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THE POSSIBLE PROTECTIVE ROLE OF NIGELLA SATIVA METHONELIC EXTRACTION AGAINST IONIZING RADIATION INDUCED CERTAIN HEMATOLOGICAL STRUCTURAL AND CONFORMATIONAL ALTERATION IN RATS

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ABSTRACT
The present work aimed to investigate the protective effect of Nigella sativa methonelic extraction against gamma radiation effect on the hematological parameters in male rats. **Method:** Sixty Male rats divided into 12 groups, 5 each, were used in this work. Average weighing 200-250 g were used. The rats were kept in in special designed cages and maintained under constant air flow and illumination during the experimental periods. Group 1 served as vehicle control, Group 2 served as NS group. Five Groups were injected intravenously with Tc-99m, while other five groups were orally administrated with Nigella sativa (400 mg/kg) then injected intravenously with Tc-99m. All these groups were decapitated after 1, 7, 14, 30 and 60 days respectively. Some hematological parameters were determined for all groups, including red blood cells (RBCs), white blood cells (WBCs), platelets (PLT), Hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). **RESULTS**: The findings results showed a disturbance in the levels of the estimated hematological parameters for all groups ($P < 0.05$) compared to the control group. The results of UV spectrum show partial photo dissociation. showed a decrease in intensity, all irradiated serum sample intensity was less than control sample; this result indicates that there is photo degradation happened to the blood components, this causes changes in the structure and conformational changes. Also Results revealed that administration of Nigella sativa methonelic extraction has protective effects against radiation-induced damage. **CONCLUTION**: The present study clarified that oral administration of Nigella sativa methonelic extraction is beneficial to prevent alterations induced by gamma radiation.

KEYWORDS: Gamma radiation, hematological parameters, Nigella sativa, UV-spectroscopy.

1. INTRODUCTION
Electromagnetic waves are a form of energy that include radio waves, infrared and visible light as well as high energy rays such as ultraviolet, x ray and gamma ray. The toxic effects of high energy rays are mediated by transferring energy to atoms/molecules, leading to their excitation and ionization. This leads to free radicals and reactive oxygen species (ROS) formation and subsequently results in the oxidation of essential macromolecules (protein, DNA, un-saturated fatty acid), cellular oxidative stress.[1-3]

The medical isotope metastable technetium-99 (Tc-99m) emits gamma rays that physicians heavily rely upon to examine how organs such as hearts, lungs, and kidneys function. Tc-99m is so beneficial to the medical community that it's used in approximately 80-85 percent of the world's diagnostic imaging procedures (cardiac perfusion scans and bone scans among them). 6-hour half-life of technetium 99m gives researchers enough time to put these nuclei to use. In order to leave the excited state, technetium-99m nuclei emits 140 keV characteristic gamma rays without accompanying beta rays. This property makes them highly desirable in medicine. Gamma rays are absorbed far from the examined organ, minimizing the danger posed to living matter.

Plants have been an important source of medicine for thousands of years. Nigella sativa (NS), commonly known as "black cumin", is an erect, herbaceous annual plant.[4] Several beneficial pharmacological effects, including anti histaminergic, anti-hypertensive, hypoglycemic, antimicrobial, mast cell stabilizing and

anti-inflammatory activities, have been attributed to various crude and purified components of black seeds.[5] Experimental studies have indicated the polar fraction of these seeds, extracted by methanol, also have antioxidant and anti-inflammatory potential. Recently its radioprotection role has also been reported. It protects radiation induced intestinal damage, improves survival, body weight recovery and normalization of intestinal villi after irradiation. These therapeutic properties are attributed to its prominent phytochemicals e.g thymoquinone, thymol, carvacrol, nigellicine and its derivatives, myristic acid, saturated and unsaturated fatty acids and various trace elements and minerals.[6]

The blood examination includes evaluation of hematological factors such as white cell count (WBC), differential count and platelet count, red blood cell (RBC) count, mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), hemoglobin (Hb) and mean cell hemoglobin (MCH). A complete blood count gives important information about types and numbers of cells in the blood especially RBCs. CBC helps in diagnosing conditions. The presence of abnormal or excessive numbers of immature blood cells should be noted. The hematopoietic system is highly sensitive to radiation and the peripheral blood count may well serve as a biological indicator of such damage.[7] The blood cell count remains a time-honored method in the hematological analysis in healthy persons and is affected by many factors including occupational threats.[8] The importance of blood cell count was discussed in numerous studies that showed the effects of partial or total body irradiation on peripheral blood cell count and most of the studies were focused on high dose radiation received accidentally or therapeutically.[9-10] The UV-Visible spectroscopy is a powerful tool used in the field of Medical science for qualitative analysis of biological samples such as blood plasma and sera or tissues. Implementation of these technique reduces time, resources and cuts cost. Spectroscopy is emerging as a potential diagnostic tool in the medical and pharmacological fields to provide information about the different chemical and morphological structures. Blood being the chief circulatory medium of our body, reflects the physiological and pathological changes that take place in the tissues, which leads to the changes in the various plasma and cellular constituents. Spectroscopy has received quite a lot of attention not only for understanding the biological nature of the disease, but also for the diagnosis of the disease in recent years. Almost in all diseases the blood undergoes major changes in chemical and biochemical properties. The application of spectroscopy for the study of biomedical compounds has increased tremendously in recent years since this gives the metabolic photography of the subject.[11,12]

The present study aims to evaluate the effect of gamma radiation on the structural and conformational characteristics of the blood also the protective role of Nigella sativa against radiation.

2. MATERIALS AND METHODS

2.1. Collection of Plant material and extraction

The Nigella sativa seeds were purchased and Sample specimens have been kept at the Faculty of Science, Zoology Department for future reference. The seeds of were powdered in a mixer. 20 g of the powdered seeds were added to 400 ml of distilled water and extraction was carried out by steam distillation. The distillation process was continued until about 200 ml of distillate was collected. The distillate was extracted three times with chloroform. Moisture was removed by anhydrous sodium sulphate and the resultant extract was evaporated using a 40 °C water bath leading to the appearance of the volatile oil. 500 mg of the volatile oil were dissolved in 1 ml of dimethyl sulphoxide (DMSO) then 9 ml of physiological saline was added to yield a concentration of 50 mg volatile oil per 1 ml solution.

2.2. Animals

Sixty Adult male rats, average weighing 250 g were used. Animals were kept under normal conditions throughout the experiment, they were housed in special designed cages and maintained under constant air flow and illumination during the experimental periods. All rats had access free to food and water throughout the experimental period.

2.3. Mode of administration

The NS methanolic extraction was freshly dissolved in double distilled water (DDW) and administered orally using an oral gavage.

2.4. Radio protective effect

The radio protective effect of NS was determined by dividing the animals into the following groups:

- The control group (n=5), this group doesn't receive any radiation.
- The NS control group(n=5), this group administered with 400 mg/kg of NS methonlic extraction
- Injected radiation groups (5 groups): The animals of these groups were injected by gamma radiation.
- Nigella sativa + Injected radiation groups (5 groups): This group of animals was administered with NS orally for 2 consecutive weeks before injection of radiation.

2.5. Irradiation

The animals were injected intravenously by the same dose of TC-99mm radiation, 5.65mCi.h.

2.6. Blood collection

The experiments complied with the laboratory animal disposal regulations of the Animal Ethics Committee. Animals were sacrificed after 1, 7, 14, 30 and 60 days from injection with radiation. The blood samples were collected by jugular venipuncture into EDTA-vacutainer test tubes. Further analyses of the samples were

performed at the laboratory within three hours from the collection.

2.7. Hematological and dimensional measurements

A hematological auto-analyzer were used to determine different hematological and dimensional parameters. These parameters were WBCs, RBCs, HB, HCT, MCV, MCH, MCHC and PLT.

2.8. UV-Visible spectrophotometric measurements

The structure characteristics of the blood from all groups were studied by recording the UV -Vis spectra. Blood were diluted with normal saline and placed in Kartell disposable polystyrene cuvette of 10 mm path length. The cuvette is placed Shimadzu UV – Vis. Spectrometer (Japan). UV –Vis spectrophotometer for analysis the spectra were scanned in the region between 190 nm to1000 nm. The blood shows a characteristic absorption spectrum in the UV- visible range, which depends on the valence and spin state of the heme and can be modified by changes in ligand bonds between the heme group and globin part.

2.9. Statistical evaluation

Statistical analysis for evaluation of the results was done by calculating arithmetic mean and standard deviation for Hematological parameters. All these measurements had been done for all groups. Results were expressed as mean ± standard deviation for each group. The results were evaluated by Student's unpaired t-tests.

3. RESULTS AND DISCUSSION

CBC analysis is a useful screening test in routine medical check-up. A high or low blood cells count even in a healthy-looking subject lead to the suspicion of disease and it should prompt further investigations.[13-14]

3.1. Effect of gamma radiation

Table (1) showed that the effect of gamma radiation on the different hematological parameters in rats. In this study, there is a significant decrease($P<0.05$) in RBCs count, WBCs count, Hb, and HCT showed a significant decrease after injected radiation doses compared with the control. The dimensional blood indices MCH, MCHC and MCV showed a significant increase ($P<0.05$) with Gamma-radiation groups compared with the control (table1). Platelets count decreased significantly ($P<0.05$) in the group treated with gamma to radiation.

There is a slow increase ($P< 0.05$) in the recovery rate in those parameters after 60 days.Red blood cell is the number of red blood cells; these are important because they deliver oxygen through body. They also help carry carbon dioxide. Hemoglobin is the protein in your blood that holds the oxygen. Hematocrit its value provides information about how much of your blood is comprised of red blood cells. WBCs appeared to be sensitive to irradiation. The damage from IR causes a significant reduction in WBC counts which may be considered a potential health risk during irradiation. Also irradiation induced leucopenia[15] and reduces lymphocytes, neutrophils and monocytes count.[16,17] The decreases could be attributed to high radio sensitivity of hematopoietic tissue[18] and a reduction in the viability of spleen hematopoietic stem cells.[19] A low RBCs count or low Hb or HCT may suggest anemia, which can be attributed to many causes. The damage effect of radiation on RBCs count may be attributed to the cessation of erythrocyte production in bone marrow, the loss of cells from the circulation by hemorrhage or leakage through capillary walls and/or direct destruction of mature circulation cells.[8,9,10,11,12. and 13] The reduction in hemoglobin due to radiation may be attributed to the incorporation of iron into hemoglobin due to disturbance in the bio generation structure of the hemoglobin molecule as evidenced by pronounced hyper ferraemia post-irradiation and oxidation of hemoglobin molecule.[16] It is generally agreed that hematopoietic organs, i.e., spleen, thymus and bone marrow are markedly sensitive to the ionizing radiation.[17] The clinical symptoms, which are largely due to damage in the radiosensitive hematopoietic organs[14], a very small dose of radiations to a blood-forming organ causes an arrest of the hematopoiesis with changes in the peripheral blood.[18] Meanwhile PLT is the blood cells that help your blood clot and avoid excess bleeding, decreased levels of platelets increase the risk for clotting. Mean Cell Hemoglobin (MCH) is the average amount of hemoglobin in an average red blood cell. Mean Cell Hemoglobin Concentration(MCHC) represent The concentration of hemoglobin in an average red blood cell that helps distinguish normal-colored red cells from pale-colored red blood cells. Mean Cell Volume (MCV) measure the average size of the red blood cell. MCH, MCHC and MCV shows a significant increase. due to The formation of superoxide partially accounts for the well-known oxygen enhancement of radiation induced biochemical changes and cell damage.[20]

Table 1: Effect of Gamma radiation on red blood cells (RBCs) count and Blood indices Hb, MCH, MCHC, MCV, WBCs and Platelets.

Blood indices	Control	One day later	7 days later	14 days later	30 days later	60 days later
Red blood cells count	5.08±0.28	5±0.47	4.70±0.47	4.61±0.37	4.86±0.19	4.87±0.34
White blood cells count	7.8±1.34	7.52±1.43	6.43±1.36	5.93±0.75	5.46±1.53	5.66±0.41
Platelets count	394.3±127	385±128	385±130	366.33±143	367.33±123.06	370±130.12
Hematocrit (HCT %)	40.86±2.7	39.5±2.5	38.8±1.51	39.2±1.65	37.1±0.2	38.96±4.76
Mean Corpuscular Hemoglobin (MCH)	20.87±0.8	21.5±0.84	20.5±0.87	20.03±0.5	19.46±0.40	18.66±1.19
Mean Corpuscular Hemoglobin Concentration (MCHC)	36.46±0.9	41±0.92	39.03±0.8	38.76±2.8	37.8±0.62	36.96±1.53
Mean Corpuscular Volume (MCV)	51.3±3.21	56±3.3	55.26±1.5	54.76±6.5	53.33±1.72	52.56± 5.31
Hemoglobin (Hb)	13.8±0.87	12.3±0.87	11.86±0.43	11.86±0.8	11.9±0.25	12.66±1.28

3.2. Role of *Nigella sativa* treatment against radiation

As shown in Table (2) the change in Hematologic parameters of controls and treated rats with radiation and *Nigella sativa* treatments. There is a small increase in MCH, MCHC and MCV parameters with ($P< 0.05$). While a reduction in PLT, RBC, Hb and HCT with ($P< 0.05$). The changes in Hematologic parameters due to the Nigella sativa seed extract due to its radio protective activity it involves different signaling pathways simultaneously to achieve the goal of prevention of cell death due to radiation. Pretreatment of NS seed extract protect the tissues from oxidative stress induced by irradiation. Furthermore, Nigella sativa is a powerful antioxidant that is able to induce some antioxidant enzymes and eliminate oxygen free radicals.[21] It is also reported the methanolic extracts of NS seeds significantly enhanced antioxidant properties of irradiated rats by reducing lipid peroxidation. when rats are treated with Nigella sativa. It has potential to directly scavenge the active free radicals from the system through electron donation and delocalization in the electrons in the aromatic rings.[22-23] Antioxidant are considered to be a cellular defense against oxidative damage. The effects of oxidant molecules are active in the defense against oxidative cell injury thanks to the fact that they are free radical scavengers.[24] NS improved anemia resulted from increased non enzymatic glycosylation in membrane proteins of RBC in diabetics. This plant also decreases anemia via lipid peroxidation reduction in RBC hemolysis and does not affect RBC levels directly. Results of this research confirmed that NS does not have an effect on blood cells directly. Although the effects of NS on homeostasis is known in traditional medicine, the pharmacological investigations on the effects of NS on homeostasis are few. Al-Jishi did not find any changes in blood cells when NS oil was given to normal rats.[25] Previous studies clearly showed that chronic treatment with NS fixed oil effectively influenced blood homeostasis in rats and seemed toinduce transient changes in coagulation activity[26].[27] In a study by Al-Hader et al., while NS fixed oil significantly decreased serum lipids and glucose levels, and leukocytes and platelets counts in rats, it significantly increased hematocrit and hemoglobin concentrations.[28] However, treatment with NS extract has been reported to significantly protect mice and rats from cisplatin induced falls in leukocytes counts,[29] and to influence leukocytes activities.[30] Biologic modifiers targeting oxidative damage for radioprotection have been studied for decades with limited success. Hence, there is a need for better and more potent compounds, especially on herbal origin to boost antioxidant defense. Nowadays there is much more attention paid to the use of plants as therapeutics because of lower adverse effects. Nigella sativa is a plant that is grown worldwide primarily in the Middle East, Mediterranean regions, Southern Europe, India, Pakistan, Syria, Saudi Arabia, and Turkey. For centuries, medicinal plants have taken part in the treatment of many diseases in various medicinal branches and also in traditional medicine.[31] Nigella sativa has been widely used for more than two thousand years as a curative and preventive substance against many diseases in Central Asia and some other Asian countries.[32] Nigella sativa is acknowledged to be a miraculous plant due to its rich history and religious background[33] and N. sativa is given even greater importance especially in Islamic countries, due to its many different beneficial properties.[34] Recently, clinical and animal studies have shown that the extracts of the black seeds have many therapeutic effects such as immunomodulative, antibacterial, antidiabetic, hepatoprotective, gastroprotective, antihistaminic and ant oxidative and neuroprotective ones.[35] The results of comparing NS group to the control group there is increased platelet count and non-significant increases in RBC and MCV and HCT. The other blood indices such as WBC, HGB, MCH, MCHC shows a non-significant decrease. After 7~60 days of irradiation a decrease has been observed for RBC, HGB, MCV and HCT while increase in WBC, PLT and MCHC. Many studies have been carried out in recent years on the pharmacological effects of black seed.[36] The black seed has analgesic, antimicrobial, anti-neoplastic, anti-inflammatory and immunological effects[37] The use of NS causes hematological changes. This plant has been shown to modify chaos in hematological parameters and increase defensive mechanisms of body[38] For instance, NS improved anemia resulted from increased nonenzymic

glycosylation in membrane proteins of RBC in diabetics. This plant also decreases anemia via lipid peroxidation reduction in RBC hemolysis and does not affect RBC levels directly. Results of this work confirmed that NS does not have an effect on blood cells directly. Since PLT counts increased in this research, NS might have increased coagulation activity. The observed significant elevation in WBC's count in this study may be due to active materials known as nigllone thymoquinone and thymohydroquinone in Nigella sativa.[39-40]

Table 2: Effect of Nigella Sativa methanloic extraction &Gamma radiation on red blood cells (RBCs) count and Blood indices Hb, MCH, MCHC, MCV, and WBCs and Platelets.

Blood indices	Control	One day later	7 days later	14 days later	30 days later	60 days later
Red blood cells count	5.69±0.77	5.7±0.53	4.98±0.53	4.65±0.24	4.87±0.66	5.48±0.47
White blood cells count	7.96±0.28	6.0±0.28	4.83±0.96	4.96±0.65	5.8±1.05	7.75±1.4
Platelets count	510.66±160.36	510±172.04	490±162.04	490.66±152.19	498.33±110.58	505.66±150.99
Hematocrit (HCT %)	38.2±3.63	37.2±4.37	34.23±4.37	35.8±5.84	36.16±2.12	37.95±3.92
Mean Corpuscular Hemoglobin (MCH)	18.8±0.51	21.6±0.3	20±0.36	19.33±0.81	18.6±0.95	18.7±0.78
Mean Corpuscular Hemoglobin Concentration (MCHC)	37.66±1.32	40±2.2	39.93±2.26	38±4.07	36.53±0.58	37.1±0.88
Mean Corpuscular Volume (MCV)	53.13±0.63	53±2.35	52.96±2.35	52.46±3.91	53.93±2.04	53.6±2.91
Hemoglobin (Hb)	13.93±1.7898	13.0±0.7638	12.71±0.7638	12.46±2.6058	12.86±0.7234	14.2±1.3229

3.3. UV-VIS. SPECTRUM OF BLOOD

3.3.1. Effect of Gamma radiation

The characteristic absorption spectra of whole blood recorded in the range of 200–800 nm from all studied groups were shown in figure (1). The absorption band at 250-300 nm (α band) is due to the delocalized electrons of the aromatic side chains of the amino acids; phenylalanine, tyrosine and trypotophan.[41] In the wavelength interval 300-800 nm, the interaction between iron and the proximal histidine exhibits transition at 340 nm.[42] The intense band at 415 nm (Soret band) and the other two bands at 540 nm (β band, resulted from nitrogen- iron bond) and 575 nm (γ band, which resulted from the heme-heme interaction) are characteristics to porphyrin ring.[43] According to figure (1), the spectra of all irradiated groups show the same number of characteristic absorption bands as the normal pattern. The data show a gradual change in the absorption intensity with a time elapsed. Different samples are analyzed quantitatively by calculating the intensities among the absorption peaks which is show decrease intensity, all irradiated sample less than control sample. This results were supported by the review of.[44] The minimum light absorption occurred at 7 days after irradiation with the fewer intensities recorded. The decrease in absorbance might be attributed to destruction, fragmentation in the aliphatic and aromatic amino acids, which are concomitant with.[45] The irradiation caused disruption of the ordered structure of the protein molecules as well as the degradation, cross-linking and aggregation of polypeptide chains.[45] Martel, 2010 has demonstrated that the irradiation produced extensive dose-dependent serum protein breakdown. These results indicate to that there is photo degradation happened to the blood components. radiation interacts with blood at the molecular level. Hemoglobin is a blood photoreceptor that Absorption intensity slightly decreases for all peaks at, due to increasing ligand electronegativity.[9] Due to the radiation, a decrease in the protein absorbance can be attributed to the unusual unfolding as well as the random motion of proteins under the different degrees of oxidative stresses. Thus, the absorption spectra of the blood serum are highly dependent on the radiation gamma dose, and the highest radiation gamma dose might be accompanied with the highest oxidative stress.[46-48]

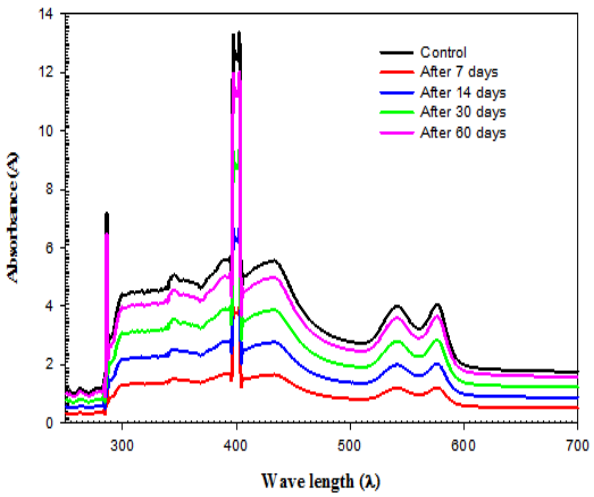

Figure (1): UV-Vis. spectra of the bloodfor control and other groups.

3.3.2. Role of Nigella sativa methanolic extraction

Radio-protectors should selectively protect the normal tissues during irradiation. In present work we tested the radio protective activity of known antioxidants, namely, Nigella sativa. The seed oil of NS is well known for its strong antioxidant properties.[49] Previous studies have documented that pre-treatment with TQ, the main active

constituents in seed oil, protected organs against oxidative damage induced by a variety of free radical generating agents, such as carbon tetrachloride[50] and including the alkylating agents, cisplatin[51], and doxorubicin.[52] We investigated only those changes in the absorption spectra of the whole blood of rats that orally administrated with *Nigella sativa* extract then injected with radiation that was detected for all of the samples studied. As shown in figure (2) shows the spectrum of non- irradiated blood sample (control). The spectrum of non- irradiated blood sample (control specified by peaks at (576.0, 542.0, 416.0 and 340.0) nm with intensities 0.793, 0.755, 2.604 and 1.253 respectively. The absorption spectra of the whole blood recorded in the range of 300–800 nm Figure (2) Contain absorption bands with λ_{max}= 340, 416 nm, a doublet band with λ_{max} = 542 and 576 nm. Due to the all the tested compounds from NS exerted strong antioxidant effects; thymol acted as singlet oxygen quencher the UV-visible absorption spectrum of other samples shows most intense absorption band at 416 nm. Also, TQ and dithymoquinone showed superoxide dismutase (SOD)-like activity.[53] In addition, it was[54] revealed that both TQ and dithymoquinone acted not only as superoxide anion scavengers, but also as general free radical scavengers so the light with this wavelength that strikes these biological tissues will be highly absorbed. The free radical scavenging effects of TQ, dithymoquinone, and thymol were tested against several reactive oxygen species (ROS).[53] This phenomenon is the key for the desired effect on the tissues.[55-56]

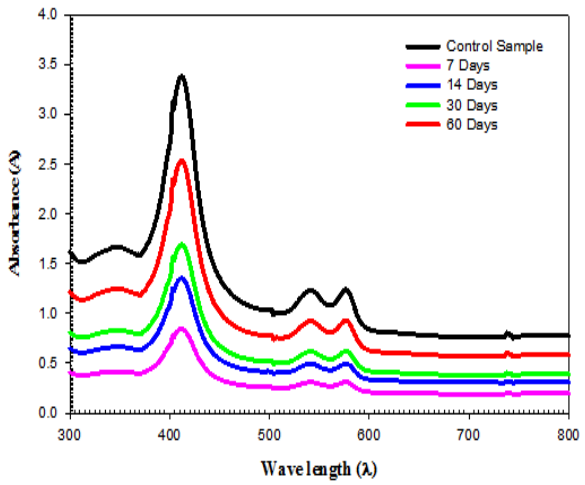

Figure (2): UV-Vis. spectra of the blood after NS treatment for control and other-groups.

4. CONCLUSIONS

The results from the present study show the hematological parameters showed a significant change with the -radiation compared with the control. A low RBCs count or low Hb or HCT may suggest anemia. The damages effect of Tc99m-radiation on RBCs count may be attributed to the cessation of erythrocytes' production in the bone marrow, the loss of cells from the circulation by hemorrhage or leakage through the capillary walls, and the direct destruction of mature circulation cells. The dimensional blood indices MCH, MCHC, MCV showed a significant increase compared with the control. This work demonstrates The decrease in absorbance in UV-vis absorption indicates to the destruction and the accumulation that might occur in response to the gamma radiation also the spectra of blood are highly dependent on the gamma radiation, and after 7 days of gamma radiation might be accompanied with the highest oxidative stress. Also The present study clarified that oral administration of Nigella sativa methonelic extraction is beneficial to prevent alterations induced by gamma radiation.

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