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RESEARCH ARTICLE

Effect of Ethanolic Ziziphus (*Ziziphus mauritiana* Lam.) Leaves Extract as Radioprotector on Some Biochemical Parameters of γ-Irradiated Male Albino Rats

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Abstract

The biological activities of ethanolic ziziphus leaves extract as radioprotector agent on some biochemical parameters of male albino rats treated with gamma irradiation doses 4 and 6 Gy were investigated. Experimental results showed a significant increase in liver enzymes (AST, ALT and ALP) and kidney functions (creatinine and urea) in rats serum treated with 4 and 6 Gy. While, significant decrease in total protein, albumin and hematological parameters was observed. Orally administration of ethanolic ziziphus leaves extract for 2 weeks has been found to improve the biological parameters activities. High recovery percentages in liver enzymes and kidney functions were noticed in γ-irradiated rats after treatment. Also, hematological parameters, total protein and serum albumin levels were increased significantly after treatment. The ethanolic extract of ziziphus leaves that orally administrated has been found to be used as a radioprotector antioxidant to avoid the hazards of γ-irradiation that could happened to albino rats.

1. INTRODUCTION

(Nias 1998) stated that radiation is a potent mutagen and carcinogen; however, it is also used in the diagnosis and treatment of human diseases. All organisms (e.g., bacteria, plants, or animals, including humans) are exposed everyday to varying amounts of ionizing radiation that interact with molecules, cells, and tissues, causing reversible deviations from homeostatic equilibrium or irreversible damage.

(Mikulikova and Popes 2001) cleared that most organisms are dependent on breakdown of adenosine triphosphate (ATP) to produce energy which is used to power all body function. Generation of free radicals or reactive oxygen species (ROS) during metabolism or as a result of exposure to gamma radiation beyond the antioxidant capacity of a biological system gives rise to oxidative stress, which leads to a decrease in mitochondrial ATP synthesis, causing liver and muscle dysfunction and cell death. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process **(Sian 2003)**.

(Alasalvar et al., 2005) studied that ROS are tightly controlled by antioxidant defense systems, including non-enzymatic radical scavengers as reduced glutathione and enzymes that can either directly detoxify ROS or indirectly regulate their levels. Dietary antioxidants oppose the oxidative damage and lower risk of degenerative disease and thus arises a necessity to extract these antioxidants from the plant matrices. These plant-based dietary antioxidants are believed to have an important role in the maintenance of human health because the endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species **(Fridovich 1998)**.

Search for effective and less toxic radioprotectors lead to increasing interest on natural compounds from dietary ingredients to medicinal herbs. The crude extracts of these plants and their preparation constitute several effective radioprotective drugs. They also work as antioxidants and significantly prevent the cellular damage in terms of lipid peroxidation, free radical scavenging activity, protein oxidation, etc.

The Medicinal plants as illustrated in **WHO 1993** played a key role in the human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material. **(Gupta 1994)** investigated a good number of medicinal plants indicating that promising phytochemicals can be developed for many health problems**.**

Ziziphus mauritiana Lam. belongs to the family *Ramnaceae*. It is called jujube tree or Indian jujube **(Morton 1987; Michel 2002).** The plant is commonly known as magarya in Hause and Whuya in Kilba (Nigeria). The leaves of the plant are used in the treatment of diarrhea, wounds, abscesses, swelling and gonorrhea **(Michel 2002).** They are also used in the treatment of liver diseases, asthma and fever **(Morton 1987). Lawton R.M. 1985** stated that the genus Ziziphus belongs to the buckthorn family (*Rhamnaceae*).

The plant has been studied by **(Ikram et al., 1976; Aynehchi and Mahoodian 1973)** and its chemical composition is well-known **(Younes et al., 1996; Mahran et al., 1996)**. The main constituents of the essential oil were alpha-terpineol (16.4%) and linalool (11.5%). The main neutral hydrocarbons were n-pentacosane forms (81%). Methyl esters isolated from leaves included methyl palmitate, methyl stearate and methyl myristate. beta-Sitosterol, oleanolic acid and maslinic acid were the main aglycones of the glycosides present in leaves. Sugars present in leaves included lactose, glucose, galactose, arabinose, xylose and rhamnose. The plant also contains four saponin glycosides **(Mahran et al., 1996)**. The highest flavonoid content was found in the leaves (0.66%).

Yamini, K. and Gopal, V. (2010) cleared that the possible prevention of the effects of ionizing radiations on biological systems by phytochemicals, plants and herbal extracts are known as "Natural Radioprotectants". However, to reduce the toxic effects of synthetic compounds, there is a need to explore the compounds; which could be less toxic and highly effective at non-toxic dose. An intensive area of research promotes such type of compounds lies in the use of natural compounds. The use of natural compounds for improving human's health has augmented in modern time. Therefore, it is quite efficient that the choice of alternative radioprotectors falls on plants/ plant products. But, their use as radioprotectors needs scientific evaluation and validation. Once this is done, natural radioprotectors could be more successful than synthetic chemicals **(Weiss J.F. and Landauer M.R., 2003).**

(Monig et al., 1990) cleared that radioprotectors could be identified as chemical compounds capable of ameliorating the biological influences of ionizing radiation when administered before and after 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**.**

The aim of this study was to investigate the potential radioprotective effects of ethanolic ziziphus leaves extract on some biochemical parameters to prevent the oxidative damages caused by gamma irradiation (4 and 6 Gy) in irradiated male albino rats.

2. MATERIALS AND METHODS

Ziziphus (*Ziziphus mauritiana* Lam.) leaves were obtained from the farms of Atomic Energy Authority, Inshas. All chemicals used in these experiments were provided from Sigma Chemical Co. of high quality and purity.

2.1 Irradiation Source and Technique:

The source of irradiation was ^{137}Cs gamma cell 40, installed in the National Center for Research and Radiation Technology (NCRRT), Nasr City, Cairo, Egypt. Rats were placed into cages. These cages were placed in the irradiation chamber of the gamma cell. The doses used for animals were 4 and 6 Gy at a dose rate = 0.475 Gy/min throughout the period of irradiation.

2.2 Experimental animals:

A total of 105 male albino rats "Swiss strain" weighting 120-150 gm per each were used in this study. Rats were provided from the rats' farm of National Research Centre, Dokki, Giza and aminated for 14 days in the animals' house of Nuclear Research Centre, Atomic Energy Authority under normal conditions. Animals allowed free access of water and fed on a diet at according to **Reeves et al., (1993).**

2.3 Experimental Design:

The experiment was carried out to study the curative and radioprotective effect of ethanolic extracts of ziziphus leaves on γ-irradiated rats at both doses of 4 and 6Gy.

Orally administrated doses for rats were prepared by dissolving 12.5 gm of ziziphus leaves extract in 50 ml distilled water at a concentration of 250 mg/ml just before experimental use. This suspension was given to γ-irradiated rats orally by gauge for 2 weeks and every rat was received ethanolic ziziphus leaves extract in concentration of 200 mg/kg body weight. The selective doses were literature based on **(Thirunavukkarasu C. et al., 2005)**. Rats were received orally the desired doses of ethanolic extract in volume of 0.96 - 140 µl/dose for 14 consecutive days before γ-irradiation. Animals were divided randomly into equal 7 groups (15 rats for each) as follows:

Group 1: Rats were fed on the diet as control group.

Group 2: Irradiated rats were fed on the diet daily for 2 weeks and γ-irradiated at a dose of 4Gy.

Group 3: Irradiated rats were fed on the diet daily for 2 weeks and γ-irradiated at a dose of 6Gy.

Group 4: Rats were fed on the diet and received orally ethanolic ziziphus leaves extract daily for 2 weeks at the dose of (200) mg/kg B.W. and γ-irradiated at a dose of 4Gy.

Group 5: Rats were fed on the diet and received orally ethanolic ziziphus leaves extract daily for 2 weeks at the dose of (200) mg/kg b.w. and γ-irradiated at a dose of 6Gy.

2.4 Identification of chemical composition in ethanolic ziziphus leaves extracts:

Aglient 6890 gas chromatograph equipped with an Aglient mass spectrometric detector was used for identification.

2.5 Ethanolic extraction of ziziphus leaves:

The ziziphus leaves were collected from a piece of land at Inshas. The leaves samples were washed then air dried at room temperature and blended to a mesh size of 1mm. The blended samples (1kg) put in 4 liters of 70% ethanol for 48 hours filtered and concentrated to dryness using rotary evaporator. The ethanolic extract was kept in the refrigerator until usage **(Japon-Lujan, R. and Luque de Castro, M.D. 2006).**

2.6 Determination of total phenolic compounds:

Total phenolics in ethanolic extract of ziziphus leaves were determined according to the Folin-Ciocalteu procedure **(Waterman and Simon 1994)**. His calculation as gallic acid equivalents (GAE) in mg per kg of dry plant material.

2.7 Blood Sampling:

Blood samples were collected after one day, one week and 2 weeks from 3 groups of rats (control, γ-irradiated and γ-irradiated rats treated with extract). Blood samples were obtained from the retro-orbital plexus veins from the individual rat according to the procedure described by **(Schermer 1967)** by means of fine capillary heparinized tubes. Blood was divided into two parts; one was collected into a plain centrifuge tube for serum preparation to assay the biochemical parameters of blood including liver function tests, kidney function tests and biochemical parameters. The second part was collected into heparinized tube for assay the complete blood picture. Serum was prepared after blood collection and separated to 16 individual ependorf tubes stored at -40°C until biochemical measurements.

2.8 Hematological Analysis:

Whole blood samples (12 µl) were evaluated using the ABC Vet Hematology Analyzer (Heska, Waukesha, WI). White blood cell (WBC), red blood cell (RBC), platelets count and hemoglobin concentrations were determined according to **Tawfik et al., (2009).**

2.9 Biochemical Analysis:

2.9.1 Determination of Serum ALT and AST:

Two transaminases namely alanine transaminase (ALT) and aspartate transaminase (AST) were determined according to **Reitman and Frankel (1957)**.

2.9.2 Determination of Alkaline phosphatase (ALP):

The alkaline phosphatase acts upon the AMP-buffered sodium thymolpthalein monophosphate. The addition of an alkaline reagents stop enzymes activity and simultaneously develops a blue chromogen, which is measured photometrically at 590 nm. **Young, D.S., (1975)**.

2.9.3 Determination of Serum Total Protein:

Serum total proteins were determined using Biuret method performed by **Doumas (1975)**. Proteins form a violet – blue complex with copper ions in alkaline solution. The complex color was measured at 546 nm.

2.9.4 Determination of Serum albumin:

Albumin was determined in serum according to the method described by **Doumas et al., (1971)**. Albumin reacts with bromocresol green to yield green color which measured at 628 nm.

2.9.5 Determination of Serum urea:

The enzymatic colorimetric method for urea assay in blood was measured according to the method described by **Tabaaco et al., (1979)**. Urea is hydrolyzed by urea's enzyme to form ammonia ions which react with a mixture of salicylate, sodium nitroproside and hypochlorite to yield a blue-green color which measured at 630 nm.

2.9.6 Determination of Serum Creatinine:

Creatinine reacts with picric acid in alkaline medium to form a red-orange color which measured at 628 nm according to the method of **Henry et al., (1974)**.

2.10 Statistical analysis of data:

Data were analyzed using SAS program. Differences between means were tested ($P < 0.05$) based on Duncon's method using PROC MEANS (SAS, 1996).

3. RESULTS AND DISCUSSION

3.1 Phenolic compounds and DPPH scavenging activity of ethanolic ziziphus leaves extract:

The ethanolic extract of ziziphus leaves was 13.24%. Total phenolic content of ziziphus leaves was 0.33%. These results are good in agreement with those reported by **Yu et al. (2006) and De Leonardis et al. (2008)**.

3.2 Identification of some antioxidant components of ethanolic ziziphus leaves extract by GC-MS:

Both the molecular weight and the size of the side chain of these molecules can be determined to some extent by **Biemann, K. (1962)**.

Ethanolic extract of ziziphus leaves were introduced separately to GC-MS apparatus model Aglient 6890 gas chromatograph equipped with an Aglient mass spectrometric detector. Each ethanolic extract was scanned for its abundance and its appearance time on the chromatogram. Fragment data on the spectra might offered some explanation of the cleavage processes based on predominant rupture of highly substituted or allylic bonds.

The ethanolic extract of ziziphus leaves showed three main peaks in the region of the resulted spectra Fig. (1). The major peak was pointed out after 6.92 min., while the second one was relatively moderate and appeared at 10.45 min., and the third peak at 21.42 min. Four other very small peaks appeared at 5.05min., 7.97 min., 19.19min., and 20.05min.

3.3 Effect of ethanolic ziziphus leaves extract on some hematological parameters in γ-irradiated rats:

3.3.1 Blood Hemoglobin (HGB):

The data given in Table (1) showed the mean values of blood hemoglobin concentrations for non-irradiated (control), and all γ-irradiated treated groups over a period of 2 weeks. The results showed a highly significant ($P<0.05$) decrease in blood hemoglobin levels in γ-irradiated rats to 9.12 ± 1.58 and 5.98 ± 0.54 g/dl at a dose level of 4 and 6 Gy, respectively after 2 weeks post γ-irradiation. The decrement percentage was about 28.30% and 61.95%, respectively, as compared with corresponding control rats.

Meanwhile, treatment with ethanolic extract of ziziphus leaves for 2 weeks increased the hemoglobin levels to 13.46 ± 0.46 and 8.7 ± 1.32 g/dl with decrement percentage about 6.26% and 48.21% at doses 4 and 6 Gy, respectively in compare with γ-irradiated group.

It was noticed that the radioprotective role of orally administration of ethanolic ziziphus leaves extract before γ-irradiation was more pronounced than γ-irradiated rats group, after 2 weeks of treatment. Results showed that the recovery percentage in dose 4 Gy of orally administrated group with ziziphus leaves extract was higher than in dose 6 Gy.

3.3.2 Red Blood Cells Count (RBC's):

Data shown in Table (2) represented the values of red blood cells count for control (non-irradiated), γ-irradiated and γ-irradiated treated group with ethanolic ziziphus leaves extracts over a period of 2 weeks. The results showed a significant decrease in the red blood cells count at 4 and 6 Gy dose level in **γ**-irradiated rats. The decrease was 3.78 ± 0.72 and 2.88 ± 0.28 $10^6/mm^3$, respectively compared with control group 6.58 ± 0.56 $10^6/mm^3$. After treatment with ethanolic ziziphus extract for 2 weeks, the RBC's count increased significantly to 6.63 ± 0.08 and 4.1 ± 0.58 $10^6/mm^3$ with recovery percentage of 42.98 and 29.75% for 4 and 6 Gy respectively, in compare with **γ**-irradiated group.

It can be concluded that orally administration of ethanolic ziziphus leaves extract before γ-irradiation showed radioprotective effect against γ-irradiation damage on the red blood cells count. **(Pecaut et al., 2002)** showed that sufficient depression in red blood cell numbers and characteristics after γ-irradiation may result in anemia**,** which may be either a decrease in erythropoietic activity or an increase in RBC's destruction. The decrement of RBC's which induced by γ-irradiation exposure may be attributed to the cessation of erythrocytic production in bone marrow loss of cells from the circulation by hemorrhage or leakage through capillary walls and/or direct destruction of mature circulation cells. In the same view, **Nikishkin et al., (1992)** reported that increased permeability in the hemolytic process and the erythrocytic membrane stability was major because of the expressed drop in red blood cells count following **γ**-irradiation.

3.3.3 White blood cells (WBC's):

The obtained results given in Table (3) showed a highly significant decrease in blood total leucocytes count in γ-irradiated rats only at a dose level of 4 Gy and 6 Gy at 2 weeks by 3.16 ± 0.33 and 2.22 ± 0.21$10^6/mm^3$ as compared with corresponding control rats 9.90 ± 0.53 $10^6/mm^3$. While orally treatment with ethanolic ziziphus extract for γ-irradiated rats for 2 weeks showed a significant increase in WBC's count to 6.62 ± 1.04 and 9.7 ± 2.131$0^6/mm^3$ at doses 4 and 6 Gy, with recovery percentage of 52.26 and 77.11%, respectively. It can be concluded that the ziziphus leaves extract showed higher radioprotective effect on γ-irradiated rats.

The observed decline in the total leucocytic count after γ-radiation exposure is in good agreement with **El-Gabry et al., (2003)** who stated that the decline could be attributed to decrease in lymphocytes and neutrophils and/or inhibition of the bone marrow precursors. **(Anwar et al., 1999)** reported that WBC's leucocytes values were higher at single dose of 5Gy gamma radiation doses, which indicate a significant protection of leucocytes by this plant extract. The initial rapid fall in leukocytes count may be due to a fast decline of lymphocytes in peripheral blood that are the most radiosensitive as revealed by differential leukocyte count. **Samarth and kumar (2003)** have reported a depression in the number of leucocytes of gamma irradiated mice. From the obtained results, it can be seen that ethanolic ziziphus leaves extract that was orally administrated before γ-irradiation to rats showed a radioprotective effect against γ-irradiation damage in WBC's count.

3.3.4 Platelets (PT):

Data presented in Table (4) showed that platelets concentrations were dramatically decreased post γ-irradiation in γ-irradiated group from 394.2 ± 55.18 and 301.2 ± 25.15 to 144.4 ± 30.5 and 44 ± 9.86 in doses 4 and 6 Gy, respectively in compare with the control group. While, treatment with ethanolic ziziphus leaves for 2 weeks increased also the platelets concentrations in compare with γ-irradiated group with recovery percentage of 68.83 and 53.48% after 2 weeks of treatment at 4 and 6 Gy, respectively.

(Casarett A.P., 1968) cleared out that the decrease in platelets count is accompanied with decrease in erythrocyte count. Whole-body gamma-irradiation induced direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage, or leakage through capillary walls and

reduced cell production**.** The decrease in the values of hematological parameters following radiation exposure may be assigned to direct damage caused by a lethal dose of radiation.

3.4 Effect of ethanolic ziziphus leaves extract on some biochemical parameters in γ-irradiated rats:

3.4.1 Serum total protein levels:

Serum total protein levels for γ-irradiated group only and γ-irradiated treated groups over a period of 2 weeks of treatment are presented in Table (5). Results showed a significant decrease ($p<0.05$) in serum total protein levels in γ-irradiated rats at a dose level of 4 and 6 Gy by 3.17 ± 0.14 and 2.60 ± 0.21g/dl, respectively as compared with corresponding control rats 6.05 ± 0.06 g/dl after 2 weeks.

Meanwhile, the γ-irradiated rats that orally received ethanolic ziziphus leaves for 2 weeks showed also an elevation in the total protein levels from 3.17 ± 0.14 to 5.81 ± 0.18 g/dl with recovery percentage of 45.43% and from 2.6 ± 0.21 to 5.74 ± 0.25 g/dl with recovery percentage of 54.70% at dose 6 Gy after 2 weeks of treatment.

(Roushdy et al., 1984) stated that marked decrease in total proteins may be attributed to the damage of vital biological processes or due to change in the permeability of liver, kidney and other tissues resulting in leakage of protein via the kidney.

The results are in agreement with **Abbady et al., 2000** who documented a decline in the level of serum total protein in animals exposed to whole body gamma irradiation at different doses and different times of exposure. A significant depression was also noticed in serum Alb content **(Karalenka et al., 1993)**. The remarkable decrease in the serum T.P. and Alb as a result of rat irradiation may be owing to impairment in the synthesis of liver proteins because of hepatic cells damage, loss from circulation by leakage to the urine and/or increment proteins degradation **(Mahdy, 1991)**.

3.4.2 Serum albumin levels:

Data in Table (6) represent the values of serum albumin levels for γ-irradiated and γ-irradiated treated group over a period of 2 weeks of treatment with ethanolic extracts of ziziphus leaves. From the obtained results, it can be shown that a non-significant decrease in serum albumin levels in γ-irradiated rats at a dose of 4 Gy after 2 weeks. While, the serum albumin levels decreased in γ-irradiated rats at dose 6 Gy to 1.99 ± 0.03 g/dl in comparison with control group.

Administration of ethanolic ziziphus leaves extract after γ-irradiation showed an increase in albumin levels to 4.10 ± 0.21 and 3.47 ± 0.08 g/dl with recovery percentage of 7.07 and 42.65% for 4 and 6 Gy, respectively in compare with γ-irradiated group.

Keren (1994) stated that the decrease in concentration of serum albumin might result from excessive loss through injury to the kidneys or gastrointestinal tract or from thermal injury to the skin. It also occurs in hypercatabolic states. In addition, **(Choldhari and Chakrabati, 1983)** stated that this decrease could be related in part to hepatic dysfunction and decreased protein synthesis. These phenomena might be, at least partially responsible for protein loss after irradiation.

3.5 Liver enzymes:

Results Shown in Tables (7, 8, and 9) showed a remarkable increase in serum liver function of γ-irradiated group by 119 ± 1.41, 95 ± 3.54 µ/ml and 104.85 ± 0.86 IU/L for AST, ALT and ALP, respectively at dose 4 Gy. While, results were 134 ± 1.73, 118.2 ± 2.11 µ/ml and 121 ± 1.41 IU/L for AST, ALT and ALP at dose 6 Gy after 2 weeks in compare with control groups. The administration with ziziphus leaves extract for 2 weeks showed a remarkable decrease in AST, ALT and ALP activities at 4 and 6 Gy. The reduction was by 48.9 % and 47.61 % for AST activity and by 36 % and 40.8 % for ALT and by 45.8 % and 44.29 % for ALP activity in comparison with the γ-irradiated group.

It can be concluded that, orally administration of ethanolic ziziphus leaves extracts for successive days resulted in a significant decrease in level of ALT and AST enzymes in serum of γ-irradiated rats.

Similar results were obtained by **El-Gabry et al., (2003)** who found that **t**hese increments in serum enzymes reflected clearly the lesions occurred in liver function after its cellular damage by γ-irradiation and consequently the elaboration of its intracellular enzymes into the blood stream. These recorded elevations could be also due to a hypoxia state in the parenchymal liver cells and increased permeability of cell membrane **(Ghanem 1984)** or mitochondrial membrane **(Todorov and Damianov 1985)** causing the release of intracellular enzymes into circulation.

3.6 Effect of ethanolic ziziphus leaves extract on some kidney functions in γ-irradiated rats:

The data presented in Table (10) and (11) represent the values of serum urea, and creatinine levels for γ-irradiated rats only and γ-irradiated treated groups over a period of 2 weeks. From these data it was shown a highly significant ($p<0.01$) increase in serum urea levels in γ-irradiated rats at a dose level of 4 and 6 Gy for 2 weeks post γ-irradiation as 48.80 ± 1.16 and 56.00 ± 1.52 mg/dl, respectively, compared with corresponding control rats 35.60 ± 1.21mg/dl.

Treatment with ziziphus leaves extract for γ-irradiation rats for 2 weeks led to a decrease in the urea levels to 36.2 ± 0.58 and 40.4 ± 0.81 mg/dl with recovery percentage of 25.81 and 27.85% for doses 4 and 6 Gy, respectively in compare with γ-irradiated group.

Data shown in Table (11) show significant ($p<0.05$) increase in serum creatinine levels in γ-irradiated rats only at a dose level of 4 Gy and 6 Gy for 2 weeks post γ-irradiation were found to be 1.1 ± 0.06 and 1.87 ± 0.15 mg/100ml as compared with corresponding control rats 0.63 ± 0.03 mg/100ml while the treated rats group that received orally ethanolic extract of ziziphus leaves showed a reduction in creatinine levels at doses 4 and 6 Gy by 0.72 ± 0.06 and 0.8 ± 0.06 mg/100ml with recovery percentage of 34.54 and 57.21%, respectively in compare with γ-irradiated group.

From the previous results it can be concluded that orally administration of ethanolic ziziphus leaves extract before γ-irradiation decreased the level of serum urea and creatinine. It means that this antioxidant was directed inside the body to protect the kidney cells activity. These results confirmed with **Montilla et al., (1998)** who investigated the high power of ziziphus leaves ethanolic extract as an antioxidant which neutralized the damaging effect of the both free radicals (hydroxyl and peroxyl) and regulated the antioxidative defense enzyme system in the kidney tissues and these are in agreement with.

The current study depicted that γ-irradiation at a dose of 6 Gy induced significant increase in the serum levels of the non-protein nitrogen compound represent by urea and creatinine, as indices for kidney function. These results come similar to previous investigations by **Badr El-Din, (2004).** These increments could be considered as a reflection of deteriorating renal performance as reported by **(Geraci et al., 1990)** due to the ammonia formed by deamination of amino acids in the liver, which converted to urea. As creatinine is formed largely in muscles and occurs freely in blood, its increased levels in serum serve as an index of renal function impairment **(Farag, 1994)**.

Table (1): Effect of ethanolic ziziphus leaves extract on HGB concentrations (g/dl) in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	14.86±0.72	12.72±0.41	15.72±0.47	14.36±0.52	16.8±0.18
One week	14.94±0.70	11.32±0.77	10.50±0.49	12.58±0.07	11.5±0.92
Two weeks	15.08±0.72	9.12±1.58*	5.98±0.54*	13.46±0.46	8.7±1.32

*LSD (0.05) = 1.99

Table (2): Effect of ethanolic ziziphus leaves extract on RBC's count (10^6/mm^3) in γ-irradiated rats

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	6.58±0.56	6.34±0.31	7.56±0.12	7.92±0.11	7.50±0.19
One week	6.58±0.56	5.31±0.31	5.52±0.17	6.39±0.07	5.68±0.47
Two weeks	6.58±0.56	3.78±0.72*	2.88±0.28*	6.63±0.08*	4.10±0.58*

*LSD (0.05) = 1.19

Table (3): Effect of ethanolic ziziphus leaves extracts on WBC's count (10^3/mm^3) in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	9.68±0.58	1.88±0.13	6.16±1.02	2.98±0.62	5.74±2.24
One week	9.86±0.55	3.44±0.51	1.94±0.48	9.38±3.64	2.20±0.88
Two weeks	9.9±0.53	3.16±0.33*	2.22±0.21*	6.62±1.04*	9.70±2.13*

*LSD (0.05) = 3.13

Table (4): Effect of ethanolic ziziphus leaves extract on Platelets concentrations in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	269±26.72	394.20±55.18	301.20±25.15	478.80±37.86	282.60±14.73
One week	269±26.72	128.20±28.03	49.40±4.55	210.40±10.16	52.20±9.30
Two weeks	269±26.72	144.40±30.50*	44.00±9.86*	463.40±55.38	94.60±15.88

*LSD (0.05) = 88.46

Table (5): Effect of ethanolic ziziphus leaves extract on serum total protein (g/dl) in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	6.12±0.27	5.69±0.18	5.98±0.06	5.90±0.33	5.72±0.09
One week	5.86±0.09	4.07±0.21	4.05±0.05	4.93±0.11	4.16±0.32
Two weeks	6.05±0.06	3.17±0.14*	2.60±0.21*	5.81±0.18	5.74±0.25

* LSD (0.05) = 0.52

Table (6): Effect of ethanolic ziziphus leaves extract on serum albumin levels (g/dl) in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	3.71±0.15	3.71±0.15	3.52±0.15	3.39±0.16	3.46±0.10
One week	3.90±0.06	3.90±0.06	2.37±0.08	2.76±0.12	3.27±0.33
Two weeks	3.81±0.06	3.81±0.06	1.99±0.03*	4.10±0.21	3.47±0.08

* LSD (0.05) = 0.39

Table (7): Effect of ethanolic ziziphus leaves extract on AST activity (µ/ml) in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	45.6±0.68	52.60±0.68	56.80±0.58	47.00±0.89	50.40±0.51
One week	46.2±0.73	109.20±0.86	122.80±1.77	66.20±1.59	74.00±1.52
Two weeks	47.2±0.37	119.00±1.41*	134.00±1.73*	60.80±0.86	70.20±0.8

* LSD (0.05) = 2.71

Table (8): Effect of ethanolic ziziphus leaves extracts on ALT activity (µ/ml) in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	33.20±1.28	40.20±1.28	43.40±0.75	40.00±1.22	45.00±1.00
One week	32.40±0.93	83.20±0.73	105.40±2.01	62.20±1.56	68.40±1.44
Two weeks	34.40±1.08	95.00±3.54*	118.60±2.11*	60.80±1.24	70.20±1.24

* LSD (0.05) = 4.08

Table (9): Effect of ethanolic ziziphus leaves extract on ALP activity (IU/L) in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	37.24±0.58	44.80±0.37	49.40±0.51	41.20±0.58	42.20±0.86
One week	40.00±0.71	85.00±1.41	101.00±2.21	61.60±0.93	72.00±1.05
Two weeks	41.60±0.58	104.8±0.86*	121.00±1.41*	56.80±0.73	67.40±0.93

* LSD (0.05) = 2.38

Table (10): Effect of ethanolic ziziphus leaves extract on urea levels (mg/dl) in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	36.2±1.24	34.8±0.86	36.6±1.36	32.6±0.51	37.2±0.58
One week	37.0±0.84	44.0±0.71	48.4±0.51	43.0±1.30	46.0±0.71
Two weeks	35.6±1.21	48.8±1.16*	56.0±1.52*	36.2±0.58	40.4±0.81

*LSD (0.05) = 2.70

Table (11): Effect of ethanolic ziziphus leaves extract on creatinine levels (mg/100ml) in γ-irradiated rats:

Treat. / Period	Control	γ-Irradiated		Ethanolic Ziziphus Leaves Extract	
		4 Gy	6 Gy	4 Gy	6 Gy
One day	0.60±0.06	0.73±0.03	0.80±0.06	0.53±0.03	0.67±0.03
One week	0.63±0.03	0.87±0.03	1.23±0.12	0.78±0.04	0.89±0.05
Two weeks	0.63±0.03	1.10±0.06*	1.87±0.15*	0.72±0.06	0.80±0.06

*LSD (0.05) = 0.12

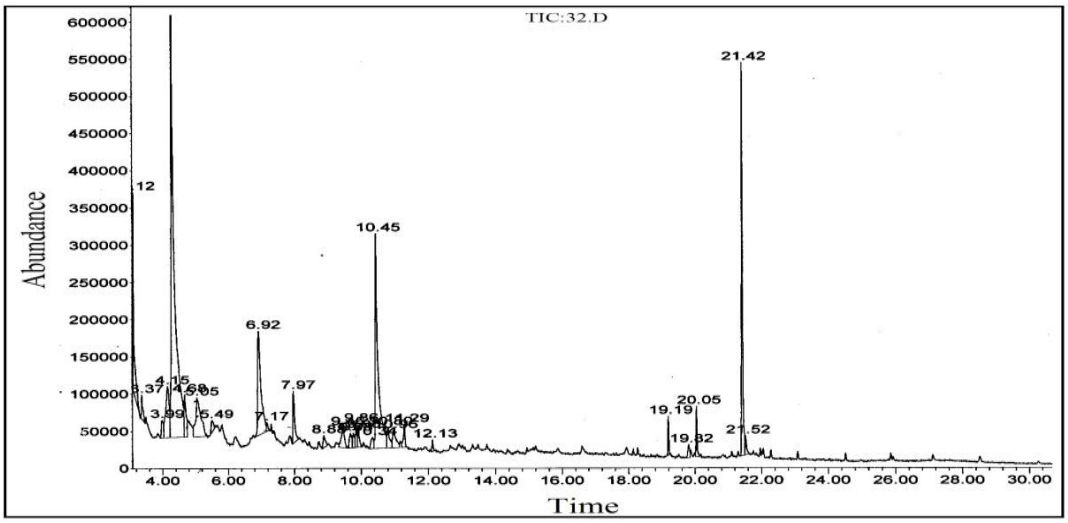

Fig. (1): separation of ziziphus leaves by GC-MS.

4. CONCLUSION

From the above mentioned results it is recommended to use the natural antioxidant e.g. ethanolic extract of ziziphus leaves as radioprotector agent in order to avoid the different hazards which can happen due to γ-irradiation effects.

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