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Protective Capacity of *Fusarium oxysporum* Extract Against Gamma Radiation: A Study for some Biochemical Parameters in Albino Male Rats

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ABSTRACT

The present work Aims to investigate the protective capacity of *Fusarium oxysporum* phosphate buffer extract (FO) against gamma radiation (ɣ-IR) based on evaluating the changes in some blood components in rat model. The variations in haematological and pairs of biochemical parameters were determined for 40 albino male rats divided into five groups : negative control , positive control, i.e. irradiated only , and three treated groups administrated increasing dose of (FO) before single four Gray (Gy) gamma irradiation dose. Three doses of FO extract (0.15, 0.3 and 0.6 mg/g body mass) were chosen, as protective doses, for subsequent evaluating of its antioxidant activity and ameliorating capacity toward radiation-induced oxidative stress. The changes in the chosen parameters, due to administration of the FO and then 4Gy irradiation, were computed after 1, 2, 3 and 7 days. Total serum protein, albumin, total globulin, besides some parameters of complete blood count (CBC) were determined and the data obtained were analyzed to evaluate the capacity of FO to compensate the gamma irradiation injures.

The present work is assuming that biocomponents extracted from FO fungi have a protective role against gamma radiation which can establish protocols for medical management of radiation injuries based on natural medication.

INTRODUCTION

The impact of exposure to irradiation hazards is of great concern to the space exploration community as well as to patients subjected to radiotherapy in medical centers.[1] Commonly, man exposure to radiation happens as a result of radiotherapy treatments, working in nuclear establishments, nuclear battlefields and nuclear accidents.[2] This is in addition to the natural resources including cosmic rays from the surrounding ecologies which constitute about 82 % of total exposure .Ionizing radiation (IR) harms biological tissues by exciting or ionizing their atoms and molecules. According to radiation dose and the biochemical processes, injures can be prompt that could be detected within minutes to weeks post exposure or delayed to several months to years later. [3] When living cells are exposed to ionizing radiation, they react in different ways which vary quantitatively and qualitatively based on the absorbed dose and the cell type and function that generally reflect injuries caused to well-defined cellular components and molecular structures. [4]

The mechanisms by which the gamma rays cause their harm effects can be attributed to the deterioration in cellular large molecules,[5] imbalance in ionic equilibrium and generation of reactive oxygen species (ROS).[6] Radiation damage is, to a large extent, caused by the overproduction of ROS, including superoxide anion ($O^{2\bullet-}$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2), that decrease the levels of antioxidants, resulting in oxidative stress and cellular damage. ROS cause damage by reacting with cellular macromolecules such as nucleotides in nucleic acids, polyunsaturated fatty acids found in cellular membranes, and sulfhydryl bonds in proteins. If this damage is irreparable, then injury, mutagenesis, carcinogenesis, accelerated senescence, and cell death can occur.[7] Ionizing radiations interact with biological systems, also, through free radicals generated by water radiolysis. This indirect

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action plays an important role in the induction of oxidative stress leading to cellular damage and organ dysfunction [8-10]. However, in medicine, there is a limited knowledge on the levels of chemicals, enzymes, and organic waste products that are normally found in the blood after irradiating with different gamma radiation doses.[11] Plants and all living organisms produce countless natural materials addressing their survival, defence, nutrition, and even growth.[12-14] Among those materials are the phenolic compounds such as phenols, flavonoids, coumarins, quinones, saponins, and xanthones, the most abundant, besides alkaloids, lectins, polypeptides, terpenoids, and essential oils.[14-17] Published studies indicated that the aqueous extracts of some plants and fungi have potent antioxidant activity.[18] The antioxidant activity is attributed to the wide range ofthe phenolic compounds in their extracts.[19-23] Fungal -derived extracts and related phytochemicals have gained a particular attention by many researchers concerning their protective role against ionizing radiation.

The CBC and pairs of biochemical parameters can be applied to predict the gamma irradiation exposure injures and the protective improvements due to FO administrations. Those tests are inexpensive, widely available, and easy to interpret.

The objective of this study is to evaluate the protective capacity of *Fusarium oxysporum* phosphate *buffer* extract, (*FO*), against the gamma irradiation impacts in albino male rats.

EXPERIMENTAL APPROACH

Fusarium oxysporum fungus

Fusarium oxysporum fungi were collected from a near soil. The isolated strains were checked up using optical microscope (MEIJI modal ML 2100). The fungi were characterized by their white colours and distinguished colonies' morphology. Moreover, the fungi were characterized by presences of their spores and the unique shape of the cells.

Cultivation of *Fusarium oxysporum* fungi

Erlenmeyer flask (250 ml capacity) containing 100 ml Czapek's sucrose liquid medium was inoculated with *Fusarium oxysporum* fungi disc (~0.5 cm diameter). The flask was incubated at 25 ^{o}C for ten days, then filtrated and the acquired mycelia were washed thoroughly with distilled water many times before their filtration using filter paper Whatman GF/C circle 47 mm (Cat #1822 047), England. The obtained mycelia were kept for the subsequent treatment.

Phosphate Buffer Extraction of *Fusarium Oxysporum* components

Fusarium oxysporum fungal mats (one g each) were mixed with 10 ml of 0.01M phosphate buffer (pH 7.0) in a glass mortar and completely ground. The obtained mixtures were squeezed through several layers of cheese cloth. The filtrates were further clarified by centrifugation at 10,000 rpm for 20 minutes at - 4 ^{o}C using Sigma 2k15-USA centrifuge. The reached clear filtrates were stored at -10 ^{o}C for subsequent use.

Gamma Irradiation Source

A radiocesium gamma cell used for animals' irradiation was the cell-40 at the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), based on Cs-137 as a gamma source at 0.84 Gy/ min. dose rate.

Grouping and Treatment of Adult Male Albino Rats

Forty adult albino male rats, *Rattus norvegicus*, weighing 160-180 g, were purchased from the National Research Center animal house, Giza, Egypt. The rats were let to steadiness for one week before grouping. The rats were then, randomly arranged into five groups; eight animals each, housed in a plastic cage. At the end of equilibrium period, the rat groups were subjected to various treatments following Table (1).

Post irradiation, the animals in the all 5 groups were returned back to their cages and followed up to one week, during this period, blood samples were collected after one day, two, three and seven days. A CBC in addition to total serum protein, serum albumin and serum total globulin contents were determined after 1, 2, 3 and then 7 days from irradiation and FO extract administration to all groups.

Table (1): The various rat groups handled

Rat group	Treatment
Group 1	Control group, fed on normal diet and drink tap water freely
Group 2	Subjected to 4 Gy single dose γ- Irradiation
Group 3	Administrated 0.15mg/g* single dose *FO* phosphate extract , irradiated**
Group 4	Administrated 0.3mg/g* single dose *FO* phosphate extract, irradiated**
Group 5	Administrated 0.6mg/g* single dose *FO* phosphate extract, irradiated**

** Rat's body mass.*

*** Subjected to 4 Gy single dose γ- Irradiation.*

2.3 Evaluation of the Biochemical Parameters

The red blood cells (RBCs) and white blood cells (WBCs) counts were evaluated based on the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to Jain procedure (Jain,1986) [24] using cyanomethaemoglobin technique . Schilling method of differential leukocytes count was applied to evaluate the distribution of the various WBCs fractions. Mitruka & Rawnsley approach was ,also, applied to compute the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).[25] On the other hand , total serum protein , albumin and total globulin were evaluated followingthe method described by Kaplan and Savory.[26]

Ethical Clearance and Approval

Procedures involving animals and their care were conducted in conformity with international laws and policies.The rats were handled according to the International Guiding Principles for Biochemical Research Involving Animals -2012, and the experimental procedures were approved by the Research Ethics Committee of the National Center for Radiation Research and Technology (REC-NCRRT), Egyptian Atomic Energy Authority.

The rats were housed in clean plastic cages, having wood saw dust mats, in conventional animal house that permit 100% fresh air circulation. The mean ambienttemperature in the housing facility was 28 ºC, ranging between 26 – 32 ºC, and the mean relative humidity was 60% (50 – 70 %) with 12 hours light /12 hours dark cycle. The animals were freely fed on a normal rodent pallets diet and clean water offered by *ad-libitum* throughout the whole experimental period.

Statistical Analysis

All data were expressed as the mean ± standard error of mean (SEM) from three independent samples. The experimental data obtained were fitted based on polynomial trend using R-square regression analysis.

The goodness of the model can be checked by the determination of the coefficients (R^2). The values of the determination coefficient $R^2$when near unity suggested that the total variations in data are attributed to the independent variables and only very less of the total variation cannot be explained by the model.

RESULTS AND DISCUSSIONS

Fusarium oxysporum extract assumes to enhance therats' immune systems as it has many antioxidant potentialingredients. It can mitigate the irradiation-induced alterations and can heal many of the harmful injures due to gamma ray exposure.Therefore, *Fusarium oxysporum* extract can be applied as an adjuvant biomedication during radiotherapy treatment for example.

Figures (1-11) show the changes in most interesting haematological parameters in male rats as function of 4 Gy single dose gamma irradiation and administrating increasing doses of *Fusarium oxysporum* extract (mg/g rat's body mass).

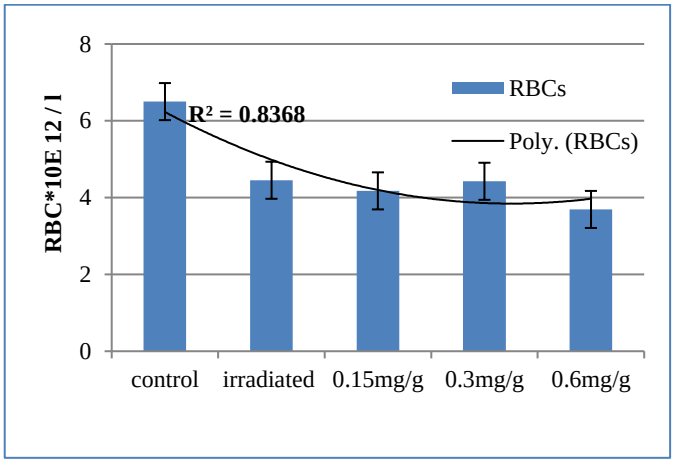

Fig. (1): The changes in the red blood cells (counts /l) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values

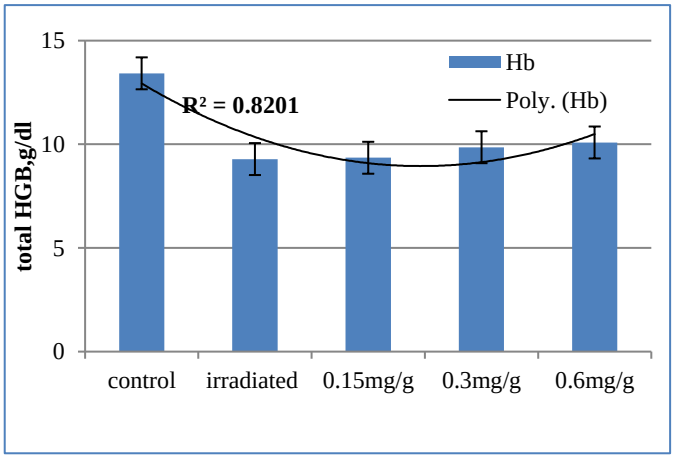

Fig. (2): The changes in the haemoglobin content (g/dl) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

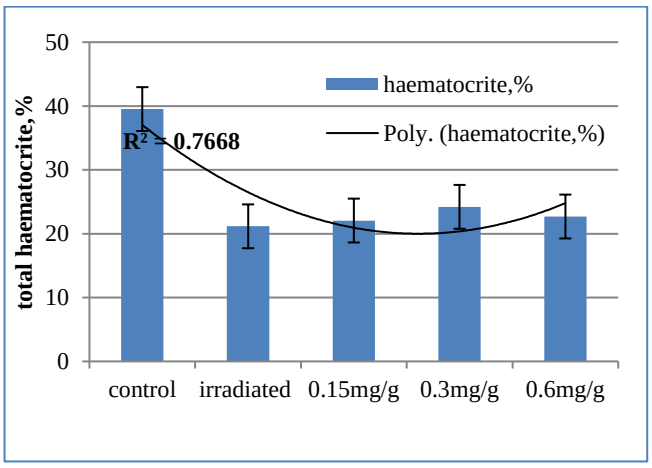

Fig. (3): The changes in the total haematocrite (%) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

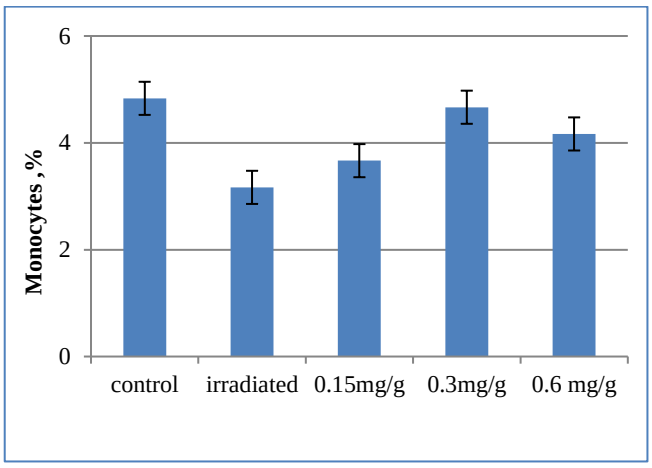

Fig. (6): The changes in the monocytes (%) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

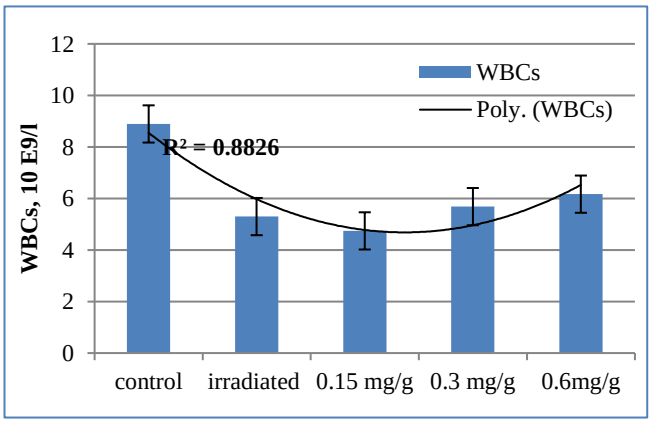

Fig. (4): The changes in the white blood cells (10 E9/l) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

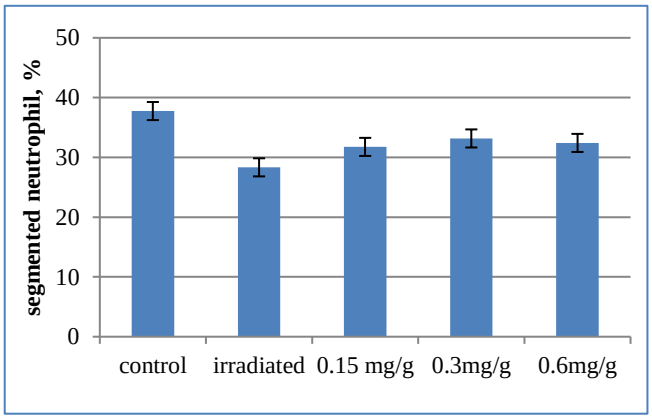

Fig. (7): The changes in the Segmented Neutrophil (%) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum*extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

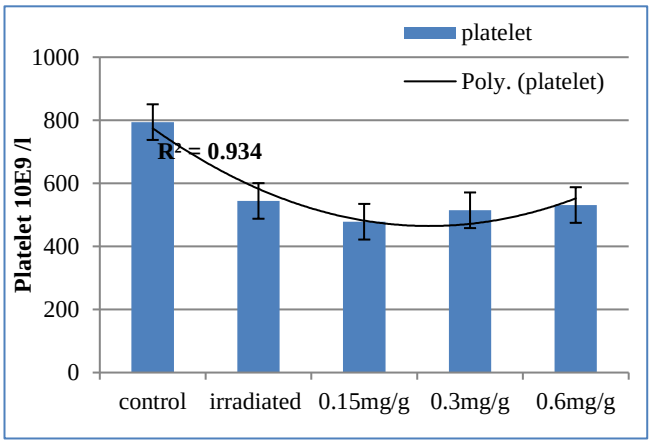

Fig. (5): The changes in the platelet count (10 E9/l) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

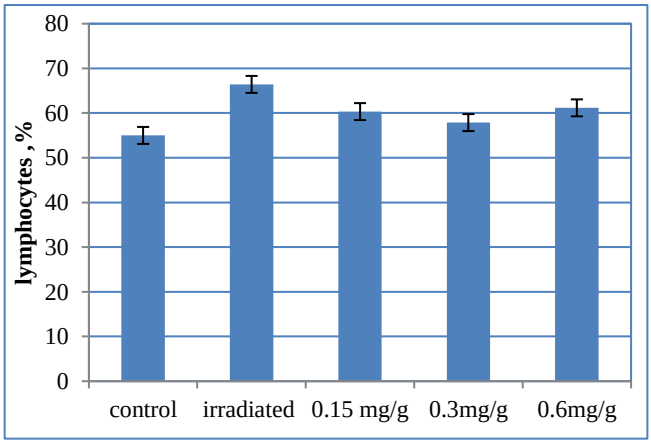

Fig. (8): The changes in the lymphocytes (%) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

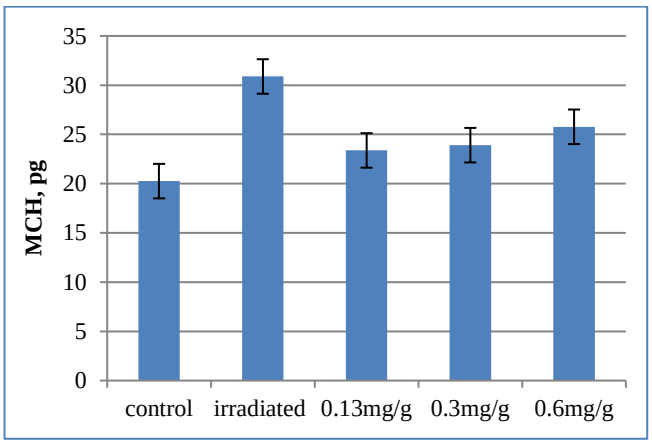

Fig. (9): The changes in the mean corpuscular haemoglobin (pg) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

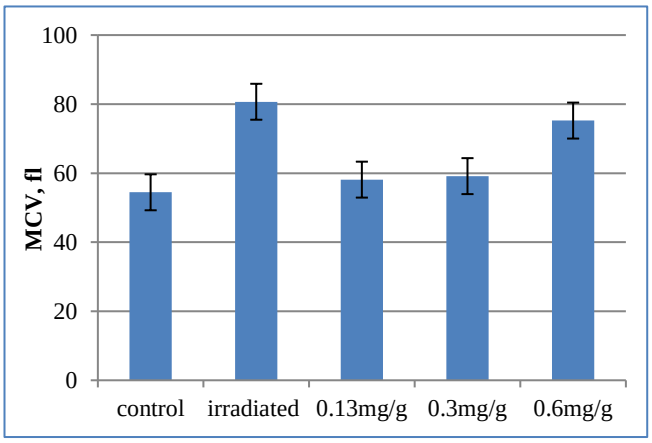

Fig. (10): The changes in the mean corpuscular volume (fl) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

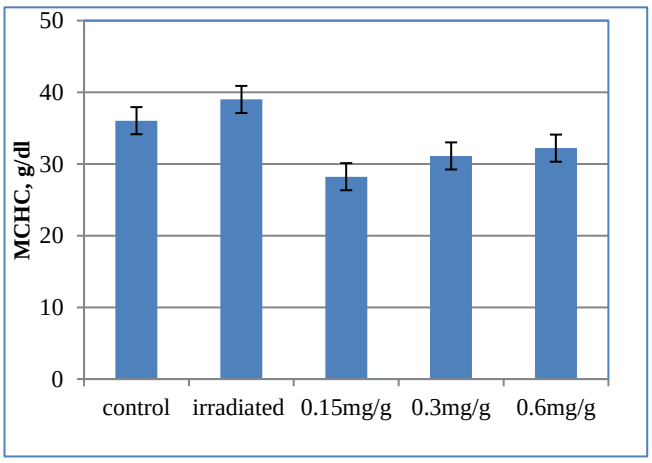

Fig. (11): The changes in the mean corpuscular haemoglobin concentration (g/dl) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

The irradiation induced injures ,in the most cases, started within the first week .[27] There were detectable decreases in RBCs, HGB , Ht, WBCs, platelets, Monocytes and segmented neutrophils in all rat groups exposed to 4 Gy gamma irradiation when compared to the positive control group . On the other hand, lymphocytes, MCV, and MCH increased significantly as the rats exposed to the single irradiation dose, while MCHC indicated non-significant changes due to irradiation treatment (Fig. 11).

In this research, the value of R^2, almost in all relations, was near or greater than 0.8, indicating a high degree of correlation between the observed and predicted values.

The injure effects of γ-radiation on the RBCs can be attributed to the suspension in erythrocytes' production in bone marrow, loss of cells from the circulation by haemorrhage or leakage through capillary walls and/or direct destruction of mature circulating cells. Nikishkin *et al.* [28] reported that increased permeability in the haemolytic process and the erythrocytes' membrane stability was major reason for the detected drop in RBCs count following γ-irradiation. According to Down *et al.*[29] , the haemolysis was the main cause of the developed anaemia following whole body γ-irradiation . Administration the FO extract post γ- irradiation showed clear ameliorations in the RBCs count (Figure 1).

The reduction in haemoglobin content post γ-irradiation can be attributed to the disturbance in the haemoglobin molecule regeneration. This can be correlated to pronounced hyperferraemia after ɣ-irradiation [30], in addition to oxidation of haemoglobin molecule.[31]

On administering FO extract, the rats showed a significant increase in their blood haemoglobin levels as compared with the negative control group. These results are in a good agreement with those reported by Chlebovsky *et al.* [32]

The diminish in haemoglobin and haematocrite contents, (Figs. 2&3), could be attributed to the reduction in RBCs count, and /or a results of erythropoiesis failure, injures of mature cells, escalation in plasma volume or the reduction in peripheral blood elements due to a bone marrow syndrome.[9,33,34]

A detectable diminishing in the total leucocytes count in the irradiated rats reached near 40 % as compared to the positive control animals. Treating the irradiated rats with increasing doses of FO extract accompanied with non-significant enhancement in the WBCs, but the rate of cells regeneration was slower than the rate of leucocytolysis due to ɣ-rays injures. FO is assumed to have ameliorative role against severe reduction in blood

leukocytes by screening injure of the bone marrow. Fig.(4). The decrease in WBCs count in the irradiated rats could be the result of radiation-induced lipid peroxidation, and damage of their cell membranes. In man, it was reported that the recovery of lymphocytes usually begins shortly after completion of irradiation treatment .The total lymphocyte counts can be restored to normal levels in less than 2 yr. The relatively slow recovery of cell mediated immunity after irradiation indicated that total lymphoid irradiation can be subjected to impact of long term immunosuppression.[35]

The decrease in platelets count is co-existed with a decrease in RBCs count (Fig 5). The whole-body single dose ɣ-irradiation can induce a direct destruction of mature circulating cells, loss of cells from the circulation by haemorrhage, or leakage through capillary walls and reduced cell production as stated before. [9] Sanzari *et al.*[1] suggested that radiation-induced coagulopathies and decreased platelet counts are present in animals exposed to the radiation doses up to 2 Gy. The WBCs decreased significantly due to irradiation, as stated, and partially recovered with administration of FO extract, whereas the decrease in platelet counts did not show the same recover as the WBCs even past the extract treatment within the same period i.e. seven days. (Figs. 4 &5). Irradiation can induce leucopenia. [37]

In differential leucocytes count, a significant decline in monocytes and segmented neutrophil percentages were detected in 4 Gy irradiated rat groups as compared to control values. In gamma irradiated rats and administering FO extract, there were pronounced increases in monocytes and segmented neutrophil percentages relative to the irradiated rats, but still lower than the control group animals. (Figs. 6&7).

It is well known that the lymphocytes are an essential part of innate immunity, the early increase in their count post ɣ irradiation could be mostly due to rapid recruitment of these cells from the bone marrow. Administration of FO extract accompanied with a detectable recovery, but not significant, in the lymphocytes percentage within 7 days (Fig. 8).

MCV and MCH increased significantly with irradiation doses compared to the control group, while MCHC% did not change significantly in most treated groups (Figures 9-11). Similar trend was reported by Abojassim *et. al.*[38] to the most cases, the decrease in the values of the haematological parameters following radiation exposure can be also, attributed to their direct damage. [39] The cellular elements of the blood are particularly sensitive to oxidative stress since their plasma membranes contain a high percentage of polyunsaturated fatty acids.[40] IR has a sufficient amount of energy to induce physical symptomatology within minutes of exposure, appearing as the acute radiation syndrome (ARS). Hematopoietic cell loss was observed as early as 3 hours after the conclusion of radiation exposure, with a more profound complications observed at the 48-hour time point. [1]

However, the time needed for recovery of all the studied haematological parameters, in the whole, is very long. Consequently, the rate of repairing the disturbance took place in the blood components can be very low compared the rate of their damage. [39] Therefore, it is worth to state that the application of different doses of FO extract to the irradiated rats manifested a tendency towards recovery during the seven days from the administration onset and, mostly, the dose at 0.3 mg/g is characteristically the effective one as a radiation protective agent.

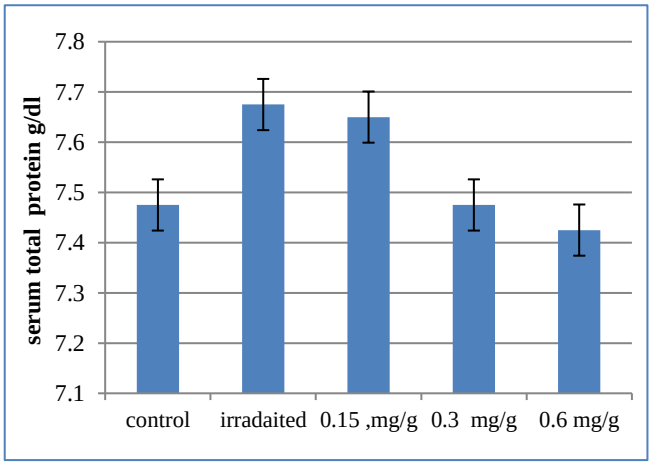

Fig. (12):The changes in the total serum protein (g/dl) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

A detectable elevation in serum total protein levels in γ-irradiated rats was found to be ~ 2.3%, as compared with the corresponding control rats. This marked increase in total protein may be attributed to preexisting complicationsin vital biological processes e.g. dehydration, and/ or due to the change in the permeability of rats' tissues resulting in leakage of protein to the blood serum. Administration of FO extract post γ-irradiation showed a protective effect against γ-radiation disturbance in serum total proteins production and permeability (Fig. 12).

The hyperproteinemia after stimulation of gamma irradiation of the rats can occur mainly due to the increase in the concentration of total globulin, figure (13), whereby the raise in total globulins can be

attributed to the elevation in α- globulin synthesis as impact of gamma irradiation. Globulin levels in serum of irradiated rats seemed to be rehabilitated and dropped near the control values as these irradiated rats were supplemented with FO extract at doses of 0.3 mg /g and 0.6 mg/g.

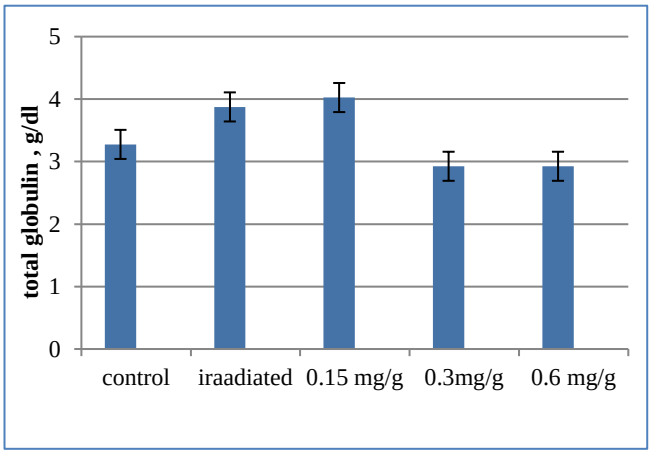

Fig. (13): The changes in the total globulin (g/dl) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

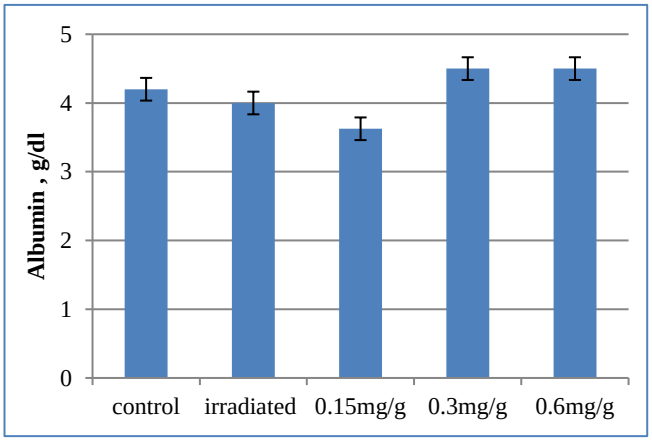

Fig. (14): The changes in the serum albumin (g/dl) as functions of gamma irradiation and post administration of increasing doses of *Fusarium oxysporum* extract (mg/ g rat's body mass). Error bars indicate standard error of mean values.

The data presented in Figure (14) showed a slight decline in serum albumin levels in the γ-irradiated rats as compared with the corresponding control ones. The diminishing in the albumin concentration exist to the rats administrated FO at 0.15 mg/g body mass post irradiation. The decrease in the level of albumin concentration could be attributed to degradation in addition to the loss of albumin through the gastrointestinal tract.[42] Moreover, the interruptions of the lymphoid organs following γ- irradiation was probably related to the drop in serum globulin levels.[30] However, rats administrated FO at dose 0.15 mg/g were unable to recover the dropped albumin values compared to its control levels i.e. the rate of albumin degradation is still greater than its rate of recovery. Whereas the rats administrated FO extract at doses of 0.3 mg/ g and 0.6 mg/g post exposure to ɣ-radiation restored the albumin levels nearly to the control values or a little bit higher within one week ,viz that the rate of recovery exceeded the rate of degradation.

It was reported that the water-extracted mycelial polysaccharide (WPS) from entophytic fungus *Fusarium oxysporum* species demonstrated antioxidant activity. [43,44] The protective effect of FO against the oxidative injures can attributed to various anti- toxicants that assumed to be found in FO extract, e.g. phenolic compounds, anthocyanins, flavonoid glycosides , procyanidins and others which are responsible for the observed antioxidant activity. In the fungi extract, these natural materials can detoxify the free radicals such as superoxide and hydroxyl radicals evoked due to gamma irradiation, consequently inhibiting lipid peroxidation and the other ionizing impacts. Similar explanations were reported for similar plant extracts.[45,46] This extract could also hinder the detrimental effects of free radicals by preventing the oxidation of cell membrane.[47] The data obtained demonstrated that FO extract ameliorated the tissue damage induced by whole body irradiation of rats as evidenced by improving most of the studied haematological parameters.

FO extract could alleviate the toxic impacts of ionizing radiation and thus it aids as a radioprotective in radiotherapy regimen.[2] *Fusarium oxysporum* extracts, as natural products, can be considered less or non-toxic with free radical scavenging capability, antioxidant capacity, immune stimulatory impacts and have the advantages, as biomaterials, over their synthetic counterparts.[27]

CONCLUSION

The analysis of the data of the present work assumed that injures due to ɣ-IR causes a significant reduction in pairs of biomedical parameters and blood components post the single irradiation dose, while the treatment with FO showed detectable improvements depending on the FO doses, which can be considered a potential health protection during exposure to irradiation.

According to the obtained results, it can be concluded that the clinically reported injures in albino male rats due to the gamma irradiation exposure can be handed by FO administration, mainly, through the impacts of its antioxidant components. The radiation protective effect of FO extract could reduce the dependence on chemically based synthetic drugs, and subdue human exposure to chemical residues related to the side effects and toxicity as a result of administrating such medications. Nevertheless, Fusarium oxysporum extract administration still needs to be optimized in terms of the proper dosage and period of administration for human application.

Generally, natural materials can be securely applied for numerous human abnormalities due to irradiation exposures. They can focus on clinical traits to offer safer and more effective gamma irradiation protective capacity. However, extensive additional studies will be necessary before the results of such and similar works can be fully assessed. More researches and much effort should be considered and focused on authorizing protocols for medical management of radiation injuries based on natural products.

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CONFLICT OF INTEREST

Authors confirmed that they have no conflict of interest regarding the content of this article.

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