of protein carbonyl only in the cytoplasm and mitochondria and this was followed by activation of histone – specific proteases in nuclei of the irradiated rats. The proteins are responsible for a specific biological process, so due to the difference in protein bands between all the treated samples, the biological processes may also be differed. The separation and characterization of the individual proteins facilitate study of the chemical nature and physiological function of each protein[64].

During the current study, irradiation caused alterations in the native proteins detected electrophoretically. This was in accordance with Davies and Delsignore (1987)[65] who documented that irradiation caused irreversible changes at the molecular level by breakage of the covalent bonds of the polypeptide chains due to generation of the hydroxy and superoxide anion radicals which modify the primary structure of the proteins resulting in distortions of the secondary and tertiary structures. The exposure of proteins to oxygen radicals resulted in both non-random and random fragmentations[66]. It was reported that irradiation caused aggregation and cross-linking of proteins. Covalent cross linkages are formed between free amino acids and proteins, and between peptides and proteins in solution after irradiation[66,67].

Data in the present study indicated that specific protein bands in spleen tissue of irradiated rats differed (through disappearence in some protein bands or appearance of new ones). Disappearance of some protein bands in treated rats may be attributed to the effects of irradiation which inhibits the synthesis and expression process of these deleted proteins (qualitative effect). In addition, even the band remained after irradiation, it usually differs in the amount of protein, and this may be explained by that irradiation could not inhibit the synthesis of this protein type, but it may be affected only on the quantitative level.

Giometti *et al*. (1987)[68] postulated that different mutations were detected by the appearance of new proteins or by the quantitative decrease in abundance of normally occurring proteins and the electrophoresis can be used to detect the mutations reflected as quantitative changes in the protein expression.

The difference in protein pattern may act as a tool to identify the similarity index and genetic distance between the control and the other treated samples. The chemical changes of the proteins that are caused by irradiation are fragmentation, cross-linking, aggregation, and oxidation by oxygen radicals that are generated in the radiolysis of water[69].

Lipoproteins are lipid–protein complexes that contain large insoluble glycerides and cholesterol with a superficial coating of phospholipids and proteins synthesized in the liver[70]. All lipoproteins carry all types of lipid, but in different proportions, so that the density is directly proportional to the protein content and inversely proportional to the lipid content[71].

In the present study, irradiation caused alterations in the electrophoretic lipoprotein pattern. This was in agreement with Tsumura *et al*. (2001)[72] who reported that the lipoproteins were more susceptible to oxidative modifications resulting in small lipoproteins.

Bonnefont-Rousselot (2004)[73] mentioned that the ROS can initiate one-electron oxidation or one-electron reduction reactions on numerous biological systems. The oxidative hypothesis classically admits the involvement of the lipoproteins oxidation radiolytically.

There was natural binding between protein and lipoproteins in the rat tissues. These two tissues known to be involved in the processing of the lipoproteins. The lipoproteins-binding protein has previously been identified in adrenal cortical plasma membranes and concentration of the binding protein was strongest in kidneys[74]. So the alterations in the protein pattern were associated with altering the lipoprotein pattern in these tissues. The alterations in the lipoprotein pattern may refer to the disturbances in the cholesteryl esterase required or cholesterol hydrolysis[75].

Esterases are very large class of enzymes. They can break an ester bond in the presence of water molecule[76]. The esterase activity stimulated breakdown of acetylcholine liberated during nervous stimulation**.** They are very polymorphic, tissue-specific and variable in populations of rats. Esterase zymograms showed that intensity and number of the nonspecific esterase bands are very variable[77]. Esterases are found associated with membrane structures. There was correlation between different esterases and the total esterase activity in the different tissues[78].

According to results of the present study, irradiation caused electrophoretic qualitative and quantitative alterations in the electrophoretic esterase pattern in the spleen tissue. This may refer to effect of irradiation on the protein pattern[79] or the disturbances occurred in the cholesterol metabolism as a result of radiation exposure. The total esterase activities were correlated to the cholesterol responses in rats[80].

As regards changes in electrophoretic mobility demonstrated in the present study, it seemed that free radicals affect the integrity of the polypeptide chain in the protein molecule causing fragmentation of the polypeptide chain due to sulfhydral-mediated cross linking of the labile amino acids as claimed by Bedwell *et al*. (1989)[79]**.** The changes in the fractional activity of different isoenzymes seemed to be correlated with changes in the rate of protein expression secondary to DNA damage initiated by free radicals[81].

During the current experiment, irradiation caused alterations in the electrophoretic catalase and peroxidase patterns. This was in agreement with Li *et al*. (2007)[82] who showed that irradiation decreased the peroxidase activity which may be due to that irradiation-induced ROS markedly alters the physical, chemical and immunologic properties of endogenetic antioxidant enzymes (CAT and GPx), which further increase oxidative damage in cells.

The study showed that the decrease in CAT and GPx activity could be attributed to the uncontrolled production of ROS and accumulation of H_2O_2 whereby oxidative damage to enzymes can cause a modification of their activity[83,84].

Bhatia and Manda (2004)[85] reported that the electrophoretic disturbances occurred as a result of irradiation in the peroxidase pattern. This might be due to irradiation-induced depletion in the level of reduced GSH, as well as GSH peroxidase. This leads to elevation of the hydrogen peroxide and hence generation of the free radicals[86]. GPx utilizes GSH as a substrate to catalyse the reduction of organic hydroperoxides and H_2O_2[87].

Salicin and salicylic acid belonged to the phenolic compounds which showed antioxidant activity due to their ability to scavenge free radicals[88]. The maintenance of normal protein levels after the treatment with salicin may be due to trapping of these free radicals by this compound, thus preventing DNA damage. Salicin was able to overcome the disturbances in the protein pattern in the spleen tissue. It showed protective effect against the irradiation due to its antioxidative effect against attack of the free radicals. It prevented the alterations in the proteins and hence the lipoproteins and isozymes in the spleen tissue.

The current results are in line with that obtained by Cetin *et al*. (2008)[89] who suggested that salicin treatment considerably increased the formation of antioxidant products in different tissues. Salicin treatment minimized the irradiation effect and this may refer to its effect on stimulation of activities of the different enzymes. The mRNA expression levels of the enzymes increased after administration of salicin whicjh may play role in regulation of these enzymes on a transcriptional level[90].

6 Conclusions

The study concluded that salicin minimized the irradiation effect and showed radioprotective effect against irradiation induced ultrastructural and different electrophoretic changes in spleen tissue of male rats.

7 Competing interest

The present study aimed to optimize salicin as a radioprotector against effect of gamma irradiation on the spleen tissue in the hope that this compound may be further explored as novel antioxidative radioprotector.

8 Author's contributions

MALAK and MSA carried out literature review and draft the manuscript. HMS participated in collection of data and arranged in tabular form. IA and WMK carried out the experimental work. All authors read and approved the final manuscript.

9 References

1. Grupen C, Cowan G, Eidelman SD and Stroh T. Astroparticle Physics. Springer-Verlag Berlinand aheidelberg GmbH & Co.K. 2005; pp: 109.

2. Lett JT. Damage to cellular DNA from particulate radiation, the efficacy of its processing and the radiosensitivity of mammalian cells. Emphasis on DNA strand breaks and chromatin break. Radiat. Environ. Biophys. 1992; 31: 257-277.
3. Daniniak N and Tann BJ. Utility of biological membranes as indicators for radiation exposure: alterations in membrane structure and function over time. Stem Cells. 1995; 13: 142-152.
4. Spitz DR, Azzam EI, Li JJ and Gius D. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: a unifying concept in stress response biology. Cancer Metastasis Rev. 2004; 23 (3-4): 311-22.
5. Fedorova M, Kuleva N and Hoffmann R. Identification, quantification, and functional aspects of skeletal muscle protein-carbonylation in vivo during acute oxidative stress. J. Proteome Res. 2010; 9 (5):2516 - 2526.
6. Umegaki K, Aoki S and Esashi T. Whole body X-ray irradiation to mice decreases ascorbic acid concentrations in bone marrow: comparison between ascorbic acid and vitamin E. Free Radic. Biol. Med. 1995; 19 (4):493 - 7.
7. Koc M, Taysi S, Buyukokuroglu M and Bakan, N. The effect of melatonin against oxidative damage during total-body irradiation in rats. Radiat. Res. 2003; 160: 251-255.
8. Nwozo SO, Okameme PE and Oyinloye BE. Potential of Piper guineense and Aframomum longiscapum to reduce radiation induced hepatic damage in male Wistar rats. Radiats Biol. Radioecol. 2012 ; 52(4): 363-369.
9. Witztum J. Splenic immunity and atherosclerosis: a glimpse into a novel paradigm? J. Clin. Invest. 2002; 109: 721-724.
10. Hawas AM. The biosensitivity of certain organs in rats exposed to low doses of γ-radiation. Journal of Radiation Research and Applied Sciences. 2013; 6 (2) : 56 – 62.
11. Otsuka K and Sakai K. Effects of low dose-rate long-term gamma-ray irradiation on DNA damage in mouse spleen. International Congress Series. 2005; 1276 : 258-259.
12. Koo HJ, Jang S, Yang K, Kang SC, Namkoong S, Kim T, Hang DTT and Sohn E. Effects of red ginseng on the regulation of cyclooxygenase-2 of spleen cells in whole-body gamma irradiated mice. Food and Chemical Toxicology. 2013; 62 : 839-846.
13. Ezz MK. The Ameliorative Effect of Echinacea Purpurea Against Gamma Radiation Induced Oxidative Stress and Immune Responses in Male Rats. Australian Journal of Basic and Applied Sciences. 2011; 5(10): 506-512.
14. Mohamed MI. Sterility and some associated physiological changes in the adult cowpea weevil, Callosobruchus maculatus (F.). Ph. D. Thesis, Dept. Entomol., Fac, Sci. Ain Shams Univ. 1990.
15. Hawkins CL, Morgan PE and Davies MJ. Quantification of protein modification by oxidants. Free Radical Biology and Medicine. 2009; 46: 965 - 988.
16. Hassan Heba A and AbdEl- Hafez Hanan F. The comparison effects of two acetylcholine receptor modulator on some biological aspects, protein pattern and detoxification enzyme of the cotton leafworm, spodoptera littoralis. Egypt. J. Agric. Res. 2009; 87 (2): 103-117.
17. Moon S and Song KB. Effect of gamma-irradiation on the molecular properties of ovalbumin and ovomucoid and protection by ascorbic acid. Food Chem. 2001; 74: 479-483.
18. Smutná M, Beňová K, Dvořák P, Nekvapil T, Kopřiva V and Maté D. Protein carbonyls and traditional biomarkers in pigs exposed to low-dose γ-radiation. Research in Veterinary Science. 2013; 94 (2): 214-218.
19. Matés M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology. 2000; 153(1–3):83–104.
20. Halliwell B and Gutteridge JMC. Free radicals in biology and medicine. 4th ed. New York: Oxford University Press. 2007.
21. Attia AA, ElMazoudy RH and El-Shenawy NS. Antioxidant role of propolis extract against oxidative damage of testicular tissue induced by insecticide chlorpyrifos in rats. Pesticide Biochemistry and Physiology, 2012; 103: 87–93.
22. Gupta RC. Toxicology of organophosphates and carbamate compounds, Elsevier Academic Press. 2006.
23. La Falci VS, Yrjö-Koskinen AE, Fazeli A, Holt WV and Watson PF. Antioxidant combinations are no more beneficial than individual components in combating ram sperm oxidative stress during storage at 5 °C. Anim. Reprod. Sci. 2011; 129(3-4): 180-187.
24. Strzezek R, Koziorowska-Gilun M and Stawiszynska M. Cryopreservation of canine semen: the effect of two extender variants on the quality and antioxidant properties of spermatozoa. Pol. J. Vet. Sci. 2012; 15(4): 721-726.
25. Kim SH, Kim HJ, Lee HO and Ryu SY. Apoptosis in growing hair follicles following gamma irradiation and application for the evaluation of radioprotective agents. In Vivo. 2003; 17 : 211 – 214.
26. Elshazly SA, Ahmed MM, Hassan HE and Ibrahim ZS. Protective effect of L-carnitine against γ-rays irradiation-induced tissue damage in mice. American Journal of Biochemistry and Molecular Biology. 2012; 2 (3): 120 – 132.

27. Weiss JF and Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology. 2003; 189 (1-2): 1–20.
28. Jourdier S. A Miracle Drug. 1999; http://www.chemsoc.org/chembytes/ezine.
29. Vane JR, Flower RJ and Botting RM. History of aspirin and its mechanism of action. Stroke. 1990; 21: IV12-23.
30. Akao T, Yoshino T, Kobashi K and Hattori M. Evaluation of salicin as an antipyretic prodrug that does not cause gastric injury. Planta. Med. 2002; 68: 714-718.
31. Singh BN, Singh BR, Singh RL, Prakash D, Dhakarey R, Upadhyay G and Singh HB. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of Moringa oleifera. Food Chem. Toxicol. 2009; 47: 1109–1116.
32. Nzaramba MN, Reddivari L, Bamberg JB and Creighton MJ. Antiproliferative activity and cytotoxicity of Solanum jamesii tuber extracts on human colon and prostate cancer cells in vitro. J. Agric. Food Chem. 2009; 57: 8308–8315.
33. Mabry TJ, Markham KR and Thomaas MB. The Systematic Identification of flavonoids, Springer-Verlag, Berlin. 1970.
34. Kur'yanov AA, Bondarenko LT, Kurkin VA, Zapesochnaya GG, Dubichev AA and Vorontsov ED. Determination of the biologically active components of the rhizomes of Rhodiola rosea. Translated from Khimiya Prirodnykh Soedinenii. 1991; 3:320-323.
35. Paget and Barnes. Evaluation of drug activities pharmacometrics. Vol. (1), Edited by Laurence, D.R. and Bacharach, A.L. Academic Press, London and New York. 1974; 135.
36. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979; 95 : 351 – 358.
37. Tánaka K. High resolution scanning electron microscopy of the cell. Biology of the Cell. 1989; 65: 89-98.
38. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976; 72: 248-254.
39. Laemmli UK. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature. 1970 ; 227: 680-685.
40. Hames BD. One-dimensional polyacrylamide gel electrophoresis. In: Gel electrophoresis of proteins: B.D. Hames B.D. and Rickwood D., 2nd ed.. Oxford university press, NY. 1990; 1-147.
41. Chippendale GM and Beak SD. Haemolymph proteins of Osirinla nubilalis (Hubner): during diapauses prepupa differentiation . J. Insect Physiolo. 1966; 12 : 1629-1638.
42. Rescigno A, Sanjust E, Montanari L, Sollai F, Soddu G, Rinaldi AC, Oliva S and Rinaldi A. Detection of laccase, peroxidase, and polyphenol oxidase on a single polyacrylamide gel electrophoresis, Anal. Lett. 1997; 30 (12): 2211.
43. Siciliano MJ and Shaw CR. Separation and visualization of enzymes on gels, in Chromatographic and Electrophoretic Techniques, Vol. 2, Zone Electrophoresis, Smith, I., Ed., Heinemann, London. 1976; p. 185.
44. Baker CMA and Manwell C. Heterozygosity of the sheep: Polymorphism of 'malic enzyme', isocitrate dehydrogenase (NADP+), catalase and esterase. Aust. J. Biol. Sci. 1977; 30 (1-2) : 127-40.
45. Nei M and Li WS. Mathematical model for studing genetic variation in terms of restriction endonuclease. Proc. Natl. Acad. Sci., USA. 1979; 76 : 5269 – 5273.
46. Dixit AK, Bhatnagar D, Kumar V, Chawla D, Fakhruddin K and Bhatnagar D. Antioxidant potential and radioprotective effect of soy isoflavone against gamma irradiation-induced oxidative stress. J. Funct. Foods. 2012; 4: 196–206.
47. Maurel A, Hernandez C and Kunduzova O. Age-dependent increase in hydrogen peroxide production by cardiac monoamine oxidase A in rats. Am. J. Physiol. Heart Circ. Physiol. 2003; 284: H1460 – H1467.
48. El Habit OHM, Saada HN, Azab KS, Abdel Rahman M and El Malah DF. The modifying effect of B-carotene on gamma radiation-induced elevation of oxidative reactions and genotoxicity in male rats. Mutation Research. 2000; 466: 179-186.
49. Kergonou J, Bernard P, Braquet M and Rocquet G. Effect of whole-body gamma irradiation on lipid peroxidation in rat tissues. Biochimie. (1981). 63 (6): 555-559.
50. Vijayalaxmi RJ, Reiter DX, Tan TS, Herman CR and Thomas J. Melatonin as a radioprotective agent: a review, Int. J. Radiat. Oncol. Biol. Phys. 2004; 59: 639–653.
51. Richi B, Kale RK and Tiku AB. Radio-modulatory effects of Green Tea Catechin EGCG on pBR322 plasmid DNA and murine splenocytes against gamma-radiation induced damage. Mutation Research. 2012; 747: 62– 70.
52. Ramanathan R and Misra UK. Spleen lipids: effect of whole body gamma irradiation and radioprotective chemicals. Biochem. Exp. Biol. 1979; 15(4):361-9.
53. Shahwar D, Sana U and Ahmad N. Synthesis and evaluation of acetylcholineesterase inhibitory potential and antioxidant

activity of benzothiazine derivatives. Turkish Journal of Chemistry. 2013; 37: 262 – 270.

54. Hamzaa RG, El Shahat AN and Mekawey HMS. The Antioxidant Role of Mulberry (Morusalba L.) Fruits in Ameliorating the Oxidative Stress Induced in γ-Irradiated Male Rats. Biochem. Anal. Biochem. 2012; 1(8): 122.
55. Chrubasik S and Eisenberg E. Willow Bark. 2004; <http://www.rzuser. uni-heidelberg.de/~cn6/iasp-sig-rp/willow.html> (accessed 11.03.04).
56. De La Cruz JP, Guerrero A, Gonzalez-Correa JA, Arrebola MM and Sanchez de la Cuesta F. Antioxidant Effect of Acetylsalicylic and Salicylic Acid in Rat Brain Slices Subjected to Hypoxia. Journal of Neuroscience Research. 2004; 75: 280–290.
57. Sagone AL and Husney RM. Oxidation of salicylates by stimulated granulocytes: evidence that these drugs act as free radical scavengers in biological systems. J. Immunol. 1987; 138: 2177–2183.
58. Li PA, Liu GJ, He QP, Floyd RA and Siesjo BK. Production of hydroxyl free radicals by brain tissues in hyperglycemic rats subjected to transient forebrain ischemia. Free Radic. Biol. Med. 1999 ; 27:1033–1040.
59. Rebouche CJ and Seim H. Carnitine metabolism and its regulation in microorganisms and mammals. Annu. Rev. Nutr. 1998; 18:9-61.
60. Ibrahim K, Seyithan T, Mustafa E, Ihsan K, Akcahan G, Orhan S and Korkmaz S. The effect of L- carnitine in the prevention of ionizing radiation induced cataracts; a rat model.Graefe _s. Archive Clinic. and Exp. Ophthalm. 2007; 245(4): 588- 594.
61. Aitken RJ, Smith TB, Lord T, Kuczera L, Koppers AJ and Naumovski N. On methods for the detection of reactive oxygen species generation by humanspermatozoa: analysis of the cellular responses to catechol oestrogen, lipid aldehyde, menadione and arachidonic acid. Andrology. 2013; 1(2): 192-205.
62. Nair CKK, Parida D and Nomura T. Radioprotectors in radiotherapy. J. Rad. Res. 2001; 42:21-37.
63. Pleshakova OV, Kutsyi MP, Sukharev SA, Sadovnikov VB and Gaziev AI. Study of protein carbonyls in subcellular fractions isolated from liver andspleen of old and γ-irradiated rats. Mechanisms of Ageing and Development. 1998; 103(1): 45-55.
64. Cheeseman K. In DNA and Free Radicals (Halliwell, B. and Aruoma, O. I., eds.), 1993; pp. 109-144, Ellis Horwood, Chichester.
65. Davies KJA and Delsignore ME. Protein damage and degradation by oxygen radicals III. Modification of secondary structure and tertiary structure. J. Biol. Chem. 1987; 262: 9908-9913.
66. Filali-Mouhim A, Audette M, St-Louis M, Thauvette L, Denoroy L, Penin F, Chen X, Rouleau N, Le Caer JP, Rossier J, Potier M and Le Maire M. Lysozyme fragmentation induced by γ-radiolysis. Int. J. Radiat. Biol. 1997; 72(1): 63-70.
67. Garrison WM. Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. Chem. Rev. 1987; 87: 381-398.
68. Giometti CS, Gemmell MA, Nance SL, Tollaksen SL and Taylor J. Detection of heritable mutations as quantitative changes in protein expression. J. Biol. Chem. 1987; 262: 12764 – 12767.
69. Cho Y and Song KB. Effect of g-irradiation on the molecular properties of BSA and b-lactoglobulin. J. Biochem. Mol. Biol. 2000; 33: 133-137.
70. Havel Rj and Kane Jp. Structure and metabolism of plasma lipoproteins. In: CR Scriver, AL Beaudet, WS Sly and D Valle, eds. The metabolic and molecular basis of inherited disease, 7th edition. McGraw- Hill, USA. 1995; 1841-1851.
71. Bass KM, Newschaffer CJ, Klag MJ and Bush TL. Plasma lipoprotein levels as predictors of cardiovascular death in women. Arch. Intern. Med. 1993; 153 (19): 2209-16.
72. Tsumura M, Kinouchi T, Ono S, Nakajima T and Komoda T. Serum lipid metabolism abnormalities and change in lipoprotein contents in patients with advanced-stage renal disease. Clinica. Chimica. Acta. 2001; 314: 27 – 37.
73. Bonnefont-Rousselot D. Gamma radiolysis as a tool to study lipoprotein oxidation mechanisms. Biochimie. 2004; 86: 903-911.
74. Fidge NH. Partial purification of a high density lipoprotein-binding protein from rat liver and kidney membranes. Federation of European Biochemical Societies. 1986; 199: 265-268.
75. Satoh T. Toxicological implications of esterases—From molecular structures to functions. Toxicology and Applied Pharmacology. 2005; 207: S11 – S18.
76. Koitka M, Höchel J, Gieschen H and Borchert H. Improving the ex vivo stability of drug ester compounds in rat and dog serum: Inhibition of the specific esterases and implications on their identity. Journal of Pharmaceutical and Biomedical Analysis. 2010; 51: 664 - 678.
77. Verimli R, Yigit N, Çolak E and Sozen M. Nonspecific Esterase Patterns of Rattusnorvegicus (Berkenhout, 1769) in Western Turkey.Turk J. Biol. 2000 ; 24 : 825–831.

78. Tegelstrom H and Ryttman H. Sex differences and androgenic regulation of esterases in the house mouse. Hereditas. 1981; 94: 189-201
79. Bedwell S, Dean RT and Jessup W. The action of defined oxygen centered free radicals on human low-density lipoprotein. Biochem. J. 1989; 262: 707-712.
80. Beynen AC, Boogaard A, Van Laack HLJM, Weinans GJB and Katan MB. Abstr. Commun.15th FEBS Meet., Brussels. 1983; p. 173.
81. El-Zayat EM. Isoenzyme Pattern and Activity in Oxidative Stress-Induced Hepatocarcinogenesis: The Protective Role of Selenium and Vitamin E. Research Journal of Medicine and Medical Sciences. 2007; 2(2): 62-71.
82. Li XL, Zhou AG and Li XM. Inhibition of Lycium barbarum polysaccharides and Ganoderma lucidum polysaccharides against oxidative injury induced by γ-irradiation in rat liver mitochondria. Carbohydrate Polymers. 2007; 69: 172–178.
83. Kregel K and Zhang H. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2007 ; 292: 18-36.
84. De Freitas RB, Augusti PR, De Andrade ER, Rother FC, Rovani BT, Quatrin A, Alves NM, Emanuelli T and Bauermann LF. J. Black Grape Juice Protects Spleen from Lipid Oxidation Induced by Gamma Radiation in Rats. Food Biochem. 2014; 38(1): 119-127.
85. Bhatia AL and Manda K. Study of pre-treatment of melatonin against radiation-induced oxidative stress in mice. Environ. Toxicol. Pharmacol. 2004; 18: 13 – 20.
86. Mills GC. Glutathione peroxidase and the destruction of hydrogen peroxide in animal tissues. Archives of Biochemistry and Biophysics. 1960; 86: 1-5.
87. Ray G and Husain SA. Oxidants, antioxidants and carcinogenesis. Ind. J. Exp. Biol. 2002; 42: 1213-1232.
88. Madrigal-Carballo S, Rodriguez G, Krueger CG, Dreher M and Reed JD. Pomegranate (Punica granatum L.) supplements: authenticity, antioxidant and polyphenol composition. J. Funct. Food. 2009; 1: 324 - 329.
89. Cetin A, Kaynar L, Kocyigit I, Hacioglu SK, Saraymen R, Ozturk A, Orhan O and Sagdic O. The effect of grape seed extract on radiation-induced oxidative stress in the rat liver. Turk. J. Gastroenterol. 2008; 19(2): 92-98.
90. Yeh CT and Yen GC. Effects of phenolic acids on human phenolsulfotransferase in relation to their antioxidant activity. J. Agric. Food Chem. 2003; 51: 1474–9.