

RADIOPROTECTIVE EFFECTS OF HIGH PROTEIN DIET, VITAMINS C AND E ON LIVER ACTIVITY, KIDNEY FUNCTION AND SERUM APOPTOSIS RATES OF IRRADIATED RATS

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ABSTRACT

This study designed to investigate the role of high protein diet, vitamin C and vitamin E as radioprotectors in rats followed exposure to 7 Gy whole body gamma irradiation. 120 male albino rats were divided into six equal groups of 20 rats each: control, irradiated, high protein (P), vitamin C, vitamin E and combination C+E+P supplemented groups. The variation in activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), urea and creatinine concentrations were estimated for all groups. Serum apoptosis detection was carried out according to the Enzyme-Linked ImmunoSorbent Assay technique (ELISA). In the irradiated group, an increase in serum ALT and AST activities, urea and creatinine concentrations and apoptosis rate were observed, whereas in almost irradiation treated groups, the reverse was occurred. Based on these biochemical observations, it was concluded that high protein, vitamin C and vitamin E treatment exerts a protective effect against irradiation damage, especially in serum AST, creatinine and apoptosis.

Keyword: Radioprotectors, vitamin C, vitamin E, soybean protein, Liver Activity, Kidney Function and Serum Apoptosis

INTRODUCTION

Military personnel and civilian population are at risk of exposure to radiation from nuclear or radiological attack. The deleterious effects of ionizing radiation in biological systems are mainly mediated through the generation of oxygen-derived intermediates such as hydroxyl radical, super oxide radical and hydrogen peroxide causing various types of tissue damage due to successive free radical reactions (Fang, 1991).

Oxygen free radicals are highly reactive chemical species generated in biological systems during numerous physiological and pathophysiological processes (Marton *et al.*, 2001). In physiological circumstances, they play a role in cellular metabolism and cellular defense systems. Meanwhile, in large amounts they are highly toxic for tissues and cells because they can oxidatively modify and damage various biological systems (Hogg, 1998). When damage is caused at the molecular level, it is usually irreversible and leads to cell death.

Radiation exposure of mammals is known to induce pronounced changes in the metabolism of biological tissues. It is known that liver and kidney are involved in these changes.

Liver is a central organ for many physiological and biochemical processes necessary for the maintenance of life. Morphological alterations

that occur in liver affect many metabolic processes in organism. Peroxide formation induced by ionizing radiation result in the release of some enzymes by interacting with cellular structure and function (Yanardag *et al.*, 2001). Thus, the serum activities of cellular enzymes such as transaminases do increase. It has been reported that radiation results increased urea and creatinine concentrations of serum as an evidence for marked impairment of kidney function (Cadenas and Cadenas, 2002).

Irradiation can induce both DNA and membrane damage (Szumiel, 1994; Haimovitz-Friedman, 1998; and Szumiel, 1998). In many cases, irradiation-induced cell death has been identified as apoptosis (Yanagihara *et al.*, 1995; Barlow *et al.*, 1997; Gobbel *et al.*, 1998a, Haimovitz-Friedman, 1998).

Apoptosis is defined as programmed cell death represents a universal mechanism (pathway) by which undesirable, unhealthy, harmful, unwanted or excess cells are eliminated during the development and maturation of most organisms, tissue homeostasis and in response to injury (Alici *et al.*, 2000; Yan *et al.*, 2000 and Tchell *et al.*, 2001). In the same context, Guido *et al.* (1995) reported that cell death is involved in the removal of superfluous and damaged cells in most organ system. It has been shown that many kinds of cells including thymocytes, circulating lymphocytes, (Cui *et al.*, 1999) and resident peritoneal macrophages (Kubota *et al.*, 2004) undergo apoptosis after exposure to clinically relevant doses of ionizing radiation. Apoptosis could be characterized by several biochemical and morphological changes, including DNA fragmentation, impairment of ATP synthesis, chromatin, endoplasmic reticulum-derived vacuoles and the "bubbling" of cytoplasm. The condensed nucleus is fragmented into membrane-enclosed "apoptotic bodies". The final biochemical characteristic of apoptosis is nuclear DNA fragmentation into oligonucleosomal subunits, that can be recognized from random cleavage observed in cells undergoing necrosis.

There are several methods defense mechanisms that protect living organisms against free radicals. Radioprotectors could be identified as chemical compounds capable of ameliorating the biological influences of ionizing radiation when administered before radiation exposure. The efficiency of these radioprotectors is greatly dependent on their chemical properties, period of treatment and the post irradiation time elapsing after radioprotectors application (Monig *et al.*, 1990).

Antioxidants such as vitamins E and C are substances that when present in low concentrations relative to the oxidizable substrate significantly delay or reduce oxidation of the substrate, or they are substances that protect other chemicals of the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body, hence hindering the process of oxidation. During this reaction the antioxidant sacrifices itself by becoming oxidized. However, antioxidant supply is not unlimited as one antioxidant molecule can only react with a single free radical. Therefore, there is a constant need to replenish antioxidant resources, whether endogenously or through supplementation (Halliwell and Gutteridge, 1995).

Soybean seed is considered as one of the best sources of plant protein because the quality of amino acids in soy protein is approximately equivalent to animal protein in spite of its deficiency in methionine (Morita *et al.*, 1997 and Anderson *et al.*, 1999). Also, soybean supplementation has an antioxidant activity which prevents the generation of oxidative stress (Abdullaev *et al.*, 2002).

Because radioactive materials in the environment continuously irradiate us with low dose rate, we should construct an appropriate experimental system to evaluate their biological effects.

Thus the present study was aimed to study the radioprotective effects of vitamins C and E and high soybean protein diet and combination of vitamins C and E and high soybean protein diet on liver and kidney functions and serum apoptosis rate of irradiated rats.

MATERIALS AND METHODS

Chemicals and Reagents:

Vitamin C and vitamin E (α -Tocopherol) were purchased from Adwin Egypt Company and ACF Chemical Company, Holland, respectively. Soybean protein were supplied from Food Technology Institute, Agricultural Research Center, Giza, Egypt. The determination of serum ALT and AST were measured using commercial kit supplied from BioMérieux Vitek, Inc., USA. The colorimetric determination of serum urea was measured using commercial kit supplied from Human Laboratory, Wiesbaden, Germany. The determination of serum Creatinine was measured using commercial kit supplied from Stanbio Laboratory, Inc. San Antonio, Texas, USA. Cell death detection (Apoptosis) was measured using a commercial kit derived from Roche Diagnostics GmbH, Roche Molecular Biochemicals, Mannheim, Germany.

Irradiation Source :

The irradiation source used in this study is Cobalt -60 (gamma -cell 220), Atomic Energy of Canada limited, installed at the Middle Eastern Regional Radioisotopes Center for the Arab Countries, Dokki, Cairo.

Animal Diet:

The balance diet consists of 60% corn, 20% soybean, 10% growth additives, 5% wheat bran and fibers, 2.75% molasses, 1.5% powdered bone, 0.5% table salt and 0.25% vitamins, supplied from National Research Centre, Giza.

Highly protein diet consists of the same composition of the balanced diet with the excess of protein as soybean to reach 30%.

Experimental Design:

One hundred and twenty male albino Sprague Dawley rats (body weight from 120 to 140 grams) were obtained from the Animal House, at the National Research Centre, Dokki, Giza. They were randomly divided into six groups of twenty animals each and housed in wire-bottom cages with

controlled ambient temperature (26-32°C) and the mean relative humidity was 60% (range from 50 to 70), at the Animal House, Radioisotopes Department, Atomic Energy Authority, Giza. All animals were freely fed on standard rodent pellets and clean water offered *ad-libitum* throughout the adaptation period.

Group I: rats received balanced diet for three weeks, served as control.

The other animals were exposed to whole body gamma irradiation (7 Gy) and divided into:

Group II: rats received balanced diet for two weeks, served as irradiation control.

Group III: rats fed daily for one week before γ – irradiation and two weeks post γ irradiation exposure on highly protein diet.

Group IV: Animal fed on balanced diet and received vitamin C daily at a dose level of 100 mg/kg body weight orally via stomach tube for one week before γ -irradiation and for two weeks post γ -irradiation exposure.

Group V: Animal fed on balanced diet and received vitamin E daily at a dose level of 50 mg/kg body weight orally via stomach tube for one week before γ -irradiation and two weeks post γ -irradiation exposure.

Group VI: combination group : Animals fed on highly protein diet, and treated with vitamin C and vitamin E at the same dose level as in group IV, V, respectively, orally via stomach tube for one week before γ -irradiation and continued for two weeks post γ -irradiation .

Blood Sampling:

Blood samples were withdrawn from optical nerve plexus at the end of every week once for three weeks from the beginning of the study .The blood samples were collected in plane test tube, then kept at 37°C till coagulation and then centrifuged for 15 minutes at 3000 rpm .The sera samples were thereafter separated and stored at – 20°C till analysis.

Biochemical Investigation:

The following are known spectrophotometric biochemical determinations. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured according to a procedure by Thefeld, (1974). Serum urea was determined by enzymatic colorimetric method described by Fawcett and Scott (1960). Determination of serum creatinine was carried out according to the method of Cook , (1975) . Serum apoptosis detection was carried out according to the Enzyme Linked ImmunoSorbent Assay technique (ELISA) established by method of Gougen and Montagnier (1993).

Statistical Analysis:

All values were presented as mean \pm S.D. significant differences among the various experimental and control groups were established by means of the student's t-test using Microsoft excel for Office 2003.

RESULTS AND DISCUSSION

Liver Function:

Serum ALT and AST Activities:

Table (1) presents the results for serum ALT activity in the experimental groups at three time intervals.

At the end of pre- γ -irradiation week, no differences were observed between control and all supplemented groups. In comparison with control at the end of 1st and 2nd weeks post γ -irradiation, a high significant increase of the serum ALT activity was observed for the irradiated group. This result is in agreement with Abdel Gawad and Amer (2001), Ramadan *et al.* (2001) and Abdel Gawad *et al.* (2003). In the groups receiving vit. C, vit. E and combination (vit. C + vit. E + P), at the end of 1st week post γ -irradiation, the same finding as in irradiated group was found with clear modulation in vit. C group at the end of 2nd week post γ -irradiation.

Table(1): Serum ALT activity(U/L) of the experimental groups within the time intervals.

Group	Control	Irradiated	Protein	Vitamin C	Vitamin E	E+C+P
Pre-irradiation						
Mean \pm S.D.	23.4 \pm 2.30		25 \pm 1.87	25.4 \pm 3.65	23.2 \pm 1.79	26.8 \pm 2.86
Change %			6.8	8.6	-0.9	14.5
1st week post-irradiation						
Mean \pm S.D.	21.2 \pm 2.86	29.6 ^{**} \pm 4.34	15.8 ^{**c} \pm 0.84	34.6 ^{***} \pm 4.34	34.2 ^{***} \pm 4.03	33.2 ^{***} \pm 3.70
Change %		39.6	-25.5	63.2	61.3	56.6
2nd week post-irradiation						
Mean \pm S.D.	20.4 \pm 2.61	34.7 ^{***} \pm 3.12	20.8 ^c \pm 2.28	28 ^{**b} \pm 3.16	35 ^{***} \pm 3.67	36 ^{***} \pm 4.06
Change %		70.1	2	37.3	71.6	76.5

* Slightly significant to control, ** Moderately significant to control, *** Highly significant to control.

a, Slightly significant to irradiated group; b, Moderately significant to irradiated group; c, Highly significant to irradiated group.

On the other hand, when compared to control, an insignificant effect was observed in serum ALT activity of high protein supplemented diet group but significant change compared to irradiated group. That means that serum ALT activity of high protein supplemented diet group was not affected by γ -irradiation, whereas had a protective effect within the experiment period against radiation.

Table (2): Serum AST activity (U/L) of the experimental groups within the time intervals.

Group	Control	Irradiated	Protein	itamin C	itamin E	E+C+P
Pre-irradiation						
Mean ± S.D.	73.76 ± 5.48		74.88 ± 4.02	71.6 ± 5.26	56.6 ^{***} ± 5.46	55.08 ^{***} ± 3.39
Change %			1.5	-2.9	-23.3	-25.3
1 st week post-irradiation						
Mean ± S.D.	73.6 ± 9.32	90.6 [*] ± 8.53	75.8 ^a ± 7.69	73.2 ^b ± 5.81	58.2 ^c ± 8.47	63.8 ^c ± 6.50
Change %		23.1	3	-0.5	-20.9	-13.3
2 nd week post-Irradiation						
Mean ± S.D.	72 ± 8.80	127 ^{***} ± 8.69	106.8 ^{***b} ± 7.01	91.8 ^{***c} ± 8.23	82.6 ^c ± 3.58	57.6 ^c ± 8.91
Change %		76.4	48.3	27.5	14.7	-20

* Slightly significant to control, ** Moderately significant to control, *** Highly significant to control. a, Slightly significant to irradiated group; b, Moderately significant to irradiated group; c, Highly significant to irradiated group.

The activity of serum AST for the irradiated group significantly was increased at 1st week post γ -irradiation compared to control and this increase became more and very highly significant at the end of the 2nd week post γ -irradiation (Table2). This result is in agreement with Abdel Gawad and Amer (2001), Ramadan *et al.* (2001) and Abdel Gawad *et al.* (2003).

When compared to control, an insignificant effect was observed in serum AST activity of the supplemented diet groups at pre- γ -irradiation week. The same result was observed at the end of 1st week post γ -irradiation in spite of exposure these groups to 7 Gy γ -irradiation. However, at the end of the 2nd week post γ -irradiation, the supplemented diet groups with vit. C, vit. E and high protein diet had significant increase in the serum AST activity compared to control. In post- γ -irradiation period, a significant reduction in serum AST activity was observed in all supplemented diet groups compared at that of the irradiated group. It is in agreement with Abdel Gawad *et al.* (2003), they reported that intraperitoneal injection of γ -irradiated rats, at a dose level of 7 Gy, with vit. E caused a significant reduction in serum activity of AST compared to irradiated rats.

The results of the present study revealed that, γ -irradiation increases both serum AST and ALT activity level which mainly due to the release of both enzymes in blood. The increases in the serum activities of these enzymes were directly proportional to the degree of cellular damage (Yanardag *et al.*, 2001). During the post γ -irradiation period, the increment of ALT activity was clearly noticed more rapid than AST activity. This may return to the fact concluded that, in acute hepatic cell injury the level of ALT activity is affected and elevated more than AST activity level. On the other hand, at the end of post γ -irradiation period, ALT activity was decreased by high protein and vit.C supplementation, while AST activity was decreased for all supplemented diet groups. This decrease may be due to the antioxidant effects which caused preventing the formation of free radicals or to quench their cell damaging effects and protect the cell against lipid peroxidation (Packer, 1991; Jaime, 2002 and Ikeda *et al.*, 2004).

Kidneys Function:

Serum Urea Concentration:

At the end of pre- γ -irradiation week, vit. C and vit. E supplemented diet groups had no differences compared to control in serum urea concentration. Conversely in both highly protein and combination groups showed highly significant increase in serum urea concentration as given in Table (3).

Table (3): Serum urea concentration (mg/dl) of the experimental groups within the time intervals.

Group	Control	Irradiated	Protein	Vitamin C	Vitamin E	E+C+P
Pre-irradiation						
Mean \pm S.D	2.8 \pm 2.16		52.54 ^{***} \pm 0.95	45.3 \pm 4.78	42.5 \pm 2.03	55.02 ^{***} \pm 1.58
Change %			22.8	5.9	-0.7	28.6
1st week post-irradiation						
Mean \pm S.D	5.52 \pm 4.34	67.58 ^{***} \pm 6.21	107.8 ^{***a} \pm 6.29	13.84 ^c \pm 2.02	16.46 ^b \pm 8.62	14.14 ^{***} \pm 5.46
Change %		48.5	77.5	-3.7	2.1	40.9
2nd week post-irradiation						
Mean \pm S.D	3.6 \pm 4.44	50.94 ^a \pm 4.57	117.4 ^{***c} \pm 11.55	55.1 ^{***c} \pm 5.29	47.98 \pm 2.74	76.9 ^{***c} \pm 5.73
Change %		16.8	110.4	73.2	10.1	76.4

* Slightly significant to control, ** Moderately significant to control, *** Highly significant to control.

a, Slightly significant to irradiated group; b, Moderately significant to irradiated group; c, Highly significant to irradiated group.

At the end of two weeks post γ -irradiation, the levels of urea of irradiated group were clearly higher than control level, also highly protein diet and combination diet groups gave the same results. In the vit. E supplemented diet group, the level of urea had no significant change compared to control through the two weeks post γ -irradiation. In contrast, when comparing to irradiated group, there was a significant decrease at the end of 1st week post γ -irradiation. Whereas in vit. C supplemented diet group gave the same results with the vit. E group at 1st week post γ -irradiation, but at 2nd week post γ -irradiation urea level became higher than that in irradiated group and control.

It is clear that the exposure of rats to 7 Gy γ -irradiation produced renal damage and increased the serum urea concentration. These results come similar to previous investigations obtained by EL-Gabry *et al.* (2003) and Badr EL-Din (2004). This increase in urea level could be considered as a reflection of deteriorating renal performance (Geraci *et al.*, 1990) due to the ammonia formed by deamination of amino acids in liver, which converted to urea. While in supplemented diet rats with only antioxidant vitamins E and C, both of vit. C, especially in 1st week post γ -irradiation, and vit. E within experiment period acts as an effective antioxidants of major importance for protection against degenerative processes caused by oxidative stress (Olas and Wachowicz, (2002) and Kanter *et al.* (2005)).

In vit. C supplemented diet group at 2nd week post γ -irradiation, the protective effect of vit. C as antioxidant which noticed at 1st week did not

continue, it could be attributed to the dose of vit. C which used in the present study (100 mg/kg bw). This finding could be in agreement with Kanter *et al.* (2005), they reported that vit. C increases tissue protection against diseases and degenerative processes caused by oxidative stress when they used 500 mg/kg bw.

On the other hand, the high increase of urea level at all the experiment period in both highly protein and combination supplemented diet rats may attribute to high protein percentage in the diet of these groups which gave urea as the exogenous end product of protein intake. Protein quality, the proportion of essential amino acids in a food relative to their proportion in proteins undergoing synthesis is, however, of critical importance. Excess amino acids are not stored. Regardless of source, those not immediately incorporated into protein are rapidly degraded (Murray *et al.*, 1993).

Serum Creatinine Concentration:

As seen in Table (4), serum creatinine level was not altered in all supplemented diet groups compared to control in the period of pre- γ -irradiation. The same observation was reported by Cay and Naziroglu (1999) when they studied the effect of vit. E intraperitoneal injection for 5 weeks in serum Creatinine level.

In the irradiated group at the end of the two weeks post γ -irradiation, the level of creatinine was clearly higher than the control level. This result is in agreement with EL-Gabry *et al.* (2003) and Badr EL-Din (2004). The levels of serum creatinine for the two supplemented diet groups with vit. C and vit. E were insignificantly different compared to control till the end of the experiment, while they had a significant decrease in creatinine level when compared to the irradiated group at the end of 2nd week post- γ -irradiation. In high protein group, there was an insignificant difference at the end of 1st week post γ -irradiation, while at the end of 2nd week post γ -irradiation the creatinine level was significantly decreased compared to control. This group showed a highly significant reduction effect compared to the irradiated group within the period of post- γ -irradiation.

Table (4): Serum Creatinine (mg/dl) concentration of the experimental groups within the time intervals.

Group	Control	Irradiated	Protein	Vitamin C	Vitamin E	E+C+P
Pre-irradiation						
Mean \pm S.D.	0.19 \pm 0.02		0.21 \pm 0.04	0.18 \pm 0.01	0.22 \pm 0.03	0.21 \pm 0.04
Change %			7.3	-5.2	14.6	7.3
1st week post-irradiation						
Mean \pm S.D.	0.2 \pm 0.02	0.25 ^{**} \pm 0.02	0.19 ^c \pm 0.01	0.23 \pm 0.05	0.21 \pm 0.04	0.30 ^{**} \pm 0.05
Change %		27	-7	13	7	52
2nd week post-irradiation						
Mean \pm S.D.	0.2 \pm 0.03	0.30 ^{**} \pm 0.05	0.12 ^{**c} \pm 0.04	0.23 ^a \pm 0.04	0.22 ^a \pm 0.04	0.22 ^a \pm 0.04
Change %		51	-42	13	12	12

* Slightly significant to control, ** Moderately significant to control, *** Highly significant to control. a, Slightly significant to irradiated group; b, Moderately significant to irradiated group; c, Highly significant to irradiated group.

The combination diet group at the end of the 1st week post γ -irradiation had the same trend that noticed in irradiated group, however in the 2nd week post γ -irradiation this trend changed to reach the level of the control.

From these results, the exposure of rats to 7 Gy γ -irradiation induced increase creatinine level in serum which serve as an index of renal function impairment (Farag, 1994).

The antioxidant supplementation diet has proven to be beneficial in decreasing the oxidative stress and preventing the renal tissue damage which induced by exposing to γ -irradiation. This finding is in agreement with Kanter *et al.* (2005), they revealed that vit. C treatment reduced serum creatinine elevation which induced as reflect of renal tissue damage and with Cadenas and Cadenas (2002), they reported that vitamins (A, C and E) are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations without harmful side effects.

Serum Apoptosis:

Table (5) represents the rates of serum apoptosis of the experimental groups. At the end of pre- γ -irradiation week, rate of apoptosis of groups which gave supplemented diet with vit. C and /or vit. E and combination (E+C+P) was decreased significantly compared to control (61.5%, 32.5% and 42.2%, respectively, $P < 0.001$). While for the high protein supplemented diet group, the apoptosis rate was near to control.

Table (5): Serum apoptosis rate of the experimental groups within the time intervals.

Group	Control	Irradiated	Protein	Vitamin C	Vitamin E	E+C+P
Pre-irradiation						
Mean \pm S.D.	0.03 \pm 0.003		0.03 \pm 0.01	0.01 [*] \pm 0.002	0.02 [*] \pm 0.003	0.02 [*] \pm 0.01
Change %			-3.6	-61.5	-32.5	-42.2
Enrichment factor			0.96	0.4	0.7	0.6
1st week post-irradiation						
Mean \pm S.D.	0.03 \pm 0.003	0.10 [*] \pm 0.005	0.02 \pm 0.001	0.01 \pm 0.001	0.05 \pm 0.002	0.05 \pm 0.002
Change %		197.6	-56.0	-63.9	-53.6	37.4
Enrichment factor		3	0.4	0.4	1.5	1.4
2nd week post-irradiation						
Mean \pm S.D.	0.03 \pm 0.002	0.05 [*] \pm 0.003	0.02 [*] \pm 0.001	0.01 \pm 0.001	0.01 [*] \pm 0.001	0.06 [*] \pm 0.002
Change %		58.3	-39.3	-60.7	-72.0	63.1
Enrichment factor		1.6	0.6	0.4	0.3	1.6

* Slightly significant to control, ** Moderately significant to control, *** Highly significant to control.

a, Slightly significant to irradiated group; b, Moderately significant to irradiated group; c, Highly significant to irradiated group.

At the end of 1st post γ -irradiation week, the irradiated group demonstrated high significant increase compared to control (197%, $P < 0.001$), while in 2nd post γ -irradiation week this change was lower but still highly significant (58.3%, $P < 0.001$).

Compared to control, apoptosis was highly significant decreased in vitamin C and high protein diet groups (63.9% and 56%, respectively, $P < 0.001$) at the end of 1st post γ -irradiation week while in vitamin E and combination diet groups, apoptosis rates were increased significantly (53.6% and 37.4%, respectively, $P < 0.001$).

On the other hand, all supplemented diet groups had lower significantly the rates of apoptosis than irradiated group at the 1st post γ -irradiation week.

As seen in Table (5), all supplemented diet groups (vitamin E, C and high protein) reduced rate of apoptosis significantly compared to control group at the end of 2nd of post γ -irradiation (72%, 60.7% and 39.3%, respectively), except the combination diet group increased the apoptosis rate significantly (63.1%, $P < 0.001$).

At the end of 2nd post γ -irradiation, all supplemented diet groups reduced rate of apoptosis significantly than irradiated group through the period of post γ -irradiation, except the combination diet group had almost the apoptosis rate in irradiated group.

γ -irradiation increases oxidative stress which alters the mitochondrial membrane integrity and releases the mitochondrial cytochrome-c into the cytosol, which increases the caspase-3 activity and apoptosis (Verhagen *et al.*, 2000; and Du *et al.*, 2000), and results in large-scale fragmentation of DNA and condensation of chromatin (Susin *et al.*, 1999 and Daugas *et al.*, 2000). This finding is in agreement with the results obtained in the present study.

While in supplemented diet groups (with vit. C, vit. E and high protein concentration), there was an antiapoptotic activity compared to control and irradiated group. This antiapoptotic activity of vitamin C and vitamin E has been reported by several authors (Straface *et al.*, 1995; Barroso *et al.*, 1997; and Mobio *et al.*, 2000).

The antiapoptotic activity of vitamins C and E may be attributed to reduce the activity of caspase 3 and maintain the Bcl-2 protein in its functional form by their membrane stabilizing action and thus inhibit the release of cytochrome-c from mitochondria. (Ramanathan *et al.*, 2005; and Vijayalakshmi, 2005).

Also, the antiapoptotic activity of high protein diet may be due to antioxidant activity of soybean protein supplementation diet, this finding is in agreement with Yerushalmi *et al.* (2001), and Abdullaev *et al.* (2002), they detected that soybean supplementation diet for old age rats has an antioxidant activity, thus reducing apoptosis by preventing the generation of oxidative stress which is a greater process occur by ageing per se and subsequent stimulation of the mitochondrial permeability transition and release of cytochrome-c from mitochondria which inhibit apoptosis process.

An ideal radioprotectant should offer significant protection against lethality from acute and long-term effects of radiation exposure; be suitable for oral administration and be rapidly absorbed and distributed throughout the

body; cause insignificant toxicological effects, particularly those on behaviour; be readily available and affordable; and be chemically stable to permit easy handling and storage. Antioxidants are one such class of agents, which are nontoxic and moderately radioprotective which include vitamins C and E and soybean protein. It is quite possible that Vitamins C and E and high soybean protein supplementation diet provide protection from γ -irradiation exposure at dose 7 Gy.

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التأثيرات الوقائية للبروتين و فيتامينات ج ، هـ من الاشعاع على نشاط الكبد
ووظائف الكلى ومعدل موت الخلايا المبرمج للفئران المشععة
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تهدف هذه الدراسة الى تقييم دور كلا من فيتامين ج وفيتامين هـ والوجبة عالية التركيز من البروتين كواقبات للاشعاع، ولقد تم ذلك عن طريق تعريض 100 من ذكور الفئران لجرعة عالية من أشعة جاما (7 جراى) حيث قامت التجربة على تقسيم 120 من ذكور الفئران الى ستة مجموعات تحوى كل مجموعة عشرون فأرا على النحو التالى:

- مجموعة ضابطة.
- مجموعة مشععة.
- مجموعة يضاف الى وجبتها الغذائية تركيز اعلى من البروتين.
- مجموعة يضاف الى وجبتها الغذائية فيتامين ج.
- مجموعة يضاف الى وجبتها الغذائية فيتامين هـ.
- مجموعة يضاف الى وجبتها الغذائية تركيز اعلى من البروتين بالاضافة الى فيتامين ج وفيتامين هـ.

تم تقدير نشاط انزيمى الألانين أمينو ترانس فيريز (ALT) والأسبراتات أمينو ترانس فيريز (AST) ، وتركيز كلا من الكرياتنين واليوريا، ومعدل موت الخلايا المبرمج تبعا لطريقة ELISA لكل المجموعات.

أظهرت النتائج أن هناك زيادة فى نشاط انزيمى الألانين أمينو ترانس فيريز والأسبراتات أمينو ترانس فيريز ، وزيادة فى تركيز كلا من الكرياتنين واليوريا، وكذلك فمعدل موت الخلايا المبرمج فى المجموعة المشععة ، بينما كانت النتائج عكس ذلك للمجموعات المعاملة بالاشعاع وامغذيات المختلفة.

من خلال هذه الدراسة يتضح أن هناك تأثير وقائى لكل من البروتين العالى وفيتامين ج ، وفيتامين هـ ضد أضرار الاشعاع وخصوصا فى نشاط انزيم الأسبراتات أمينو ترانس فيريز ، وتركيز الكرياتنين ومعدل موت الخلايا المبرمج.