

Effects of Low Dose Gamma Ray on Some Hematological Parameters in Adult Rats

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ARTICLE INFO	ABSTRACT
<p>Article type: Original Article</p>	<p>Introduction: The study aimed to find the low dose effect gamma ray on some hematological parameters in male albino rats obtained from the Mosul University, IRAQ, and also studied the radioprotective effect of black seed oil on the hematological parameters.</p>
<p>Article history: Received: Nov 10, 2018 Accepted: Apr 01, 2019</p>	<p>Material and Methods: The system is made of ²⁴¹Am as gamma source .The rats were exposed to gamma radiation for limit period .Hematological investigations in which the red blood cells (RBC) also white blood cells (WBC) were found by the result of Neubauer haemocytometer method hemoglobin (Hb) concentration obtained due to cyanomethaemoglobin test. The packed cell volume (PCV) was determined using the micro-centrifuge method. Platelets were achieved.</p>
<p>Keywords: Gamma Ray Hematological Black Seed Oil (<i>Nigella Sativa</i>) Free Radical</p>	<p>Results: All hematological parameters found significantly decreased in all groups receiving daily dose of 6 mGy/h for 7h/day for 25, and 50 days compared with those in the control group. However group 2. Treated with 400 mg/kg wt /day for 25 days, showed a significant increase in the mentioned parameters. Groups 3 and 4 were exposed to gamma ray in two stages(at a daily dose 6 mGy/h for 7h /day for 25 and 50 days) .Group 4 showed a significant enhancement in hematological parameter when treated with black seed oil , compared with group3 which had a significant decrease in these parameters compared with the control group.</p> <p>Conclusion: The present work showed the function of black seed oil considered as an advanced material in decreasing the oxidant stress on hematological parameters against gamma ray with limited dose.</p>

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Introduction

After the absorbance of radiation energy in the tissue, the continuous exposures to radiation of biological consequences are moderate with a series of physical and chemical properties with biochemical and fixing cellular responses. The power contact with shortest wavelength has a magnitude (not splitting), also with the production of derivative electron. Initial moves of power sedimentation and molecular knot separation is 10.000(GHz) on the system of frequency range. The original structure in cellular material return to the water , it is initially the water ionization that yield in the generation of petty type with increase inverse interaction and low life frequency(10GHz - GHz) like the OH,H₂O₂with minor type that middle the reaction of chemical that spoil biologically main molecules like the membrane of cell and proteins [1].

The devastating effects and transformation from radiation were originally an idea to be due firstly to direct consistence of high energy ray and particles with dashing center of microbial cells. From hydrolysis of water leading to strongly free radicals that are most dangerous parameters contributing to lethal and sublethal variations in microbial cell

[2].Several scientists are modernly study the irradiation impact on different section of rats. Also other studies showed the gamma effect, on RBCs membrane solubilization of rats erythrocytes by sodium dodecyl sulfat (SDS) [3],[4]. Asrar M. Ha studied the effect of low dose gamma rays (0.055 Gy) certain original metals, namely Fe,Zn,Cu, and Zn, and their levels in tissue [5].

Black seed oil is edible product containing beta-carotene, iron, calcium, potassium and sodium .It has eight out of nine essential amino acids which cannot be produced in our bodies. In addition to the chief active constituent in black seed oil, this seed also has thymoquinone, myristic acid ,beta sitosterol, palmitic acid , plamitoletic acid , oleic acid, stearic acid, linolenic acid, linolenic acid, arachidonic acid, protein, folic acid ,copper, zinc, phosphorous, and VitaminB1,B2and B3[6].

The present study was conducted to investigate the effects of long exposure to low dose gamma rays (i.e., 7 h a day for 25-50 days) emitted from Americime ²⁴¹Am (in mGy) on some hematological parameters in male adult rats and use advanced

material in decreasing the oxidant stress on hematological parameters (e.g. RBC, WBC, PCV, Hb and platelets) against gamma ray with limited dose that agree with the reduces that could be lead to high radio sensitivity of hematological tissue. Therefore, this substance can be used as a therapeutic option for some types of cancers, especially those under the treatment of low dose radiation, to promote the protection against the radiation effect.

Materials and Methods

The *Nigella Sativa* (black seed) was bought from the local market in Mosul City, Iraq, and seeds were filtered to remove any impurities. The Black Seed oil was produced by equipment breaking 50 gm from seed with 250 cm³ from ethanol with concentration (95%) by using break device inside the snowy bath. The mixture was shaken with an electric motor generator for 1h at room temperature. The filtration was performed using multiple layers of filter paper has pore size of 2 μm (Ahlstrom Germany GmbH company, Germany). The filtered mixture was then centrifuged at a speed of 1000 rpm for (15) min. Subsequently, the seed oil was collected from surface layers. This study was conducted on male adult albino rats with the age of 8-12 weeks and weight of 150- 200. The healthy rats were obtained from the college of veterinary of Mosul University and kept in plastic cages with plastic metal lids (20*30*15 cm). Steps were taken to ensure good hygiene. The four groups, including the control (sham exposure), black Seed, gamma ray radiation exposure, and Black Seed with gamma ray irradiation, were kept at a temperature of 26±2 C⁰. And humidity of 35% ± 5% throughout the experiment. Furthermore the sawdust was changed every week. Feeding mixture consisted of wheat (35%), corn (34%), soybean (20%), protein (10%), and dried milk (1%). The rats were also supplied with water during the experimentation [7]. The dose of the black seed oil was determined as 400 mg/kg weight of body following as active dose for the same condition depending on [8]

Americium-241 Properties

Americium has an energy of 59.5 keV and the activity of radiation of 50*10⁻⁶ Ci took from Mosul University /Physis Department with exposure constant or gamma constant level of Γ=0.013 R.m/Ci.h..This radioactive element has a half-life of 432 years.

Work System

The system is made of ²⁴¹Am as a source of gamma radiation (6 mGy) put into box plastic on the plastic cages with distance (20 cm).The rats were exposed to gamma radiation for 7 h per day for 25, 50 days .The portable Gieger counter (INNOVA GmbH company, www.alphaaix.com , Germany) was used to determine the radiation dose practically with R/h and convert it to mGy. The detector has other applications, such as the detection of the radiation dose of X-ray,alpha, and beta released from other environments in an addition to gamma ray.

Experimental Design

****Forty male rats were used in the experiment, in four groups as follows:

- 1) The first group (control) included ***10 male rats that were not exposed to gamma ray radiation (control).
- 2) The second group entailed 10 male rats that were daily exposed to 6mGy/h for 7 h for 25 days
- 3) The third group consisted of 10 male rats, orally treated daily with black seed oil (400 mg/kg body weight) for 25 days.
- 4) The fourth group included 10 male rats that were daily exposed to 6mGy/h for 7 h for 25 days and orally treated with (400 mg/kg body wieght) of black seed for the same time.
- 5) Groups (2-4) ,the number of male rats are 30 used for 50 days at a rate of 7 h daily.The work system was prepared at 6 mGy/h of radiation dose.with different exposure time for 50 days on white male rats and at period 7 hours daily.

Statistical Analysis

Alls variables were exposed as mean±S.D. The data with SAS program (SAS software, GitHub company,U.S.A.)were analyzed using the one way analysis of variance (ANOVA).In addition, the differences were determined by Duncan test at a significance level of p≤ 0.05[9].

Hematological Parameters Analysis

The investigations of some hematological measurement as red blood cells (RBC) also white blood cells (WBC) were found by the result of Neubauer haemocytometer method hemoglobin (Hb) concentration with gm/100 ml obtained due to cyanomethaemoglobin test.The packed cell volume (PCV) was determind using the micro-centrifuge method. Platelets were achieved according to the method adopted in [10].The capillary blood samples were collected from all groups at the end of the experiment by orbital venous plexus puncture. Furthermore, the blood was collected in EDTA tube a vial containing 2% ethylenediamine- tetra -acetic acid anticoagulant.

Results

Table 1 and 2 summarize the results of the present study regarding the effect of gamma ray irradiation and treatment with black seed oil for 25 and 50 days on hematological parameters in male rats.

Table 1. Effect of low dose gamma ray irradiation and Black Seed oil for 25 days on hematological parameters in male rats

Parameters of blood	Control Group 1 Mean±S.D	Blacked Seed oil with 400 mg/kg. wt Group 2 Mean±S.D	Irradiation with Gamma ray at 6 mGy/h for 7h/day Group 3 *Mean±S.D	Black Seed oil with 400 mg/kg.wt + Irradiation with Gamma ray at 6mGy/h for 7h/day Group 4 Mean±S.D	Ratio % Group2 due to group 1	Ratio % group3 due to group 1	Ratio % Group4 due to group 1
Hb (g/100 cm ³)	14.275 b ± 0.40	14.50 a ±0.41	3.22d±0.33	12.15 c ±0.45	101.75	22.55	85.11
PCV (%)	39.9625b± 1.81	42.21a± 0.74	7.33d±1.84	34.02c ±1.92	105	18.3	85.12
WBC(count/ml)	6128.8b± 1.47	6503.22a ±1.21	1000.91d±0.41	4000.87c ±0.45	105	16.34	65.27
RBC(count *10 ³ /ml)	6128.8 b±0.12	6500.34 a±0.31	1000.21d± 0.23	5000.52c ±0.27	106	16.31	81.59
Platelet (x10 ² /mi)	296.65b± 89.58	320.89a± 54.55	50.09 d± 0.91	112.23c± 41.53	108.17	16.88	37.83

Hb: hemoglobin, PCV:packed cell volume, WBC:white blood cells, RBC:red blood cells

a: High significant level between groups at (P≤0.05)

b: Medium significant level between groups at (P≤0.05)

c: Low significant level between groups at (P≤0.05)

d: Extremely low significant level between groups at (P≤0.05)

Table 2. Effect of low dose gamma ray irradiation and black seed oil for 50 days on hematological parameters in male rats

Parameters of blood	Control Group 1 Mean±S.D	Blacked Seed oil with 400 mg/kg.wt Group 2 Mean±S.D	Irradiation with Gamma ray at 6 mGy/h for 7h/day Group 3 Mean±S.D	Black Seed oil with 400 mg/kg.wt + Irradiation with Gamma ray at 6mGy/h for 7h/day Group 4 Mean±S.D	Ratio % Group2 due to group 1	Ratio % group3 due to group 1	Ratio % Group4 due to group 1
Hb (g/100 cm ³)	14.275a ±0.40	13.02b±0.42	1.121d ±0.29	11.43 c±0.41	91.20	7.85	80.07
PCV (%)	39.9625a ± 1.81	35.33b±0.77	4.54d±2.11	31.32c±4.07	88.40	11.36	78.37
WBC(count/ ml)	6128.8a± 1.47	6007b ±1.11	1000.76d±0.21	4000.16c±0.44	98	16.37	65.26
RBC(count *10 ³ /ml)	6128.8a ±0.12	5500.21b±0.26	500.56d±0.22	4000.77c±0.23	89.74	8.167	65.27
Platelet (count*10 ² /ml)	296.65a± 89.58	280.51b±41.90	22.61d±0.87	99.24c±38.43	94.53	7.62	33.45

Hb: hemoglobin

PCV: packed cell volume

WBC: white blood cells

RBC: red blood cells

Number of animals per group: 10

total number of animals: 40

Level of significance (P≤0.05) as a,b,c and d between groups.

As indicated in Table1, 7h/day irradiation by gamma ray at 6mGy/h and treatment with black seed oil at 400 mg/kg/day for 25 days resulted in a significant decrease in the hematological parameters namely RBC,WBC, Hb and platelets in groups 3 and 4, compared to that in group1.In addition ,group 2 showed a significant increase in these parameters compared with group 1.Based on the results presented in Table 2, 7 h/day gamma ray at a radiation dose of 6 mGy/h and treatment with black seed oil at 400 mg/kg/day for 50 days led to significant decrease in RBC, WBC, Hb, and platelet in groups 2, 3, and 4 compared with that in group1.

Discussion

The results of present study showed a significant decrease in the mean value of hematological parameters, namely Hb, PCV, WBC, RBC and platelet in groups 3, and 4. However, a significant increase was observed in these parameters in group 2 after being irradiated with 6 mGy/h for 25 days gamma ray for 7 h/day for 25 days

receiving treatment with 400 mg/kg/day for the same time.

The ionizing radiation which quickly causes the radiolysis of water and next products several free radicals is offered to be released in their radiated cells from the biological sources. The mitochondria reactive oxygen species level is increased by increased ionizing radiation [11]. Irradiation by ionizing radiation effects dose on hematopoietic cells not only effect on the production of the bone marrow by reduce it but also by using the apoptosis(killing cell program) of nature formed elements of the blood [12].

On the other hand, after irradiation with gamma ray for 7 h /day at a dose of 6 mGy/h and treatment with 400 mg/kg/day black seed oil for 50 days , there was a significant decrease in the hematological parameters in groups 2 received just black seed oil not irradiation,3and 4 compared with that in group1. In addition, the ratio % percentage of irradiation with 25 and 50 days showed that the values in groups 3 and 4 were less than the value in group 2.

The results of the present study revealed that the treatment of normal rats with blacked seed oil (*Nigella sativa*) at a dose of 400 mg/kg body weight resulted in a significant variation in the levels of WBC, RBC, Hb, PCV and platelets after 25 and 50 days, while treating the group irradiated with gamma ray showed a significant decrease ($P < 0.05$) in RBC, WBC, Hb, PCV and platelets after 25 and 50 days compared with the control group. These results led to the protective effect of phenolic antioxidant (important composed in black seed oil as antioxidant material) during the study of blacked seed oil (*Nigella sativa*). This antioxidant is capable of reducing leukocyte lipoxigenase enzymes and the changing consequences (change genetic expression) of their ability to release free radicals. However, putting unimpaired generation of prostaglandins, which enhance microvascular blood flow and change as immune modulators [13].

Finally, the results of this study showed that group 2 which was treated with black seed oil has a significant increase in hematological parameters, compared with the control group (group 1). These results agree with those obtained by Fabiani et al [14] who investigated the oral administration of the black seed oil (*Nigella sativa* oil, 0.04 mg/kg wt per day) at several doses (0.01-0.1) for 30 days in mice. They demonstrate that the group of mice receiving black seed oil had a significant increase in hematological parameters compared with the control group.

Our results are also in agreement with those reported by Thomas [15] showing the role of black seed oil in reducing the side effect of radiation therapy and improving the recovery after administering 1 g/kg body weight per day for 10 days, along with radiation therapy with gamma ray. In addition, El-Desouky et al [16] reported that the rats treated with a mixture of green tea and grape seed extract, as antioxidant materials, had fewer side effects of gamma ray therapy as dose fractionation of radiation effect with 4, 6, and 10 Gy for 14 days.

The results of the current study are consistent with these obtained by Eshak and Osama [17] who noticed a significant decrease in RBCs, WBC, PCV, platelet, and Hb in the group exposed to 4 and 6 Gray gamma radiation. The hematological parameters underwent a decline following radiation exposure and may return to direct damage caused by gamma ray radiation and also overproduction of reactive oxygen species that depend on gamma radiation interaction.

Conclusion

The finding of the present study demonstrated the role of black seed oil as an antioxidant substance that lead to the reduction of free radicals. Based on the results, this substance may be able to reduce the oxidative stress caused by low dose gamma ray radiation. This reduction could be attributed to the high radiosensitivity of haematopoietic tissue. Therefore, this substance can be used this as a therapeutic option for

some kinds of cancers especially those under the treatment of low dose gamma ray radiation, by enhancing protection for a long time. According to these results, black seed oil (*Nigella sativa*) is considered a good protective agent against oxidative stress when administered orally.

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