Journal of

Pharmacology and Toxicology

ISSN 1816-496X

Academic
Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 2 (1): 1-19, 2007
ISSN 1816-496X
© Academic Journals Inc., USA

Role of Anserine and/or Zinc in Modulating Nucleic Acid and Protein Disorders in Rats Exposed to Gamma Irradiation

[1]Hanan F. Ali, [2]Laila M. Faddah, [1]Maha Z. Rizk and [2]Hisham El-Ebiary
[1]Department of Medicinal Chemistry, National Research Centre, Dokki,Cairo, Egypt
[2]Department of Biochemistry, Faculty of Pharmacy, Minia University, Minia, Egypt

Abstract: In view of radiation injuries to tissues, the present study was carried out to provide information on the deteriorative effects induced by γ-irradiation (5.7Gy) after 24 h and 14 days and to determine both the prophylactic and curative influences of intraperitoneally administered anserine and/or zinc on liver nucleic acids and on total serum total protein, albumin and also different serum protein fractions, namely, prealbumin, α- and β-lipoproteins, α-1 antitrypsin, α-1 acidic glycoprotein, α-1 macroglobulin, cholinesterase, transferrin, ceruloplasmin, haptoglobin and haemopexin. The data obtained revealed that all liver and serum parameters measured significantly declined in rats exposed to whole body irradiation in the two time phases except for the lipoprotein fractions. Administration of anserine and/or zinc either pre or post radiation dose induced a significant improvement in all parameters. It could be concluded that either anserine or zinc has a pronounced effect in overcoming the disturbances induced by γ-irradiation especially when administered post irradiation and the action of each can be potentiated by supplementation of the other. It is thus recommended to use a collective combination of both anserine and zinc as radioprotectors as well as for treatment of radiation hazards.

Key words: Anserine, zinc, γ-irradiation, nucleic acids, protein

Introduction

Ionizing radiation generates reactive oxygen species resulting in oxidative damage to cell membranes, disturbance in metabolism as well as in structure and function of body organs (Rafaie *et al*.,1993; Kanshala, 2004). Excessive free radicals formed by γ-irradiation are important factors in DNA damage. This damage mainly occurs from reactions involving hydroxyl radicals that cause oxidation of deoxyribose sugar groups and base release with resultant breakage of phosphodiester bonds (Koch and Giadomenica, 1994). γ-rays cause lesions on template DNA strand which result in impaired gene transcription, therefore, the synthesis of functional m-RNA is impaired and this may alter the pattern of protein synthesis either by stimulation or by inhibition (Mahdy *et al*.,1997; El-Wakf *et al*.,1999). In addition, γ-irradiation stimulates acute phase response essential for generation and limitation of inflammation. This response is usually orchestrated through cytokines and hepatocyte stimulatory factors associated with characteristic metabolic changes in protein synthesis (Fujioke *et al*., 2001).

Carnosine (β-alanyl-L-histidine) and its methylated derivative (β-alanyl-1-methyl-L-histidine are bioactive dipeptides having the ability to modulate the immune response in human neutrophils through increasing interleukin-1 production and suppressing apoptosis (Tan and Candlish, 1998). These compounds are also considered cellular antioxidants characterized by their high potency to scavenge free radicals (Boldyrev and Abe, 1999). Zinc also has potent immune and antioxidant activities

Corresponding Author: Maha Z. Rizk, Department of Medicinal Chemistry, National Research Centre, Dokki,Cairo, Egypt Tel: (02) 3371433 Fax: (02) 3371931

(Youssef *et al*., 2002). However, earlier study on metal complexes with L-carnosine and related compounds has not been actively carried out. The natural dipeptides can form stable complexes with metal ions of biological significance as Zn and Cu. The synergistic effects and advantageous properties are expected from this complexation (Motsukara and Tanak, 2000).

In the present study, the influence of anserine and/or zinc administration on liver DNA and RNA, on total serum protein and on different serum protein fractions was investigated in rats exposed to γ-irradiation in order to provide information on the mechanisms responsible for development of tissue injury induced by γ-irradiation exposure. The influence of anserine and/or zinc administration on these parameters was studied as a means for application of these compounds in clinical medicine, based on the chemical and pharmacological features of both zinc metal and the natural dipeptide anserine.

Materials and Methods

All chemicals used were of high analytical grade, products of Sigma (USA), BDH (England) and Fluka (Switzerland). L-anserine nitrate salt was imported from Sigma and Zinc chloride was purchased from El-Nasr Chemical Industries Company.

Animals

Animals used were healthy male albino rats, ranging in weight from 100-120 g, obtained from the animal house of National Research Center (Dokki, Giza, Egypt). The animals were kept under standard conditions for 2 weeks given food and water ad libitum during the experiment. Appropriate anaesthetic and sacrifice procedures were followed ensuring that animals did not suffer at any stage of the experiments. Anaesthetic procedures are complied according to legal ethical guidelines approved by the Ethical Committee of the Federeal Legislation and National Institute of Health Guidelines in USA. An overdose of ether was given gradually and then the abdomen was opened by a mid-line incision and livers were separated.

Source of Gamma Irradiation

The source of irradiation was Cobalt-60-gamma cell 3500. This source is located at Middle Eastern Regional Radioisotopes Center for the Arab Countries MERRCAC (Dokki, Giza, Egypt). The dose of gamma radiation was 5.7 Gy (Kozurkova *et al*., 1999) and the dose rate was 2.67 rad sec^{-1}.

Experimental Design

Nintey six male albino rats were divided into five main groups as follows:

- The first group Normal Control Group: Comprised 8 animals and served as untreated normal control healthy group.
- The second group Control Treated Group: Comprised 24 animals and subdivided into three subgroups, eight rats in each subgroup.

Anserine control subgroup: I.P. injected with anserine nitrate at dose level 0.4 mg kg^{-1} (Abul-Nasr *et al*., 2001) for 14 days; Zinc control subgroup: I.P injected with zinc chloride at dose level 10 mg kg^{-1} (Roosen *et al*., 1994) for 14 days; Anserine and Zinc control subgroup: I.P. injected with combination of anserine nitrate (0.4 mg kg^{-1}) and zinc chloride (10 mg kg^{-1}) for 14 days. All rats in subgroups were scarified after 14 days.

- The 3rd group control irradiated group: Comprised of 16 animals and subdivided into two subgroups, eight rats in each subgroup.

γ_1 Control: exposed to whole body gamma at dose level 5.7Gy (Kozurkova *et al*., 1999) and scarified 24 h after radiation exposure; γ_2 control: exposed to whole body gamma radiation at dose level (5.7 Gy) and scarified 14 days after radiation exposure.

- The 4th group pre-treated irradiated group: Comprised 24 animals and subdivided into three subgroups, eight rats in each subgroup.

Anserine pre-treated irradiated subgroup: I.P. injected with anserine nitrate (0.4 mg kg^{-1}) for 14 days, then exposed to whole body gamma radiation (5.7 Gy); Zinc pre-treated irradiated subgroup: I.P injected with zinc chloride (10 mg kg^{-1}) for 14 days, then exposed to whole body gamma radiation (5.7Gy); Anserine and Zinc pre-treated irradiated subgroup: I.P injected with collective combination of anserine nitrate (0.4 mg kg^{-1}) and zinc chloride (10 mg kg^{-1}) for 14 days, then exposed to whole body gamma radiation (5.7 Gy). All rats in subgroups were scarified 24 h after radiation exposure.

- The 5th group post-treated irradiated group: Comprised 24 animals and subdivided into three subgroups, eight rats, in each subgroup.

Anserine post-treated irradiated subgroup: Exposed to whole body gamma radiation (5.7 Gy), then I.P. treated with anserine nitrate (0.4 mg kg^{-1}) for 14 days; Zinc post-treated irradiated subgroup: exposed to whole body gamma radiation (5.7 Gy), then I.P. treated with zinc chloride (10 mg kg^{-1}) for 14 days; Anserine and zinc post-treated irradiated subgroup: exposed to whole body gamma radiation (5.7 Gy), then I.P. treated with collective combination of anserine nitrate (0.4 mg kg^{-1}) and zinc chloride (10 mg kg^{-1}) for 14 days. All rats in subgroups were scarified 24 h after the last dose. The pre-treated irradiated subgroups were administrated the last dose 1 h before gamma radiation exposure, while the post-treated groups were administrated the first dose 1 h after gamma radiation exposure.

Animals were scarified, blood samples withdrawn, sera separated and liver tissue samples homogenized. Samples were subjected to the following biochemical analyses.

Total liver DNA and RNA contents, total serum protein, serum albumin, serum protein fractions separation by two dimensional immunoelectrophoresis.

Quantitative Determination of Liver DNA and RNA Contents

Liver DNA and RNA contents were estimated according to the method of Shigko *et al*. (1967) using standard RNA and DNA solutions.

Determination of Serum Total Protein

Total serum protein content was estimated according to the method of Bradford (1976). The amount of protein was calculated from a standard curve using serial concentrations of bovine serum albumin (1-10 μg).

Determination of Serum Albumin Level

Serum albumin was determined according to the method of Doumas *et al*. (1971) modified by Spencer and Price (1977). The concentration of albumin was measured at 620 nm and was calculated from a standard curve for serial concentrations of bovine serum albumin (100-1000 μg/25 μL).

Protein Separation by Gel Electrophoresis

Rabbits weighing 1.5-2.0 kg were immunized against rat serum proteins to obtain antiserum according to the method mentioned by Harlow and Lane (1988). Proteins were separated by two dimensional immunoelectrophoresis according to Laurell (1965) and later modified by Fouad *et al*. (1983).

The areas under the individual protein peaks in the electropherogram were measured using the planimeter and referred to total protein. The area enclosed by individual precipitate is proportional to antigen/antibody ratio and is determined from the concentration of the antigen sample (Scherer and Ruhenstroth, 1976). From the position of albumin and transferrin peaks in the electrophoretic plate, the other proteins can be identified according to their electrophoretic mobility (Weeke, 1970).

Statistical Analysis

Data were expressed as mean±SD. One way analysis of variance (ANOVA) followed by LSD was used to show the statistical significance among the different groups. The Statistical Package for the social Sciences (SPSS) version 8.0 was used in data analysis. The probability level of significance (P) was determined where $p<0.005$ was considered significant.

Results

Determination of Liver DNA and RNA Contents

DNA and RNA contents in the liver of normal, control treated, control irradiated and pre and post-treated irradiated groups are shown in Table 1. Careful inspection of the data indicate that, liver DNA and RNA contents are significantly elevated at $p<0.001$ in control treated groups with respect to control healthy group. Exposure to whole body gamma irradiation induced a highly significant decline in DNA and RNA liver contents at $p<0.001$at time intervals 24 h and 14 days post-irradiation compared to control healthy group. Administration of anserine or zinc and their combination before irradiation exposure caused a highly significant rise in liver DNA and RNA contents at $p<0.001$ with percentages of improvement 32.77, 15 and 41.79% for DNA and 29.15, 16.44 and 34.59% for RNA respectively. In addition, injection of anserine or zinc or their collective combination after radiation exposure significantly increased liver DNA and RNA contents at $p<0.01$ compared to control irradiated group (γ_2) with percentages of improvement 106.00, 67.51 and 130% for DNA and 85.17, 49.07 and 90.26% for RNA, respectively.

Table 1: DNA and RNA liver contents in normal control, control-treated, control-irradiated and pre and post-treated irradiated

	Parameters	
	DNA (μg DNA/g wet liver tissue)	RNA (μg RNA/g wet liver tissue)
Groups		
Normal control (1)	51.90±2.95 (7,9,11)	63.19±2.21 (1,7,8,9)
Control-treated		
Anserine (2)	79.27±1.84 (2,3,4,10)	86.76±3.83 (2,11)
Zinc (3)	69.63±7.65 (2,3,10)	75.99±0.86 (3,9,11)
Anserine +Zinc (4)	86.81±3.54 (2,4,10)	95.60±4.27 (4,10)
Control-irradiated		
γ_1 (5)	23.70±2.33 (5,6)	47.53±1.64 (5,6)
γ_2 (6)	22.75±3.59 (5,6,8)	48.68±2.43 (5,6)
Pre-treated irradiated		
Anserine (7)	40.71±6.71 (1,7,8,9)	66.22±0.94 (1,7,8,9)
Zinc (8)	31.50±1.25 (6,7,8)	57.92±4.63 (1,7,8)
Anserine +Zinc (9)	45.39±3.52 (1,7,9)	69.39±1.68 (1,3,7,9)
Post-treated irradiated		
Anserine (10)	77.77±3.56 (2,3,4,10)	101.35±6.76 (4,10)
Zinc (11)	57.79±2.50 (1,11)	79.69±1.66 (2,3)
Anserine +Zinc (12)	90.51±2.29 (4,12)	125.04±2.67 (12)
Level of significance p<	0.001	0.001

Data are expressed as $\bar{x}$±SD of eight rats in each group. Significant difference between groups is analyzed by one-way ANOVA combined with post hoc (LSD-SPSS computer program) ($p<0.001$: highly significant). Numbers between brackets indicate non-significant correlation

Determination of Serum Total Protein Level

Table 2 shows serum total protein level in normal control, treated control, irradiated control and pre and post-treated irradiated groups. The data revealed that, only combination of anserine and zinc induced a significant rise in total protein level at $p<0.001$ compared to control healthy group. It is shown that whole body gamma irradiation (5.7 Gy) caused highly significant decline in serum total protein at $p<0.001$at time intervals 24 h and 14 days post-irradiation compared to control healthy group. The data also revealed that, administration of anserine and/or zinc before and after irradiation exposure, caused a significant increase ($p<0.001$) in serum total protein compared to irradiated groups with percentages of improvement, 20.56%, 15.12 and 30.41% for pre-treated irradiated groups and 33.64, 22.36 and 56.62% for post-treated irradiated groups respectively.

Measurement of Serum Albumin Level

Serum albumin level is significantly increased in anserine and anserine and Zn control groups at $p<0.001$ compared to control healthy group, while no significant difference was observed between zinc control and control healthy group. The significant decline in serum albumin content in irradiated rats at different sampling times i.e., 24 h and 14 days is observed in Table 2. Pre and post-treatment of irradiated rats with anserine, zinc and their combination induced a significant rise in serum albumin level at $p<0.001$ compared to control irradiated groups with percentages of improvement 19.63, 5.52 and 24.53% for pre-treated irradiated groups and 31.28, 13.49 and 72.39% for post-treated irradiated groups, respectively.

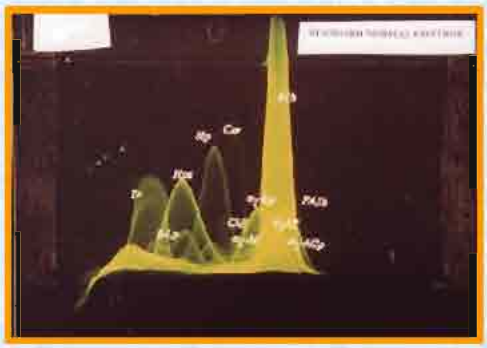
Separation of Protein Fractions by Gel Immunoelectrophoresis

Figure 1-4 reveal the electrophoretic peaks obtained on the electropherograms of control treated and untreated rat serum and of sera of rats treated with anserine, zinc, anserine+zinc pre and post irradiation dose.

Table 2: Serum levels of total protein and albumin in normal control, control-treated, control-irradiated and pre and post-treated irradiated groups

	Parameters	
	Total protein (mg mL^{-1})	Albumin (g dL^{-1})
Groups		
Normal control (1)	116.77±6.44 (1,2,3,12)	1.63±0.031 (1,3,12)
Control-treated		
Anserine (2)	125.44±5.87 (1,2,3,12)	1.85±0.045 (2,3)
Zinc (3)	125.17±5.93 (1,2,3,12)	1.73±0.044 (1,2,3,12)
Anserine +Zinc (4)	143.43±2.54 (4)	2.13±0.12 (4)
Control-irradiated		
γ_1 (5)	54.90±3.68 (5,6)	0.52±0.021 (5,6,8)
γ_2 (6)	54.08±1.06 (5,6)	0.52±0.025 (5,6,8)
Pre-treated irradiated		
Anserine (7)	78.91±4.47 (7,8,11)	0.84±0.043 (7,9,11)
Zinc (8)	72.56±2.25 (7,8,11)	0.61±0.034 (5,6,8,11)
Anserine +Zinc (9)	90.42±3.80 (9,10)	0.92±0.045 (7,9,10)
Post-treated irradiated		
Anserine (10)	92.20±2.53 (9,10)	1.03±0.067 (9,10)
Zinc (11)	80.19±1.35 (7,8,11)	0.74±0.041 (7,8,11)
Anserine +Zinc (12)	120.20±1.50 (1,2,3,12)	1.70±0.078 (1,3,12)
Level of significance p<	0.001	0.001

Data are expressed as $\bar{x}$±SD of eight rats in each group. Numbers between brackets indicate non-significant correlation, Significant difference between groups is analyzed by one-way ANOVA combined with post-hoc (LSD-SPSS computer program) ($p<0.001$: highly significant)



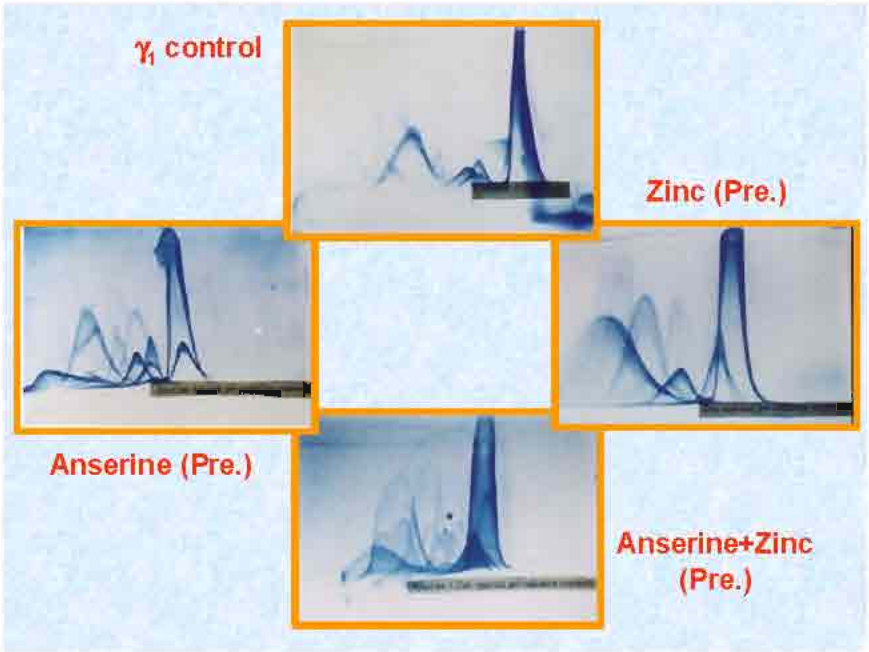
Palb= Prealbumin.
Alb = Albumin.
α_1-Lp = α_1-Lipoprotein.
α_1-M = α_1-Macroglobulin.
α_1-Agp = α_1- Acid glycoprotein.
α_1-AT = α_1-Antitrypsin.
ChE = Cholinesterase.
Cer = Ceruloplasmin.
Hpx = Haemopexin.
Hp = Haptoglobin.
Tr = Transferrin.
β-Lp = β-Lipoprotein.

CROSSED TWO DIMENSIONAL IMMUNOELECTROPHORESIS OF RAT SERUM PROTEINS.

Control Anserine

Control Zinc

Control Anserine+Zinc


γ1 control
Zinc (Pre.)
Anserine (Pre.)
Anserine+Zinc
(Pre.)

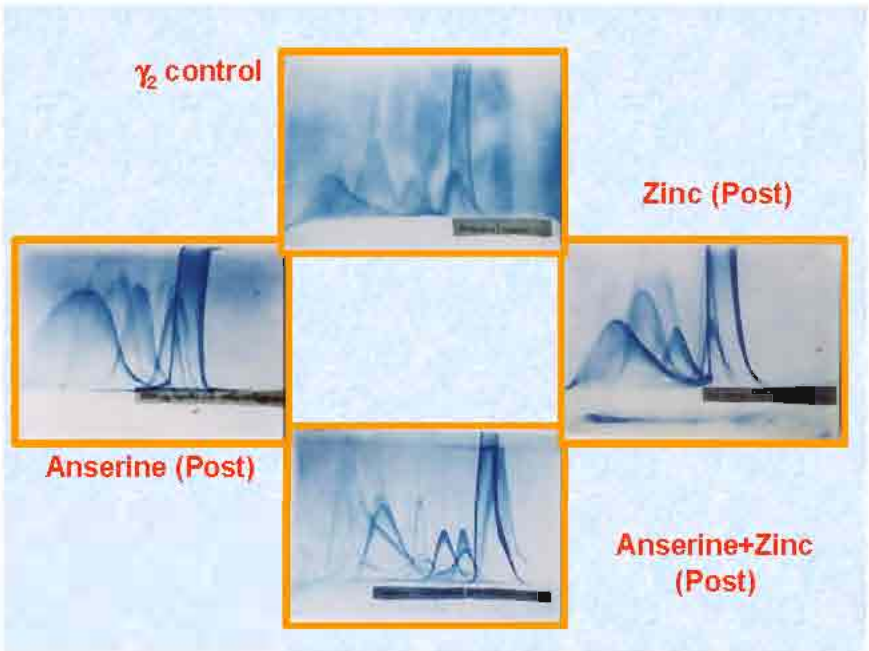

γ2 control
Zinc (Post)
Anserine (Post)
Anserine+Zinc
(Post)

Table 3: α- lipoprotein and β-lipoprotein semm levels in normal control, control-treated, control-irradiated and pre and post-treated irradiated groups

	Parameters	
	α-Lipoprotein (HDL) (mg mL^{-1})	β-Lipoprotein (LDL) (mg mL^{-1})
Groups		
Normal control (1)	2.63±0.026 (1,2,3,4)	4.34±0.054 (1,12)
Control-treated		
Anserine (2)	2.57±0.029 (1,2,3,7)	5.04±0.045 (2,4,6,9,10,11)
Zinc (3)	2.58±0.023 (1,2,3,4)	4.68±0.022 (3,9,10,12)
Anserine +Zinc (4)	2.61±0.026 (1,2,3,4,5)	5.57±0.44 (4,8)
Control-irradiated		
γ_1 (5)	7.98±0.45 (5)	6.63±0.02 (5)
γ_2 (6)	8.44±0.15 (6)	6.69±0.18 (6)
Pre-treated irradiated		
Anserine (7)	6.91±0.032 (7,9)	5.13±0.09 (2,7,9,10,11)
Zinc (8)	7.2±0.009 (7,8)	5.88±0.026 (4,8)
Anserine +Zinc (9)	5.00±0.067 (9,11)	4.86±0.40 (2,3,7,9,10)
Post-treated irradiated		
Anserine (10)	4.16±0.11 (10)	4.96±0.045 (2,3,7,9,10,11)
Zinc (11)	5.16±0.059 (9,11)	5.23±0.04 (2,7,10,11)
Anserine +Zinc (12)	3.10±0.09 (12)	4.47±0.03 (1,3)
Level of significance p<	0.001	0.001

Data are expressed as x̄±SD of eight rats in each group. Numbers between brackets indicate non-significant correlation
•Significant difference between groups is analyzed by one-way ANOVA combined with post-hoc (LSD-SPSS computer program) (p<0.001: highly significant)

Table 3 demonstrates α-lipoprotein and β-lipoprotein levels in serum of normal control, control treated, control irradiated, pre and post-treated irradiated groups. Serum α-lipoprotein concentration of control treated groups showed no significant change while a significant rise in serum β-lipoprotein level at p<0.01 was observed compared to control healthy group. On the other hand, gamma radiation exposure caused a significant rise in serum α-and β-lipoprotein levels (p<0.001) at time intervals 24 h and 14 days after radiation exposure compared to control healthy group. The data also revealed that, administration of anserine and/or zinc prior or after radiation exposure induced a significant decline in serum α- and β-lipoprotein levels at p< 0.001 compared to control irradiated groups (γ1 and γ2).

The effect of anserine and/or zinc on serum prealbumin and α-1-antitrypsin is shown in Table 4. Control treated groups show a significant rise in prealbumin level at p<0.01 compared to control healthy group. As a result of gamma radiation exposure, there is a significant decrease in serum prealbumin and α-1-antitrypsin concentration levels (p<0.001) at time intervals 24 h and 14 days post-radiation exposure compared to control healthy group. On the other hand, administration of anserine and/or zinc prior or after radiation exposure caused a significant elevation in prealbumin level and α-1-antitrypsin at p<0.001 compared to control irradiated groups.

The change in serum α-1-acid glycoprotein and α-1-macroglobulin concentrations is shown in Table 5. The data reveal a significant rise in serum α-1-acidic glycoprotein and α-1-macroglobulin in control treated groups at p<0.01 compared to control healthy group, while a significant decline of serum α-1-acidic glycoprotein and α-1-macroglobulin levels (p< 0.001) was observed at time intervals 24 h and 14 days post-radiation.

On the other hand, injection of anserine and/or zinc prior or after radiation exposure induced a significant rise in α-1-acidic glycoprotein and α-1-macroglobulin levels (p<0.001) compared to control irradiated groups.

The effect of anserine and/or zinc on serum cholinesterase and transferrrin levels is shown in Table 6. All control treated groups show a significant elevation in serum cholinesterase and transferrrin levels at (p<0.01 and p<0.001) respectively compared to control healthy group. As a result of gamma

Table 4: Prealbumin and α-1-antitrypsin serum levels in normal control, control-treated, control-irradiated and pre and posttreated irradiated groups

	Parameters	
	Prealbumin (mg mL^{-1})	α-1-Antitrypsin (mg mL^{-1})
Groups		
Normal control (1)	2.14±0.06 (1)	3.39±0.10 (1)
Control-treated		
Anserine (2)	2.63±0.032 (2)	4.07±0.068 (2,3,12)
Zinc (3)	2.47±0.098 (3)	3.98±0.02 (2,3,12)
Anserine +Zinc (4)	2.89±0.009 (12)	4.87±0.24 (4)
Control-irradiated		
γ_1 (5)	1.07±0.053 (5)	1.17±0.061 (5,6)
γ_2 (6)	1.20±0.012 (6,8)	1.25±0.042 (5,6)
Pre-treated irradiated		
Anserine (7)	1.43±0.075 (7,9)	2.01±0.55 (7,8,10,11)
Zinc (8)	1.25±0.005 (6,8)	1.82±0.053 (7,8,11)
Anserine +Zinc (9)	1.54±0.03 (7,9)	2.48±0.11 (9)
Post-treated irradiated		
Anserine (10)	2.00±0.018 (10)	2.16±0.034 (7,10,11)
Zinc (11)	1.87±0.034 (11)	1.99±0.073 (7,8,10,11)
Anserine +Zinc (12)	2.83±0.041 (4,12)	3.88±0.026 (2,3,12)
Level of significance p<	0.001	0.001

Data are expressed as $\bar{x}$±SD of eight rats in each group. Numbers between brackets indicate non-significant correlation, Significant difference between groups is analyzed by one-way ANOVA combined with post-hoc (LSD-SPSS computer program) ($p<0.001$: highly significant $p<0.01$ significant)

Table 5: Serum α-1-acidic glycoprotein and α-1-macroglobulin levels in normal control, control-treated, control-irradiated and pre and post-treated irradiated groups

	Parameters	
	α-1-Acidic glycoprotein (mg mL^{-1})	α-1-Macroglobulin (mg mL^{-1})
Groups		
Normal control (1)	1.69±0.012 (1)	1.50±0.037 (1)
Control-treated		
Anserine (2)	1.96±0.038 (2,3,12)	1.84±0.039 (2)
Zinc (3)	1.88±0.021 (2,3,12)	1.72±0.04 (3,12)
Anserine +Zinc (4)	2.15±0.052 (4)	1.97±0.018 (4)
Control-irradiated		
γ_1 (5)	0.57±0.022 (5,6)	0.47±0.027 (5,6)
γ_2 (6)	0.62±0.022 (5,6)	0.51±0.026 (5,6)
Pre-treated irradiated		
Anserine (7)	0.89±0.009 (7,11)	1.05±0.067 (7,10,11)
Zinc (8)	0.73±0.003 (8)	0.66±0.017 (8)
Anserine +Zinc (9)	1.02±0.028 (8)	1.20±0.067 (9,10)
Post-treated irradiated		
Anserine (10)	1.12±0.022 (10)	1.13±0.021 (7,9,10)
Zinc (11)	0.92±0.022 (7,11)	1.02±0.022 (7,11)
Anserine +Zinc (12)	1.91±0.077 (2,3,12)	1.67±0.023 (3,12)
Level of significance p<	0.001	0.001

Data are expressed as $\bar{x}$±SD of eight rats in each group. Significant difference between groups is analyzed by one-way ANOVA combined with post-hoc (LSD-SPSS computer program) ($p<0.001$: highly significant and $p<0.01$: significant). Numbers between brackets indicate non-significant correlation

Table 6: Cholinesterase and Transferrin serum levels in normal control, control-treated, control-irradiated and pre and post-treated irradiated groups

	Prameters	
	Cholinesterase (mg mL^{-1})	Transferrin (mg mL^{-1})
Groups		
Normal control (1)	2.22±0.062 (1)	4.57±0.029 (1)
Control-treated		
Anserine (2)	3.94±0.058 (2,12)	5.24±0.015 (2)
Zinc (3)	3.10±0.07 (3)	4.99±0.06 (3)
Anserine +Zinc (4)	4.43±0.053 (4)	6.28±0.033 ($,12)

Table 6: Continued

	Prameters	
	Cholinesterase (mg mL^{-1})	Transferrin (mg mL^{-1})
Control-irradiated		
γ_1 (5)	1.01±0.023 (2,6)	2.01±0.027 (5,6)
γ_2 (6)	1.10±0.012 (5,6)	2.06±0.047 (5,6)
Pre-treated irradiated		
Anserine (7)	1.62±0.035 (4,7,8)	2.74±0.017 (7)
Zinc (8)	1.52±0.024 (7,8)	2.62±0.034 (8)
Anserine +Zinc (9)	1.91±0.058 (9,11)	2.92±0.046 (9)
Post-treated irradiated		
Anserine (10)	2.10±0.043 (1,10,11)	4.11±0.025 (10)
Zinc (11)	2.01±0.026 (9.11)	3.31±0.012 (11)
Anserine +Zinc (12)	3.95±0.043 (2,12)	6.24±0.019 (4,12)
Level of significance p<	0.001	0.001

Data are expressed as $\bar{x}$±SD of eight rats in each group. Numbers between brackets indicate non-significant correlation significant difference between groups is analyzed by one-way ANOVA combined with post-hoc (LSD-SPSS computer program) ($p<0.001$: highly significant and $p<0.01$: significant)

Table 7: Serum ceruloplasmin, haptoglobin and haemopexin levels in normal control, control-treated, control-irradiated and pre and post-treated irradiated groups

	Parameters		
	Cemloplasmin (mg mL^{-1})	Haptoglo bin (mg mL^{-1})	Haemo pexin (mg mL^{-1})
Groups			
Normal control (1)	1.36±0.031 (1)	4.45±0.037 (1)	1.49±0.05 (1)
Control-treated			
Anserine (3)	1.75±0.047 (2)	4.97±0.064 (2)	1.71±0.035 (2)
Zinc (4)	1.58±0.029 (3)	4.74±0.057 (3)	1.63±0.047 (3)
Anserine +Zinc (5)	2.03±0.047 (4)	5.40±0.015 (4)	1.95±0.04 (4)
Control-irradiated			
γ_1	0.65±0.009 (5,6)	1.52±0.045 (5,6)	0.61±0.012 (5,6)
γ_2	0.67±0.023 (5,6)	1.57±0.020 (5,6)	0.64±0.009 (5,6)
Pre-treated irradiated			
Anserine (7)	0.98±0.023 (7,9)	2.66±0.045 (7,9)	0.91±0.025 (7,9)
Zinc (8)	0.89±0.005 (8)	2.13±0.10 (8)	0.80±0.028 (8)
Anserine+Zinc (9)	1.04±0.038 (7,9,11)	2.85±0.043 (9)	0.98±0.023 (7,9,11)
Post-treated irradiated			
Anserine (10)	1.24±0.038 (10)	3.96±0.45 (10)	1.23±0.046 (10)
Zinc (11)	1.12±0.02 (9,11)	3.13±0.10 (11)	1.02±0.024 (11)
Anserine+Zinc (12)	1.95±0.035 (12)	5.69±0.027 (12)	1.96±0.042 (12)
Level of significance p<	0.001	0.001	0.001

Data are expressed as $\bar{x}$±SD of eight rats in each groupSignificant difference between groups is analyzed by one-way ANOVA combined with post-hoc (LSD-SPSS computer program) ($p<0.001$: highly significant and $p<0.01$: significant). Numbers between brackets indicate non-significant correlation

radiation exposure, there is a significant decline in serum cholinesterase and transferrrin levels ($p<0.001$) at 24 h and 14 days after radiation exposure compared to control healthy group. On the other hand, I.P. injection of anserine and/or zinc before or after radiation induced a significant rise in both serum proteins ($p<0.001$) compared to control irradiated groups.

Data shown in Table 7 demonstrate that control treated groups show a significant elevation in serum ceruloplasmin, haptoglobin and haemopexin levels at $p<0.01$ compared to control healthy group. On the other hand, these serum proteins significantly declined in control irradiated groups at $p<0.001$ compared to control healthy group. Administration of anserine and/or zinc prior or after radiation exposure caused a significant improvement in the levels of the three proteins compared to irradiated groups at $p<0.001$.

Discussion

Because of the potential application of certain chemical and biological substances that exhibit a protective or a curative role against lethal effects of ionizing radiation, the present study has been planned in order to identify the prophylactic and curative roles of anserine, zinc or their combination, as well as the optimal administration time relative to irradiation.

The significant reduction in serum total protein and liver DNA and RNA contents in rats submitted to a single dose of gamma radiation particularly 24 h and 14 days after exposure could be attributed to the excessive free radicals i.e. ROS formed by ionizing radiation which are considered as important factors in DNA damage resulting in single or double strand breaks causing destruction of sugar moiety or in cross-links and formation of dimers. This coincides with the findings of Cai and Cherian (2003) and Ptastinska *et al*. (2004) that ionizing radiation leads to damage of living cells particularly via strand breaks of DNA or RNA. This damage is a result of misrepair of these breaks. Many authors have also explained the significant decline in serum total protein following exposure to whole body gamma irradiation to result from excessive protein loss through injury to kidney or gastro-intestinal tract or from thermal injury to skin (Keren, 1994). In addition, this decrease in serum total protein level could be related to hypercatabolic states after gamma irradiation or hepatic dysfunction and decreased protein synthesis (El-Nabarawy and Abdel-Gawad, 2001). All these phenomena might be, at least partially, responsible for protein loss after irradiation. In the present data, significant elevation in serum total protein and liver DNA and RNA contents in the groups treated with anserine or zinc was observed, while a more significant rise in these parameters was observed, when a collective combination of both anserine and zinc was administered. This could be attributed to the physiological role of the antioxidants i.e., anserine and zinc in minimizing radiation-induced injuries, particularly to cell membranes (Inanami *et al*., 1999). The detectable elevation in serum total protein as well as DNA and RNA contents may be related to stimulated DNA synthesis with subsequent enhancement of hepatic protein synthesis induced by anserine and/or zinc as previously reported (Kimball *et al*., 1995; Soliman *et al*., 2001).

These results can also be explained on the basis that anserine and anserine related compounds have the ability to protect DNA by reducing the formation of 8-hydroxyl-deoxyguanosine, a marker of DNA oxidative damage (Kantha *et al*., 1996) and their ability to protect DNA damage against non-enzymatic cross links (Hipkiss *et al*., 1998a). On the other hand, Tanaka *et al*. (2005) showed that rats exposed to 6 Gy whole body gamma radiation presented a better granulation tissue than irradiated untreated rats and closely similar to control and reported that carnosine related compounds are effective radioprotectors. Concerning the effect of zinc, Chiricola *et al*. (1993) demonstrated that after zinc supplementation, the rate of DNA repair after gamma irradiation became similar to control subjects which confirms that the element is able to correct some immune defects and to modify the biochemical steps which control the rate of DNA repair. Moreover, Kumar *et al*. (1994) stated that, zinc supplementation accelerates cellular proliferation and clonal expansion by stimulation of DNA synthesis and various enzymes synthesis during mitosis division. Also, zinc showed a significant protection against DNA induced damage better than GSH and resulted in attenuating the adverse effects on the antioxidant status following exposure to irradiation (Cai and Cherian, 2003; Vijayata and Dhawan, 2005).

Our study on protein was also extended to evaluate protein fraction abnormalities, which give useful information along with usual circumstances of their increase, decrease and change in band architecture. In the current study, exposure to gamma radiation induced a significant decline in all serum

protein fractions except for α lipoprotein and β-lipoprotein at time intervals 24 h and 14 days post-radiation exposure. These results could be attributed to the destruction of cellular structure which causes activation and liberation of proteolytic enzymes followed by autodigestion of host tissue which induces inflammatory responses usually accompanied by changes in serum proteins. Moreover, exposure to gamma radiation alters the synthesis of proteins either by stimulation or inhibition (Mahdy *et al*., 1997). Previously, Defeo *et al*. (1995) illustrated that significant reduction in serum protein fractions induced by progressive development of liver injury resulted from reducing protein synthesis and/or inducing of intracellular accumulation of secretory proteins, thus reducing their levels in serum.

Serum albumin level, in the present work, was significantly decreased in control irradiated groups (γ_1 and γ_2) with respect to control healthy group. The decline of serum albumin level after radiation exposure may be related to significant pathology either in production of albumin by the liver or in its leakage through damaged surface as reported by Mackiewicz *et al*. (1992). Abou-Seif *et al*. (2003) reported that total protein, albumin and albumin/globulin ratio declined in gamma irradiated rats. I.P. injection of anserine nitrate and/or zinc chloride before or after radiation exposure significantly improved the decline in albumin level of irradiated control groups. These results can be explained by the ability of carnosine related compounds as anserine to inhibit modification and cross-linking of ovalbumin and bovine serum albumin induced by MDA and hypochlorite (Hipkiss *et al*., 1998b). On the other hand, Yousef *et al*. (2002) reported that zinc is an essential trace element for hepatic synthesis of albumin and immunoglobulins in rats.

In addition, gamma radiation stimulates acute phase response. This response is essential for generation and limitation of inflammation. Individual acute phase reactants have different biochemical functions and hence regulatory mechanisms that are differentially observed within hours or days (Campbell *et al*., 2001). Some of acute phase reactant proteins are protease inhibitors as α-1-macroglobulin, α-glycoprotein and α-antitrypsin. Others are transporters as ceruloplasmin, transferrin, haemopexin, haptoglobin and α&β-lipoproteins (Ganong, 1999).

Concerning the effect of gamma radiation on serum α and β-lipoproteins, our results illustrated that, gamma rays caused a significant elevation in both α and β lipoprotein fractions at time intervals 24 h and 14 days post-radiation exposure. Our results are in harmony with those of El-Nabarawy and Abdel-Gawad (2001) who found, increasing concentration of lipoprotein fractions, despite the reduction in number of their bands, in serum of irradiated rats with single dose (5.5 Gy) of gamma radiation. These results can be explained on the fact that increasing of lipid synthesis and serum release of α-lipoprotein, which is more concerned with high density lipoprotein (HDL) and β-lipoprotein, which is more concerned with low density lipoprotein (LDL) by cytokines could be a possible protective not deleterious part of acute phase response as reported by Feingold *et al*. (1989). On the contrary Feugard *et al*. (1998) found that serum lipoproteins could be involved in radiation-induced vascular damage due to their susceptibility to peroxidation.

Prealbumin, also known as transthyretin, is increased in cases of severe reual and hepatic failure (Castaneda and Ruiz, 1994). In the current study, the significant decline of prealbumin after radiation exposure may be attributed to tissue injury induced by gamma radiation leading to decrease of prealbumin production by the hepatocytes (Dickson *et al*., 1982).

The significant decreased levels of α-1-antitrypsin and after radiation exposure at times 24 h and 14 days could be related to tissue injury induced by gamma irradiation as reported by Moota *et al*. (2001).

In the present study, the significant decline of α-1-glycoprotein in control irradiated groups with respect to control healthy group coincides with the studies of Kirpichenok *et al*. (1997) who found increasing of α-antiproteinase inhibitor and decreasing of α-1-acid glycoprotein plasma level on 7th and 14th days after gamma irradiation.

In the present study, gamma irradiation also induced a significant decline in α-1-macroglobulin at time intervals 24 h and 14 days after radiation exposure. These results are in agreement with Vershinina *et al*. (1985) who suggested that the effect of gamma radiation on the metabolic pool of blood amino acids and inhibitory capacity of α-1-macroglobulin depends upon type and dose of radiation. In addition, they found a significant correlation between early changes in metabolic pool of amino acid, α-antiprotease activity and degree of life span after irradiation. Collectively, Fouad *et al*. (1983) reported that proteins of acute phase α-1-macroglobulin deficiency is associated with α-1-antitrypsin deficiency of liver damage and referred the reduction of serum α-1-macroglobulin to decrease in its production by hepatocytes.

Although, Roushdy *et al*. (1997) reported that the use of serum enzymes as a measure of organ damage is based on the premise that the enzymes released from injured cells into extracellular fluid find their way into blood, the present study recorded a significant decease of cholinesterase level in serum of rats subjected to whole body gamma rays at time intervals 24 h and 14 days post-radiation. Present results are in accordance with Hedayat and Abdel-Hamid (1999) who found that gamma irradiation at a dose level 7 Gy caused a significant inhibition of cholinesterase in serum and liver, whereas, no change was observed in brain. This decline in serum cholinesterase level could be attributed to effect of gamma irradiation on the enzymes involved in the biosynthesis of neurotransmitter as stated by Roushdy *et al*. (1995). Moreover, Hedayat and Abdel-Hamid (1999) added that the decline in cholinesterase level by gamma irradiation may be due to disturbance in the protein synthesis function of the hepatic parenchyma and in the metabolism of nucleoprotein in the cell.

Transferrin functions to transport non-heme free iron from the gastro-intestinal tract to the bone marrow (Trinder *et al*., 2000).Transferrin showed a marked significant decrease at 24 h and 14 days post-irradiation with respect to control healthy group. Transferrin is usually decreased in chronic liver diseases and thermal injuries as stated by Keren (1994) and this might explain the pronounced decrease of transferrin in control irradiated groups.

Moreover, the decline in iron utilization by bone marrow or the decrease in bound iron with increased unsaturated iron-binding capacity may also explain the decline in serum transferrin level after radiation exposure as reported by Edress *et al*. (1995). On the contrary, Maiti *et al*. (2001) stated that transferrin messenger RNA (mRNA) expression dramatically increased in response to gamma irradiation.

Ceruloplasmin (CER) is an important extracellular antioxidant that functions coordinately with transferrin to promote iron binding and prevent metal-catalyzed free radical reactions (Goldstein *et al*., 1982). Our results showed that serum CER level significantly declined after radiation exposure at time intervals 24 h and 14 days. These results were in accordance with the findings of Pulatova *et al*. (1992) who found ceruloplasmin and plasma copper levels declined in 3rd day after radiation exposure. In addition, the dynamics of changes in transferrin and ceruloplasmin pools were phase dependent with the maxima at 2nd, 6th and 10th days after irradiation. These results can be explained by consumption of ceruloplasmin as antioxidant to inhibit lipid peroxidation induced by gamma radition as reported by Honcharuk *et al*. (1995). Furthermore, Sharygin *et al*. (2003 and 2005) demonstrated that Fe^{3+} tranferrin pool is one of the most informative, highly radiosensitive and rapidly reactive marker against irradiation since it controls a rate-limiting iron-dependent stage for DNA synthesis, the synthesis of dNTP.

Haptoglobin is an acute phase protein and in addition, initiated to restore homeostasis after trauma. The present study revealed a significant reduction in serum haptoglobin level after irradiation. These results could be attributed to decreased synthesis of haptoglobin by injured liver or due to intravascular hemolysis or decreased erythropioesis as described by El-Nabarawy and Abdel-Gawad (2001). In addition, the decrease in serum haptoglobin level was also previously reported by Jeschke *et al*. (2000) in cases of internal injury.

Haemopexin is considered one of extracellular antioxidants. It binds to heme proteins to minimize its ability to catalyze free radical damage (Halliwell and Gutteridge, 1990). In addition, Ganong (1999) reported that, of the porphyrinis, haemopexin is particularly specified for heme carriage and recycling. These functions indicated the native selectivity of acute phase reaction biological machineries in condition of injury. Present study revealed a significant decline of serum haemopexin level after 24 h and 14 days of radiation exposure. These results could be explained by excessive hematological disorders and depression in haemoglobin content induced by gamma irradiation as stated by Edrees *et al*. (1995).

In the present study, healthy control treated groups demonstrated a significant increase in all protein fractions except α-lipoprotein compared to the normal healthy non-treated group, but combination of anserine and zinc induced a more significant enhancement of protein fractions than either anserine or zinc alone. In addition, pre and post intraperitoneal injection of anserine nitrate and/or zinc chloride prior or after radiation exposure caused a significant improvement of all protein fraction except α and β-lipoproteins compared to control irradiated groups (γ_1 and γ_2), however combination of anserine and zinc post-radiation treatment showed the most potent action and amelioration of all serum protein fractions. The stimulation of protein synthesis in healthy control group and pre and post-treated irradiated groups may be related to the protein promoting effect of anserine. Fontana *et al*. (2002) and Kang *et al*. (2002b) reported the ability of naturally-occurring dipeptides as anserine and carnosine to protect tyrosine against nitration, α-1-antiproteinase against inactivation, human low density lipoproteins against modification by peroxynitrite and ceruloplasmin (CER) against H_2O_2-mediated oxidative damage through cupper chelation and free radical scavenging mechanisms. Also, Hipkiss and Chana (1998) proved the ability of anserine and related compounds to protect the already formed proteins against modification induced by hexoses, pentoses, trioses and the highly reactive lipid peroxidation end product (MDA). Furthermore, carnosine and related compounds can inhibit Cu^{2+} promoted low density lipoprotein oxidation (Decker *et al*., 2001) and can inhibit non-enzymatic glycosylation of proteins (Halliday and Mc-Farland, 2000).

On the other hand, the promoting effect of zinc on serum protein fractions of control treated, pre and post-treated irradiated groups may be related to the antioxidant effect of zinc, induction of metallothionein by exogenous zinc, regulatory function of zinc on protein synthesis and immuno-suppressive effects of zinc.

The antioxidant action of zinc is mediated through different mechanisms, it can regulate cellular redox potential involving glutathione (GSH) (Nakatani *et al*., 2000), it plays an important role in the antioxidant cellular defenses being a structural element of the non-mitochondrial form of superoxide dismutase enzyme (SOD) (Virgili *et al*., 1999) and zinc can also induce release of metallothionein (MT), which can scavenge free oxygen and nitrogenous radicals (Ogawa and Minura, 1999). Metallothionein, as an acute or stress response protein, is related to inflammation and cellular protection from oxidative damage (Cai *et al*., 2000, 2004). Also, Cai and Cherian (2003) found that, metallothionein, induced by zinc, is a high capacity antioxidant to protect radiation-induced DNA protein damage. Sharygin *et al*. (2003 and 2005) also reported that radioprotectants and antioxidants

activate protein synthesis and increase pools of Fe^{3+} transferrin and Cu^{2+} ceruloplasmin in blood, which activate dNTP and DNA and protein synthesis in organs. These increased pools of blood proteins underlay the formation of body resistance to DNA-damaging factors.

As shown from the previous discussion, the obtained data might be useful to identify the development of rat tissue injury following gamma irradiation at a dose level (5.7 Gy). The underlying mechanisms of such injury involve release of reactive oxygen species, damaging effects on nucleic acids and inhibition of protein synthesis Furthermore, administration of anserine, zinc and especially their collective combination prior or after gamma radiation exposure have protective and curative actions against the deleterious effects of gamma rays. Metal complexes offer a new approach to overcome the pathological effects of ionizing radiation and it is suggested their use as physiological treatments to prevent or facilitate recovery from radiation injuries.

References

Abul-Nasr, S., M. El-Shafey and M. Osfor, 2001. Carnosine protection against cadmium-induced nephrotoxicity in male albino rats. Sci. Agric. Bohem., 32: 53-77.

Abu-Seif, M.A., M.M. El-Naggar, M. El-Far, M. Ramadan and N. Salah, 2003. Prevention of biochemical changes in γ-irradiated rats by some metal complexes. Clin. Chem. Lab. Med., 41: 926-933.

Boldyrev, A. and H. Abe, 1999. Metobolic transsformation of neuropeptide carnosine modifies its biological activity. Cell. Mol. Neurobiol., 19: 163-175.

Bradford, M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Cai, L., D. Deng, S. Chen, R. Zhong, M. Cherian and S. Chakrabarti, 2000. Induction of metallothionein with preservation of testicular function in rats following long term renal transplation. Urol. Res., 28: 79-103.

Cai, L., S. Ishander, M. Cherian and R.R. Hammond, 2004. Zn or Cd pre-induced metallothionein protect human CNS cells and astrocytes from radiation induced apoptosis. Toxicol. Lett., 146: 217-226.

Cai, L. and M. Cherian, 2003. Zn-MT protects from DNA-damage induced by radiation better than GSH and copper or cadmium metallothioneins. Toxicol. Lett., 13: 193-198.

Campbell, J., L. Prichard, J. Schmitz, F. Stephenson, M. Rosenfeld, G. Argast, P. Heindrich and N. Fawsto, 2001. Expression of suppressors of cytokines signaling during liver regneration. J. Clin. Invest., 107: 1285-1292.

Castaneda, L. and P. Ruiz, 1994. Duplicate prealbumin bands in urine: Association with hepatorenal failure. J. Clin. Pathol., 12: 475-77.

Chiricola, M., A.R. Musa, D. Monti, M. Zannotti and C. Franceschi, 1993. Enhanced DNA repair in lymphocytes of Down syndrome patients: Influence of Zn nutritional supplementation. Mut. Res., 295: 105-111.

Decker, E., V. Ivanov, B. Zhu, B. Frei and B. Zhu, 2001. Inhibition of LDL oxidation by carnosine and histidine. J. Agric. Food. Chem., 49: 511-516.

Defeo, P., E. Volpi, P. Lucidi, F. Monacchia, F. Santeusanio, G. Balli and P. Brunetti, 1995. Ethanol impairs post-prandial hepatic protein metabolism. Am. Soc. Clin. Inv., 95: 1472-479.

Dickson, P., G. Howlett and G. Schreiber, 1982. Metabolism of prealbumin in rats and changes induced by acute inflammation. Eur. J. Biochem., 129: 289-293.

Doumas, B., W. Watson and H.G. Biggs, 1971. Albumin standards and measurement of serum albumin with bromocresol. Clini. Chim. Acta, 31: 87-96.

Edrees, G., Y. Megahed M. Amer and M. El-Sayed, 1995. Prophylactic and curative effects of colostrum and alkoxyglycerol against radiation induced disorders in hematological values, blood forming organs and hormonal levels in male albino mice. Isotope Rad. Res., 27: 127-136.

El-Nabarawy, S.K. and I.I. Abdel-Gawad, 2001. Electrophoretic protein analysis and some biochemical studies on the effect of selenium and vitamin E in rats subjected to whole body gamma irradiation. Isotope Rad. Res., 33: 295-317.

El-Wakf, A.M., 1999. Protection of rat skin against X-radiation injury by glutathione treatment. Isotope Rad. Res., 3: 35-45.

Feingold, K., M. Soued and M. Serio, 1989. Multiple cytokines stimulate hepatic lipid synthesis *in vivo*. Endocrinology, 125: 279-285.

Feugard, C., D. Bayle, C. Serougne, A. Mazur, C. Lutton, J. Aigueperse, P. Gourmelon and D. Mathe, 1998. Effect of ionizing radiation on plasma lipids and lipoproteins in rats. Radiat. Res., 150: 43-51.

Fontana, M., F. Pinnen and I. Pecci, 2002. Prevention of peroxy dependent damage by carnosine and related sulphonamido pseudodipeptides. Cell. Life Sci., 59: 546-551.

Fouad, F.M., M. Goldberg and B.G. Ruhen-Stroth, 1983. Plasma protein determination as clinical probe for liver injury in rats induced by thioacetamide, alloxan or ixoten. J. Clin. Chem. Clin. Biochem., 21: 203-207.

Fujioke, T., M. Murakami, T. Niiya, Y. Enami and M. Kusano, 2001. Effect of methyl prednisolsone on the kinetics of cytokines and liver function of regenerating liver in rats. Hepatol. Res., 19: 60-73.

Ganong, W.F., 1999. Review of Medical Physiology. 19th Edn. Appleton and Lange Norwalk, California, pp: 516-517.

Goldstein, I.M., H.B. Kaplan, H.S. Edelson and G. Weismann, 1982. Ceruloplasmin: An acute phase reactant that scavenge oxygen-derived free radicals. Ann. NY. Acd. Sci., 389: 368-375.

Halliwell, B. and J. Gutteridge, 1990. Role of free radicals and catalytic metal ions in human disease: An overview. Method Enzymol., 186: 1.

Harlow, E. and D. Lane, 1988. Antibodies. A laboratory Mannual. Cold Spring Harpor, USA., pp: 55-136.

Hedayat, I. and F. Abdel-Hamid, 1999. Some liver and brain disorders induced by anticholinesterase organophosphorus pesticide in rats subjected to gamma irradiation. Isotope Rad. Res., 31: 71-77.

Hipkiss, A. and H. Chana, 1998. Carnosine protects proteins against methylglyoxal-mediated modifications. Biochem. Biophys. Res. Comm., 248: 28-32.

Hipkiss, A., J. Preston, D. Himsworth, M. Keown and J. Michaelis, 1998a. Pluripotent protective effects of carnosine, a naturally occurring dipeptide. Ann. NY. Acad. Sci., 854: 37-53.

Hipkiss, A., V. Worthington, D. Himsworth and W. Herwig, 1998b. Protective effects of carnosine against protein modification by MDA and hypochlorite. Biochem. Biophys. Acta, 12: 46-54.

Holliday, R. and G. Mc-Farland, 2000. Review: A role of carnosine in cellular maintenance. Biochemistry (Moscow), 65: 843-848.

Honcharuk, L., V. Bardov, A. Kartysh, M. Korshun and O. Verpivs, 1995. An experimental study of the mechanism of the combined action on the body of ionizing radiation, pesticides, nitrates and lead and cadmium salts. Lik. Sprova., 5: 7-12.

Inanami, O., K. Takahashi and M. Kuwabara, 1999. Attenuation of caspase-3-dependent apotosis by Trolox post-treatment of X-irradiated MOLT-4 cells. Intl. Radiat. Biol., 75: 155-63.

Jeschke, M., D. Herndon and R. Barrow, 2000. Insulin like growth factor I in combination with insulin like growth factor binding protein III affects the hepatic acute phase response and hepatic morphology in thermally injured rats. Ann. Surg., 231: 408-416.

Kang, J.H., K.S. Kim, S.Y. Choi, H.Y. Kwon, M.H. Won and T.C. Kang, 2002b. Protection by carnosine-related dipeptides against hydrogen peroxide-mediated ceruloplasmin modification. Mol. Cells., 13: 107-112.

Kanshala, P., 2004. Cell membrane oxidative damage induced by γ-radiation and apoptotic sensitivity. J. Environ. Pathol. Toxicol. Oncol., 23: 1-7.

Kantha, S., S. Wada, H. Tanaka and H. Oehi, 1996. Carnosine sustains the retention of cell morphology in continuous fibroblast culture subjected to nutritional insult. Biochem. Biophys. Res. Commun., 223: 278-282.

Keren, D.F., 1994. High Resolution Electrophoresis and Immunofixation Techniques and Interpretation. 2nd Edn. Butterworth-Heinmann, USA,. pp: 41.

Kimball, S., S. Chen, R. Risica. and L. Jefferson, 1995. Effects of zinc deficiency on protein synthesis and expression of specific mRNAs in rat liver. Metabolism, 44: 126-133.

Kirpichenok, L., L. Gidranovich and V. Kheidorov, 1997. The joint action of nitrates and gamma radiation on the plasma proteinase-inhibiting and antioxidative systems in rats. Radiats. Biol. Radioecol., 37: 297-302.

Koch, C. and A. Giandomenica, 1994. Do low doses of radiation induce a response modulating induction or repair of DNA single-strand breaks? Rad. Res., 138: 89-92.

Kozurkova, M., E. Misurova, K. Kropacova and H. Hakova, 1999. Effect of essential on histones and nucleic acids in liver and blood-forming tissues of rats irradiated with gamma rays. Radioecologiia, 39: 388-393.

Kumar, P., G. Rai and S. Flora, 1994. Immunomodulation following zinc supplementation during chelation of lead in male rats. Biometals, 7: 41-44.

Laurell, C.B., 1965. Antigen-antibody cross electrophoresis. Anal. Biochem., 10: 385-91.

Mackiewiez, A., H. Schooltink, P. Heinrich and S. Rose-John, 1992. Complex of soluble human II-6 receptor/II-6 up-regulates expression of acute phase proteins. J. Immunol., 149: 2021-2027.

Mahdy, A.M., H.N. Saada, A.M. El-Naggar, A.M. Abdel-Salam and Z.S. Osama, 1997. The combined effect of vitamin C and single or fractionated gamma irradiation on serum contents of proteins and urea in albino rats. Isotope Rad. Res., 29: 31-37.

Maiti, S., G. Wilson, G. Shetty, M. Marcelli, P. Morris and M. Wilkinson, 2001. Irradiation selectively inhibits expression from the androgen-dependent pem homoeobox gene. Endocrinology, 142: 1567-1577.

Matsukara,T. and H. Tanak, 2000. Applicability of Zn complex of L-carnosine for medical use. Biochemistry (Moscow), 65: 817-823.

Moota, M., I. Giugno, P. Ruello, G. Pistone and M. Malaguarnera, 2001. Lipoprotein (a) behaviour in patients with hepatocellular carcinoma. Minerva. Med., 92: 301-305.

Nakatani, T., K. Opare, A. Kojima and Y.I. Maatsui, 2000. Apoptosis induced by chelation of intracellular zinc is associated with depletion of cellular GSH level in rat hepatocytes. Chem. Biol. Interact, 125: 151-463.

Ogawa, T. and Y. Minura, 1999. Antioxidant effect of zinc on acute renal failure induced by ischemia-reperfusion injury in rats. Am. J. Nephrol., 19: 609-614.

Ptastinska, S., S. Denifl, P. Candori, S. Yoon, P. Scheier, P. Illenberger and T.D. Mark, 2004. Radiation damage of nucleosides by low energy electrons. J.Chem. Phys., 120: 8505-8510.

Pulatova, M., D. Filatov, V. Sharygin, V. Beletskii and G. Chernov, 1992. The dose dependence of the development of compensatory-restorative body reactions to irradiation. IZV. Akad. Nauk., 2: 215-233.

Refaie, F.M., F.M. Abdel-Hamid and R.M. Yousri, 1993. Effects of irradiation on liver and kidney functions in rats subjected to cadmium or mercury injection. Isotope Rad. Res., 25: 19-26.

Roosen, N., F. Doz, K. Yeomans and M. Rosenblum, 1994. Effect of pharmacologic doses of zinc on the therapeutic index of brain tumor chemotherapy with carmustine. Cancer Chemother. Pharmacol., 34: 385-392.

Roushdy, H.M., F.M. Abdel-Hamid and A.R. Abu-Ghadir, 1995. Impairment of liver and kidney function in gamma irradiated rats suffering pesticide toxicity. Proceedings of the 2nd Arab Conference on the Peaceful Uses of Atomic Energy, Oct., Part IIA., pp: 653-660.

Roushdy, H., F. Abdel-Hamid and A.R. Abu-Ghadir, 1997. Impairment of liver and kidney functions in gamma irradiated rats suffering from pesticide toxicity. Isotope and Rad. Res., 29: 11-422.

Scherer, R. and G. Ruhenstroth-Bauer, 1976. Plasma protein profiling: The diagnostic evaluation of disorders in plasma protein composition by a new immunoelectrophoretic method. Clin.Chim.Acta, 66: 417-433.

Sharygin, V.L., M.K. Pulatova, T.G. Shliakova and I.N. Todorov, 2003. Time and dose dependent post irradiation changes of Fe^{+3} transferrin and Cu^{+2} ceruloplasmin pools in blood, their influence on ribonucleotide reductase activity in animal tissues and the effect of radioprotectors. Rad Biol. Radioecol., 43: 662-677.

Sharygin, V.L., M.K. Pulatova, T.G. Shliakova, I. I. Mitrokhinand, I.N. Todorov, 2005. Activation of deoxyribonucleotide synthesis by radioprotectants and antioxidants as a key stage in formation of body resistance to DNA-damaging factors. Izv. Akad. Nauk. Ser. Biol., 4: 401-422.

Shigko, P., C. Korivistoenen, A. Tratnyek, C. Newball and L. Friedman, 1967. Quantative estimation of DNA and RNA in blood and liver homogenate. Anal. Biochem., 19: 514-519.

Soliman, K.M., A. El-Ansary and A.M. Mohamed, 2001. Effect of carnosine administration on certain metabolic parameters in bilharzial infected hamsters. Comp. Biochem. Physiol., 129: 157-464.

Spencer, K. and C.P. Price, 1977. Influence of reagent quality and reaction conditions on the determination of serum albumin by the bromocresol green dye-binding method. Ann. Clin. Biochem., 14: 105-115.

Tan, K.M. and J.K. Candlish, 1998. Carnosine and anserine as modulators of neutrophil function. Clin. Lab. Hematol., 20: 239-244.

Tanaka, R.A., F.M. Ramos, S.M. Almeida, M.R. Vizioli and F.N. Boscolo, 2005. Evaluation of radioprotective effect of carnosine on wound healing in rats. J. Applied Oral Sci., 13: 253-258.

Trinder, D., P. Oates, C. Thomas and E. Morgan, 2000. Localization of divalent transporter 1 to the microvillus membrane of rat doudenal enterocytes in iron deficiency, but to hepatocytes in iron overload. Gut, 46: 270-276.

Vershinina, S., L. Konnovo and E. Zherbin, 1985. Effect of gamma and gamma-neutron irradiation on the composition of the metabolic amino acid pool, blood antiprotease activity and the lifespan of animals. Radiobiologia, 25: 241-245.

Vijayta, D. and D.K. Dhawan, 2005. Radioprotective role of zinc following single radioiodine exposure to red blood cells of rats. Ind. J. Med. Res., 122: 338-342.

Virgili, F., R. Canal, F. Figus, F. Nobili and E. Mengheri, 1999. Intestinal damage induced by Zn deficiency is associated with enhanced SOD activity in rats. Free Radic. Biol. Med., 26: 1194-201.

Weeke, B., 1970. The serum proteins identified by mean of Laurel's crossed-electrophoresis. Scand. J. Clin. Lab. Invest., 25: 269-275.

Yousef, M., H. El-Hendy, F. El-Demerdash and E. Elagamy, 2002. Dietary zinc deficiency induced changes in the activity and levels of free radicals, lipids and protein electrophoretic behavior in growing rats. Toxicology, 175: 223-34.