Effect of Chitosan on Oxidative Stress and Metabolic Disorders Induced In Rats Exposed to Radiation

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Abstract: Radiation is one of the most widespread sources of environmental stress in living environment which cause oxidative stress and metabolic changes. Chitosan is widely distributed in nature as a component of bacterial cell walls and exoskeletons of crustaceans and insects. The present study aims to evaluate the antioxidant effect of chitosan against gamma rays induced oxidative stress and metabolic disorders in rats. The study was conducted on forty eight (48) female rats which were classified into four equal groups. Group1: Control group, rats administrated orally 1.0 ml vehicle solution for forty days Group. 2: Chitosan group, rats administrated orally (intragastric intubation) 1.0 ml of chitosan solution (100mg/kg b.wt. / day for 40 days).Group3: Irradiated rats, rats were subjected to whole body γ–irradiation to dose 4 Gy delivered as single exposure dose. Group 4: Combined treatment: rats administrated orally 1.0 ml of chitosan solution (100mg/kg b.wt. / day) for 40 days. At day 35 of chitosan treatment the rats were irradiated at dose level of 4Gy. Rats inspected after 1^{th} and 5^{th} days post irradiation and liver, spleen, lung and blood samples were collected. The animals exposed to gamma radiation had significant increase in TBARS, LDH, glucose, cholesterol, triglycerides, LDL-C, copper, iron, urea, creatinine, AFP and non significant increase in Mg. Also, significant decrease in GSH, CAT, HDL-C and estradiol was recorded. Administration of chitosan to rats prior and post gamma radiation improved the tested parameters so it is a therapeutic alternative for oxidative stress, hyperlipidaemia and hormonal changes. In this way, chitosan may be contributed to the prevention of atherogenic processes and contribute as safe functional fiber food.
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1. Introduction:

Human exposed to ionizing radiation has become inevitable with its vast application in diagnosis and industry. Everyone on earth exposed to radiation either natural background radiation or some of the population has the occasional medical or dental X-ray (Stevenson, 2001). Radiation damage, is to a large extent caused by over production of reactive oxygen species(ROS) which cause disruption of membrane lipids leading to subsequent formation of peroxide radicals(Rajapakse *et al.*,2007). Oxygen free radicals are highly reactive and may cause cell and tissue damage by interacting with cell membranes and organelles, lipid peroxidation is a ubiquitous phenomenon in the body under the influence of oxidative stress (Ozturk *et al.*, 2003). Reactive oxygen species are constantly generated in aerobic organisms during normal metabolism and in response to both internal and external stimuli as a result of water radiolysis (Kamat *et al.*, 2000). ROS have been implicated as major initiators of tissue damage and can up regulate enzyme activity, signal transcription, and gene expression (Massafra *et al.*, 2000).

Evidences have shown that over production of ROS in both intra and extra cellular spaces results as an imbalance between pro-oxidants and antioxidants that cause ROS production exceed the activity of endogenous antioxidants which increase oxidative stress. Once this imbalance takes place cellular molecules such as nucleic acids, proteins, structural carbohydrates, and lipids may be damaged by oxidative modifications (Ornoy, 2007). Also,ROS play a causative role in numerous disease pathologies such as cancer, ischemia, and degenerative disease such as aging, atherosclerosis, arthritis and neurodegeneration (Nelson and Melendez,2004)and cytotoxicity, metabolic and morphologic changes in animals and humans(Fang *et al.*,2002).

Marine organisms produce many bioactive substances, which are having a lot of potential applications. Chitosan, is the deacetylated form of chitin of marine origin is extracted from the shells of crustaceans (Sini *et al.*, 2005).

Chitosan (CS), is non toxic copolymer consisting of β-(1, 4)-2- amino-2 deoxy-D-glucose (β1, 4-linked polymer of glucosamine) and lesser amounts of N-acetylglucosamine. It is a naturally occurring biodegradable and biocompatible cationic polysaccharide derived from the N-deacetylation of chitin which is the most abundant natural structure polysaccharide after cellulose. It can be found in exoskeleton of crustaceans which can be obtained from the shell waste of the crab, shrimp and craw fish during processing industries and in fungal cell walls (Shahdat *et al.*, 2007). As a natural renewable

resource, chitosan has both reactive amino and hydroxyl groups that can be used under mild reaction for biomedical application.Chitosan is an attractive agent for drug development given its function in the gastrointestinal tract and its intrinsic safety when taken orally (Murata *et al.*, 2009).

Chitosan is the most abundant natural amino polysaccharide and is being used as a new source of dietary fiber (Liao *et al.*, 2007). Chitosan has a number of unique properties such as clarification, purification, antimicrobial activity, non toxicity, biocompatibility and biodegradability, which attract scientific and industrial interest in fields of biotechnology, pharmaceutics, waste water treatment, cosmetics, agriculture, food science nutrition, paper and textiles to use it (Hua and Wang, 2009).Also, it combined with calcium phosphates for use as bone graft substitutes (Geffre *et al.*, 2010). Chitosan has many advantages due to its nontoxicity and biodegradability without damaging the environment. It is a biocompatible material that breaks down slowly into a harmless product, glucosamine, which is absorbed completely by the body (FDA, 2002).

Chitosan has been reported to possess immunological (Mori *et al.*, 1997), antibacterial (Tokura *et al.*,1997) and wound healing (Okamoto *et al.*,1993) properties.Both hard and soft contact lenses can be made from chitosan(Santhosh *et al.*,2006).Sapelli *et al.(*1986) studied the application of chitosan in dentistry.

The present study designed to establish the putative protective, antioxidant and restorative effect of chitosan against gamma radiation induced oxidative stress which alternate the antioxidant status in hepatic, spleen and lung tissues and metabolic disturbances in serum.

2-Material and Methods

2.1.1-Materials

Chitosan with high molecular weight, Brookfield viscosity 800.000 cPS (Aldrich product of USA) was dissolved in 1% glacial acetic acid solution.

2.1.2- Animals

Female albino rats (130-140g) were obtained from the animal breeding house of Nuclear Research Center, Atomic Energy Authority, Inshas,Egypt. The animals were kept in isolated cages, under standard laboratory conditions including all hygienic measures with constant illumination and ventilation and normal conditions of temperature and humidity. Animals were maintained on a standard laboratory pellets containing all nutritive elements and free access to tap water was available. The animals were allowed to acclimatize for two weeks before the experiment.

2.1.3- Radiation Facility:

Whole body gamma irradiation was performed using gamma cell –60(Cobalt-60) unit at the Middle Eastern Regional Radioisotopes Center for The Arab Countries (MERRCAC), Dokky, Cairo, Egypt. Animals were exposed to 4.0 Gy applied as a single shot dose.

2.1.4-Experimental design

The animals were randomly assigned into 4 groups (12 rats for each group).

Group1: Control group, rats administrated orally 1.0 ml vehicle solution (1% glacial acetic acid in disttiled water) for forty days. Group 2: Chitosan group, rats administrated orally 1.0 ml of chitosan solution contains dose level of 100mg/kg b.wt. / day for forty days using stomach tube .Group3: Irradiated rats, rats were subjected to whole body γ- irradiation to dose 4 Gy delivered as single exposure dose. Group 4: Combined treatment: rats administrated orally (intragastric intubation) 1.0 ml of chitosan solution contains dose level of 100mg/kg b.wt. / day for 40 days. At day 35 of chitosan treatment the rats were irradiated at dose level of 4Gy .

2.1.5-Sample Collection

Animals were fasted overnight prior to sacrificing. Six rats from different animal groups sacrificed at 1st and 5th days after the irradiation. Blood samples were collected in two types of tubes the first contain sodium fluoride for glucose determination and the second plain tube to separate serum. Liver, spleen and lung were rapidly excised, washes with physiological saline solution, dried, weighed and homogenized in phosphate buffer (pH7.4) and kept frozen until used for biochemical assays.

2.2- Biochemical parameters

Lipid peroxidation content was determined by quantifying thiobarbituric acid reactive substance (TBARS) in tissue homogenates according to the colorimetric method described by Yoshioka *et al. (*1979), Catalase(CAT) activity was determined according to the method described by Johansson and Hakan Borg(1988). Determination of reduced glutathione (GSH) content was performed according to Beutler *et al.* (1963). Serum lactate dehydrogenase (LDH) estimated according to Young (2001). Serum levels of total cholesterol, triglycerides and high density lipoprotein (HDL-C) according to Allain *et al.* (1974), Fossati and Principe (1982) and Demacer *et al. (*1980) respectively. Low density lipoprotein (LDL-C) according to Friedewald *et al. (*1972). Serum urea and creatinine were estimated according to Young (1990) and Henery(1974) respectively. Serum copper, iron and magnesium determined

colorimetrically using commercial spectrum diagnostic kits (Germany). Plasma glucose determined according to Teitz (1986). Estimated sera and plasma parameters were colorimetrically methods performed by using spectrophotometer (Milton Roy Spectronic 1201). Serum alpha feto protein (AFP) and estradiol (E2) were determined using radioimmunoassay kit purchased from Immunotech A Bechman Coulter Company ,France ,using solid phase radioimmunoassay technique(RIA). The day before the animals were sacrificed vaginal smear was done to determine the oestrus cycle stage, so the data of estradiol (E2) represent the mean values of different oestrus cycle.

2.3-Statistical analysis

Data were recorded as mean ± SE. The results were submitted to one way analysis of variance(ANOVA) according to Snedecore and Cochron(1989) and means were compared between groups by Duncan multiple range test(Duncan,1955).

3. Results

There were no significant differences in behavior or external appearance and food consumption between control and rats administrated chitosan. In addition, no significant difference in blood biochemistry was found in both control and treated rats with chitosan.

In the present study the TBARS levels in hepatic, spleen and lung tissues were significantly ($P<0.05$) increased, while GSH content and CAT activity significantly ($P<0.05$) decreased in irradiated rats group, when compared to control. The TBARS levels in hepatic, spleen and lung tissues showed a significant ($P<0.05$) decrease in rats treated with chitosan prior and post whole body γ- irradiation, when compared to irradiated group.In addition, significant ($P<0.05$) increases in the GSH content and the activity of CAT were observed in hepatic,spleen and lung tissues in rats administrated chitosan prior and post irradiation as compared to irradiated rats (Table 1).

Table (1): Effect of chitosan on lipid peroxidation (TBARS), reduced glutathione (GSH) content and catalase (CAT) activity in hepatic, spleen and lung homogenate tissues in different groups(M±SE).

Parameters	Experimental Period (days)	Groups(n=6)			
		Control	Chitosan	Irradiated	Chitosan+Irradiatd
Liver TBARS (µmol/g wet tissue)	1	9.74±0.83^{c}	9.46±0.49^{c}	17.38±0.82^{a}	14.27±0.48^{b}
	5	10.57±0.17^{c}	8.43±0.37^{d}	13.28±0.36^{a}	11.98±0.22^{b}
GSH (mg/g wet tissue)	1	19.21±0.43^{a}	19.66±0.74^{ab}	15.89±0.72^{c}	17.82±0.44^{b}
	5	18.55±0.98^{a}	19.14±0.78^{a}	14.38±0.34^{c}	17.78±0.79^{a}
CAT (U/g wet tissue)	1	17.37±0.44^{a}	18.43±0.42^{a}	12.65±0.87^{c}	14.95±0.47^{b}
	5	17.29±0.59^{ab}	18.38±0.53^{a}	13.69±1.01^{c}	16.98±0.16^{b}
Spleen TBARS (µmol/g wet tissue)	1	28.35±2.08^{c}	26.59±1.49^{c}	53.32±2.84^{a}	39.75±2.92^{b}
	5	28.90±1.53^{c}	24.40±1.41^{c}	43.17±1.90^{a}	35.88±2.33^{b}
GSH (mg/g wet tissue)	1	18.59±0.60^{a}	19.09±0.55^{a}	13.65±0.67^{b}	17.62±0.49^{a}
	5	19.24±0.96^{a}	20.10±1.09^{a}	14.94±0.50^{b}	18.43±0.77^{a}
CAT (U/g wet tissue)	1	16.25±0.38^{a}	17.31±0.63^{a}	10.42±0.44^{c}	13.45±0.74^{b}
	5	16.55±1.56^{a}	17.92±1.14^{a}	12.28±1.11^{b}	15.75±1.08^{a}
Lung TBARS (µmol/g wet tissue)	1	14.37±1.10^{c}	13.55±0.81^{c}	35.19±1.21^{a}	28.32±1.14^{b}
	5	13.40±1.13^{c}	12.59±0.98^{c}	29.33±1.48^{a}	20.88±1.05^{b}
GSH (mg/g wet tissue)	1	17.65±0.56^{a}	17.73±0.57^{a}	13.33±0.53^{c}	15.65±0.67^{b}
	5	16.98±0.23^{b}	18.25±0.51^{a}	14.80±0.28^{c}	17.44±0.38^{ba}
CAT (U/g wet tissue)	1	18.10±0.78^{a}	19.24±0.47^{a}	12.06±0.65^{c}	15.71±0.49^{b}
	5	17.10±0.41^{b}	19.55±0.34^{a}	13.08±1.08^{c}	16.48±0.57^{b}

Data are presented as mean ±SE

The different small letters in the same row are significantly differ at $P<0.05$

The results presented in Table (2) showed that the serum activities of LDH was not significantly ($P>0.05$) affected by chitosan administration compared to the control group. However, γ-irradiation leads to significant ($P<0.05$) increase in the serum levels of these myocardial injury marker compared to the control group. The prior administration of chitosan for thirty five days before whole body γ- irradiation and five days post irradiation at dose of 4 Gy maintained the activities of this enzyme close to their normal activity as compared to control group (Table 2).

Table (2): Effect of chitosan on serum lactate dehydrogenase(LDH),plasma glucose and serum levels of lipid profile in different groups(M±SE).

Parameters	Experimental Period (days)	Groups(n=6)			
		Control	Chitosan	Irradiated	Chitosan+Irradiatd
LDH U/L	1	364.69±34.06^{b}	361.25±15.86^{b}	484.97±23.22^{a}	399.98±29.58^{b}
	5	355.12±33.81^{b}	349.50±26.73^{b}	469.12±24.04^{a}	378.84±21.34^{b}
Glucose mg/dL	1	103.09±3.27^{c}	99.11±4.87^{c}	138.45±5.33^{a}	121.43±2.65^{b}
	5	101.36±3.80^{c}	92.74±7.09^{c}	127.56±3.28^{a}	116.07±3.17^{b}
Cholesterol mg/dL	1	105.51±2.85^{c}	90.75±3.56^{d}	136.52±2.96^{a}	124.54±4.48^{b}
	5	109.33±3.39^{b}	91.45±5.44^{c}	128.25±5.12^{a}	112.91±2.62^{b}
Triglycerides mg/dL	1	93.78±3.62^{c}	90.02±2.58^{c}	137.32±1.62^{a}	129.24±1.79^{b}
	5	90.13±3.44^{c}	71.09±4.46^{d}	125.85±3.81^{a}	107.61±4.92^{b}
LDL-c mg/dL	1	72.48±2.19^{c}	55.69±0.82^{d}	100.82±1.98^{a}	81.61±2.23^{b}
	5	76.25±1.87^{b}	59.73±3.78^{c}	94.49±3.92^{a}	75.69±5.18^{b}
HDL-c mg/dL	1	14.27±1.89^{a}	17.05±1.64^{a}	8.24±1.95^{b}	17.08±1.41^{a}
	5	15.05±1.43^{a}	17.50±1.13^{a}	10.59±1.29^{b}	15.69±1.08^{a}

Data are presented as mean ±SE
The different small letters in the same row are significantly differ at P<0.05

As shown in (Table 2), γ- irradiation significantly (P<0.05) increase plasma glucose and altered serum lipid profile. The levels of total cholesterol, triglycerides and LDL-C were significantly (P<0.05) increased and HDL-C was significantly decreased in the irradiated rats group when compared to control group. The prolonged administration of chitosan prior and post exposure to single shot dose body γ-irradiation showed significantly (P<0.05) lower the alteration in the plasma glucose and lipid profile levels when compared to the irradiated rats.

Results in Table (3) show that irradiation caused significant (P<0.05) increase in serum Cu, Fe, urea, creatinine and non significant increase in Mg as compared to control group. Chitosan administration prior and post irradiation discerned significantly (P<0.05) lower levels than irradiated group and non significant decrease in Mg as compared to control group.

Table (4) show no significant changes in serum AFP and E2 levels in rats treated with chitosan when compared to control group. A significant (P<0.05) increase was observed in serum AFP level in the irradiated group compared to control group. While the irradiated group discerned significant (P<0.05) decrease in E2 compared to the control group. The prolonged administration of chitosan prior and post whole body exposure to single dose of 4 Gy γ-irradiation showed significant (P<0.05) decrease in AFP and significant (P<0.05) increase in E2 levels as compared to the irradiated group

Table (3): Effect of chitosan on serum levels of copper (Cu),iron(Fe), magnesium(Mg), urea and creatinine in different groups(M±SE).

Parameters	Experimental Period (days)	Groups(n=6)			
		Control	Chitosan	Irradiated	Chitosan+Irradiatd
Cu µg/dL	1	90.73±1.82^{c}	86.37±1.44^{c}	109.44±1.29^{a}	100.87±1.67^{b}
	5	84.16±1.06^{c}	84.65±1.73^{cb}	94.49±1.29^{a}	89.37±1.87^{b}
Fe µg/dL	1	323.74±0.51^{c}	325.87±1.24^{c}	418.42±2.49^{a}	376.00±1.79^{b}
	5	368.73±3.08^{c}	372.07±2.34^{cb}	392.50±1.94^{a}	378.14±2.79^{b}
Mg µg/dL	1	2.29±0.29^{a}	2.32±0.22^{a}	2.89±0.16^{a}	2.51±0.39^{a}
	5	2.33±0.20^{a}	2.36±0.21^{a}	2.69±0.11^{a}	2.42±0.15^{a}
Urea mg/dL	1	44.61±1.32^{bc}	41.89±0.44^{c}	55.12±2.19^{a}	47.51±1.55^{b}
	5	40.06±1.88^{b}	38.52±2.00^{b}	48.07±1.02^{a}	42.55±1.56^{b}
Creatinine mg/dL	1	0.73±0.02^{c}	0.71±0.01^{c}	1.10±0.06^{a}	0.89±0.03^{b}
	5	0.75±0.03^{b}	0.72±0.03^{b}	0.89±0.04^{a}	0.74±0.02^{b}

Data are presented as mean ±SE
The different small letters in the same row are significantly differ at P<0.05

Table (4): Effect of chitosan on serum alpha feto protein (AFP) and estradiol (E2) levels in different groups(M±SE).

Parameters	Experimental Period (days)	Groups(n=6)			
		Control	Chitosan	Irradiated	Chitosan+Irradiatd
AFP IU/mL	1	0.35 ± 0.09^{c}	0.30 ± 0.04^{c}	0.94 ± 0.09^{a}	0.65 ± 0.08^{b}
	5	0.33 ± 0.03^{c}	0.34 ± 0.09^{cb}	0.75 ± 0.07^{a}	0.46 ± 0.04^{b}
E2 ng/mL	1	16.61 ± 1.07^{a}	16.75 ± 1.17^{a}	13.19 ± 1.04^{b}	16.98 ± 0.92^{a}
	5	19.47 ± 0.50^{a}	19.93 ± 0.45^{a}	15.18 ± 0.71^{c}	17.63 ± 0.58^{b}

Data are presented as mean ±SE

The different small letters in the same row are significantly differ at $P<0.05$.

4. Discussion

Lipid peroxidation, a process induced by free radicals leads to oxidative deterioration of polyunsaturated lipids (Català, 2009). Under normal physiological conditions, only low levels of lipid peroxides occur in body tissues. The excessive generation of free radicals leads to peroxidative changes that ultimeately result in enhanced lipid peroxidation(Joshi *et al.,*2007). Radiation exposure has been reported to be associated with increased disruption of membrane lipids leading to subsequent formation of peroxide radicals (Rajapakse *et al.*, 2007).

In the present investigation, such a disruption of membrane lipids possibly accumulated for the observed increase in TBARS levels in the hepatic,spleen and lung tissues of irradiated rats. In addition, insufficient levels of antioxidants to scavenge peroxy-radicals during radiation could also have contributed to the elevated level of TBARS in irradiated rats (Manda *et al*., 2007).

Glutathione is the most abundant nonprotein sulfhydryl containing compound and constitutes the largest component of the endogenous thiol buffer (Holmgren *et al.*, 2005). Assessment of GSH in biological samples is essential for evaluation the redox homeostasis and detoxification status of cells in relation to its protective role against oxidative and free radical mediated cell injury(Rossi *et al.*,2005). The present study recorded a significant depletion of GSH content in hepatic, spleen and lung tissues in irradiated animals as compared to control group due to oxidative stress. The depletion of GSH content in irradiated rats might be due to enhanced utilization during detoxification process. The resultant reduction in GSH level may thus increase susceptibility of the tissue to oxidative damage including lipid peroxidation. However, Glutathione has diverse cellular functions in addition to its antioxidant properties including enzymatic conjugation through the glutathione- S transferase family of proteins and non enzymatic conjugation to cytotoxic compounds. Glutathione may react with H_2O_2 and lipid peroxides by action of GSH-PX to reduce their toxicity (Davis *et al.*, 2001).

Depletion in GSH level after radiation exposure might be resulted from diffusion through impaired cellular membranes and ⁄ or inhibition of GSH synthetase. Also, the decrease in the content of organs GSH might result from a diminished activity of glutathione reductase and a deficiency of NADPH which is necessary to change the oxidized glutathione to its reduced form (Pulpanova *et al.*, 1982). GSH can function as an antioxidant in many ways. It can react chemically with singlet oxygen, superoxide and hydroxyl radicals and therefore function directly as a free radical scavenger. GSH may stabilize membrane structure by removing acyl peroxides formed by lipid peroxidation reactions (Price *et al.*, 1990).

The prolonged administration of chitosan prior irradiation induced significant decrease in TBARS and significant elevation in GSH content and CAT activity in all tissues investigated as compared to irradiated group throughout the experiment. These results became in accordance with (Jeon *et al.*, 2003) who reported that chitosan administration significantly decreased liver thiobarbituric acid reactive substances (TBARS) and increased antioxidant enzyme activities catalase and superoxide dismutase

Antioxidants are necessary for preventing the formation of free radicals and they inhibit some of the deleterious actions of reactive oxygen species on lipids,DNA and proteins(Boojar and Shockravi, 2007). The observed reduction in the TBARS level in irradiated rats following administration of chitosan is one indicator of the antioxidant activity of chitosan as illustrated by (Chiang *et al.*, 2000).

Animals treated with chitosan alone did not record any abnormal changes as compared to the control group (Table 1). Xie *et al.* (2001) showed that the scavenging effect of chitosan on hydroxyl radicals inhibits lipid peroxidation of phosphatidylcholine and linoleate lipiosomes *in vitro.* Harish *et al.* (2007) reported that chitosan showed scavenging of ·OH and O_2^- radicals and offered

protection against calf thymus DNA damage. The free radical quenching property of this marine polysaccharide has been reported (Xing *et al.*,2005).The results obtained are in agreement with De Jesuse Valle *et al.*(2008) who reported a protective effect against oxidative stress in lung tissue which might even be attributed to chitosan inhalation.

The decrease in the activities of CAT could be due to a feed back inhibition or oxidative inactivation of enzyme protein caused by ROS generation (Ohta *et al.*, 2004) the current study recorded significant depletion of CAT activity in irradiated rats. However, CAT is one of three families of primary antioxidant enzymes in mammalian cells which are critical to peroxide removal. So the recorded depletion of enzymatic activity of CAT may be due to the increased utilization of this antioxidant to counteract lipid peroxidation production, however CAT removing H_2O_2 which occurred (Kalpana and Menon, 2004). Also, the decrease in the activity of CAT might be attributed to excess of ·OH resulting from water radiolysis after exposure to ionizing radiation which causes oxidative damage to enzymes that lead to the modification of the activity of CAT(Kregal and Zhang, 2007).

Radiation is associated with a decrease in antioxidants and an increase in oxidant free radicals resulting in increased oxidative stress which is followed by development of a variety of sub cellular changes in the myocardium, typical of radiation induced cardiac injury (Diniz *et al.*, 2003). The results obtained showed significant elevations in the activities of LDH in the serum of irradiated rats which indicate the severity of radiation induced necrotic damage of the myocardial membrane and the release of LDH enzyme from damaged heart tissue into the blood stream and an alterations in dynamic permeability of cardiac cell membranes due to the excessive production of free radicals and lipid peroxides that caused cellular membrane damage and leakage of cytosolic enzymes(Sridharan and Shyamaladevi, 2002).

In the present study, the prior– administration of chitosan was found to significantly lower the radiation induced elevation in the levels of diagnostic marker enzyme. This could be due to the free radical scavenging property of chitosan (Santhosh *et al.*,2007).

Major precursors of atheriosclerosis (hyperglycaemia,hypercholesterolaemia, hypertriglycerolaemia and even the process of aging) all induce mitochondrial dysfunction. Chronic overproduction of mitochondrial ROS leads to destruction of pancreatic β-cells increased oxidation of LDL-C and dysfunction of endothelial cells. Factors that promote arteriosclerosis (Nageswara *et al.*, 2007). In the present study, the elevated level of plasma glucose in γ- irradiated group may be correlated with hepatic gluconeogensis and glycogenolysis (Verspohl *et al.*, 2003).

Radiation induced hyperglycemia could be attributed to the diminished utilization of glucose by irradiated tissues. Irradiation could induce the transport of certain amino acids and thus increased glucose formation through the processes of deamination and transamination(Alhersova *et al.*,1981) as well as acceleration of gluconeogenesis, which resulted as an indirect effect of radiation exposure(Sedlakova *et al.*,1998). The increase in glucose level may also be related to endocrine abnormalities induced by irradiation which promote the secretion of biologically active peptide as ACTH which has well documented relation to carbohydrate metabolism by promoting gluconeogesis in the liver (Harper *et al.*, 1977). Elevated glucose levels in the blood causes the sugar to chemically react with proteins of the blood vessel walls and form glycosylated proteins that subsequently causes the capillaries to swell and get easily broken (Jean-Luc and Schmidt, 2004).

Administration of chitosan prior and post irradiation cause significant decrease in plasma glucose level. This result became in accordance with Yao *et al. (*2008). Other study demonstrated that oral chitosan microcapsulated insulin has the antihyperglycemic effect on the blood glucose level of streptozotcin diabetic rats(Huang *et al.*,2001).

The hyperlipidaemic state observed after irradiation could be attributed to the mobilization of fats from the adipose tissue to the blood stream (Chajek- Shaul *et al.*, 1989), in addition to mitochondrial dysfunction (Nageswara *et al.*,2007). Hyercholesterolaemia is an important risk factor for cardiovascular disease (Bokura and Kobayashi, 2003). Oxidation of LDL-C accelerates the growth of fatty streaks in blood vessel walls and the formation of plaque (Klebanv *et al.*, 1998). Toxic aldehydes formed in lipid oxidation react with the apo lipoprotein B of the LDL particle to produce a novel epitope that is recognized by macrophage receptors,resulting in the formation of foam cells and arteriosclerotic plaques and increased risk of heart disease and stroke (Burcham *et al.*,2002).

Irradiation induces hyperlipidemia through cell membrane destruction, enhancement of lipid metabolism, cholesterol release and triglycerides synthesis (Bodwen *et al.*,1989). Free radicals destruct cell membranes and enhance cholesterol release and increase lipid peroxidation(Karbowink and Reiter, 2000).

Increased triglycerides after irradiation might result from inhibition of lipoprotein lipase activity leading to reduction in uptake of triglycerides by adipose cells (Sedlakova *et al.*, 1998). The hypercholesterolemia and hypertriglyceridemia might be attributed to an increase in the activity of 3-hydroxyl methyl glutaryl COA, as an early reaction necessary for the restoration of biomembranes (Kolomijtseva, 1986). In addition to decreased fatty acid oxidation (Clark, 2001). Moreover, radiation enhanced the process of lipid peroxidation which results in cell membrane damage and the release of fats from peripheral and adipose tissues to the blood (Sedlakova *et al.*,1998). This increase may be due to increased lipolysis of depot triglycerides liberates free fatty acids from adipose tissue stores and the free fatty acids librated by the adipose tissue are also, taken up by the liver tissue, leading to the hypertriglyceridemic condition (Sedlakova *et al.*, 1998).

The data obtained demonstrated that chitosan administration to rats prior and post gamma irradiation resulted in amelioration of hyperlipidemic status. Such a protective effect was ascribed by Ylitalo *et al. (*2002). One mechanism by which CS might decrease cholesterol levels is by adsorption of bile acids (BAs) (Shields *et al.*, 2003).BAs are secreted in the gastrointestinal tract, primarily as glycine or taurine conjugates (primary BAs), after which secondary BAs, such as deoxycholate are produced from primary BAs by intestinal microorganisms. BAs are re-cycled via the enterohepatic circulation. Chitosan combined bile acids in the digestive tract and combined its products (primary bile acids, cholic acid and chenodeoxycholic) was excreted into the feces. Therefore, the oral administration of an anion – exchange resin, such as cholestyramine, inhibits the entrohepatic circulation of BAs, thereby decreasing serum cholesterol levels (Homma *et al.*, 1997). Also, Murata *et al. (*2009) reported that hpercholesterolamic rats fed a diet containing chitosan significantly had increased fecal fat and cholesterol excretion,reduced the lipid level in plasma and liver and tended to relieve the degenerated fatty liver tissue. These results suggest that chitosan reduced the absorption of dietary fat and cholesterol *in vivo* and could effectively improve hypercholesterolemia in rats.

Animals administrated chitosan did not alter the plasma glucose concentration as compared to the control group. This result became in agreement with (Yao *et al.*, 2006), who reported that diabetic rats administered chitosan had lower levels of plasma glucose, cholesterol, higher fecal cholesterol concentration and plasma TBARS was significantly decreased in diabetic rats. Also, Xia et al. (2010) reported that the supplementation of chitosan to rats fed on high fat diet reduced effectively the serum lipid levels such as total cholesterol,LDL-c and triglycerides which were regarded as to cause the cardiovascular disease moreover it elevated effectively HDL-c value which was regarded protect cardiovascular diseases. This result suggested that chitosan could effectively prevent hypercholesterolemia with a high fat diet and a longer treatment time brought a greater hypercholesterolemic effect. These results are in agreement with Zhou *et al.* (2008) who reported that chitosan was thought to possess hypocholesterolemic properties. Xu *et al, (*2007) suggested that chitosan improve lipid metabolism by regulating cholesterol and LDL by upregulating of hepatic LDL receptor mRNA expression, increasing the excretion of fecal bile acids.

The results obtained suggest that the lipid lowering effects of chitosan may be mediated by increases in fecal fat and ⁄ or bile acid excretion resulting from the binding of bile acids and by a decrease in the absorption of dietary lipids from the small intestine as a result of the inhibition of pancreatic lipase activity. These results are in agreement with Santhosh *et al. (*2007). Other study by Shahdat *et al. (*2007) suggested that dietary chitosan decreses the atherogenic lipid profiles of both normocholesterolaemic and hypercholesterolaemic rats and reduces the body weight gain of hypercholesterolaemic rats.

Increased cholesterol levels in the liver might be due to increased uptake of LDL from the blood by the tissues (Kissler *et al.*, 2005). The abnormal cholesterol deposition is favored by the dangerous tendency of cholesterol to passive exchange between the plasma lipoproteins and the cell membranes (Brown and Goldstein 1986).The hepatoprotective effect of chitosan is probably related to its ability to inhibit the lipid accumulation in the liver tissue by its antilipidemic property(Xing *et al.*,2005). Moreover, Anraku *et al. (*2011) reported that chitosan reduces the levels of pro- oxidants such as cholesterol and uremic toxins in the gastrointestinal tract, thereby inhibiting the subsequent development of oxidative stress in the system circulation.

Copper is absorbed into the intestine and transported by albumin to the liver. Copper is carried mostly in the blood stream on a plasma protein called ceruloplasmin. Exposure to gamma irradiation is associated with an increase in serum levels of Cu as compared to control rats. Hepatic Cu overload leads to progressive liver injury and eventually cirrhosis (Dashti *et al.*, 1992). Increased Cu level

may due to oxidative stress inducing proteolytic modification of ceruloplasmin (Kemal *et al.*, 2003).

In the present study the increased iron level in irradiated rats may be due to oxidative stress inducing proteolytic modification of ferritin and transferrin (Trinder *et al.,* 2000). Iron overload is associated with liver damage characterized by massive iron deposition in hepatic parenchymal cells, leading to fibrosis and eventually to hepatic cirrhosis. Accumulation of iron induced hepatotoxicity might be attributed to its role in enhancing lipid peroxidation. Free iron or low molecular iron or chelatable iron pool facilitates the decomposition of lipid hydroperoxides resulting in lipid peroxidation and induces the generation of •OH radicals and also accelerates the non enzymatic oxidation of glutathione to form $O_2^{\bullet-}$ radicals(Pulla and Lokesh, 1996).

Administration of chitosan prior and post irradiation was observed to prevent the generation of strongly oxidating hydroxyl radical by chelating of transitional metals ions,Cu and Fe. Furthermore, chitosan was shown to act as antioxidant (Chiang *et al.,* 2000) and prevent lipid peroxidation of cell membranes (Santhosh *et al.,* 2007).

Peroxidation of lipids can disturb the assembly of the membrane, causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes (Nigam and Schewe, 2000). The serum level of magnesium was decreased in irradiated rats supplemented with chitosan. This results in agreement with Liao *et al. (*2007) who reported that chitosan supplementation over 8 weeks lowered blood lipids and maintain normal calcium, magnesium and iron status in elderly hyperlipidemic patients.

The exposure to single dose of γ- radiation leads to a significant increase in serum urea and creatinine levels due to the increased protein breakdown as the urea is the end product of protein catabolism however, the degradation of protein by exposure to ionizing radiation is accompanied by an increase in the serum urea and different tissues free ammonia (Moulder *et al*., 2002). Also, ionizing radiation induce extensive retention in daily excreted urine that lead to increased creatinine and urea levels in the blood (Robbins *et al.*, 2001) and increased production of ROS and oxidative stress (Ogeturk *et al.,* 2005). The findings of Robbins *et al. (*2002) indicated that kidney irradiation clearly leads to a progressive reduction in function associated with concomitant glomerulosclerosis and ⁄ or tubulointerstitial fibrosis with many of pathologic changes. The results obtained indicated that water soluble chitosan did not cause liver, spleen,lung or kidney damage so that it is safe functional food (Sumiyoshi and Kimura, 2006) and improve kidney function due to its antioxidant effect (Chiang *et al.,* 2000).

The serum level of AFP was significantly increased after irradiation which may be due to hepatic damage as a result of lipid peroxidation increased after irradiation. In the present study AFP showed significant increases in irradiated animals group concomitant with increased level of TBARS, marker of lipid peroxidation have high toxicity and an inhibitory action on protective enzyme and so it acts as a tumor promoter and carcinogenic agent (Taysi *et al*., 2002). Alpha- feto protein (AFP) may be elevated due to hepatocellular carcinoma, as well as chronic hepatitis and liver cirrhosis (Kawai *et al.,* 2005).

Also, estradiol decrease in irradiated animals due to damage effect of radiation on gonad. The treatment with chitosan prior irradiation revealed significant modulatory effect of the studied parameters. A high molecular weight water soluble chitosan had significant effects on the ovulation rate, both the *in vivo* and *in vitro* fertilization rates and embryonic development. These results indicate an improvement in the ovarian and oviduct dysfunctions caused by obesity and suggest an adjustment in the internal secretions and metabolic functions (Choi *et al.,* 2002).

All rats appear healthy and remain active after oral administration of chitosan therefore chitosan is safe dietary fibers to inhibit hypercholeaterolmia. The results obtained are in agreement with *Tao et al. (*2011*).*

In conclusion, the results of the present study indicate that the administration of chitosan prior and post irradiation may prevent the deleterious effects of irradiation. The overall hepatoprotective effect of chitosan is probably due to a counteraction of free radicals by its antioxidant nature which decreaseTBARS production and increase antioxidant GSH content, enzyme CAT activity and⁄or to its ability to inhibit lipid accumulation by its antilipidemic property. Therefore, it is greatly recommended to incorporate chitosan in the diet as a nutritional fiber supplement before and during radiotherapy to prevent the oxidative daily damage induced by radiotherapy.

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