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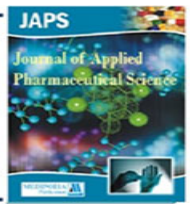

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Evaluation of the radioprotective action of anserine along with zinc in albino rats exposed to gamma-radiation

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ABSTRACT

Administration of dietary antioxidants has been suggested to protect against the subsequent liver tissue damage. The present data to explore the hepatoprotective and antioxidant effect of anserine nitrate and /or zinc chloride against γ-irradiation induced hepatotoxicity. Healthy male albino rats were exposed to γ-irradiation from Co60 gamma cell 3500 at dose level (5.7 Gy) at a dose rate 2.67 rad/sec after 24 h and 14 days on the liver and to determine both prophylactic and therapeutic role of intraperitoneally administrated. Exposure to γ- irradiation induced a significant increase in levels of ALP, ALT and AST, while levels of glucose, total proteins, albumin, triglycerides, cholesterol, LDL-C, HDL-C, total, direct and indirect bilirubin and serum fractions were significantly decreased except for total lipids level which was almost not affected. Administration of anserine and/or zinc prior or after radiation exposure was found to offer protection against γ-irradiation induced hepatocellular damage and oxidative stress in rats, probably by exerting a protective effect against hepatocellular necrosis via its free radical scavenging and membrane stabilizing ability.

Keywords: Anserine, Gamma radiation, albumin, protein, lipid profile, liver.

INTRODUCTION

Histidine-containing dipeptides include Carnosine (beta-alanyl-L-histidine) and carnosine related compounds (CRCs) homocarnosine (gamma-amino-butyryl-L-histidine) and anserine (beta-alanyl-1-methyl-L-histidine) (Kang *et al*., 2002). Carnosine and its derivatives were compared in terms of their protecting action on the oxidation of serum lipoproteins by hydroxyl radicals and their abilities to decrease the levels of ROS, anserine demonstrates the highest degree. This effect may attribute to the protective potential of anserine against free radical damage (Boldyrev *et al*., 1997). Many studies show that it is a good scavenger of the hydroxyl radical (-OH) (Aruoma *et al*., 1989). Anserine is thought to inhibit lipid oxidation by a combination of free radical scavenging and metal chelation (Chan &Decker 1994) Thus, modification of carnosine via methylation in to anserine may serve as a regulator of the intracellular reactive oxygen species ROS level (Boldyrev, 1999; Reinheckel et al., 2000 and Boldyrev *et al*., 2004). Zinc is an essential metal with numerous functions in biology (Hatakeyama *et al*., 2002), the ability of zinc to retard oxidative processes has been recognized for many years (Powell *et al.*,1999).

The risk from ionizing radiation always limits the use of radiation and nuclear medicine in both diagnostic and therapeutic domains (Jagetia & Baliga, 2004) Liver, which is the major haemopoietic organ, is highly sensitive to radiation damage (Devi & Hossain 2000) Plasma proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. In addition, proteins help to balance the osmotic pressure of the blood and tissue; Proteins play a major role in maintaining the delicate acid- alkaline balance of blood (Ali *et al.,* 2006). Serum proteins profiling pattern are a useful tool for diagnosis of liver diseases such as cirrhosis, thousands of individual serum proteins which vary in their physiological functions enable the discovery of disease biomarkers (Theodore *et al.,* 2005).

MATERIAL AND METHODS

Material

All chemicals used were of high analytical grade, products of Sigma (USA) and Randox (United Kingdom). L-anserine nitrate salt was imported from Sigma and Zinc chloride was purchased from El-Nasr chemical Industries Company.

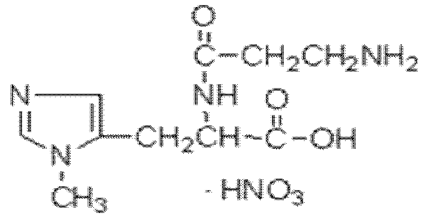

Fig. 1: The chemical structure of L-anserine nitrate.

Animals

Adult male Albino rats ranging in weight 180-120 gm, supplied from the animal house of National Research Center (Dokki, Giza, Egypt) were used for experimental investigation in this study. Animals were kept for 2 weeks to accommodate on laboratory conditions. They were maintained under controlled conditions of light and temperature (25°±2°C) and were provided with standard laboratory pellet diet and distilled water *ad libitum*. Appropriate anesthetic and sacrifice procedures were followed ensuring that animals did not suffer at any stage of the experiments. Anesthetic procedures complied with the legal ethical guidelines approved by the Ethical Committee of the Federal Legislation and National Institutes of Health Guidelines in USA were approved by the ethical committee of the National Research Centre in Egypt. An overdose of ether was given gradually to rats, blood was withdrawn and serum separated then the abdomen was opened by a mid-line incision and livers were removed.

Experimental Design

96 male albino rats were divided into five main groups. The first group "Normal Control Group": served as untreated normal control healthy group. The second group "Control treated group": subdivided into three subgroups to determine the effects of anserine nitrate (0.4 mg /Kg body weight) given intraperitoneally (I.P) as a single daily dose for 14 successive days (Abul-nasr *et al*., 2001) and/or Zinc (10 mg / Kg body weight) (I.P) as a single daily dose for 14 successive days (Roosen *et al*.,1994) on normal healthy rats. The third group 'Control Irradiated Group': subdivided into two subgroups to determine the effect of whole body gamma irradiation delivered from $Cobalt^{60}$ gamma cell 3500 at dose level (5.7 Gy) at a dose rate 2.67 rad/sec (Kozurkova & Misurova, 1999) at different time intervals i.e. 24 hrs (γ_1) and 14 days (γ_2) radiation exposure This source is located at the Middle Eastern Regional Radioisotopes Center for the Arab Countries "MERRCAC" (Dokki, Giza, Egypt).

The fourth group "Pre-Treated Irradiated group": subdivided into three subgroups to evaluate the protective effect of anserine and/or zinc against gamma radiation-induced hepatotoxicity, the pre treated irradiated were administered the last dose one hour before irradiated, all rats were sacrificed 24h after irradiation exposure . The fifth group "Post-Treated Irradiated Group": subdivided into three subgroups (administered the first dose one hour after gamma irradiation exposure) to evaluate the curative effect of anserine and/or zinc against gamma radiation-induced hepatotoxicity. Animals were fasted 24 hrs before scarifying, liver tissue and blood samples were collected at time intervals 24 hrs and 14 days post radiation exposure.

Preparation of samples

Animals were fasted 24 hrs before scarifying, liver tissue samples were collected at time intervals 24 hrs and 14 days post radiation exposure, liver tissue were weighed and homogenized with 5ml of saline, Liver and blood samples were centrifuged at 4000 rpm for 15 minutes, the supernatant was collected and stored into aliquots in eppendorff tubes, kept at (- 80°c).

Mean outcome measures

The parameters of (ALP), (ALT), (AST), glucose, total proteins, albumin, total bilirubin, direct bilirubin, indirect bilirubin, total lipids (HDL-C) and standard protein fractions as α_2-Macroglogulin (180 KDa), β-Galactosidase (116 KDa), Phosphorylase b (97.4 KDa), serum albumin (66 KDa) Fumarase, (48.5 KDa), Carbonic anhydrase (29 KDa), β-Lactoglobulin (18.4 KDa), α-Lactalbumin (14.2 KDa), Aprotinin (6.2 KDa) were measured in all groups.

Experimental procedures in liver tissues

Determination of Alkaline phosphatase (ALP) activity

ALP acts upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically (Kochmar and Moss, 1976).

Determination of Aspartate Aminotransferase (AST, Alanine Aminotransferase (ALT) Activities

AST and ALT activities were measured by monitoring the concentration of oxaloacetate or pyruvate hydrazone formed with dinitrophenyhydrazine (DNP) in alkaline medium (Reitman and Frankel 1957).

Determination of Albumin Level

This was performed according to Doumas *et al*., (1971) in a buffered solution of bromocresol green dye; a blue color appears and is proportional to the albumin concentration in the sample. This color is measured photometrically.

Determination of bilirubin levels

Bilirubin is determined by reaction with the diazotized sulphanilic acid, in the presence of caffeine, which releases albumin bound bilirubin with the final production of an azo pigment (Jendrassik & Grof, 1938).

Determination of glucose level

Glucose is determined after enzymatic oxidation in the presence of glucose (GOD) The hydrogen peroxide formed reacts, under catalysis of peroxide, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator (Barham & Trinder, 1972).

Determination of total protein

Total proteins were reacted with Bradford reagent to give a blue complex which is measured colorimetrically at wavelength 595 nm (Bradford, 1976).

Determination of Total lipids

Lipids are hydrolysed by sulphoric acid, then treated with phosphor-vanillin mixture to produce sulpho- phosphovanillin complex of rose coloration (Zollner &Kirsch, 1962)

Determination of total cholesterol (TC) levels

The cholesterol is determined after enzymatic hydrolysis and oxidation .The quinoneimin is formed from hydrogen peroxide and 4- aminoantipyrine in the presence of phenol and peroxidase (Roeschlau *et al.*,1974).

Determination of Triglycerides levels

Triglycerides were determined after enzymatic hydrolysis with lipase enzyme. The indicator was a quinoneimine formed from hydrogen peroxide, 4- aminophenazone and 4- chlorophenol under the catalytic influence of peroxidase (Fossati, 1982).

Determination of High and Low density Lipoprotein – cholesterol (HDL-c and LDL-c) levels

LDL and VLDL and chylomicron fractions are precipitated quantitatively by the addition of phosphtungestic acid in presence of magnesium ions. After centrifugation, the cholesterol concentration in HDL fraction, which remains in the supernatant, is determined (Gordon & Amer, 1977).

Determination of serum protein fractions

Using 4-30% gradiant polyacrylamide gel electrophoresis in the presence of SDS. The gel stained with Coomassie Brilliant Blue R250 followed with silver stain, which is more sensitive than C.B.B. R250 alone (De Moreno *et al.*, 1985) Individual standard protein fractions (180 KDa, 116 KDa, 97.4 KDa, 66 KDa, 48. 5 KDa, carbonic 29 KDa, 18.4 KDa, 14,200 KDa and 6.5 KDa, were used as standards (Laemmli.1970). The dry gel can be handled like a piece of paper (Jaung *et al.*,1984). The dry gel was scanned at 575 nm with Ultrascan Laser Densitometer. The electrophoretic results recorded as percentage to the concentration of total protein expressed as mg protein/ml. Helena France scanner at 600 nm measured electrophoretic separation bands.

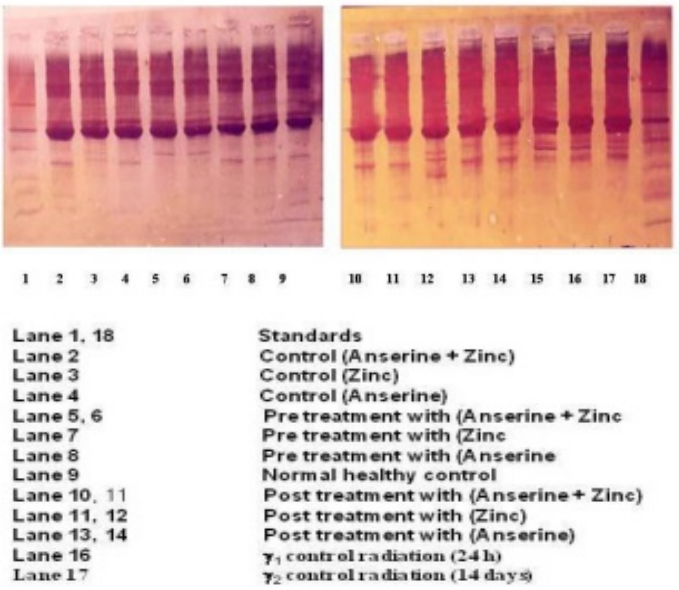

Fig 2: Electrophoretic profile of serum proteins of rats on polyacrylamide gel electrophoresis "PAGE" one dimensional Electrophoresis.

Statistical analysis

All data obtained was performed with the SPSS software package for Windows (Version 8.0), differences between groups were assessed by one-way analysis of variance (ANOVA). The values are expressed as mean±SD for eight animals in each group. Post hoc testing was performed for intergroup comparisons using the least significance difference (LSD) test. The results are expressed as the median for nine animals in each group, significance was set at $P < 0.05$ level.

RESULTS

The data in table (1) illustrate that ALP, ALT, AST, Total bilirubin, direct bilirubin and indirect bilirubin showed a significant increase in rats groups at different sampling time's i.e. prior radiation, 24 hrs and 14 days post radiation exposure. On the other hand, administration of anserine, zinc or their combination induced a significant decrease in these levels before and after radiation exposure, except for Zinc pre treated group which showed mild significant increase. While, Glucose, Total proteins, Albumin, Cholesterol, Total lipids, Triglycerides, HDL and LDL showed a significant decrease at different sampling times i.e. prior radiation 24 hrs and 14 days post radiation exposure. On the other hand, administration of anserine, zinc or their combination induced a significant increase in all mentioned parameters before and after radiation exposure except for Zinc pre treated group

which showed mild significant increase in case of total protein and cholesterol, as show in table (2). The fractions of serum 180 KDa, 116 KDa, 97.4 KDa, 66 KDa (albumin) 48.5 KDa, 29 KDa, 18.4 KDa, 14.2 KDa and 6.2K Da show a significant decrease at different sampling times i.e. 24 hrs and 14 days post radiation exposure. On the other hand, administration of anserine, zinc or their combination induced a significant increase all fractions of serum protein before and after radiation exposure, as show table (3). The percent change of the improvement in all these parameters and in serum protein fractions are shown in table (4) a&b as compared to control groups which considered as 100 % illustrating the levels of enhancements in all testing parameters.

Table. 1: Levels of ALP, AST, ALT, albumin, total bilirubin, direct bilirubin, indirect bilirubin in liver rats in different groups.

Parameters	Normal control (1)	Treated Control			Irradiated Control		Treated Pre-Irradiated γ_1			Treated Post-Irradiated γ_2		
		Anserine (2)	Zinc (3)	Anserine +Zn (4)	γ_1 (5)	γ_2 (6)	Anserine (7)	Zinc (8)	Anserine +Zn *(9)*	Anserine *(10)*	Zinc *(11)*	Anserine +Zn *(12)*
ALP	11.44 ± 1.89 (2,3,4,5,6)	12.33 ±1.93 (1)*	14.64 ±1.54 (1)*	14.69 ±1.59 (1)*	46.13 ±3.35 (1)***	50.82 ±3.84 (1)***	9.62 ±1.66 (5)***	15.88 ±1.17 (5)***	15.08 ±1.74 (5)***	24.89 ±1.92 (6)***	15.09 ±1.74 (6)***	33.77 ±1.96 (6)***
AST	12.51 ±1.87 (2,3,4,5,6)	12.29 ±1.13 (1) ns	8.48 ±1.44 (1)*	8.21 ±1.85 (1)*	20.02 ±1.21 (1)**	31.40 ±1.09 (1)***	13.78 ±1.75 (5)**	13.29 ±1.76 (5)**	11.63 ±1.44 (5)***	30.09 ±1.46 (6) ns	15.78 ±1.34 (6)***	15.41 ±1.67 (6)***
ALT	7.64 ±1.79 (2,3,4,5,6)	8.79 ±1.22 (1) ns	7.57 ±1.48 (1) ns	8.98 ±1.07 (1) ns	10.19 ±1.39 (1)**	14.70 ±2.35 (1)***	7.91 ±0.44 (5)**	8.79 ±1.28 (5)*	6.82 ±1.29 (5)***	12.89 ±1.31 (6)*	12.82 ±1.92 (6)*	12.22 ±1.124 (6)*
Albumin	7.71 ±0.17 (2,3,4,5,6)	7.71 ±0.17 (1) ns	7.66 ±0.23 (1) ns	7.72 ±0.15 (1) ns	5.60 ±0.19 (1)***	4.74 ±0.24 (1)***	6.93 ±0.15 (5)***	6.31 ±0.15 (5)***	7.34 ±0.13 (5)***	6.49 ±0.13 (6)***	5.92 ±0.12 (6)***	6.37 ±0.12 (6)***
Total bilirubin	0.048 ±0.01 (2,3,4,5,6)	0.041 ±0.011 (1)n.s	0.045 ±0007 (1)n.s	0.04 ±0.011 (1)n.s	0.077 ±0.006 (1)***	0.088 ±0.005 (1)***	0.046 ±0.003 (5)***	0.027 ±0.004 (5)**	0.049 ±0.008 (5)***	0.038 ±0.007 (6)***	0.05 ±0.006 (6)***	0.044 ±0.001 (6)***
Direct bilirubin	0.012 ±0.003 (2,3,4,5,6)	0.01 ±0.002 (1)n.s	0.011 ±0.002 (1)n.s	0.01 ±0.003 (1)ns	0.020 ±0.001 (1)***	0.025± 0.001 (1)***	0.02 ±0.005 (5)***	0.014 ±0.001 (5)**	0.012 ±0.002 (5)**	0.01 ±0.002 (6)**	0.012 ±0.002 (6)*	0.011 ±0.002 (6)***
Indirect bilirubin	0036 ±0.005 (2,3,4,5,6)	0.03 ±0.002 (1)n.s	0.033 ±0.002 (1)n.s	0.03 ±0.003 (1)ns	0.057 ±0.001 (1)***	0.063 ±0.001 (1)***	0.034 ±0.002 (5)***	0013 ±0.001 (5)*	0.037 ±0.003 (5)***	0.028 ±0.002 (6)***	0.038 ±0.004 (6)***	0.033 ±0003 (6)***

Data are expressed as X ± SD of eight rats in each group. ALP, AST, ALT are expressed as U/L/gm tissue – Albumin is expressed as gm/dL/gm tissue –Total, direct, indirect bilirubin are expressed as mg/dL/gm tissue Significance difference between groups in analyzed by one way ANOVA, where: (***P< 0.001 : highly significant, **P< 0.01: significant, *P < 0.05: mild significant, ns P > 0.05:not significant.

Table. 2: Levels of glucose, total protein, total lipid, cholesterol, triglyceride, HDL-C, LDL-C, in liver rats in different groups.

Parameters	Normal control	Treated Control			Irradiated Control		Treated Pre-Irradiated γ_1			Treated Post-Irradiated γ_2		
		Anserine	Zinc	Anserine +Zn	γ_1	γ_2	Anserine	Zinc	Anserine +Zn	Anserine	Zinc	Anserine +Zn
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
Glucose	161.1 ±14.7 (2,3,4,5,6)	156.7 ±15.3 (1) ns	167.52 ±16.0 (1) ns	163.3 ±18.3 (1) ns	89.23 ±6.81 (1)***	64.24 ±5.29 (1)***	97.96 ±11.69 (5)**	130.0 ±11.5 (5)***	108.84 ±15.92 (5)***	119.8 ±13.3 (6)***	97.84 ±13.16 (6)***	169.8 ±15.5 (6)***
Total protein	5.55 ±0.54 (2,3,4,5,6)	5.24 ±0.56 (1) ns	5.20 ±0.55 (1) ns	5.71 ±0.17 (1) ns	2.48 ±0.52 (1)***	1.29 ±0..48 (1)***	4.69 ±0.48 (5)***	4.00 ±0.29 (5)*	5.48 ±0.20 (5)***	3.58 ±0.26 (6)**	2.96 ±0.27 (6)**	3.55 ±0.24 (6)**
Total lipid	1.40 ±0.09 (2,3,4,5,6)	1.36 ±0.13 (1) ns	1.33 ±0.13 (1) ns	1.34 ±0.15 (1)ns	1.22 ±0.17 (1)*	1.10 ±0.10 (1)*	1.32 ±0.083 (5)*	1.29 ±0.15 (5)ns	1.36 ±0.04 (5)***	1.29 ±0.09 (6)**	1.27 ±0.07 (6)**	1.34 ±0.05 (6)**
Cholesterol	24.34 ±1.67 (2,3,4,5,6)	24.03 ±175 (1) ^{ns}	23.41 ±2.49 (1) ^{ns}	25.25 ±1.42 (1) ns	20.06 ±0.95 (1)***	19.40 ±1.17 (1)***	23.45 ±226 (5)**	22.05 ±1.58 (5)**	25.00 ±1.47 (5)***	22.17 ±1.46 (6)***	20.79 ±0.64 (6)**	23.79 ±1.68 (6)***
Triglyceride	50.6 ±3.2 (2,3,4,5,6)	51.0 ±3.2 (1)ns	50.4 ±5.1 (1)ns	49.8 ±4.45 (1)*	35.0 ±2.27 (1)***	33.6 ±1.87 (1)***	45.0 ±5.8 (5)***	44.2 ±2.26 (5)***	49.0 ±4.4 (5)***	40.4 ±2.1 (6)***	41.2 ±3.1 (6)***	44.6 ±2.8 (6)***
HDL-C	7.01 ±0.87 (2,3,4,5,6)	6.94 ±0.11 (1)ns	6.9 ±0.73 (1)*	6.82 ±0.59 (1)ns	5.47 ±0.109 (1)***	5.06 ±0.93 (1)***	6.52 ±0.13 (5)**	6.16 ±0.94 (5)**	6.84 ±0.63 (5)**	6.07 ±0.82 (6)*	5.99 ±0.73 (6)ns	6.18 ±0.51 (6)*
LDL-C	7.24 ±0.19 (2,3,4,5,6)	6.87 ±0.24 (1)*	6.4 ±0.32 (1)*	8.2 ±0.23 (1)$^{n.s}$	7.55 ±0.14 (1)$^{n.s}$	7.8 ±0.10 (1)*	7.89 ±031 (5)$^{n.s}$	6.72 ±0.15 (5)*	8.42 ±0.19 (5)*	8.02 ±0.15 (6)*	6.56 ±0.06 (6)*	8.69 ±0.20 (6)*

Data are expressed as X ± SD of eight rats in each group. Total protein & total lipid are expressed as gm /dl/gm tissue --glucose, cholesterol, triglyceride, HDL-C and LDL-C are expressed as mg/dl/gm tissue Significance difference between groups in analyzed by one way ANOVA, where: (***P< 0.001: highly significant, **P< 0.01: significant, *P < 0.05: mild significant, ns P > 0.05: not significant.

Table. 3: Levels of serum protein fractions in different groups.

Parameters	Normal control	Treated Control			Irradiated Control		Treated Pre-Irradiated γ_1			Treated Post-Irradiated γ_2		
		Anserine	Zinc	Anserine +Zn	γ_1	γ_2	Anserine	Zinc	Anserine +Zn	Anserine	Zinc	Anserine +Zn
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	*(9)*	*(10)*	*(11)*	*(12)*
180 KDa	8.15 ±0.81 -	6.69 ±0.60 (3,7,9)	6.15 ±0.44 (2,4,9,12)	5.65 ±0.54 (3,8,11,12)	3.83 ±0.66 (6)	3.87 ±0.59 (5)	7.01 ±0.44 (2,9)	4.74 ±0.51 (11)	6.53 ±0.46 (2,3,7)	8.82 ±0.52 -	5.17 ±0.44 (4,8)	5.85 ±0.53 (3,4)
116 KDa	5.82 ±0.53 (2)	5.94 ±0.62 (1)	8.14 ±0.50 -	11.95 ±0.66 (12)	3.90 ±0.73 (6,8)	3.94 ±0.70 (5,8,10)	2.99 ±0.51	4.30 ±0.47 (5,6,9,10,11)	4.87 ±0.65 (7,10,11)	4.62 ±0.38 (6,8,9,11)	4.88 ±0.73 (8,9,10)	11.96 ±053 (4)
97.4 KDa	7.93 ±0.44 (7,11)	6.30 ±0.83 (3,8,9,10)	6.24 ±0.65 (2,8,9,10)	12.42 ±0.53 -	4.97 ±0.79 (6)	4.78 ±0.63 (5)	8.21 ±068 (1,11)	6.66 ±0.47 (2,3,9,10)	6.058 ±0.63 (2,3,8,10)	6.74 ±0.49 (2,3,8,9)	7.72 ±0.59 (1,7)	13.79 ±0.48 -
66 KDa	48.05 ±0.69 -	52.71 ±1.81 (3)	51.72 ±1.19 (2)	61.47 ±0.89 -	19.74 ±1.32 (6)	18.15 ±0.53 (5)	26.62 ±0.65 -	23.62 ±0.79 -	31.91 ±1.47 -	41.12 ±1.43 -	28.27 ±0.91 -	39.24 ±0.86 -
48.5 KDa	8.10 ±0.67 (2)	7.58 ±0.55 (1)	9.85 ±0.62 -	12.56 ±0.64 -	4.13 ±0.82 (7,10)	5.27 ±0.93 (10)	3.76 ±0.48 (5)	6.80 ±0.53 (9,11)	6.46 ±0.49 (8,11,12)	4.67 ±0.47 (5,6)	6.13 ±0.72 (8,9,12)	6.04 ±0.61 (9,11)
29 KDa	8.33 ±0.89 -	6.80 ±0.63 (3,7,9,11)	6.74 ±0.69 (2,7,9,10,11)	12.07 ±0.84 -	5.29 ±0.92 (10,12)	4.19 ±0.48 (8,12)	6.82 ±0.65 (2,3,9,11)	3.70 ±0.58 (6)	7.01 ±0.48 (2,3,7,11)	5.89 ±0.71 (5)	6.88 ±0.67 (2,3,7,9)	4.84 ±0.59 (5,6)
18.4 KDa	7.65 ±0.69 (8,11)	9.04 ±0.35 (9,11)	12.05 ±0.64 (4,12)	11.32 ±0.87 (3,10,12)	3.96 ±0.69 (7)	4.97 ±0.57 (7)	4.29 ±0.66 (5,6)	7.07 ±0.44 (1)	8.83 ±0.70 (2,11)	10.90 ±0.63 (4)	8.29 ±0.90 (1,2,9)	11.67 ±0.52 (3,4)
14.2 KDa	8.66 ±0.0.81 (3,7,12)	11.59 ±0.68 -	8.60 ±0.74 (1,7)	6.16 ±0.72 (6,8,11)	7.83 ±0.58 (9,10)	6.84 ±0.60 (4,8,9,11)	8.80 ±0.72 (1,3,12)	6.85 ±0.56 (4,6,9,11)	7.26 ±0.35 (5,6,8,10,11	7.58 ±0.37 (5,9)	6.67 ±0.51 (4,6,8,9)	9.33 ±0.43 (1,7)
6.5 KDa	8.63 ±0.66 (9,11)	13.64 ±0.74 (3)	13.12 ±0.75 (2,12)	9.77 ±0.78 -	5.98 ±0.74 (6,10)	5.39 ±0.83 (5)	7.63 ±0.51 (8,9,11)	7.10 ±0.34 (7)	8.33 ±0.37 (1,7,11)	6.15 ±0.45 (5)	8.26 ±0.90 (1,7,9)	12.81 ±0.54 (3)

Data are expressed as X ± SD of eight rats in each group. Mwt of fractions of serum protein are expressed as mg/ml/serum. Significance difference between groups in analyzed by one way ANOVA, where: Number between brackets indicate non significant correlation between groups.

Table. 4a: % Improvement in the levels of different parameters in the treated groups according to control group which considered as 100 %.

Parameters	Treated 14 day pre-irradiated γ1			Treated 14 day post-irradiated γ2		
	Anserine	Zinc	Anserine+Zinc	Anserine	Zinc	Anserine+Zinc
ALP	- 219	- 164.4	- 171.4	- 126.7	-212.3	- 49
AST	50.1	46.2	32.9	89.5	- 24.9	- 27.8
ALT	70.2	81.7	55.9	76.1	75.4	67.54
Albumin	117.25	109.20	122.56	122.69	115.3	121.14
T.bilirubin	35.4	-4	41.7	- 4	20.8	8.3
Dir-bilirubin	0.0	50	33.3	- 25	**- 8**	**- 16.7**
Ind-bilirubin	36.1	-22	44.4	2.8	30.6	16.67
Glucose	105.4	125.3	112.17	134.5	120.6	165.52
T.protein	139.8	127.4	154.1	141.3	130.1	140.7
T.lipid	107.14	105	110	113.6	112.14	117.14
Cholesterol	113.9	108.2	120.3	111.38	105.71	118.03
triglyceride	119.8	118.2	127.7	113.4	115.01	121.7
HDL	114.9	109.8	119.5	114.4	113.3	115.97
LDL	104.7	88.5	112.01	103.03	82.88	112.3

Table. 4b: % Improvement in serum protein fractions in the treated groups according to control group which considered as 100 %.

protein Fractions	Treated 14 day pre-irradiated γ1			Treated 14 day post-irradiated γ2		
	Anserine	Zinc	Anserine+Zinc	Anserine	Zinc	Anserine+Zinc
180 KDa	124.3	115.9	160.7	133	111.2	130
116 KDa	84.4	106.9	116.7	111.7	116.2	237.8
97.4 KDa	140.9	121.3	113.7	124.7	137.1	329
66 KDa	114.3	108.1	125.3	147.8	121.1	143.9
48.5 KDa	95.4	132.9	128.8	92.6	110.6	109.5
29 KDa	118.4	80.9	120.6	120.5	132.3	107.8
18.4 KDa	104.3	140.7	163.7	177.5	143.4	187.6
14.2 KDa	114.6	88.7	93.4	108.5	98.1	128.75
6.2 KDa	119.11	112.9	127.2	108.8	133.3	185.9

DISCUSSION

Gamma-irradiation induced an increase in ALP activity in liver tissue on the first and seventh post-exposure day. Khamis & Roushdy (1991) confirmed an increase in ALP activity in blood serum 1 day after exposure to 5.7 Gy, This may be attributed to the possible release of this enzyme from different tissues associated with the obstruction of the blood stream to the liver (Khamis &Roushdy, 1991) Administration of anserine alone or in combination with zinc 14 days prior and after radiation exposure induced a significant decline in ALP levels in liver tissue. The potency of supplemented zinc to keep more or less normal liver functions as levels of serum and hepatic activity of ALP and AST; ALP; ACP; LDH and total protein (El-Zayat *et al.,* 1996). The irradiated rats showed a significant increase in the activities of both liver ALT and AST through the time intervals. The increase in aminotransferase activities by radiation may be due to the damage of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the aminotransferase activities in liver and blood serum (Ramadan *et al.*,2002).

Administration of anserine, zinc or their combination significantly reversed the radiation-induced increase in ALT and AST levels in both pre-treated and post-treated groups. Zinc has a protective effect against different types of hepatotoxicities; this protection is attributable to the 10- to 50-fold induction of hepatic metallothionein, it acts as a free radical scavenger protecting against oxidative damage (Sato & Bremner, 1993).

γ- Irradiation induced significant decrease in glucose level in both pre and post-irradiated groups; it causes destruction of alpha cells of pancreas decreasing the level of glucagon hormone which responsible for increasing the level of glucose so, the increase in blood glucose and decrease in blood insulin were effectively suppressed by irradiation (Takahashi et al.,2000).Administration of anserine, zinc or their combination caused significant increase in glucose levels within normal range. Concentration of blood glucose in irradiated rats did not practically differ from that of control animals during the whole period of investigation (Ahlersova *et al.,* 1980). Zinc plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells. The abilities to synthesize and secrete insulin and utilization of glucose are impaired in the zinc deficient state (Song *et al.,* 1998).

γ - Irradiation produced a significant reduction in the contents of hepatic total proteins in rats submitted to single dose of gamma radiation. This decline may be attributed to excessive protein loss through injury of kidney or gastro-intestinal tract or from thermal injury to skin (Keren, 1994).Administration of anserine, zinc or their combination significantly improved the radiation-induced decline in total protein level in liver tissue in both irradiated groups. This could be attributed to the physiological role of anserine and zinc as antioxidants in minimizing radiation-induced injuries, particularly to cell membrane (Inanami *et al.,* 1999) In addition, zinc had an apparent effect on liver protein content through its ability to regulate hepatic protein synthesis in rats via regulation the relative abundance of their m-RNAs (Kimball *et al.,* 1995).

γ - Irradiation induced a significant reduction in albumin content. This decline may be related to significant pathology either in production of albumin by the liver or its leakage through damaged surface (Mackiewiez *et al*.,1992).Intraperitoneal injection of anserine, zinc or their combination significantly reversed the decline in albumin content before and after radiation exposure. These results can be explained by the ability of anserine to inhibit modification and cross-linking of ova albumin and bovine serum albumin induced by malondialdehyde and hypochlorite (Hipkiss *et al.,*1998). Zinc is an essential trace element for hepatic synthesis of albumin and immunoglobulins in rats (Yousef *et al.,* 2002).

Lipid profile includes total lipids as cholesterol, trigycerides and lipoproteins as HDL-C, LDL-C (Dahlen *el al.,*1986) Most lipids circulate through the bloodstream as lipoproteins. Lipoproteins are lipid–protein complexes that contain large insoluble glycerides and cholesterol with a superficial coating of phospholipids and proteins synthesized in the liver (Havel and Kane ,1995).All lipoproteins carry all types of lipid, but in different proportions, so that the density is directly proportional to the protein content and inversely proportional to the lipid content (Bass *et al.,*1993). In the present study, γ - irradiation induced significant decrease in cholesterol and triglycerides level at different time intervals, while total lipids were almost not affected. Ardesta *et al.,* (2002) stated that whole gamma body irradiation decreased the level of cholesterol at 24 hours after irradiation, but opposing their findings which state that whole gamma body irradiation significantly increased liver fatty acids as well as triglycerides. This effect may be related to the oxidative stresses induced by gamma radiation which cause increase in lipid peroxidation and subsequent tissue damage through free radical production (Morcillo *et al.,* 2000). Single whole-body gamma-irradiation of rats results in plasma and liver microsomal membrane lipid peroxidation, and impairments of microsomal membrane structure and function (Zavodnik, 2003).

Administration of anserine, zinc or their combination induced a significant increase in total lipids, cholesterol and triglycerides levels. This effect is in agreement with findings of Naumova *et al.,* (1992) which confirmed the ability of carnosine to provide effective protection against post-radioactive increase of LPO in irradiated organisms. In the present study, gamma radiation induced a significant decrease in HDL level, while it caused a slight significant decrease in LDL level. This effect is in agreement with the findings of Ardesta *et al.,* (2002) which proved that rats exposed to gamma radiation were highly affected especially their livers, it was noticed a large accumulation of lipid peroxidation products.

Administration of anserine, zinc or their combination induced significant increase in HDL-C and LDL-C level at different time intervals. CRCs and polyunsaturated fatty acids enriched diet contributed to a decrease of LPO products content in the blood serum and ApoB lipoproteins as well as to the inhibition

of lipoprotein oxidation during their synthesis in liver cells; the diet may be recommended for the prophylaxis and treatment of atherosclerosis (Gariballa , 2000). Administration of anserine, zinc or their combination induced significant decrease in all propotions of bilirubin. This effect may be attributed to the hepatoprotective effect of CRCs and zinc. Carnosine enhanced the liver function and accelerated the metabolism of stress-related substances (Guiotto *et al*., 2005). Measurements of total protein reflect liver disease, nutritional state, kidney disease and others. A decreased value of total protein may indicate liver or kidney disease (Sharpe et al., 1996). Albumin is the most abundant serum protein; it constitutes 30-35% of the total proteins in animals and 60-67% of total serum proteins in human respectively (Kaneko,1997).It is synthesized by the liver using dietary protein; its concentration reflects the functional capacity of the liver (Bernheim, 2005). Serum albumin has different physiological functions including nutrition, contribution of about 80% of plasma osmotic pressure and serum carrier protein of calcium, bilirubin, free fatty acids, drugs and steroids (Sherwin and Sobenes,1996). It is a very strong predictor of health; low albumin is a sign of poor health and a predictor of a bad outcome (Avidan, 2005) Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. A reversed A/G Ratio may be a helpful indicator for early diagnosis of liver damage (Delcourt *et al.*, 2005). Gamma irradiation induced a significant decrease in different fractions of serum proteins at different time intervals. This in agreement with findings of Abou-Seif *et al.,* (2003) which confirmed that the average values of albumin and the albumin/globulin ratio were decreased in gamma-irradiated rats. This effect may be due to the reactive species generated by gamma-irradiation that cause oxidation and glycation of albumin (Traverso *et al.*, 1996). Zinc and anserine may play an important role in the maintenance of the antioxidant system, in agreement with Haiying *et al.*, (2009)

CONCLUSION

From all the results obtained in the present study, we can conclude that anserine, a naturally histidine dipeptide, has a remarkable radio and hepatoprotective effect against gamma radiation-induced hepatotoxicity and may be other types of hepatotoxicities. Also zinc has proved to have a potentiating effect along with anserine and this confirm its hepatoprotective and antioxidant effect. Finally, it is better to recommend a collective combination of anserine and zinc in treatment of as well as protection against radiation induced or other types of hepatotoxicities. Hence we encourage further investigation on anserine and anserine related compounds with other trace elements having antioxidant or hepatoprotective effect to find a better combination that gives the best prophylactic or curative effect against gamma radiation-induced hepatotoxicity.

DECLARATION OF INTEREST

The authors have no declarations of interest to report this original paper.

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Abbreviations:

Sodium dodocyl sulphate	SDS
Alkaline phosphatase	ALP
total protein	T.P
total cholesterol	TC
High density Lipoprotein –cholesterol	HDL-c
Lowdensity Lipoprotein –cholesterol	LDL-c
Coomassie Brilliant Blue R250	C.B.B. R250