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Ameliorating Effects of Yeast Glucan with Zinc Bisglycinate on Histological and Biochemical Changes in γ-Irradiated Rats

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ABSTRACT

The present study aimed to examine the radioprotective effect of a nutraceutical supplement of yeast glucan with Zn bisglycinate against histological and biochemical changes induced by γ-irradiation. Animals received orally in water suspension (65 mg Glucan + 100 mg Zn bisglycinate kg^{-1} body weight for 5 consecutive days before irradiation & 7 days during the period of radiation exposure) (2 Gy x 4 delivered every other day). Results revealed that the major part of yeast extract was 1,3-β-D-glucan and confirm the existence of chelation in Zn bisglycinate. Histological examinations of different tissues (liver, heart, kidney and testis) showed that administration of the nutraceutical supplement has attenuated radiation-induced damaged and improved tissue architecture. The histological amelioration in the different tissues was accompanied by a remarkable decrease of their lipid peroxide levels. Moreover, the metabolic disorders accompanying tissue damage were significantly ameliorated. Changes in serum aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (γ-GT), acid phosphatase (ACP), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities, urea, creatinine glucose content and lipid profile were significantly ameliorated, compared to corresponding values in irradiated rats. According to the obtained results 1,3-β-D-glucan with zinc bisglycinate might be considered a potent nutraceutical supplement with a remarkable radioprotective capacity.

Key Words: Yeast glucan; Zinc bisglycinate; γ-irradiation; FT-IR; Microanalysis; Liver; Heart kidney; Testis

INTRODUCTION

1,3-β-D-glucan is a natural polysaccharide derived from the cell walls of Baker's yeast, fungi and bacteria that bind to pattern recognition receptors and modulate innate immunity (Fabrick *et al.,* 2003). Yeast glucan (YG) passes the stomach virtually un-changed. In the intestine, there are macrophages that inhabit the intestinal wall and are able to pick YG particles through beta glucan receptors via phagocytic transport mechanism (Lowe *et al.,* 2002).

A wide spectrum of beneficiary of the internal application of beta glucan has been reported. It lowers blood pressure in hypertensive patients and is also useful in lowering low density lipoprotein (LDL-Cholesterol). Reversal of cirrhosis of the liver is noted in patients treated with the oral administration of 1,3-β-D-glucan (1500 mg day^{-1}) (Keller & Pharm, 2000). The purified form of 1,3-β-D-glucan provide efficient protection of animals against infections by virus, bacteria, fungi and parasites. Such enhanced protection is obtained after injection as well as after oral or mucosal administration (Enjstad *et al.,* 2002). It also counteracts the toxic effects of bacterial endotoxins and enhances the body's capacity to destroy cancer cells. The experimental data suggest that glucan can also function as an effective free radical scavenger (Azab & El-Dawi, 2005).

Minerals are essential to life. All minerals must be absorbed into the body from the outside. Inorganic sources of minerals are known to induce irritation of the gastrointestinal tract in nutritional quantities and toxicity in higher quantities. Metals, chelated with amino acids, have shown greater bioavailability and utilization in human and animals. Because chelates possess five-membered heterocyclic rings that are strong enough to resist cleavage by phosphate, phytates and other dietary scavengers of free metals, yet not so strong as to resist cleavage and use-fullness for the body (Ashmead, 2003).

Zinc (Zn) is essentially required in humans and animals for many physiological functions, including immune and antioxidant function, growth and reproduction (Sun *et al.,* 2005). It protects various membrane systems from peroxidative damages induced by heavy metals and high oxygen tension and stabilize the membrane perturbations. Furthermore, the protective effects of zinc against radiation hazards have been reported in many investigations (Azab *et al.,* 2004).

Glycine is unique among the amino acids in its lack of a side chain. It was found to play a role in preventing inflammation (Li *et al.,* 2001) and was reported also to be an

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important therapeutic resource among diabetics to avoid the characteristic immuno deficiencies of this disease (Lezcano *et al.,* 2006). The biological consequences each of 1,3-β-D-glucan, zinc and glycine encourage the design of this a nutraceutical supplement containing yeast 1,3-β-D-glucan and zinc in its organic form as zinc bisglycinate. This study was undertaken to evaluate its radio protective capacity on radiation-induced structural changes in the liver, testes, heart and kidney tissues, associated to some metabolic disturbances.

MATERIALS AND METHODS

All animals studies were conducted in accordance with criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals.

Experimental animals. Male albino rats (120-150 g) obtained from the Egyptian Holding Company for Biological Products and Vaccines were used as experimental animals. Twenty four Animals were kept under standard conditions along the experimental period and fed on pellet concentrated diet containing all the necessary nutritive elements. Liberal water intakes were available.

Radiation facility. Whole body γ-irradiation was performed with a Canadian gamma cell-40, (^{137}Cs) at the National Center for Radiation Research and Technology, Cairo, Egypt at dose rate 0.48 Gy min^{-1}. Rats were exposed to fractionated γ-irradiation administered as 2 Gy every other day up to total dose of 8 Gy.

Preparation of yeast 1,3-β-D-glucan. It was prepared from *Saccharomyces cerevisiae* (local isolate) yeast cell walls by autolysis of the yeast suspensions (10% w/w solid content & pH 5.5 at 50 ˚C for 24 h). The autolysate was then centrifuged and the yeast cell wall collected and alkaline extraction done by using 1 M NaOH at 90±5ºC for 1 h. The insoluble cell wall fraction was collected by centrifugation. This was followed by 0.1 M acetic acid extraction. The solids were collected and rinsed twice with deionized water to remove any residual acid as well as any yeast degradation products. Then acetone was added to process the extraction and eliminate the non-polar lipids and hydrophobic proteins. The resulting wet solid were dried in vacuum oven at 40ºC for about 3 days. The yielded powder collected consists of approximately 85% β-glucan as whole glucan particles (identified according to the method of Whistler & Wolfrom, 1962) and 4.5% protein content (identified according to the method of Bradford, 1976). Triglycerides and total cholesterol were estimated according to the methods of (Allain *et al.,* 1974; Fossati & Prencipe, 1982), respectively.

Ultrasonication of the hydrated aggregated β-glucan particles was performed in an ice bath using ultrasound generator to obtain micro particulate β-glucan homogeneous suspension particles that was sterilized by UV-radiation. Elemental analysis and FT-IR spectroscopy were applied for characterization of the glucans (El-Batal & Fadel, 2002; Hunter *et al.,* 2002; Hromodkova *et al.,* 2003).

Preparation of Zinc bisglycinate sulphate. Zinc-amino acid chelate formation, comprising zinc ion as $ZnSO_4.7H_2O$ being chelated by an amino acid ligand comprising glycine, where ligand to zinc molar ratio is 2:1. The Sulphate anion in zinc sulphate heptahydrated will not form a complex with the produced amino acid chelate. Physical characteristics such as melting point, solubility, elemental analysis including atomic absorption spectroscopy and FT-IR spectroscopy were performed to identify and confirm the existence of chelation (El-Batal, 1985; Ashmead, 2003).

Experimental design. Animals were divided into 4 groups (n=6):

Control. Rats of this group have not received the nutraceutical supplement and were not irradiated.

Supplemented. Rats of this group were given the supplement (65 mg yeast glucan+30 mg Zn in zinc bisglycinate) for 12 consecutive days.

Irradiated. Rats of this group were exposed to fractionated γ-irradiation administered as 2 Gy every other day up to a total dose of 8 Gy.

Supplemented irradiated. Rats of this group received the supplement for 5 successive days before irradiation and 7 days during the exposure of rats to fractionated irradiation.

Animals were sacrificed one day after the last irradiation dose. Blood samples were collected via heart puncture. Liver, testis, kidney and heart were removed completely to run the biochemical and histological investigations.

Biochemical analysis. The lipid peroxidation products of polyunsaturated fatty acids of cellular membrane were estimated as thiobarbituric acid reactive substances, TBARS (Yoshioka *et al.,* 1979). Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were determined following the method of Reitmen and Frankel (1957). Gamma glutamyl transferase (γ-GT) and acid phosphatase (ACP) activities were determined according to Szasz *et al.* (1974) and Seiler *et al.* (1983), respectively. The concentrations of urea and creatinine in serum were estimated by the methods of Marsh *et al. (*1975) and Bord and Sirota (1976), respectively. Total activity of serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK), were measured according to the methods of Klin (1972) and Rec (1977), respectively. Serum triglycerides (TG) were measured according to Fossati and Prencipe (1982). The contents of serum total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) were estimated according to the methods of Allain *et al.* (1974) and Demacker *et al.* (1980), respectively. Low density lipoprotein cholesterol (LDL-C) content was calculated according to Friedewald *et al. (*1972). Serum glucose levels were measured after Trinder (1969).

Histological analysis. Samples of the different tissues (liver, heart, kidney & testis) were immediately excised, fixed in buffered formal, processed routinely for paraffin embedding and sectioned at 5 µm. Sections were stained

with haematoxylin, and eosin (HE) and mounted with Canada balsam. Sections were examined by Olympus light microscope to detect the histological and histopathological changes induced by any of the treatments described previously.

Statistical analysis. The SPSS/PC computer program was used for statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA) The data were expressed as mean ± standard error. Differences were considered significant at ($P \leq 0.05$).

RESULTS

In the present study, structural and characterizations of the water insoluble 1,3 β -D-glucan isolated from the yeast biomass by consecutive alkaline/acid extraction and purification steps were performed using different analytical methods. Treatment with alkali hydrolyzed and solublized the cellular protein, nucleic acids, mannans, soluble glucans and polar lipids into the supernatant fraction and deacetylated chitin to chitosan in the cell wall. On the other hand, extraction by acetic acid was performed to complete the removal of glycogen, chitin, chitosan and remaining proteins. The extraction process by acetone was used to eliminate the non-polar lipids and hydrophobic proteins.

Glucan derived from yeast has been described to be soluble solely in dimethyl sulfoxide (DMSO), but not in water, alkali, acid or other organic solvent. In the present study, a homogeneous water suspension of micro particulates β-glucan was obtained by sonication to facilitate the phagocytic transport mechanism of β-glucan following oral administration.

In the present study, verification of the 1,3-β-D-glucan structure was performed using FT-IR spectra. The glucan sample obtained showed the same spectral pattern typical of a 1,3-β-D-glucan i.e., contained absorption bands arising from the υ(C-C) and the υ(C-O-C) stretching vibration at 1160 cm^{-1}, two partially overlapped bands at 1078 cm^{-1} and 1048 cm^{-1} attributable to ring (C-OH) side group stretching and a band at 890 cm^{-1} assigned to the β-glycosidic (C_1-H) deformation mode. The sample lacked strong absorption in 2955 to 2855 cm^{-1} (indicative of saturated fatty acids), 1650 cm^{-1} (indicative of protein), 850 cm^{-1} (indicative of glycogen α-linked), or 930 cm^{-1} (indicative of β(1-4) polysaccharides e.g., chitin). Finally, 1,3-β-D-glucan was found with strong absorbance in the 890 cm^{-1}.

For the verification of the chelation between zinc and glycine; the physical characteristics above (solubility, melting point & elemental analysis e.g., percent of nitrogen and zinc & approximate molecular weight) do not confirm the existence of chelation. The only way to verify that an amino acid chelate exists is to look at the bonds (FT-IR spectroscopy). The bands characteristics of amino acid chelate bonds are what set it apart as a highly bioavailable mineral source, because the nature of those bands is specific to the chelate and are not present in other mineral sources.

Fig. 1. Photomicrograph of a section in the liver of rats (x 200)

a) Control, b) Yeast glucan+zinc bisglycinate: normal architecture of hepatic trabeculae, normal sinusoids, normal hepatic portal area (pa), many binucleated hepatic cells (bn), *c)Irradiated:* vacuolated cytoplasm, severe rupture and shrinkage hepatocytes, necrotic (n), pyknotic (p), karyolytic (k) nuclei, severe dilating and widening portal vein (pv) *d)Yeast glucan+zinc bisglycinate-irradiated:* normal portal area (pa), ameliorated hepatic trabeculae and improved sinusoids

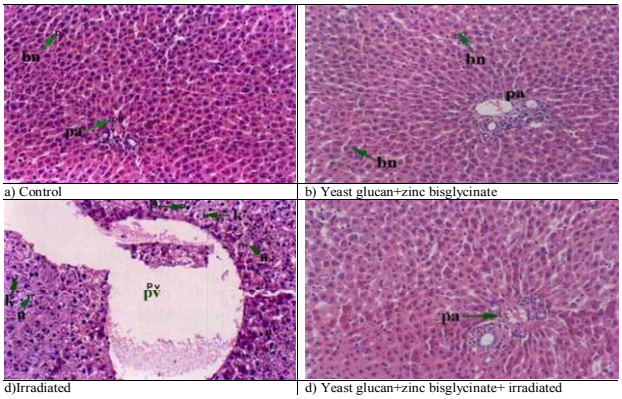
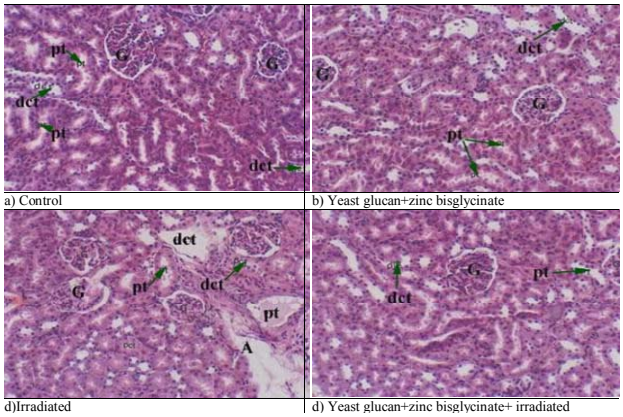


Fig. 2. Photomicrograph of a section in the renal cortex of rats (x 200)

a) Control, b) Yeast glucan+zinc bisglycinate: normal architecture of glomeruli (G), proximal tubule (pt), distal convoluted tubules (dct) *c)Irradiated:* degenerated destructed glomeruli (G), ruptured, dilated and abnormal shaped proximal tubule (pt) and distal convoluted tubules (dct), dilated arteriole (a) *d)Yeast glucan+zinc bisglycinate-irradiated:* ameliorated architecture of glomeruli (G), proximal tubule (pt) and distal convoluted tubules (dct)



Therefore, evaluation of these bonds gives a unique and definitive answer to whether or not a mineral is chelated. By using infrared spectroscopy to measure the energies emitted by molecules the structure can be determined. Two bonding energies are in the chelation of glycine to a zinc metal that of the carbon and the nitrogen.

The FT-IR scan of a zinc bisglycinate chelate (16% Zn metal) showed a NH_2 broad band stretch indicating amine bond to zinc at 3500-3000 cm^{-1}. Also symmetric COO^- shift at 1395 cm^{-1} indicative bonding of carboxyl and shoulder at 1643 cm^{-1} evidence of ring formation. There is no peak present at 504 cm^{-1} indicating the carboxyl is bound to the zinc and the absence of a strong peak at 2100 cm^{-1}, which is

characteristics of alpha amino acids. Mineral glycine chelate have been shown to be stable and bioavailable.

Animals fed a normal diet and given a daily dose of the nutraceutical supplement for 12 consecutive days via oral tube, showed no significant changes in the concentration of TBARS in liver, testis, kidney and heart tissues (Table I). Blood serum enzymatic activities and metabolites concentrations observed in the present study showed approximately normal ranges (Table II & III).

The histological study on tissues of control and supplemented treated rats displayed normal structure of hepatic trabeculae, normal sinusoids, normal hepatic portal area (portal vein, lymph & bile duct). Many binucleated hepatocytes were noticed (Fig. 1a). Photomicrographic analysis on kidney sections showed normal configuration of glomeruli and both proximal and distal convoluted tubules. (Fig. 2a). Testis sections subjected to histological observations demonstrated normal dense fibrous membrane tunica albuginea, seminiferous tubules, normal tubuli recti and normal interstitial cells laying in the loose connective tissue between the seminiferous tubules (Fig. 3a). Cardiac muscles sections exhibited normal structure of cardiac muscle fibers branch which anatomized with other fibers to form a network, each cardiac muscle cell have its own nucleus located centrally as well as normal endomysium capillaries (Fig. 4a).

In the present work, whole body γ-irradiated rats showed a significant increase ($P \leq 0.05$) in TBARS concentrations in liver, testis, kidney and heart tissues (Table I). Furthermore, exposure to fractionated γ-irradiation induced significant increase of serum glucose, urea, creatinine, TG, TC and LDL-C concentrations, while HDL-C showed a decrease. Serum ALT, AST, ACP, CPK and LDH showed significant increase in the serum of irradiated rats compared to control animals (Table II & III).

In addition, clear histopathological changes were noticed in liver, heart, kidney and testis. Extremely dilated and widened portal vein, degenerated and ruptured hepatocytes, necrotic, pyknotic and karyolytic hepatic nuclei and vacuolated cytoplasm (Fig. 1c) were observed. Histological disorders of kidney resulted in the form of collapsed glomeruli, large hemorrhagic area with fibroblastic invasion between the degenerated tubules, most of renal tubules appeared necrotic and degenerated epithelial cells and dilated renal arterial (Fig. 2c). Abnormal changes occurred as ruptured tunica albuginea, destructed and degenerated seminiferous tubules, ill-defined shape tubuli recti and degenerated interstitial cells (Fig. 3c). Abnormal structures of cardiac muscles were found as ill –defined shape, necrotic, karyolitic nuclei. Severe dilated, widened and inflammated capillaries in endomysium (Fig. 4c).

Oral administration of the supplement to rats for 5 consecutive days, before exposure to the first dose of radiation and daily within the period of radiation exposure led to a decrease in lipid peroxides (TBARS) induction in the investigated tissues associated with remarkable

Fig. 3. Photomicrograph of a section in the testis of rats (x 200)

a)Control, b)Yeast glucan+zinc bisglycinate: normal configuration of tunica albuginea (T), seminiferous tubules(st), tubuli recti (tr), normal interstitial cells (i) *c)Irradiated:* ruptured tunica albuginea (T), destructed and degenerated seminiferous tubules(st), ill defined shape of tubuli recti (tr), degenerated and ruptured interstitial cells (i) *d)Yeast glucan+zinc bisglycinate-irradiated:* recovery of tunica albuginea (T), regenerated seminiferous tubules(st), regenerated tubuli recti (tr), and regenerated normal interstitial cells (i).

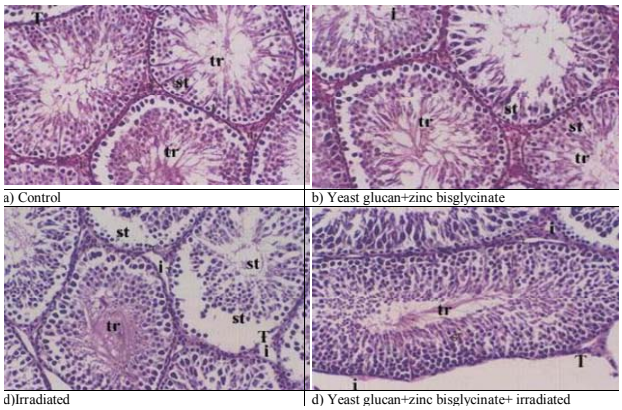
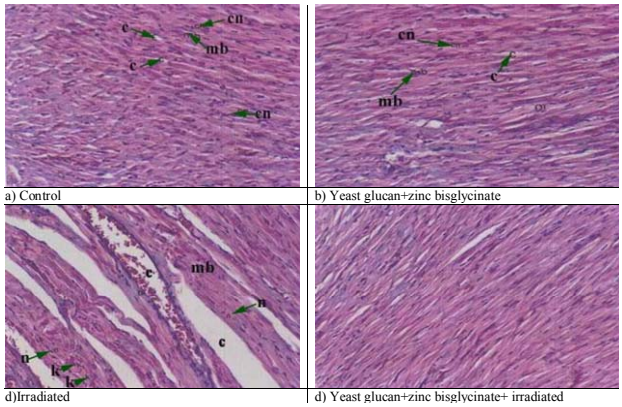


Fig. 4. Photomicrograph of a section in the cardiac muscle of rats (x 200)

a)Control, b)Yeast glucan+zinc bisglycinate: normal architecture of cardiac muscle fibres branch (mb) and anastomose, normal capillary in endomysium (c), normal central nuclei (cn), *c)Irradiated:* ruptured, sticky and ill-defined shape of cardiac muscle branch (mb), necrotic (n), karyolytic (k) nuclei, severe dilated widened and inflamated capillaries in endomysium (c) *d)Yeast glucan+zinc bisglycinate-irradiated:* ameliorated structure of cardiac muscle fibres, improved nuclei and ameliorated capillaries in endomysium



improvement in serum enzymes activities and metabolites concentrations. Amelioration of hepatic trabeculae, hepatic portal area and sinusoids was observed (Fig. 1d). Most glomeruli and renal convoluted tubules tend to be normal (Fig. 2d). In testis, well defined shape tunica albuginea, regenerated seminiferous tubules, well-defined shape tubuli recti and ameliorated interstitial cells were observed (Fig. 3d). In cardiac muscle structure, improved nuclei and ameliorating features of capillaries in endomysium were noticed. (Fig. 4d).

Table I. TBARS levels in liver, kidney, testis and heart of different animals groups at one day after the last irradiation fraction

Animal groups	Tissues TBARS (nmol/mg protein)			
	Liver	Spleen	Heart	Kidney
Control	1.66±0.084[a]	2.73±0.142[a]	3.78±0.196[a]	3.90±0.211[a]
Supplemented	1.46±0.082[a]	2.27±0.161[a]	3.98±0.216[a]	3.89±0.252[a]
Irradiated	2.94±0.182[b]	3.97±0.183[b]	5.01±0.311[b]	5.30±0.331[b]
Supplemented Irradiated	2.21±0.12[c]	3.22±0.201[c]	3.52±0.261[a]	3.99±0.282[a]

Values are means ± SE (n = 6)
Values with unlike superscript in the same column are significantly different at $P \leq 0.05$

Table II. Serum enzyme activities of different animals groups one day after the last irradiation fraction

Serum enzyme activities	Animals groups			
	Control	Supplemented	Irradiated	Supplemented Irradiated
ALT (U/ml)	19.5±1.02[a]	20.1±1.12[a]	41.2±2.21[b]	29.1±1.88[c]
AST(U/ml)	135.2±6.33[a]	129.8±6.94[a]	169.8±7.84[b]	134.2±6.55[a]
γGT(U/ml)	3.22±0.147[a]	3.18±0.154[a]	6.21±0.201[b]	5.03±0.194[c]
LDH (U/l)	342±14.2[a]	326±14.9[a]	822±24.8[b]	568±21.5[c]
CPK (U/l)	110±4.81[a]	108±4.65[a]	320±7.42[b]	214±6.12[c]
ACP (U/l)	28.1±1.18[a]	26.3±1.21[a]	47.6±1.85[b]	36.8±1.64[c]

Values are means ± SE (n = 6)
Values with unlike superscript in the same row are significantly different at $P \leq 0.05$

Table III. Serum metabolites levels (mg/100 mL) of different animals groups at one day after the last irradiation fraction

Serum metabolites	Animals groups			
	Control	Supplemented	Irradiated	Supplemented Irradiated
Glucose	120±5.74[a]	118±6.55a	74±2.33[b]	115±4.33[a]
Creatinine	0.301±0.014[a]	0.282±0.016[a]	0.211±0.017[b]	0.301±0.019[a]
Urea	48.5±2.42[a]	47.3±2.24[a]	71.5±2.98[b]	61.5±2.68[c]
Triglycerides	168±6.12[a]	169±7.01[a]	184±6.53[b]	169±6.47[a]
Total cholesterol	129±4.81[a]	121±4.62[a]	157±5.13[b]	133±5.11[a]
HDL-cholesterol	36±1.47[a]	35±1.54[a]	43±1.56[b]	47±1.58[b]
LDL-Cholesterol	52±1.63[a]	53±1.66[a]	77±1.75[b]	59±1.68[a]

Values are means ± SE (n = 6).
Values with unlike superscript in the same row are significantly different at $P \leq 0.05$.

DISCUSSION

Evidence have shown that the over production of reactive oxygen species (ROS) in both intra and extracellular spaces upon exposure of cells or individuals to hyperoxia, certain chemicals, radiation, or local tissue inflammation, results in oxidative stress defined as imbalance between pro oxidants and antioxidants. Once this imbalance takes place, cellular molecules may be damaged by the predominant free radicals. This leads to oxidative modifications of cellular molecules (Romero *et al.,* 1998). There has recently been an increasing interest in finding more effective and safe biological agents to minimize oxidative stress and control radiation hazards. In the present study, we have examined an assembly between yeast glucan and Zn bisglycinate against changes in biochemical and histological aspects associated with radiation exposure.

In the present study, the radiation-induced changes in the configuration and architecture of liver, kidney, testis and cardiac tissues were associated to significant elevation in the content of lipid peroxides and significant metabolic disturbances. Increased lipid peroxidation might be due to the over production of ROS in the aqueous media of the cells and the interaction of the OH radicals with the polyunsaturated fatty acids of cell membranes phospholipids initiating the process of lipid peroxidation and the consequent damage of cell membranes (Saada & Azab, 2001).

The aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are sensitive indicators of liver cell injury. The enzymes are normally present in serum in low concentrations. ALT is generally concentrated in liver cells. On the other hand, AST is found in cardiovascular areas, skeletal muscle, kidneys, brain, pancreas, lungs, white blood cells, red blood cells and liver. The rise of ALT and AST in the serum is usually related to the injury of tissues and membrane permeability. Elevated ALT and AST activities is observed in acute and chronic hepatitis, cirrhosis, infectious mononucleosis, heart failure, various infections, metastatic carcinoma and alcohol related liver damage. ALT is best used to monitor the progress of present and on-going liver inflammation and a trended to decrease is a good sign that fibrosis resulting from inflammation is being controlled (Quinn & Johnston, 1997). The significant increases in serum activities of ALT, AST, γ-GT as reported in the present study results from rupture of hepatocytes and necrotic degeneration of liver cells (Fig. 1c

& Table II). The elevated γ-GT activity recorded in this study after radiation-exposure pointed to the destruction of cell membrane where the enzyme is attached. γ-GT, is a membrane-bound enzyme that initiates the degradation of extracellular glutathione (GSH) forming γ-glutamyl amino acid and cysteinylglycine. Cysteinylglycine is then degraded by dipeptidases into cysteine and glycine, both of which can be taken up by cells and used for *de novo* GSH synthesis. The utilization of extracellular cysteine has been shown to be dependent on the γ-GT activity in human endothelial cells (Cotgreave *et al.,* 1994). GSH plays an important role in natural antioxidant defense mechanisms against the attack of ROS and free radicals induced post radiation exposure. γ-GT is important for oxidant-challenged cells to maintain their intracellular GSH concentration and participates in the GSH synthesis salvage pathway (Shi *et al.,* 1993).

The experimental results of the present study revealed significant increases of serum of LDH, CPK activities concomitant with abnormal cardiac muscles (Table III & Fig. 2c). These abnormalities could be explained on the bases that ionizing radiation instigates the alterations in dynamic permeability of membranes allowing leakage of biologically active material out of the injured cells (Hedayat & Azab, 2004).

The results obtained showed that exposure to ionizing radiation induced significant elevation in serum concentration of urea and creatinine associated with kidney histological disorders and that could be attributed to the destruction and malfunction of kidney cells due to the action of ROS released post radiation exposures (Table III & Fig. 3c). Urine formation begins with filtration of the blood at the glomerulus, further processing of the filtrate by the renal tubules and elimination of the formed urine by the renal collecting system. Alterations of any of these processes can result in the picture of rapidly deteriorating renal function (Anderson *et al.,* 1997). Acute renal failure (ARF) result in failure of urinary elimination of nitrogenous waste products (urea nitrogen & creatinine), which results in a rise of their content in the blood. Ionizing radiation impairs glomerular filtration, which could be considered a sign of renal failure and kidney malfunction. In addition, the increase in serum acid phosphatase activity was associated to distortion of testis integrity coupled with testis abnormal structures after radiation exposure (Table II & Fig. 4c) that could be attributed to widespread free radical hazards which cover tissues and organs of various radio sensitivity.

The significant increase of serum glucose might be attributed to radiation-induced gluconeogenesis and inhibition of glucose uptake by peripheral tissues. In the present study hyperglycemia was associated with hyperlipedimia. Experimental studies have shown that a decline in skeletal muscle glucose utilization and/or an excessive hepatic glucose production, constitutes a major pathogenic importance in dyslipidemia and may be one of the driving factors behind elevated triglyceride levels (Schwarz *et al.,* 2003). Hyperglycemia promote LDL-C rise and HDL-C drop. On the other side, the hyperlipidemic state observed in the serum of irradiated rats might result from increased fat mobilization from adipose tissues resulting from radiation-induced injury to cellular biomembranes (Saada *et al.,* 2003). In addition, a decrease in lipoprotein lipase activity (clearing factor) reduces the uptake of lipids by adipose cells. The elevated level of total cholesterol might result from increased synthesis as an early reaction necessary for the restoration of biomembranes. One must consider also, that alteration in lipid metabolism are probably due to radiation induced liver injury.

In this study, animals receiving the nutraceutical supplement (yeast glucan + zinc bisglycinate) before whole body γ-irradiation and during the period of radiation exposure showed significant recovery of radiation-induced structural changes in liver, kidney, testis and cardiac tissues with significant reduction in the amount of TBARS. Furthermore, significant amelioration in the levels of serum metabolites (glucose, lipid profile, urea, creatinine), as well as serum enzymatic activities (ALT, AST, GGT, ACP), were recorded in supplemented irradiated rats, compared to their corresponding values in irradiated rats.

Ingestion of beta-glucans has been shown to improve the pattern of lipids in humans and experimental animals with elevated serum cholesterol (hypercholesterolemia) (Nicolosi *et al.,* 1999). Several mechanisms have been proposed for the cholesterol-lowering effect of beta-glucan. Beta-glucans help the fermentation of lipids by intestinal bacteria to short-chain fatty acids which are easily absorbed, in addition, beta glucans inhibit hepatic cholesterol synthesis. Beta glucans reduce the intestinal absorption of both cholesterol and bile acids by binding to the glucans, shifting the liver from cholesterol synthesis to bile acid production (Nicolosi *et al.,* 1999). On the other hand, the anti-diabetic effect of beta glucans was attributed to its activation of macrophages the main source IL-1 in the body, which increases insulin production resulting in lowering of blood glucose level (Lang & Dobrescu, 1989). Beta-glucans could modulate the autoimmune mechanisms directed to pancreatic islets and inhibit the development of diabetes in rats. Furthermore, it could reduced carbohydrate absorption from the gut (Battilana *et al.,* 2001).

In vitro studies have demonstrated that beta-glucan inhibits lipid peroxidation in human red blood cells hemolysates). It has been shown that zinc administration minimize oxidative damage and reduce the elevated levels of malondialdehyde in the testis of of Cd-exposed rats. Furthermore, zinc act as antiperoxidative agent following iodine-131 induced changes on the antioxidant system and the morphology of red blood cells (Amara *et al.,* 2008).

It is worthy to mention that, the modulatory action of the supplement might be attributed to the synergistic effect of its constituents. Yeast glucan has been reported to scavenge free radicals (Azab & El-Dawi, 2005), mainly OH radicals and to inhibit lipid peroxidation. Zinc was found to minimize oxidative damage and reduce the elevated levels

of malondialdehyde in the testis of of Cd-exposed rats (Amara *et al.,* 2008). In addition it protects various membrane systems from peroxidative damages and stabilize the membrane perturbations. Furthermore, glycine maintains membrane continuity and prevents severe structural damage (Javadpour *et al.,* 1999).

From the results it is concluded that this supplement by modulating radiation-induced lipid peroxidation would preserve membrane integrity and tissues normal structures and ameliorate metabolic disorders.

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