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The Radioprotective Potential of *Spinacia oleracea* and *Aesculuc hippocastanum* Aganist Ionizing Radiation with Their Antioxidant and Antimicrobial Properties

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Abstract: The present study, was designed to determine the possible protective effects of *Spinacia oleracea* L. extract (S.E) and *Aesculuc hippocastanum* L. extract (AE) against oxidative tissue damage induced by radiation as well as examinig their *in vitro* antibacterial potential. The animals were divided totally 4 groups as Control (C), Radiation (R), S.E Extract + Radiation (SER) and AE Extract + Radiation (AER) group. AER and SER groups were also separated to 2 subgroups as SER 1, SER 15, AER 1 and AER 15. C group was administered by 1 mL 0,9 % saline every other day during 20 days. The R group rats were received 1 mL q 0,9% saline every other day i.p. and other day 0.5 Gy radiation during 20 days (n = 10). The other groups, SER and AER were administered by 50 mg kg^{-1} i.p. S.E and AE extract every other day and other day 0.5 Gy radiations, respectively for 20 days. At the end of experimental period, the animals sacrificed by anesthetizing at 1 (group-C, R, SER 1 and AER 1) and 15 days (group-SER 15 and AER 15) postirradiation. Malondialdehyde (MDA) and reduced Glutathione (GSH) levels in tissues and *in vitro* antimicrobial activity in the plant extracts were determined. The results indicate that S.E and AE treatment decreases the tissue oxidative stress in irradiation-induced oxidative tissue damage by maintaining the GSH recycling activity and free radical scavenging potential. Moreover, our results demonstrate that, in animals exposed to irradiation, S.E and AE could provide great advantages against to systemic infection from endogenous and exogenous organisms increased after exposure to ionizing radiation. Consequently, the natural compound found in *S. oleracea* and *A. hippocastanum* including antioxidants, antimicrobials and other phytonutrients, substantially could be protect the tissue from radiation damage and its complications.

Key words: *Aesculuc hippocastanum* L., *Spinacia oleracea* L., lipid peroxidation, antimicrobial activity, radiation

INTRODUCTION

Radiation therapy is considered to be one of the most popular and important therapeutic modalities for the cure of cancer (Mackillop *et al*., 1997). Despite its benefits, radiation is known to induce oxidative stress through generation of free radicals, resulting in imbalance of pro-oxidants and antioxidants in the cells, which is suggested to culminate in cell death (Katz *et al*., 1996; Kaur *et al*., 2000). On the other hand, infection probably plays a major role in radiation death (Benacerraf, 1960; Klainer *et al*., 1967). As quoted by Benacerraf (1960), germfree rats survive under lethal doses of radiation significantly longer than conventional animals. Likewise, since animals and humans can not live in a germ free environment, their defense system will be weakened and lethal complication will occur as a consequence of exposure to radiation. It is important to seek possible radioprotectants to modify the normal response of biological systems to radiation-induced toxicity or lethality (Hosseinimehr *et al*., 2003). With regard of this, interest has recently increased in the development of plant origin potential drugs for the modification of radiation effects. Plant extract such as garlic and ginseng have been found to have radioprotective effects in mammals (Samarth and Kumar, 2003).

Naturally, occurring antioxidants are only 1 class of radioprotectors. Many of phytochemicals have significant

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antioxidant capacities associated with lower occurrence and mortality rates of several diseases (Weiss and Landauer, 2003). It is well known, that many natural substances in plants contain a wide variety of free radical-scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, saponins and some other endogenous metabolites, which are rich in antioxidant activity. Phenolic compounds can play an important role in preventing body cells from injuries by hydrogen peroxide. Prevention of cells by these compounds is realized by lipid peroxides and neutralizing free radicals (Changwei *et al*., 2008). During the last few years, antimicrobial properties have been reported in a wide range of plant extracts and natural products attempting to discover new chemical classes of antibiotics that could resolve the problems such as the appearance of undesirable side effects and the emergence of previously uncommon infections (Alviano *et al*., 2008), *Aesculus hippocastacanum*, commonly known as horse chestnut, is origined from the Hippocastanacae family of plants (Peirce, 1999). Seeds and bark of horse chestnut tree, have been widely used in European traditional medicine since 16th century. The horse chestnut seed contains several active chemicals such as aescin, coumarin derivatives (aesculin, fraxin, scopolin), flavonoids (quercetin, kaempferol, astragalin, isoquercetrin, rutin, leucocyanidine), essential oils (oleic acid, linoleic acid) and other chemicals (amino acidsallantoin, argyrin, carotin, choline, citric acid, epicatechin, leucodelphinidin, phyosterol, resin, scopoletin, tannin and uric acid. The principal extract and medicinal constituent of horse chestnut seed is aescin, a mixture of triterpenoid saponin glycosides (Vertuani *et al*., 2004; Sato *et al*., 2006). *Spinacia oleracea* L. (Spinach, Hindi, Punjabi-Palak) is an important dietary vegetable. Cultivated globally, it is an important raw-material in the food processing industry. Spinach belongs to Chenopodiaceae family and is commonly reported to be a good source of minerals, vitamin B complex, ascorbic acid, carotenoids (β-carotene, lutein, zeaxanthine), flavonoids, apocyanin and p-coumaric acid. The biological activities of spinach polyphenols have been reported widely (Aehle *et al*., 2004; Bhatia and Jain, 2004).

The present study has been undertaken to determine the possible protective role of *Aesculus hippocastacanum* and *Spinacia oleracea* against radiation-induced oxidative tissue damage as well as examining their *in vitro* antibacterial potential.

MATERIALS AND METHODS

Chemicals: Thiobarbituric acid, trichloroacetic acid and acetic acid were purchased from Fluka (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). Glutathione (reduced form), was obtained from Merck (Darmstadt, Germany). Other chemicals used in the study were purchased from Sigma-Aldrich Chemical.

Plant materials and preparation of plant extracts: Plant materials were collected from different localities in Afyonkarahisar, Turkey. Voucher specimens were authenticated by Assistant Prof. Dr. Mehmet Temel of Department of Biology, Faculty of Arts and Science, Afyon Kocatepe University, were deposited in the Herbarium of Faculty of Arts and Science, Afyon Kocatepe University, Afyon, Turkey. Collection sites, parts used and herbarium numbers of plant materials are described.

- *A. hippocastanum* L. seed (Hippocastanaceae): Afyon, Atatürk stadium, aerial parts [AKU 4345].
- *S. oleracea* L. (Chenopodiaceae): Afyon, Cayirbag, aerialparts [AKU 4435].

Each plant material was dried under shade and powdered to a fine grade by using a laboratory scale mill. Dried plant material (4×50 g) was extracted with 100% ethyl acetate at 40°C for 2 times (×500 mL). The combined ethyl acetate layers were filtered and evaporated to dryness in vacuo to give crude extract. The obtaining residue was dissolved with 100% methanol and saponin in the residue suspended over the methanol then saponin was taken from the solution. The resulting methanol extracts were filtrated and evaporated to dryness. Extract yields for the plant materials were as follows (wt/wt, %): *A. hippocastanum* seed: 3.42; *S. oleracea*: 2.08.

Animals and experimental design: Male albinoWistar rats, 6-8 weeks old, weighing about 180-250 g, from an inbred colony, were purchased from the animal breeding laboratories of Faculty of Medicine, Ege University (Izmir, Turkey). All animals were acclimatized to our laboratory conditions for 2 weeks before the beginning of the experiments and housed under standard conditions of temperature (23±2°C), humidity and dark-light cycle (lights on from 6:00 am to 6:00 pm). They were provided standard rat feed supplied by Bil-Yem Ltd. (Turkey). Tap water was available *ad libitum*. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the University of Afyonkarahisar Kocatepe Animal Ethics Committee.

The rats were placed in plastic cages (10×10×10 cm) for exposure and were irradiated using a Indico 100 Rab X-ray instrument (CPI, Ontario, Canada). The rats in the irradiated groups were exposed to 5 Gy of radiation at a dose rate of 3 Gy min^{-1} at a focus on 10 cm away from the

skin during 20 days. Experimental animals were randomly divided into 4 groups. Experimental groups are described.

Control (C) group: Rats were received intraperitoneal (i.p.) 1 mL 0,9% saline every other day during 20 days (n = 10).

Radiation (R) group: Rats were received i.p. 1 mL 0.9% saline every other day and other day 0.5 Gy radiation during 20 days (n = 10). C and R groups animal were sacrificed at 1 day after experimental period.

S.E Extract + Radiation (SER) group: S.E extract given i.p., dose of 50 mg kg^{-1} every other day and other day 0.5 Gy radiation during 20 days.

SER 1: The animals were sacrificed at 1 day after experimental period (n = 10).

SER 15: The animals were sacrificed 15 days post-exposure last radiationdose (n = 10).

AE Extract + Radiation (AER) group: AE extract given i.p., dose of 50 mg kg^{-1} every other day and other day 0.5 Gy radiation during 20 days.

AER 1: The animals were sacrificed at 1 day after experimental period (n = 10).

AER 15: The animals were sacrificed 15 days post-exposure last radiation dose (n = 10).

Biochemical estimation: At the end of the experimental period, the rats were anaesthetized and killed by cervical dislocation. The liver, kidney and testis tissue were removed immediately and washed in ice-cold saline for examination of clinical biochemistry. Tissues were homogenized 1.40 w/v in 0.1 M phosphate buffer, pH 7,4, containing 1 mM EDTA. After centrifugation at 18000×g for 15 min at 4°C, the supernatant was extracted and kept at -30°C in advance of assays. In the tissue homogenates the levels of MDA and GSH were assayed.

Determination of tissue malondialdehyde levels: MDA levels, an index of lipid peroxidation, were measured by the method of Okhawa *et al.* (1979). The method is based on spectrophotometric measurement of the purple color generated by the reaction of TBA with MDA. MDA reacts with thiobarbituric acid to form a colored complex that has maximum absorbance at 532 nm. MDA levels as nmol g^{-1} wet tissue was expressed.

Determination of tissue reduced glutathione levels: The tissues GSH concentration was measured using the method described by Beutler *et al.* (1963). Briefly, 0.2 mL supernatant was added to 1.8 mL distilled water. Precipitating solution of 3 mL (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 mL distilled water) was mixed with haemolysate. The mixture was allowed to stand for approximately 5 min and then filtered (Whatman No. 42). Filtrate of 2 mL was taken and added into another tube and then 8 mL of the phosphate solution (0.3 M disodium hydrogen phosphate) and 1 mL DTNB were added. A blank was prepared with 8 mL of phosphate solution; 2 mL diluted precipitating solution (three parts to two parts distilled water) and 1 mL DTNB reagent. A standard solution of the GSH was prepared (40 mg 100 mL^{-1}). The optical density was measured at 412 nm in the spectrophotometer. Results were communicated as mg g^{-1} wet tissue.

Statistical analysis: All data were presented as mean ± S.E for parametric variables. Parametric variables were compared using one-way analysis of variance with post-hoc analysis using the Duncan test. Data were analyzed using the SPSS® for Windows computing program (Version 10.0) and $p<0.05$, was considered statistically significant (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

The results of all determination for the experimental and the control group tissues are given in Table 1 and 2.

The marker of lipid peroxidation MDA levels in the liver, kidney and testis of the rats significantly increased in R group compared to the C group ($p<0.05$). The liver MDA levels significantly decreased in SER 15 group ($p<0.05$). Whereas, the mean levels of liver MDA in SER1 group didn't show any significant change compared to the R group. The kidney MDA levels was significantly lower in the S.E-extract treatment groups in contrast to the R group ($p<0.05$). Thus, it was observed that S.E-extract application causes a significant decrease in kidney MDA levels. Testis MDA levels was significantly higher in the S.E-extract groups in contrast to the R and control animals ($p<0.05$). Moreover, MDA level was higher in SER 1 group as compared to the SER 15 and R groups ($p<0.05$). The liver, kidney and testis GSH concentrations significantly decreased in R group compared to control group ($p<0.05$). It was observed that radiation causes a significant decrease in tissue GSH. The liver and testis GSH levels were significantly higher in the S.E-extract treatment

Table 1: Levels of biochemical parameters in control, radiation and *Spinacia oleracea* L. extract groups

				SER ($\bar{X}$ ±S.E)	
Tissue	Parameters	Control ($\bar{X}$ ±S.E)	R ($\bar{X}$ ±S.E)	SER 1	SER 15
Liver	MDA (nmol g^{-1})	2.11±0.12^{b*}	3.14±0.09^{a*}	2.80±0.05^{a*}	2.36±0.17^{b*}
	GSH (mg g^{-1})	115.0±1.2^{a*}	94.51±0.51^{c*}	105.38±3.30^{b*}	114.10±0.95^{a*}
Kidney	MDA (nmol g^{-1})	2.46±0.14^{c*}	3.96±0.18^{a*}	3.29±0.13^{b*}	2.80±0.07^{c*}
	GSH (mg g^{-1})	99.04±1.3^{a*}	78.41±7.10^{b*}	80.64±4.97^{b*}	80.76±5.00^{b*}
Testes	MDA (nmol g^{-1})	2.93±0.16^{b*}	3.99±0.28^{a,b*}	5.4±1.23^{a*}	4.34±0.32^{a,b*}
	GSH (mg g^{-1})	89.92±2.37^{a*}	35.74±1.67^{c*}	58.07±6.70^{b*}	69.92±3.97^{b*}

Values with different letters show statistically significant differences (*: $p<0.05$)

Table 2: Levels of biochemical parameters in control, radiation and *Aesculuc hippocastanum* L. extract groups

				AER ($\bar{X}$ ±S.E)	
Tissue	Parameters	Control ($\bar{X}$ ±S.E)	R ($\bar{X}$ ±S.E)	AER 1	AER 15
Liver	MDA (nmol g^{-1})	2.11±0.12^{c*}	3.14±0.09^{a*}	3.86±0.36^{a*}	2.08±0.13^{c*}
	GSH (mg g^{-1})	115.0±1.2^{a*}	94.51±0.51^{b*}	102.63±6.08^{b,c*}	107.09±2.67^{a,b*}
Kidney	MDA (nmol g^{-1})	2.46±0.14^{c*}	3.96±0.18^{a*}	3.45±0.14^{b*}	2.58±0.21^{c*}
	GSH (mg g^{-1})	99.04±1.3^{a*}	78.41±7.10^{b*}	94.76±2.45^{a*}	103.75±3.51^{a*}
Testes	MDA (nmol g^{-1})	2.93±0.16^{c*}	3.99±0.28^{a,b*}	4.24±0.25^{a*}	3.38±0.27^{b,c*}
	GSH (mg g^{-1})	89.92±2.37^{a*}	35.74±1.67^{d*}	50.72±4.59^{c*}	75.07±7.76^{b*}

Values with different letters show statistically significant differences (*: $p<0.05$)

Table 3: Antibacterial activity of methanol extracts of *Aesculuc hippocastanum* L. and *Spinacia oleracea* L. expressed as inhibition zones (mm)

	Extracts (200 µg $disc^{-1}$)		Antibiotics (control)			
Test microorganisms	AE	S.E.	Penicilin 10 µg	Amikacin 30 µg	Moxifloxacin 5 µg	Teicoplanin 30 µg
Gram (+)						
Bacillus subtilis NRS 744	10	12	22	20	43	20
Bacillus cereus ATCC 11778	10	22	-	24	31	22
Enterococcus faecalis ATCC 29212	-	12	-	13	34	22
Listeria monocytogenes ATCC 7644	-	10	-	20	33	-
Staphylococcus aureus NRRL-B767	10	12	30	20	40	23
Gram (-)						
Pseudomonas aeruginosa ATCC 10145	9	12	-	21	18	-
*Salmonella typhimurium**	-	-	12	22	40	-
*Klebsiella pneumonia**	-	8	-	20	33	-
*Proteus vulgaria**	-	16	-	13	34	25
*Yersinia enterocolitica**	11	-	-	23	30	28

-: absence of inhibition,*: clinical isolated

groups in contrast to the R group ($p<0.05$). However, the kidney GSH levels of SER1and SER15 groups didn't show any significant change compared to the R group.

The liver MDA levels significantly decreased in AER15 group compared to the R and AER1 groups ($p<0.05$). The mean of the kidney MDA levels was significantly lower in the AE-extract treatment groups in contrast to the R group. Thus, it was observed that AE-extract application causes a significant decrease in kidney MDA levels. Testis MDA levels was significantly higher in the AER1 group in contrast to the R and AER 15 group animals ($p<0.05$). However, MDA level in testis was lower in AER 15 group as compared to the R groups ($p<0.05$). The liver, kidney and testis GSH levels were significantly higher in the AE-extract treatment groups in contrast to the R group ($p<0.05$). It was observed that AE-extract application causes a significant increase in tissue GSH.

The antimicrobial activities of *A. hippocastanum* and *S. oleracea* extracts were compared with standard antibiotics such as penicillin, amikacin, moxifloxacin and teicoplanin being employed as positive controls. Results from the antibacterial disc diffusion assay are summarized in Table 3. The gram-positive strains displayed a variable degree of susceptibility against the investigated plant extracts. The data indicated that *B. cereus* was the most sensitive strain of those tested with the S.E, with the strongest inhibition zone of 22 mm. The S.E also exhibited high antimicrobial activity against *B. subtilis* (12 mm), *E. faecalis* (12 mm), *S. aureus* (12 mm) and *L. monocytogenes* (11 mm). The S.E also showed gram-negative antimicrobial activity against *P. vulgaria* (16 mm), *P. aeruginosa* (12 mm) with modest activity against *K. pneumonia* (8 mm). The sensitivity of AE against gram-positive *B. subtilis, B. cereus* and *S. aureus* was found equal quantity, with the inhibition zone of

10 mm. Amongst these, the gram-negative strains also displayed against AE. *Y. enterocolitica* and *P. aeruginosa* found to be sensitive among the gram-negative bacteria tested with an inhibition zone of 11 and 9 mm, respectively. The data obtained from the disc diffusion method indicated that the AE and S.E had more activity against gram-positive strains than the gram-negative strains tested.

The interest in ROS in medicine has been increased because of their strong relationship with aging and disease processes (Cao *et al*., 1995). ROS are produced as a normal product of cellular metabolism. The continual formation of ROS and other free radicals is important for normal physiological functions and cellular redox reactions under normal conditions. However, excessive generation of free radicals can occur due to endogenous biological or exogenous environmental factors, such as exposure to radiation, pollution or chemical substances (Misra and Fridovich, 1972). A cell defends itself against ROS by elaborating systems of biological defence. In spite of numerous biological defense systems, increased free radical generation has the potential to result in oxidative stress. Oxidative stress may result from an imbalance between ROS and antioxidants levels (Lightboy *et al*., 2001). It is well known that, when the organism cannot balance free radical generation with the defense systems, a cellular injury and tissue damage might occur. The main damage induced by ROS results in alterations of cellular macromolecules such as membrane lipids, proteins and DNA and changes in intracellular calcium and intracellular pH, or cell death (Dorval *et al*., 2003; Fidan and Dundar, 2008). The 2 main molecular components of the membrane are lipids and proteins. As lipids are prone to oxidation of unsaturated bonds, it is perhaps reasonable to advocate lipid peroxidation as a significant event in the development of membrane damage (Yagi, 1993). The impact made by free radicals on lipids is named as Lipid Peroxidation (LP). LP is a complicated radical chain reaction leading to the formation of various products including lipid hydroperoxides, conjugated dienes and malondialdehyde. Detection of lipid hydroperoxides and conjugated dienes and thiobarbituric acid-reactive substances (TBARS) such as MDA, are often applied to the study of lipid peroxidation reactions (Diplock, 1994; Enginar *et al*., 2006). LP is 1 of the main manifestations of oxidative damage in cells and tissues. LP produces a progressive loss of cell membrane integity, imparement in membrane transport function and disruption of cellular ion homeostasis (Bano and Bhatt, 2007). Thus, the presence of MDA is considered as an indicator of free-radical damage through membrane lipid peroxidation (Katz *et al*., 1996; Enginar *et al*., 2006).

Under normal conditions, numerous cellular antioxidant systems exist to defend against oxidant stress and maintain the redox balance of the cell. ROS are cleared from the cell by enzymatic systems including superoxide dismutases, catalase and glutathione peroxidase, or the nonenzymatic system including alpha-tocopherol, ascorbic acid, glutathione and uric acid. Glutathione peroxidase plays an important role as defense mechanism in mammals, against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate. In addition to its role as a substrate in GSH redox cycle, glutathione, also act as a direct endogenous scavenger of hydroxyl radicals, involved in detoxification and metabolism of a number of substances in the liver (Sen, 2000). As a consequence of GSH deficiency, a number of related functions may be impaired such as a decrease in reducing capacity, protein biosynthesis, immune function, accumulations of lipid peroxidation products and detoxification capacity. A reduced detoxification capacity in the liver may lead to the accumulation of hepatotoxic metabolites leading to liver damage (Hayes and McLellan, 1999; Sen, 2000).

The most important action of ionizing, radiation on water and water solutions is that ions excited molecules and the obtained free radicals are able to irreversible cell membrane damage. The reactive free radical can oxidize bio molecules and lead to cell death and tissue injury. Therefore, cytotoxic effects of ionizing radiation may be related to oxidative stress (Enginar *et al*., 2006). These ROS can stimulate oxidative damage to essential cellular molecules and structures including DNA, lipids, proteins and membranes (Cadet *et al*., 2004). Products of lipid peroxidation such as MDA and 4NHE (4-hydroxynonenal) have the ability to interact with and alter macromolecules, possibly resulting in diseases (Petersen and Doorn, 2004). Radiotherapy combined with chemotherapy has been reported to reduce the antioxidant status of cancer patients (Bhuvarahamurthy *et al*., 1996), through free radical-mediated reactions. Numerous studies have ascertained the delay in coetaneous wound healing after radiotherapy, probably through the free radical-mediated cell damage during wound healing. The use of antioxidants has been observed to improve coetaneous wound healing significantly (Jagetia *et al*., 2003). Our results showed radiation treatment (X-rays) has induced significant increases of tissue MDA concentrations after giving 5 Gy radiations, indicating lipid peroxidation due to radiation treatment. Those results showed that lipid peroxidation occurred this dose of radiation.

Plants have been used in many parts of the world for hundreds of years as herbal medicines with broad ranges of nutritional and therapeutic values. Epidemiological studies have demonstrated the beneficial effects on human and animal health from consumption of vegetables and fruits. The antioxidant composition and capacity of vegetables and fruits relative to intake data are important to understand the health implications of various dietary patterns. It has been reported that vegetables ranked in the top 10 in an antioxidant assay included sweet potato leaf, ginger, amaranth, spinach, eggplant, pak choi, leafy Chinese cabbage, tomato and onion (Thomas, 2008). Moreover, previous studies demonstrated that AE and S.E contain antioxidant principles and could limit the extent of lipid peroxidation (Yalinkilic and Enginar, 2008; Bhatia and Jain, 2004). In the present study, the liver MDA levels significantly decreased in SER 15 and AER 15 groups. The kidney MDA level was significantly lower in the S.E-extract and AE-extract treatment groups in contrast to the R group. These results suggested that AE and S.E supplementation exhibited direct antioxidant by reducing basal MDA formation and protective antioxidant effect towards ionizing radiation-induced cell damage. MDA level in testis decreased only in AER 15 group as compared to the R groups. Depending on the acquired results, new studies should be carried out to understand this mechanism in details. However, Deger *et al.* (2003), found that the supplementation with antioxidant vitamins such as vitamins C and E did not affect initial values MDA concentrations in rabbits after exposure to X-rays. The decreases of MDA concentrations could be due to the AE and S.E ability to scavenge secondary reactive radicals or to prevent formation of superoxide and/or hydrogen peroxide in response to radiation treatment.

On the other hand, ionizing radiation would quickly oxidize the thiol groups of cells. GSH scavenges O_2 and protects protein thiol groups from oxidation. Accordance to this theory, radiation decreases the cellular concentration of GSH and leads to formation of glutathione disulfide. Our result supported this theory because the results in the present study also showed that, GSH concentrations significantly decreased in liver, kidney and testis by radiation treatment, paralel to the previous studies. It was observed that radiation causes a significant decrease in tissue GSH. Radiosensitivity of cells depends on the intracellular thiol level. Administration of various thiols can protect the cells and animals against the effects of radiation (Gul *et al.*, 2000; Meister and Anderson, 1983). Several studies experimental liver cirrhosis (Kaur *et al.*, 2000; Cantürk *et al.*, 2000) has shown decreased levels of GSH in different tissues. Our results are in agreement with these previous and Bhatia and Jain (2004) research reporting low levels of GSH. Different mechanisms may contribute to the reduced activity of antioxidant enzymes (GSH) in saponin treated rats. For instance, GSH is inhibited by superoxide anion (Blum and Fridovich, 1985) and excessive lipid peroxidation can cause increased GSH consumption (Sgambato *et al.*, 2001).

In the present study, the liver and testis GSH levels were significantly higher in the S.E-extract treatment groups in contrast to the R group. As well as the liver, kidney and testis GSH levels were significantly higher in the AE-extract treatment groups in contrast to the R group. The increased GSH levels in the extract group may be related to the antioxidant and free-radical scavenging effect of saponin. Another, explanation of this significant increase in GSH levels in extract-treated rats could depend on saponin stimulation of γ-glutamylcysteine synthetase activity. This increased GSH is consistent with the protective effects of saponin and polyphenols against oxidative damage in liver, kidney and testis. Furthermore, the kidney GSH levels between R and S.E-extract treatment groups didn't show any significant change. So that, new studies should be carried out on this subject, towards understanding this mechanism.

The threat of accidental or hostile exposure to ionizing irradiation is of great concern. Destruction of the intestinal mucosal lining with ulceration and hemorrhage, destruction of lymphoid tissue and of bone marrow, severe leucopenia and thrombocytopenia and damage to capillary endothelium have been occour after irradiation. So that, susceptibility to systemic infection from endogenous and exogenous organisms increased after exposure to ionizing radiation (Kaplan *et al.*, 1952; Brook *et al.*, 1986). As quoted by Benacerraf (1960), if the radiation damage is sufficiently severe death will ensue in the absence of complicating infection. Germfree rats, however, survive lethal doses of X-radiation significantly longer than conventional animals (Benacerraf, 1960). Klainer *et al.* (1967), reported that the coliform organisms residing in the gastrointestinal tract probably played a very significant role in postirradiation infection. These and other gramnegative intestinal bacteria appear to be the species most frequently involved in postirr adiation bacteremia and death. So that, the administration of antimicrobial therapy is prevent of systemic infection from endogenous and exogenous organisms that can occur after exposure to ionizing radiation. The antimicrobial effect of the medicinal plants is well documented (Valero and Salmeron, 2003). The results of different studies provide evidence that some medicinal plants might indeed be potential sources of antibacterial agents (Kone *et al.*, 2004).

In the present study, using the disk diffusion method, the antimicrobial activities of S.E and AE were compared with standard antibiotics such as penicillin, amikacin, moxifloxacin and teicoplanin being employed as positive controls. It was observed that extracts of S.E and AE produce antibacterial activity against gram positive than gram negative pathogens. These results suggest that *Aesculuc hippocastanum* and *Spinacia oleracea* extracts have antibacterial activity *in vitro*.

CONCLUSION

Our study shows that, ionizing radiation induced oxidative stress in tissue by decreasing the activities of antioxidant enzymes and generation of free radicals in rats. Although, *S. oleracea* and *A. hippocastanum* treatment decreases the tissue oxidative stress in irradiation-induced oxidative tissue damage by maintaining the GSH recycling activity and free radical scavenging potential. Moreover, our results demonstrate that, in animals exposed to irradiation, S.E and AE could provide great advantages against to systemic infection from endogenous and exogenous organisms increased after exposure to ionizing radiation. On the other hand, it was beliaved further studies should be carried out to deterimine the relationship between S.E-extract, testis MDA and kidney GSH levels. Consequently, *S. oleracea* and *A. hippocastanum* extracts could be used as a potential antioxidant and antibacterial resource aganist ionizing radiation with their antioxidant and antimicrobial contents.

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