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Investigation of the Impact of Thermal Acute Low-level Neutron Radiation on Various Hematological Parameters and Lipid Profiles in Rats.

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FEW studies have examined low-dose occupational radiation exposure; most have focused on radiation exposure from therapy or accidents. By analyzing the lipid profile and a few hematological indicators, this study seeks to determine the possible consequences of oxidative damage on the blood of male rats exposed to Thermal Acute low-dose Neutron Radiation (TANR) from the Am-Be source. Four groups of twenty adult male rats were created. The changes in the concentration of Platelets PLTs, Mean Corpuscular Volume (MPV), Lymphocytes (LYMPH), and PLTs/LYMPH ratio were measured, in addition to the changes in total Cholesterol (CHOL), Triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in the blood taken after 14 days from irradiated rats. The results indicated that all irradiated groups, compared with the control group, showed a significant increase in the concentration of total Cholesterol, Triglycerides, LDL-c, LDL/HDL ratio, and PLTs/lymphocyte ratio. At the same time, the results showed a significant decrease in platelet numbers (PLTs), platelet volume (MPV), lymphocytes, and the concentration of HDL-c. The results showed that the low dose of TANR had certain negative effects, illustrated by the rise of Triglycerides and Cholesterol. In conclusion, the blood systems of male rats are considerably impacted by thermal acute neutron radiation.

Key Words : High let radiation, Acute exposure. Lipid profile. Thermal neutron exposure, Astronauts.

Introduction

Interaction with various sources, including the radioactivity released by naturally unstable atoms, cosmic radiation, and other artificial sources, can expose humans to ionizing radiation. Several mechanisms can be used to explain ionizing radiation-induced biological effects, such as the generation of free radicals in the irradiated area, which can cause localized or systemic oxidative stress or effects that affect the body as a whole. This continuous cell challenge may cause genomic instability and the development of cancer. [1], [2], while also inducing several DNA damage response (DDR) mechanisms, which are thought to be the primary element of the cellular radiation response [3]. Given that some of the immune system's constituents are thought to be the body's most radiosensitive substances, exposure to high-LET particles undoubtedly impairs it [4]. More specifically, it has been shown that lymphoid cells and tissues are markedly affected by high-LET radiation at relatively low doses and that some aberrations persist long after exposure, such as thymus and spleen atrophy and leucocyte population depletion [5]. Furthermore, alterations to the immune balance have been observed, including decreased functions of T cells and natural killer cells as well as elevated levels of inflammatory plasma cytokines [6]. El-Marakby et al. (2020) demonstrated that a radio-adaptive response occurs particularly after prolonged exposure to low-gamma doses before exposure to a relatively high dose[7]. The National Council on Radiation Protection (NCRP) examined the potential radiological hazards associated with radiation and recognized the need for further research into the radiobiology of neutrons [8]. Furthermore, the health effects of neutron exposure have been of great concern ever since the detonation of Hiroshima in 1945. This idea has gained momentum in tune with more radiology, Radiation Biology, Medicine, and especially space research. During deep space

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missions, astronauts are exposed to highly ionizing radiation, including neutrons, protons, and heavy ions from galactic cosmic rays (GCR), solar wind (SW), and solar energetic particles (SEP), This increases the risks for carcinogenesis, damage to the central nervous system (CNS), cardiovascular diseases, etc. Large SEP events can even cause acute radiation syndrome (ARS) [9]. The radio-adaptive phenomenon is generally noticed with low-LET radiation like Gamma radiation [10], [11], and only scarce information exists for high-LET neutrons. [12]. For the above reasons, the mechanisms by which exposure to cosmic rays can disrupt the central nervous system (CNS) are troubling space agencies (e.g., the National Aeronautics and Space Administration (NASA)), as the neurocognitive complications that may occur jeopardize the success of the mission, the safety of astronauts and their quality of life after completion of the mission [13]. This research is a component of a larger study that describes the biological effects of low-dose acute neutrons on rats' entire bodies and looks at how this amount of radiation affects rats' ability to adapt to radiation.[14]

In previous research, we studied the effect of low doses of gamma rays on the rat's blood [7]. The focus of this study is on low radiation doses of 5, 10, and 50 mSv as acute low neutron doses. We investigated the effect of these low doses on some hematological properties and different types of lipids in rats' blood. However, we also searched for a response to the following: Does radiation exposure to a very low dose of neutron (TANR) have a dangerous effect?

Materials and Methods

Animals' preparation

The animals were prepared to be irradiated under the following conditions: Animal selection and feeding of 20 male albino rats with an average weight of 130±10 g, the animals were fed with normal rat food with a balanced meal. The best types of rat feed were used and contained about 21% 10 ± protein. Considering good ventilation, regular feeding, and drinking water.

Housing for animals

The animals were housed in plastic cages and preserved at room temperature and pressure. The room light was controlled (12-h day/night cycle). The cages were cleaned daily at fixed times. The animals' housing and feeding were done for this study at the biophysics lab, faculty of science, Al-Azhar University.

Animal Grouping and Irradiation

Animals were classified into four groups (G#). Each group consists of five male mice. The first group (G1) was selected as a control group. The remaining groups were defined as G2, G3, and G4. Each one of these groups was exposed to a certain dose of neutron radiation, and the effect of each dose level on the blood of mice was studied. G2, G3, and G4 were exposed to an acute neutron dose of 5, 10, and 50 mSv, at a dose rate of 1.5 mSv/h for 3.5, 7, and 40 h, respectively. The neutron source was an Am-Be source with a dose rate of 1.5 mSv/h. The neutron source emits spectrum neutrons from 0 up to $11\ MeV$ with an average energy of around $4.5\ MeV$. All rats were irradiated to neutron with a dose rate of $1.5\ mSv/h.$ Animal treatment was approved by the Institutional Animal Care and Use Committee (IACUC) in Egypt. Neutron dose equivalents are measured using a secondary standard neutron monitor (Nuclear Enterprise, UK, NM2) at the National Institute of Standards (NIS), Giza, Egypt, which can be traced to an SI unit and calibrated at PTB, Germany [15].

Hematological parameters

The hematology analyzer (Diff3) Mek6410/ Mek-6420 was used to determine and evaluate the platelets (PLTs), the mean platelet volume (MPV), and the lymphocytes (LYMPH) within two hours after collection using Wintrobe's technique. [16].

***C**holesterol, Triglycerides, LDL-C, and HDL-C*

Cholesterol was determined according to the Kaplan and Pesce method [17]. Low-Density Lipoprotein Cholesterol (LDL-C) [18] and High-Density Lipoprotein Cholesterol (HDL-C) fractions were determined according to Nauck et al. [19]**.** Triglycerides were determined according to **Fossati (1982)** [20]**.** The serum was separated from the samples by centrifuging them for ten minutes at 3000 rpm. The serum was then kept in a refrigerator at -20 °C for one day until used.

Computational and statistical analysis

The Statistical Package for the Social Sciences application (SPSS Inc., Chicago, US) for data analysis and comparison in version 26 computed the analysis findings for the data and curves. The mean ± S.D. is used to express the results. At $p < 0.05$, the statistical significance threshold was established.

Results

Effects of Thermal Acute Neutron Radiation Exposure (TANR)

On PLTs count and volume

Figures 1 (A & B) illustrate the difference in percentage for G2, 3, and 4 compared with G1 for PLTs counts and PLTs volume. As indicated in this figure, there is a deep decrease in both PLTs count and volume as the radiation dose from the neutron source (Am-Be) increases. The difference percentages in PLTs count in G 2, 3, and 4 to G1 were (5.44% ± 6.86), (9.2%±7.18), and (20.78% ± 11.23), while they were (7.68% ±0.056), (12.89% ± 0.10), and (21.42 % ± 0.062) for PLTS volume, respectively.

On percentage of Lymphocytes

Fig. 2. shows the effect of the thermal neutron radiation dose on the percentage of lymphocytes in the blood of the rats. The percentage of lymphocytes in the control group was (90.16 ±1.69%), which is a normal value. After irradiating other groups, the percentage values of lymphocytes decreased with the increase in neutron dose radiation. The values were (85.97 ±1.02, 75.92±2.78, and 53.03±2.43) for G 2, 3, and 4, respectively.

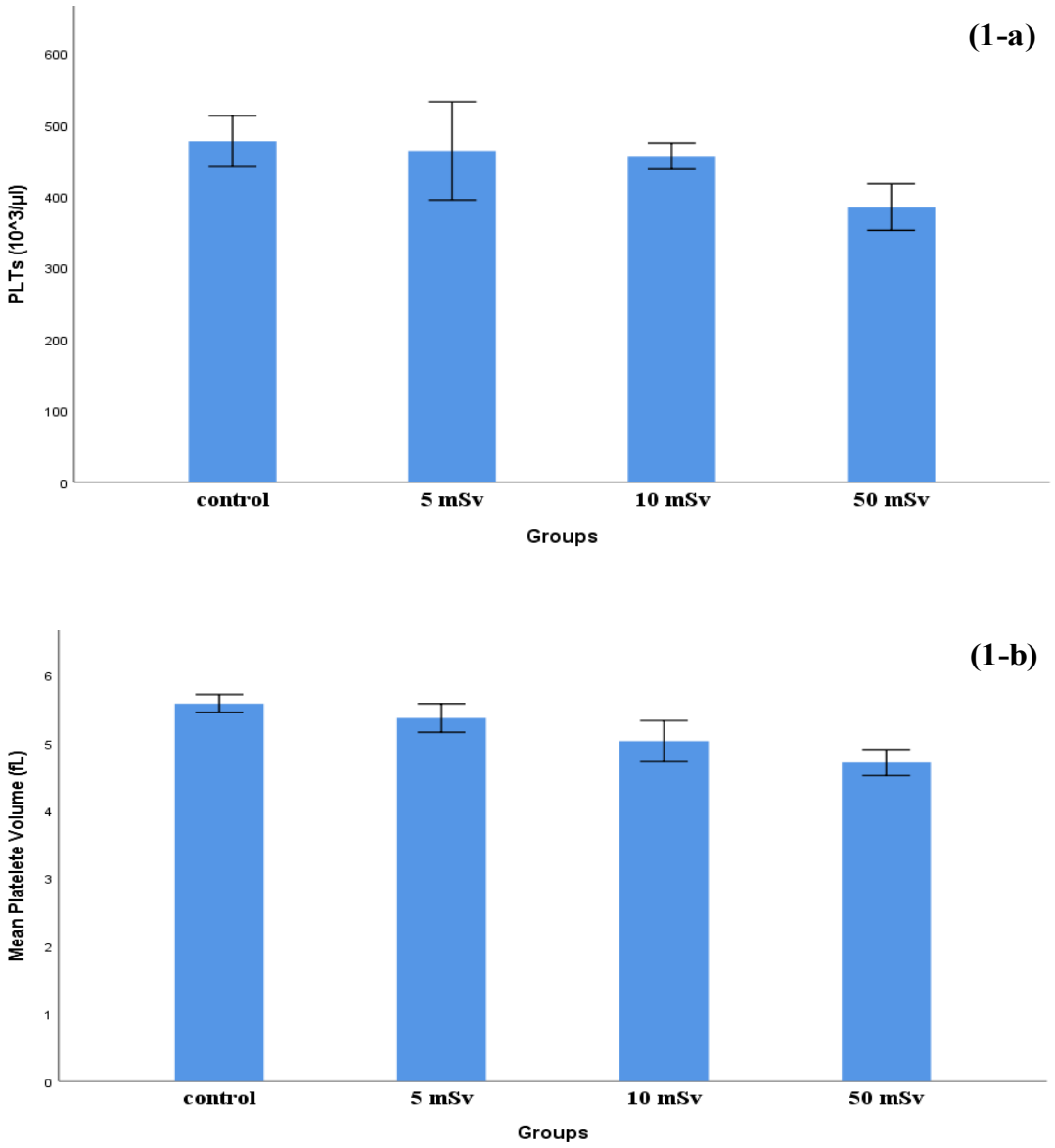

Fig. 1. Effect of neutron radiation doses on (a) Platelets [PLTs], and (b) [MPV] for all irradiated groups.

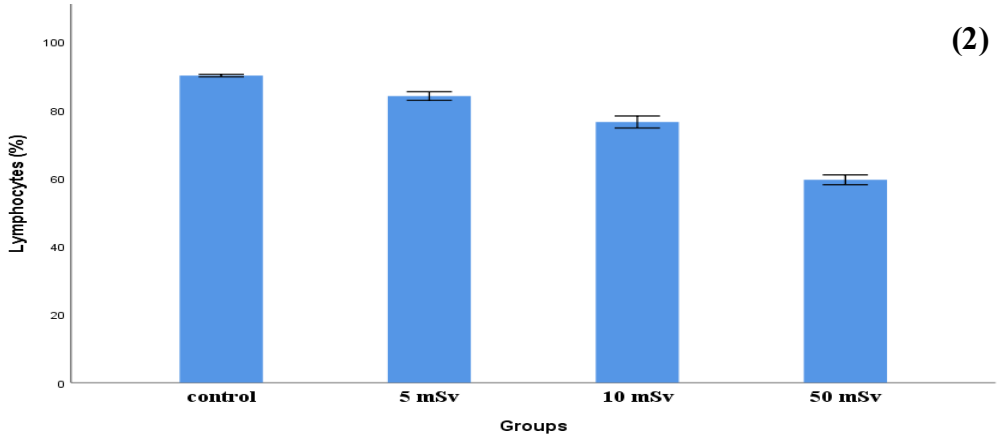

Fig. 2. Effect of neutron radiation doses on lymphocytes for all irradiated groups.

On PLTs to Lymphocyte Ratio (PLTs/ Lymphocytes)

Fig. 3. Describes the effect of radiation on the PLTs-to-lymphocyte ratio. As indicated in this figure, there was a (0.78$\pm$0.12%) difference percentage in the PLTs to lymphocyte ratio between the values of G2 as a control group and G1, which was exposed to 5 mSv from neutron radiation, as well as an increase (7.92$\pm$0.125%) for G3 and (34.83$\pm$0.32%) for G4, who were exposed to 10 mSv and 50 mSv of thermal acute neutron radiation, respectively, compared with G1.

On percentage of Cholesterol and Triglycerides concentration

Fig.4 illustrates the effect of neutron doses on the concentration of (a) Triglycerides and (b) Cholesterol for the examined groups. As shown in these figures, Triglycerides are 1.83, 13.43, and 32.16 for neutron exposure to 5, 10, and 50 mSv respectively. In addition, Cholesterol increased (4.73, 17.57, and 38.69) for neutron exposure to 5,10, and 50 mSv respectively when compared with the Control group.

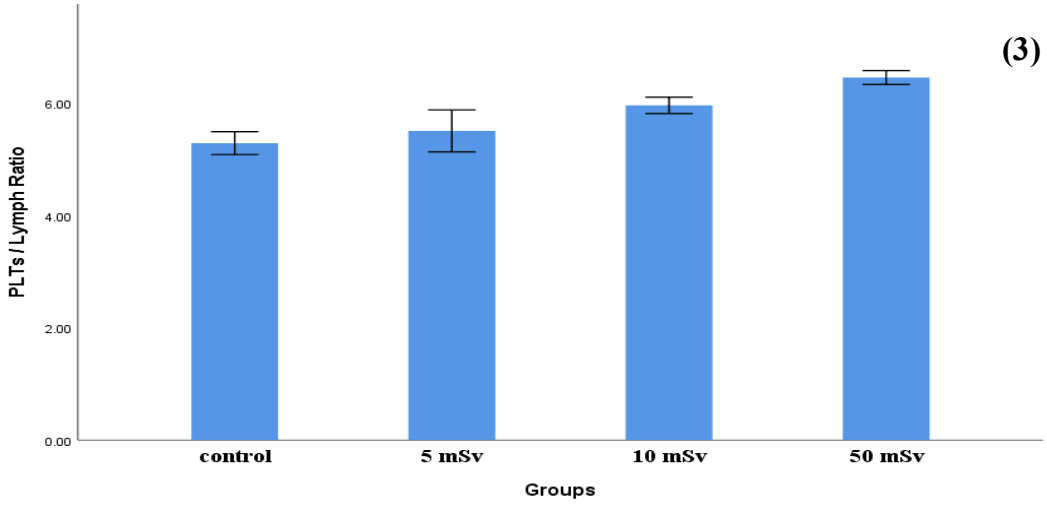

Fig. 3. The effect of neutron radiation doses on the [PLTs/ Lymph Ratio] for all irradiated groups.

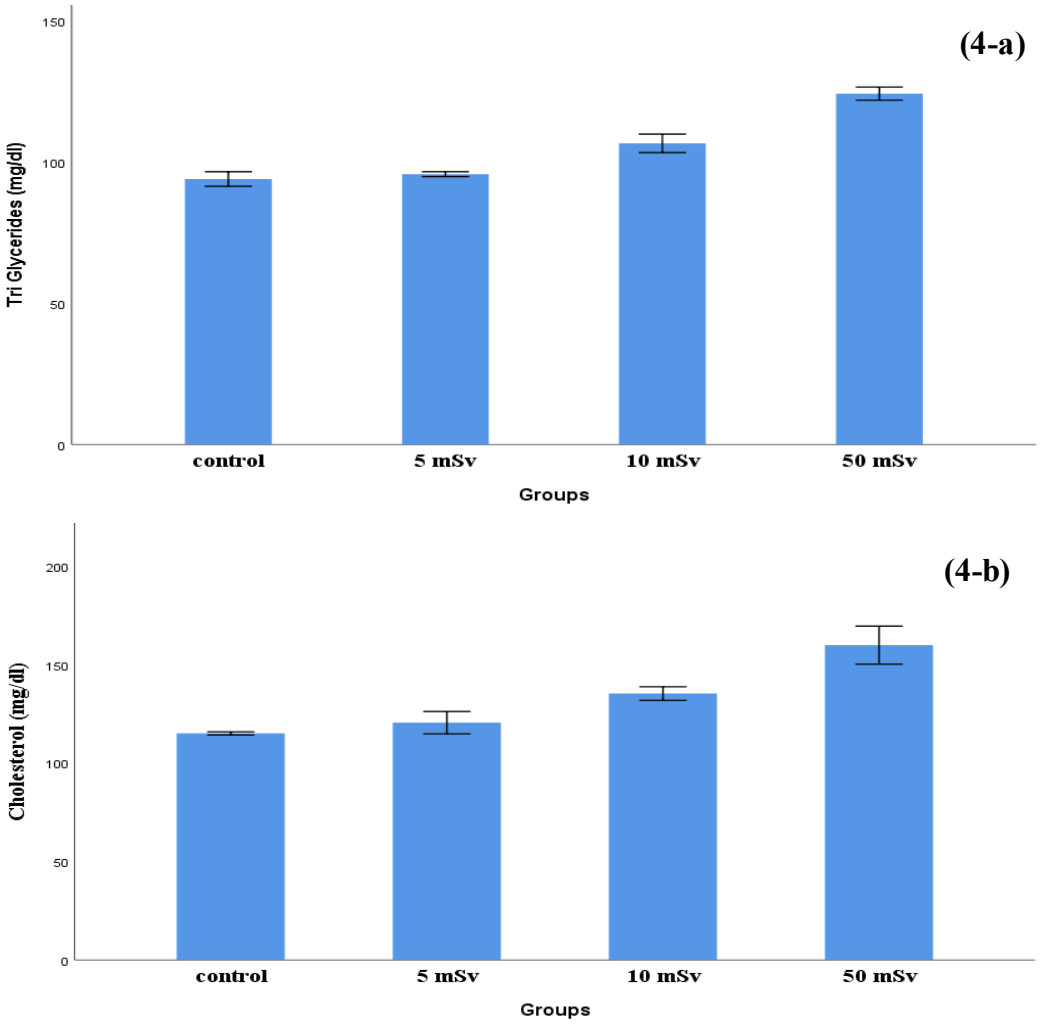

Fig. 4. The effect of neutron radiation doses on (a) Triglycerides and (b) Cholesterol concentration.

On the HDL-C and LDL-C concentrations

Fig. 5. shows the effect of low doses of neutron radiation on HDL-C, LDL-C concentrations, and HDL-C/LDL-C ratio of the investigated groups. As shown in this figure, the HDL-C concentration for examined groups compared with the control decreased gradually. The concentration difference percentages for G2, 3, and 4 compared with G1 were (8.49$\pm$0.96%, 12.41$\pm$2.03%, and 26.32$\pm$1.18%) respectively. Also, this figure indicates that there was a significant increase in the concentration of LDL-C and the difference percentage between G2 to G1 was (5.1$\pm$0.94%), while it was (12.03$\pm$ 1.3%) and (19.97$\pm$0.42 %) differences between G3 to G1 and G4 to G1 respectively.

On the LDL-C/HDL-C Ratio

Fig. 6. explains the effect of neutron radiation on the LDL-C/HDL-C Ratio. As indicated in this figure, there is a significant increase of 15.4 $\pm$0.03 % in the LDL-C/HDL-C ratio between the values of G1 as a control group and G2, while there is a rise of 26.7$\pm$0.1% for G3 and 62.74 $\pm$0.16% for G4, respectively, compared with G1.

Discussion

Neutron radiation has High Linear Energy Transfer (LET) and that has no charge. So, the radiobiology of neutrons is gaining importance amidst the prevailing nuclear arsenals (including H-bomb), nuclear power plants, and the unique A-bomb exposed cohorts in Hiroshima and Nagasaki. The criticality emphasizes the need for evaluating the biological response.

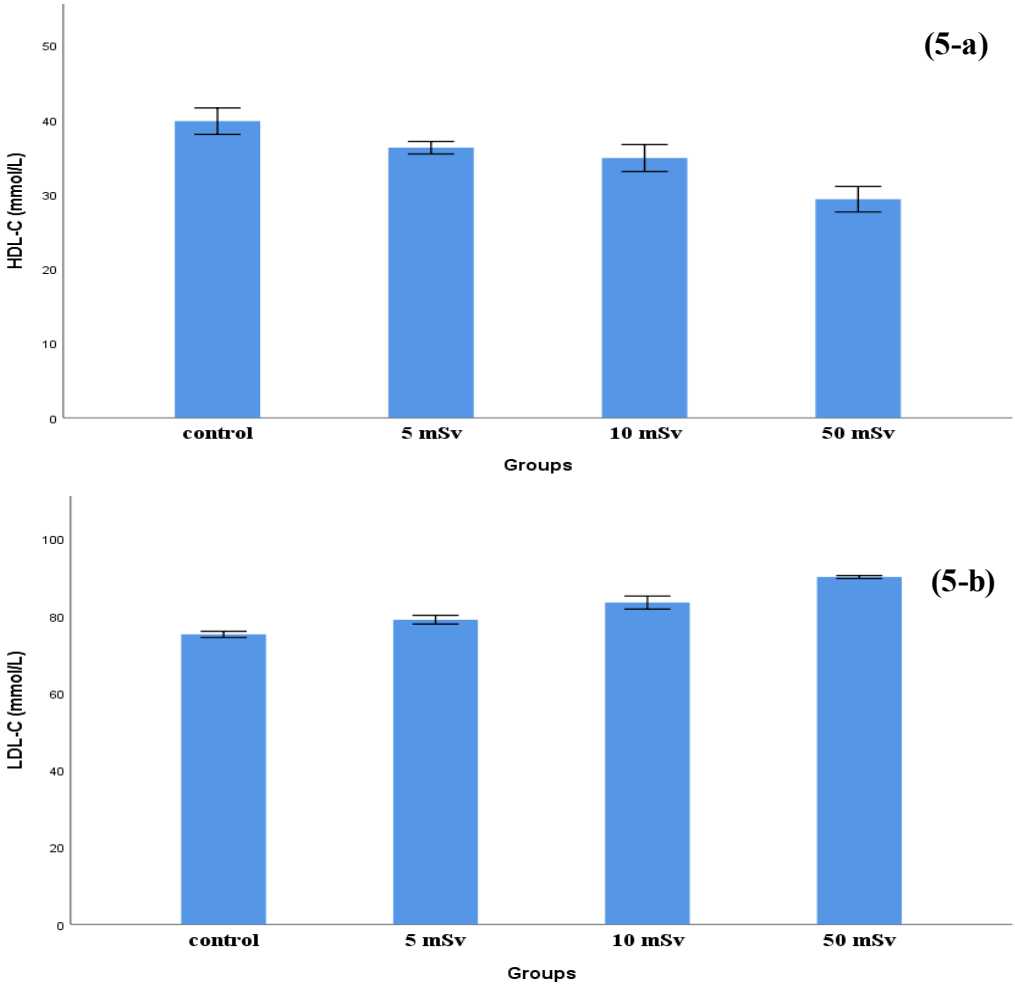

Fig 5. The effect of neutron radiation doses on (a) HDL-C and (b) LDL-C

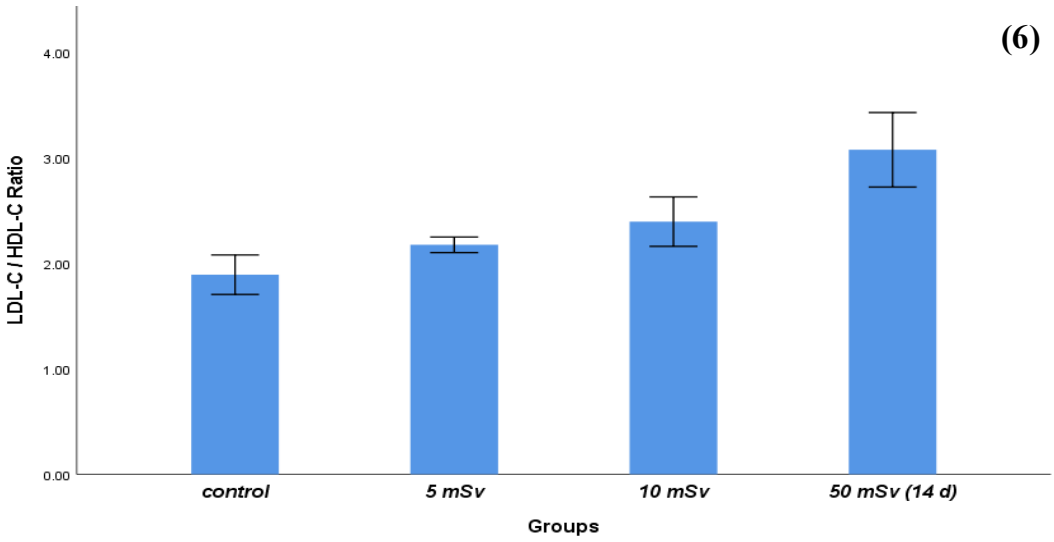

Fig. 6. The effect low doses of neutron beams on HDL/LDL Ratio for the examined groups relative to the control group.

Platelets (PLTs) are small blood with anucleate cells that stick together to make blood clots that stop or slow bleeding when there is a cut or injury. Platelets are made from animal bone marrow. The average platelet size is determined by the MPV blood test. Any change in the result of this test out of normal value may be due to a defect in the bone marrow. Therefore, this test can be used to diagnose bone marrow diseases and bleeding problems. Proplatelets in the blood are further divided into smaller platelets in the circulatory system [21]. Traditionally, the main function of platelets is to participate in hemostasis and thrombosis. Yet, increasing evidence has suggested that platelets can perform a myriad of other functions. For instance, platelets can secrete both pro-inflammatory and anti-inflammatory cytokines, thereby modulating immune system function [22][23]. There are two types of thrombocytosis: autonomous thrombocytosis, which occurs from disequilibrium in the clonal bone marrow process (e.g., hematologic malignancies), and reactive thrombocytosis, which is more common and occurs in response to endogenous processes external to the megakaryocyte cell. Causes of reactive thrombocytosis include iron deficiency, infection, inflammation, postoperative stress, and hematologic malignancy. Patients with severe iron deficiency often present with thrombocytosis [24]. The underlying mechanism for an increased platelet count is unclear but is independent of hematopoietic growth factors such as thrombopoietin and thus Thrombocytosis may occur in response to excessive bleeding (i.e., to increased coagulation potential) [25] or to prevent erythropoiesis in an iron-deficient environment [26]. As early as 1865, Armand Trousseau documented the development of venous thrombosis during the progress of pancreatic cancer, suggesting an interplay between cancer development, platelet activity, and blood clot formation [27]. Since then, more clinical data has suggested an elevated risk of venous thromboembolism in cancer patients, and the risk depends upon factors including cancer type and stage [28]. Conversely, higher cancer morbidity is observed in patients with primary deep venous thromboembolism. In cancer patients, thrombosis is associated with a poor prognosis, such as an increase in cancer progression and mortality [29]. Tumor cells can induce platelet activation and aggregation directly, either through the release of soluble platelet agonists into the bloodstream or through contact between tumor cells and platelets. Tumor cells can release classic soluble factors such as adenosine diphosphate (ADP) [30]. Moreover, the association between platelet activity and cancer has been employed in the clinical detection of cancer [31]. Many studies have shown that platelets can directly be associated with tumors of both epithelial (e.g., breast and lung) and mesenchymal (e.g., melanoma) origin. Platelets' diverse functions and their deep association with cancer progression prompt scientists to study platelets' functions in cancer progression.

The effect of ionizing radiation on the hematopoietic system must be considered due to the high exposure risk of occupational populations. So, in this study, we assessed the effect of neutron radiation on rats and the percentage of platelets in the blood. The results showed a significant decrease in the percentage of platelets with an increase in neutron dose in all acute exposure groups (G2, G3, and G4) for doses (5, 10, and 50 mSv respectively). There is a relationship between platelet count and mean platelet volume (MPV) [32] and a relationship between platelet count and spleen size [33]. Along with an elevated platelet count, indicators of iron deficiency from a complete blood count (CBC) include an increased mean platelet volume, a low hematocrit, a low mean corpuscular volume (MCV), and a high red cell distribution width.

Lymphocytes enable the body to remember antigens and to distinguish self from harmful non-self (including viruses and bacteria). Lymphocytes are a type of white blood cell that plays several roles in the immune system, including protection against bacteria, viruses, fungi, and parasites and enable the body to remember antigens and distinguish itself from harmful non-self-objects (including viruses and bacteria). Lymphocytes usually constitute 20 to 40% of all white blood cells in the bloodstream circulate in the bloodstream and lymphatic system and travel to tissues as needed. The immune system can remember every antigen it encounters because, after the encounter, some lymphocytes develop into memory cells. These cells live for a long time, for years or even decades. When memory cells encounter an antigen for the second time, they immediately recognize it and respond quickly, strongly, and specifically to that antigen. This specific immune response is why people don't get chickenpox or measles more than once, and vaccination can prevent certain disorders. Xiang Hong Li also reported that the number of

blood cells, lymphocytes, and monocytes changes with whole-body irradiation when exposed to the effect of low- and medium-dose gamma radiation on the hematopoietic system of mice at different doses from 0.5 to 3 and 5 Gy [34], [35]. There are several main categories of thrombocytopenia, grouped according to the cause of the disease: (1) immune thrombocytopenia, (2) hereditary deficiency-associated thrombocytopenia, and (3) cancer-associated thrombocytopenia, which may occur in diseases such as chronic lymphocytic leukemia and tumors. Lymphoma, prostate, breast, and ovarian cancer [36], [37]. Seed et al. reported that infrared radiation is one of the cytotoxic agents that particularly causes damage to cell regeneration systems. They also showed that lymphocytes, platelets, and neutrophil granulocytes uniformly showed an early decline over the first few days, consistent with cumulative radiation doses [38]. Previous studies in mice have shown that lymphocytes, a type of white blood cell, show the most immediate response to infrared radiation by showing a significant decrease after radiation exposure. In contrast, platelets decline more gradually over a longer period [38].

In this study, we observed fewer lymphocytes in the group exposed to acute neutron radiation at doses of 5, 10, and 50 mSv. This indicates that there are complications associated with hematopoietic syndrome. A decrease in lymphocytes is associated with a decrease in PLTs count and MPV. The detrimental effects of radiation on red blood cell count may also be linked to the bone marrow's inability to produce lymphocytes, along with the loss of cells from the circulation blood system due to bleeding or leakage through capillary walls, as well as the direct destruction of mature circulating cells. This suggests that direct damage from a lethal dose of ionizing radiation may be the source of the drop in normal blood index values after radiation exposure [39]. The decrease in the number of peripheral blood cells recorded during radiation exposure serves not only as a marker of exposure severity but also as a marker for treatment and prognosis [40].

Lipids are organic compounds that may be divided into classes of biomolecules that ensure the proper metabolism and functioning of the organism. The major groups of lipoproteins in order of size are chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). They enable the transport of multiple different fat molecules, including cholesterol (CHOL) and triglycerides (TG). Any malfunctioning of the anabolic or catabolic processes of lipoproteins may lead to the development of pathological processes in cells. Disorders in lipid and lipoprotein metabolism can be a result of the metabolic syndrome—overweight and obesity—which can be associated with a higher risk of cancer and can have an impact on the prognosis in cancer patients [41]. A lot of previous research noticed that CHOL accumulates in malignant tissues. CHOL is the precursor in steroidogenesis [42], in which androgens are produced, which in turn stimulate the proliferation of prostate cancer (PCa) cells. Other authors point out the importance of factors such as a healthy endothelium and a very strong antioxidative system in cancer progression. LDL protects the endothelium against oxidation, and oxidatively modified LDL is an atherogenic risk factor. LDL is also proven to downregulate lysyl oxidase (LOX), which is a kind of protein crucial in cancer invasion. The metastases and advancement of diseases correlate with angiogenesis (a cancer growth and metastasis factor). In the studies of Rokicka., et al on cervical cancer [43] and Ghahremanfard., et al on breast, colon, gastric, and ovarian cancers, it was observed that a statistically significant increase of CHOL and LDL values correlates with the advancement of the diseases [44]. LDL is one of many factors that take part in the process of angiogenesis and can inhibit this process. The growth of tumors and metastases can be stopped by inhibiting angiogenesis [45]. In this study, an increase in LDL after exposure to TANR was shown, and it was observed to increase at higher concentrations of dose. indicate that a higher risk of cancer can have an impact on the prognosis of cancer patients [41].

Conclusion

This study attempted to assess the impact of 5, 10, and 50 mSv doses of thermal acute neutron radiation from the Am-Be source on several lipids and blood indices in rats, including triglycerides, cholesterol, HDL-C, and LDL-C, PLTs, and MPV. The results indicated that there were significant changes in the biological process in these parameters. Acute neutron exposure at low doses of 5, 10, and 50 mSv produced a lot of free radicals, which raised total cholesterol and had negative effects on red blood cells. Based on our results, there are harmful effects on blood indices and concentrations of cholesterol

and triglycerides in rat blood according to the neutron dose. To properly evaluate this "effect," a broader group of studies would be needed with an adjustment for confounding factors including time exposure, time response, type of radiation, and so forth. We need a broader study that furthers this. The following Table 1 provides an overview of the notable alterations in blood parameters and lipid profiles observed during the study.

TABLE 1: summarizing the key results of the study, emphasizing the changes observed in blood parameters and lipid profiles:

Parameter	*Control Group*	*Low Dose (5 mSv)*	*Medium Dose (10 mSv)*	*High Dose (50 mSv)*	*Significance*
Platelet Count	Normal	Decreased	Decreased	Decreased	$p < 0.05$
Platelet Volume	Normal	Decreased	Decreased	Decreased	$p < 0.05$
Lymphocytes (%)	Normal	Decreased	Decreased	Decreased	$p < 0.05$
Plts/ Lymph Ratio	Normal	Increased	Increased	Increased	$p < 0.05$
Total Cholesterol	Normal	Increased	Increased	Increased	$p < 0.05$
Triglycerides	Normal	Increased	Increased	Increased	$p < 0.05$
LDL-C	Normal	Increased	Increased	Increased	$p < 0.05$
HDL-C	Normal	Decreased	Decreased	Decreased	$p < 0.05$

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"دراسة تأثير الإشعاع النيوتروني منخفض المستوى على بعض مؤشرات الدم وملف الدهون في الجرذان"

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تناولت العديد من الدراسات التعرض للإشعاع، سواء عن طريق الحوادث أو من خلال العلاج، مع وجود عدد قليل من الدراسات التي تنطوي على التعرض المهني لجرعات منخفضة.

الهدف من الدراسة هو:
التحقيق في التأثيرات المحتملة للتلف التأكسدي على دم الفئران الذكور الناجم عن الإشعاع النيوتروني الحراري ذو التعرض الحاد منخفض الجرعة (TANR) المنبعث من مصدر Am–Be من خلال فحص نسبه الدهون الثلاثية والكوليسترول وبعض المعايير الدموية.

المواد والطرق:
تم تقسيم عشرين فأرًا إلى أربع مجموعات. تم قياس التغيرات في تركيز الصفائح الدموية PLTs ومتوسط حجم الصفائح الدموية (MPV) ونسبه الخلايا الليمفاوية (LYMPH) ونسبةPLTs / LYMPH ، بالإضافة إلى التغيير في الكوليسترول الكلي (CHOL) والدهون الثلاثية (TG) والبروتين الدهني عالي الكثافة(HDL) والبروتين الدهني منخفض الكثافة (LDL) في الدم المأخوذ بعد 14 يومًا من الفئران المعرضة للإشعاع.

النتائج والمناقشة:
أشارت النتائج إلى أن جميع المجموعات المشعة، مقارنة بمجموعة التحكم، أظهرت زيادة كبيرة في تركيز الكوليسترول الكلي والدهون الثلاثية وLDL–c ونسبة LDL/HDL ونسبة PLTs/lymphocytes وفي الوقت نفسه، أظهرت النتائج انخفاضًا كبيرًا في أعداد الصفائح الدموية (PLTs) ومتوسط حجم الصفائح الدموية (MPV)والخلايا الليمفاوية وتركيزHDL–c وأظهرت النتائج أن الجرعة المنخفضة من TANR كان لها بعض التأثيرات السلبية، والتي يتضح من ارتفاع الدهون الثلاثية والكوليسترول. وفي الختام، تتأثر أنظمة دم ذكور الفئران بشكل كبير بالإشعاع النيوتروني الحاد الحراري. تم تقسيم عشرين فأرًا إلى أربع مجموعات. تم قياس التغيرات في تركيز PLTs وحجم الكريات المتوسطة (MPV) والخلايا الليمفاوية (LYMPH) ونسبةPLTs / LYMPH ، بالإضافة إلى التغيير في الكوليسترول الكلي (CHOL) والدهون الثلاثية (TG) والبروتين الدهني عالي الكثافة (HDL)والبروتين الدهني منخفض الكثافة (LDL) في الدم المأخوذ بعد 14 يومًا من الفئران المعرضة للإشعاع.
أشارت النتائج إلى أن جميع المجموعات المشعة، مقارنة بمجموعة التحكم، أظهرت زيادة كبيرة في تركيز الكوليسترول الكلي والدهون الثلاثية و LDL–cونسبة LDL/HDL ونسبة .PLTs/lymphocytes وفي الوقت نفسه، أظهرت النتائج انخفاضًا كبيرًا في أعداد الصفائح الدموية (PLTs) وحجم الصفائح الدموية(MPV) والخلايا الليمفاوية وتركيز .HDL–c وأظهرت النتائج أن الجرعة المنخفضة من TANR كان لها بعض التأثيرات السلبية، والتي يتضح من ارتفاع الدهون الثلاثية والكوليسترول.

استنتاج:
تتأثر أنظمة دم ذكور الفئران بشكل كبير بالإشعاع النيوتروني الحاد الحراري.