



The efficiency of *Nigella sativa* against gamma radiation on ionized serum Ca^{+2} , K^{+} and Na^{+} levels in rats

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Abstract An acquaintance of the deliberation of sodium, potassium, and calcium in the blood is essential in association with a variety of problems. There are about 24 elements in the body, the sodium, calcium and potassium considered to be the most important elements. Ionizing radiation may lead to irregular concentration of the concentration of the above-mentioned ions may effect cardiac arrhythmias, muscle contraction disorders, conflicts of neuronal activity. This study had been planned to evaluate the hazards of ionizing gamma radiation on rats. Also to test the efficiency of *Nigella sativa* methonelic extract as a radio-protector. Methonelic extract were orally administrated for two weeks before injection of radiation. Serum levels were investigated. The obtained results showed that ionizing radiation disturb the levels of sodium, calcium and potassium concentration. Meanwhile administration of the *Nigella sativa* methonelic extract effects the concentration of these ions. The *Nigella sativa* methonelic extract created a modulation against oxidative stress made by ionizing gamma radiation.

Keywords serum, blood, potassium, calcium, sodium, gamma radiation, *Nigella sativa*

1. Introduction

A worldwide anxiety about the use of ionization radiations in many fields. Radiation are being comprehensively used in analysis as well as in therapeutic use for cancer patients. Ionization radiations effect human health as they disturb chemical bonds of the molecules and destroy DNA by the creation of free radicals and hence proliferative cells can undergo apoptosis [1]. Stem cells are found to be radiosensitive and their mutilation by radiations can result into non-performance of the functional cells. Ionizing radiation can affect proliferating cells [2]. Ionizing radiation is dangerous for those tissues (i.e., bone marrow) which turn over rapidly as well as to those tissues (i.e., central nervous system, lung, heart, liver, kidney and gonads) which turnover slowly [3]. On the other hand, the body contain many elements [4-6]. The most common mineral elements include calcium (Ca^{+2}), sodium and potassium (K^{+}). Na^{+} ion plays an important role for water retention by the kidneys in the control of hydration and for electrical conductivity of nerve cells, muscle tissue. The sodium ion considered to be the most important one in keeping of osmotic fluid pressure. Ionized Na^{+} plays an important role in β -cell function and modulation of insulin release. Na^{+} ion plays an important role in the binding of agonist or antagonist to the α_2 -receptor and in suppressing insulin release. The role of sodium stimulus in the pancreatic β -cell secretion. An increase in membrane permeability in excitable cells for sodium ions for construction action potential. The distribution of sodium in the body about fifty percent present in the extracellular fluid, about 45 % in bones about, and the remaining percent inside cells. Bone crystals play a reservoir role and release sodium in case of serum level deficiency [7-8].



The most essential ionic component in the intracellular fluid is potassium ions. It plays physiological role in numerous processes such as the contraction of smooth and skeletal muscles, electrical impulse conduction. Potassium ion helps cell membrane job and appropriate enzyme activity. Also play a significant role in neurons. Where resting potential depends mainly on potassium, since their membrane is the most permeable to this ion [1]. About 95 % of the body's total content of potassium ion is sited inside the cells. It considered a crucial for the maintained normal heart rate rhythm [9-10]. The kidney is the organ that maintain the levels of sodium and potassium. The ion that plays an important function such as control of ion transmission, blood clotting and activation of secretor functions and cell reduction is ionized calcium ion, also in many physiological processes in the body such as muscle contraction. About 99% of the body's calcium is stored in bones. [11]. Synchronized of calcium through exchanges with bones, excretion through the kidney, and oral intake. The balance between these electrolytes and their balance plays an important role in diagnosis and prognosis of any disease [12]. A serious factors such as ionizing radiation affecting the values of these ions must be taken into consideration in order to achieve dependable data analysis. Thus, the aim of the present work is to inspect the changes in the concentration of sodium, potassium, and calcium in the blood due to ionizing gamma radiation, as well as to find out the role of natural radio protector that minimize the effect of ionizing radiation.

2. Materials and Methods

2.1. Plant Material and Extraction

Nigella sativa seeds were purchased and Sample specimens have been kept at the Faculty of Science, Zoology Department. 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. The NS methanolic extraction were administered orally using an oral gavage.

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. Animal grouping

The animals were into the following groups where each group contain 5 animals, the control group which didn't receive any radiation. The NS control group which orally received about 400 mg/kg of NS methonlic extraction. The gamma radiation groups (5 groups), these groups were injected by gamma radiation. *Nigella sativa* & radiation groups (5 groups), were administered with NS orally for 2 consecutive weeks before injection of radiation.

2.4. Injection of radiation

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

2.5. Blood collection

The Animals were sacrificed after 1, 7, 14, 30 and 60 days from injection with radiation.

2.6. Measurements of K⁺, Na⁺ and Ca⁺²

The blood samples were collected then the changes in the concentrations of Na⁺, K⁺ and Ca⁺² of blood were determined using a hematological auto-analyzer.



2.7. Statistical evaluation

For calculating arithmetic mean and standard deviation for the obtained results a statistical analysis was performed. These results were assessed by Student's t-tests.

3. Result & Discussion

3.1. The effect of Tc^{-99m} radiation

Potassium is one of the body's ions, which transfer an electric charge when dissolved in body fluids such as blood. It is needed for cells, muscles, and nerves to function properly. Calcium ions which carry an electric charge when dissolved in body fluids such as blood. Sodium ion helps to keep the equilibrium of water in and around your cells. Calcium ion is important for proper muscle and nerve function, also to keep constant blood pressure levels. For all measured parameters levels of sodium, potassium and calcium ions concentration shows a significant differences compared to control after injection of radiation ($P < 0.01$). As shown in figure (1), The concentration of K^+ increased from 5.62 mmol/L in control samples to 7.01 mmol/L in samples that collected after 60 days from injecting with radiation ($P < 0.01$). In contrast, in figure (2) the concentration of Ca^{+2} increases from 9.3 mmol/L in the control group to 10.8 mmol/L in after 60 days from injecting with radiation. The change in Na^+ concentration was from 135 mmol/L in control sample to 122 mmol/L as shown in figure (3). The changes in all ionized ions were time-related. Many electrolytes such as sodium, potassium and calcium are present in aqueous buffer that bounded cell membrane. The distribution of K^+ , Ca^{+2} and Na^+ are not in equal manner. Also a difference in the concentrations of these ions in the intracellular and extra cellular environments. Many parameters such as electrochemical gradient, channel of electrolytes and ATPase play an important role in the distribution of K^+ , Ca^{+2} and Na^+ throughout cell membrane [13]. Our obtained results indicate that activation of ions carriers of the cell membranes such as Na^+ due to high amounts of hydrogen ions and ATP. Those high levels of H^+ ions in the intracellular disturb the Na^+-H^+ anti-transport activities within plasma membrane [14]. The gamma radiation causes activation in Na^+-K^+ pump that increase levels of intracellular potassium ion and decrease intracellular sodium ion [15]. Also the radiation cause shift of Ca^{+2} due to the changes that happen occurring respiratory chain due to photo bio-stimulation for enzymes [16]. This work suggests that the changes in (Na^+ , K^+ , Ca^{+2}) ions in plasma can be return to increase motion of intracellular molecules. The absorption of gamma radiation by intracellular chromophores causes many alterations on the movement of ions out of cells. A conformational change in protein molecules due to gamma radiation because of the damage in disulphid bonds within molecules of proteins [17]. The gamma radiation causes a various alteration in cell membrane also enzyme activities of cell membranes including acetyl cholinesterase and sodium- potassium ATPase [18-19]. The conformational changes by ionizing radiation that initiate several biochemical reactions leads to increase the function of enzymes. The enzymes absorb light from surrounding lipids and itself leads to a gradual decrease in sodium ions [20]. Also this gradual decrease in sodium concentration due to photo bio-stimulation of enzyme Na^+-K^+ ATPase that exceed the transport of Na^+ ions. A damage produced in lipid bilayer of cellular membrane due to gamma radiation. That damage appears in polyunsaturated fatty acids then membrane permeability increase [21]. The ionizing radiation absorbed by chromophores [22], that reduces the oxidation potential of cells and increase both reactive oxygen species, oxidation rate [23,24]. The lipid peroxides that produced due to the effect of ionizing radiation on cellular components originate from unsaturated fatty acids of cell membrane [25]. The respiratory chain of mitochondria effected by radiation and electron transport activity enhanced and an increase ATP was produced [26]. Many studies indicated that enzymatic activities are increase due to different doses of gamma radiation on cells [27] also many variations of ATPase activities are found. Also from the obtained results it suggests that the effect of ionizing radiation showed increase in the calcium ion. The interaction of radiation with cellular components causes hyperpolarization to occur due to release of calcium ions that activate potassium ion channels.



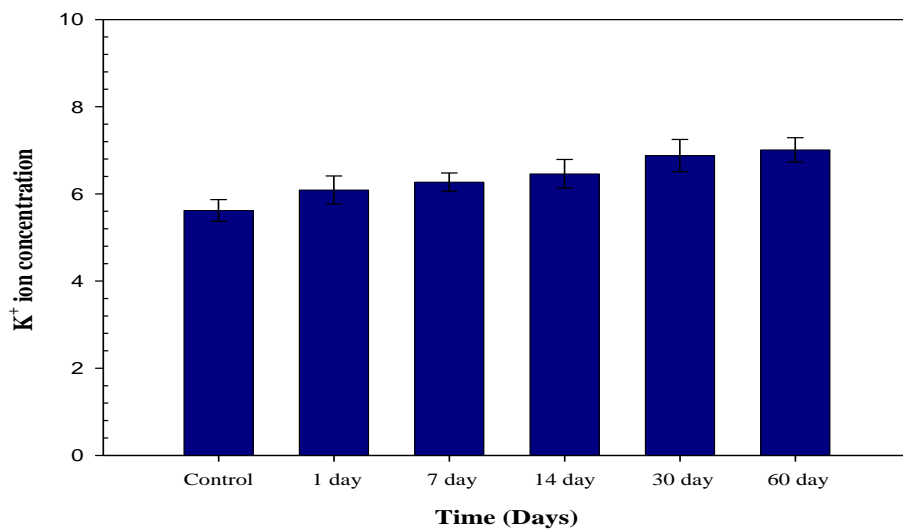


Figure 1: The effect of gamma radiation on potassium ion concentration

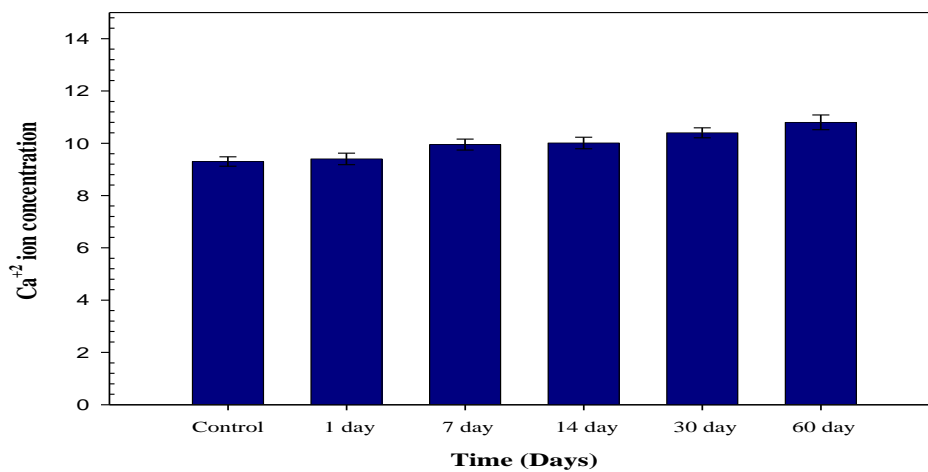


Figure 2: The effect of gamma radiation on Calcium ion concentration

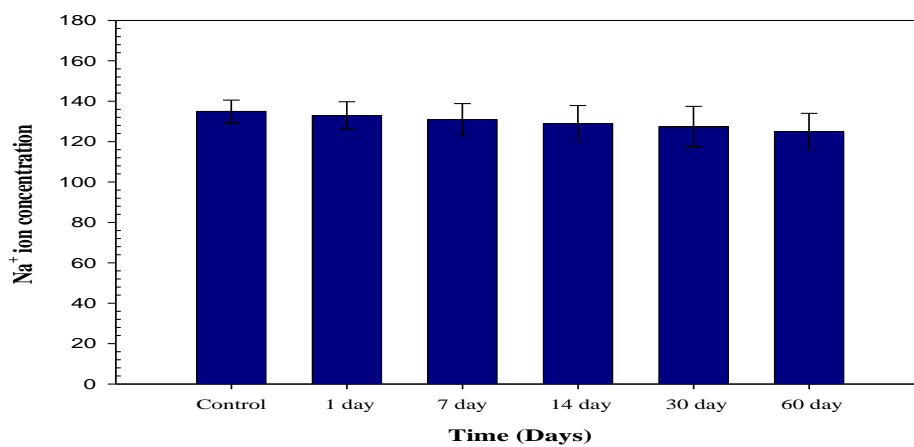


Figure 3: The effect of gamma radiation on Sodium ion concentration



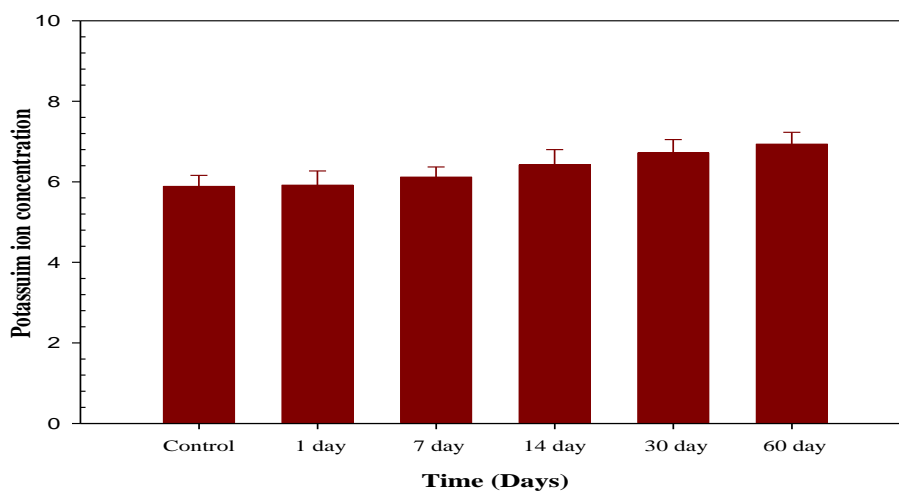


Figure 4: Role of *Nigella sativa* against radiation effect on K^+ concentration

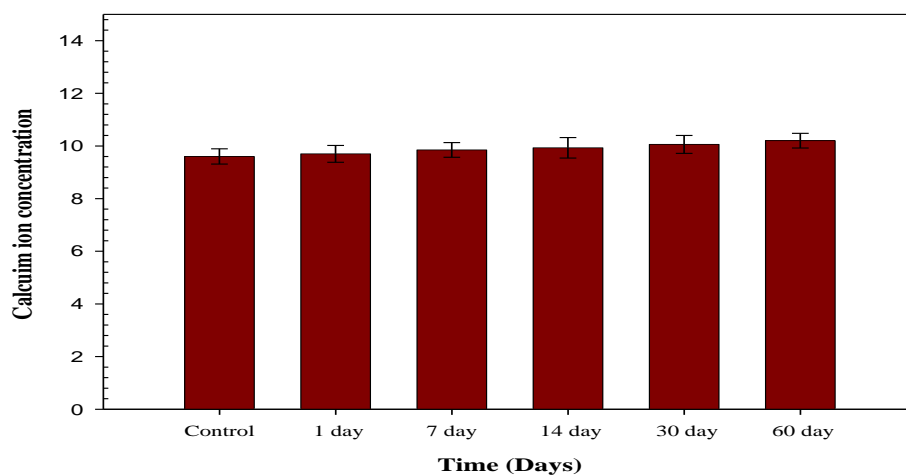


Figure 5: Role of *Nigella sativa* against radiation effect on Ca^{+2} concentration

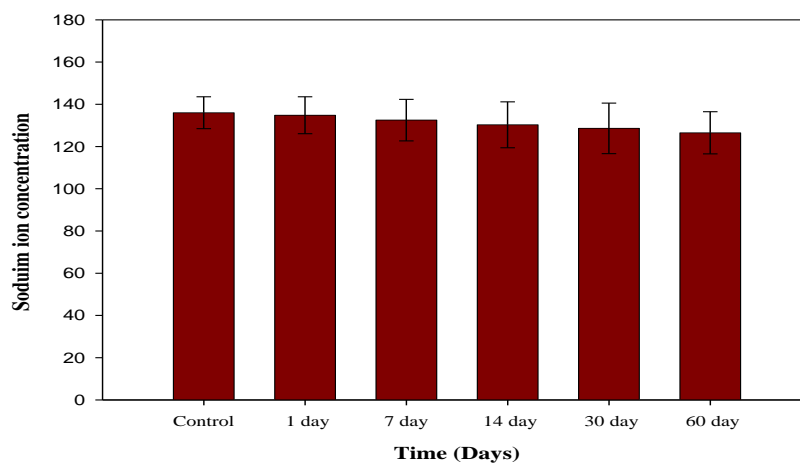


Figure 6: Role of *Nigella sativa* against radiation effect on Na^+ concentration



3.2. Starring role of *Nigella sativa* against ionizing radiation

The effect of *Nigella sativa* to minimize the effect of radiation on ionized potassium, sodium and calcium concentration were studied. As shown in figure (4) a significant change in potassium ion concentration from 5.89 to 6.94 ($P < 0.01$). Meanwhile The variation in sodium ion concentration from 136 to 126.5 ($P < 0.01$) as shown in figure (6). The calcium ions increased from 9.6 to 10.2 ($P < 0.01$) shown in figure (5). To reduce or prevent the oxidation effect of free radicals a radio- protector's materials are used [28]. The ionizing radiation produces reactive oxygen species from normal cellular metabolism [29-30]. The biological system became at risk due to impacts of reactive oxygen [31]. The balance between production of reactive oxygen species and capability of cells to protect against them can be kept up by applying several materials which have antioxidant aspects [32-34]. The radio protective effect of some chemical materials were tested. When these materials administered before injection of ionizing radiation, it reduces mortality. But they have undesirable side effects that limited their use [34]. Meanwhile natural products have benefits including: they are a safe, proven therapeutic uses and a radio protective profile. The natural antioxidant compounds support the defensive system from nourishment [35]. The extraction of antioxidants from natural products has a great importance. A variety of actives compounds were found in the *Nigella sativa* methanolic extraction. Among them carbohydrates, both fixed oils and volatile oils, amino acids, proteins, and, alkaloids, saponins, crude fiber, as well as minerals. The administration of NS methanolic extraction before injection of radiation be able to keep the balance of ion concentration [36-38]. It can be concluded that *Nigella sativa* is a marvel medicinal plant with a large healing spectrum [39-41]. The various extracts of *Nigella sativa* seeds shows a different beneficial effects [42-44].

4. Conclusion

The administration of natural antioxidants such as The *Nigella sativa* methanolic extraction mitigates γ - induced oxidative stress in rat blood. The obtained data showed that ion concentration found to be a good tool to indicated that NS has efficient antioxidant activity, which is proved experimentally. Further investigations are required to elucidate the mechanisms of *Nigella sativa* methanolic extraction actions against ionizing radiation.

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