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Assessing the Biological Effects of Irradiated Sorghum (Sorghum bicolor) Seeds in Male Wistar Rats

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ABSTRACT

Sorghum, a key cereal crop worldwide, is vital to millions of people in the semi-arid tropical regions. This study aimed to evaluate the growth rate and certain hematological parameters in male Wistar rats fed irradiated sorghum (*Sorghum bicolor*) for 2 months. Sorghum seeds were purchased locally, cleaned manually, and sorted into 4 groups, each placed in Petri dishes. 3 groups were treated with different forms of irradiation—X-ray, gamma-ray, and UV light—while the fourth group served as the control. Growth rates were determined by measuring the initial and final body weights of the rats. After 60 days, blood samples were collected via the retrorbital sinus, and renal, hematological, and liver function parameters were evaluated. The findings showed that the growth rate of rats fed irradiated sorghum was lower than that of the control group. The highest growth rate (3.57 g/day) was observed in control-sorghum-fed rats, followed by UV-sorghum-fed rats (3.4 g/day), X-ray-sorghum-fed rats (3.33 g/day), and gamma-sorghum-fed rats (3.25 g/day). In addition, irradiation affected several hematological, renal, and liver function indicators. It is important to assess the safety of food and feed exposed to irradiation before use.

Keywords: Hematological parameters, Growth rate, Wistar rats, Sorghum seeds, Irradiation

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Introduction

Wistar rats (Rodentia, Muridae: Rattus norvegicus domestica) are commonly used in scientific research, particularly as animal models for studies in psychology, biomedicine, pharmacology, and cancer research [1]. These albino rats are frequently utilized in research settings, often more than mice or other rat species [2]. Ionizing radiation, including gamma rays, X-rays, and UV light, consists of electromagnetic waves capable of ionizing atoms or molecules. This contrasts with non-ionizing radiation, such as microwaves, visible light, infrared, and radio waves [3]. Ionizing radiation is commonly employed in medical imaging, radiotherapy, and nuclear energy applications, including nuclear reactors and weapons. Exposure to such radiation can cause cell damage, with high doses resulting in radiation burns, while lower, sustained doses are linked to cancer development [4] and mutations in somatic or reproductive cells [5]. DNA is highly vulnerable to ionizing radiation, which can lead to DNA damage, potentially cause tissue harm, and disrupt biological systems [6].

Sorghum a flowering monocot grass, is cultivated for animal and human consumption [7]. This cereal is rich in carbohydrates (74.68%), fiber (1.71%), fat (4.24%), and protein (12.25%), as well as a variety of vitamins (B1, B2, B3, B5, B6, B9, C, and E) and minerals (Ca, Cu, Fe, Mg, Mn, P, K, Si, Na, and Zn). Sorghum grain is edible

in its young form and can be consumed raw, but once mature, it must be ground into flour for consumption [8, 9]. Hematology involves the study of blood-related disorders, including the diagnosis, prevention, and treatment of conditions affecting blood cells, hemoglobin, bone marrow, blood vessels, spleen, and coagulation agents. Blood tests commonly include assessments of renal function (e.g., creatinine, urea, Na, K), liver function (e.g., albumin, bilirubin, protein levels, ALP, ALT, GGT, AST), and complete blood counts (e.g., WBC, RBC, platelets) [10-12].

This study aimed to evaluate the growth rate and certain hematological parameters of male Wistar rats fed irradiated sorghum (treated with X-rays, gamma rays, and UV light) over two months.

Materials and Methods

Materials

Sorghum bicolor seeds, the primary material for this research, were manually cleaned and separated into four distinct groups in sterile Petri dishes. The first group underwent exposure to X-ray radiation (100 eV) using an X-ray apparatus, while the second group received gamma-ray exposure (200 cGY) from a Co-60 unit at the National Cancer Institute, University of Gezira, Sudan. The third group was subjected to UV light for 30 minutes at the Food Microbiology Laboratory, Faculty of Engineering and Technology, University of Gezira. The fourth group was maintained as a control. After treatment, the seeds from each group were ground individually to produce sorghum cakes, which were then used to feed the experimental rats.

Experimental animals

The study utilized twelve male Wistar albino rats, each 4 weeks old, acquired from the Biosafety Center Laboratory in Khartoum State. The rats were randomly allocated to four groups according to the type of feed. Weights were recorded at the beginning and end of the 60-day trial period under standardized conditions. The rats were housed in clean polypropylene cages, with each receiving a daily ration of 12 grams of wheat bran mixed with 12 grams of irradiated sorghum flour in a 1:1 ratio. The mixture was blended with water to form a paste, which was then molded into small pieces, compressed, and allowed to dry. No additional food or supplements were introduced during the experiment. The study was approved by the Faculty of Scientific Research's Ethical Committee at the University of Gezira (1523-October, 2018).

Blood sampling for hematological evaluation

After the study, blood samples were obtained from the retro-orbital sinus of the Wistar rats, a network of dilated veins located at the rear of the eye socket. Before blood collection, the rats were fasted overnight. No anesthesia was used during the procedure. A microhematocrit tube was gently inserted into the medial canthus, near the eye, to collect the blood. Approximately 2.5 ml of blood was drawn and placed into a sterile container containing EDTA as an anticoagulant to prevent clotting.

The blood cell counts, including white blood cells, red blood cells (RBC), and platelets, were performed using a Sysmex KX 21N analyzer. To separate the plasma, the collected samples were centrifuged at 2500 rpm for 5 minutes. The plasma was then analyzed for renal function indicators, including creatinine, urea, sodium (Na+), and potassium (K+). Liver function tests—such as total protein, albumin, bilirubin, total bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase (ALT)—were conducted at the Blood Bank Laboratory, Wad Medani Teaching Hospital.

Statistical analysis

The growth rates (initial and final weights) of the experimental Wistar rats were analyzed using the least significant difference test. For the hematological parameters, the recorded standard reference values for Wistar rats were used to assess the normal range, allowing evaluation of the effects of the irradiated sorghum on the rats fed with it.

Results and Discussion

The average initial body weights (g) of the 4-week-old Wistar rats before the start of the experiment were as follows: 81.2 ± 3.41 g in the control group, 82.2 ± 2.73 g in the X-ray group, 78.3 ± 4.52 g in the UV-treated

group, and 80.2 ± 2.84 g in the gamma-ray group. Statistical analysis using the LSD test revealed no significant differences in the initial weights across these groups. After 60 days of treatment, the final weights of the rats were recorded as 295.2 ± 4.82 g in the control group, 282 ± 4.12 g in the X-ray-treated group, 285.2 ± 3.73 g in the UV-treated group, and 275.3 ± 4.08 g in the gamma-treated group. The LSD analysis showed that the control group had a significantly higher final weight compared to the other groups. The growth rates also varied, with the control group exhibiting the highest growth rate of 3.57 g/day, after the UV group (3.4 g/day), X-ray group (3.33 g/day), and the gamma group (3.25 g/day) (**Table 1**).

Table 1. Mean \pm SE body weight (g) of the experimental Wistar rats before and after study

Stage	Control	X-ray	UV	Gamma
Before study	81.2 ± 3.41^{a}	82.2 ± 2.73^{a}	78.3 ± 4.52^{a}	80.2 ± 2.84^a
After study	295.2 ± 4.82 ^a	282.0 ± 4.12 ^b	285.2 ± 3.73 ^b	275.3 ± 4.08^{b}
Growth rate (g/day)	3.57	3.33	3.40	3.25

Different letters reflected different significant levels.

The biomedical tests for Wister rats fed on irradiated sorghums

The creatinine levels were measured at 0.70 mg/dl in the control group (C) and the X-ray irradiated sorghum-fed group (X). In the gamma-irradiated sorghum-fed group (G), the creatinine level was slightly lower at 0.65 mg/dl, while the UV-irradiated sorghum-fed group (UV) had a higher level at 0.88 mg/dl.

The urea concentration was 24.5 mg/dl in the control group, which was lower than that in the X-ray (40.3 mg/dl) and gamma (36 mg/dl) irradiated groups, but notably lower in the UV group at 12 mg/dl.

Sodium (Na) levels were similar between the control and X-ray groups at 145.0 mmol/L. In the gamma group, the level was higher at 148.4 mmol/L, while the UV group had a slightly lower concentration at 144.0 mmol/L. Potassium (K) concentrations were found to be 5.3 mmol/L in both the control and UV groups, 5.6 mmol/L in the X-ray group, and 4.9 mmol/L in the gamma group (**Table 2**).

Table 2. Effects of irradiated sorghum seeds on renal function parameters of albino rats

Parameter	Control	X-ray	$\mathbf{U}\mathbf{V}$	Gamma
Cretin (mg/dl)	0.70	0.70	0.88	0.65
Urea (mg/dl)	24.5	40.3	12.0	36.0
Na (mmol/L)	145.0	145.0	144.0	148.4
K (mmol/L)	5.3	5.6	5.3	4.9

Liver function

Albumin levels were 4.8 mg/dl in the control group (C), higher than the 4.2 mg/dl observed in the X-ray group (X) and 4.3 mg/dl in the UV-treated group (UV). The gamma group (G) had the highest albumin level at 5.0 mg/dl.

The alkaline phosphatase (ALP) activity was 75.5 u/L in the control group, 88 u/L in the UV group, 128 u/L in the gamma group, and 230 u/L in the X-ray group.

The alanine aminotransferase (ALT) levels were 141.5 u/L in the control group, which was higher than the 128 u/L in the gamma group, but lower than the 190.7 u/L observed in the X-ray group, and the highest at 342 u/L in the UV-treated rats.

Aspartate aminotransferase (AST) was 279 u/L in the control group, and 253 u/L in the gamma group, while the X-ray group showed 410.7 u/L and the UV group had the highest level at 861 u/L.

Bilirubin levels were undetectable in all groups. Total bilirubin was recorded at 0.1 mg/dl in the control, X-ray, and gamma groups, but absent in the UV group. Total protein levels were 8.2 mg/dl in the control group, 8.8 u/L in the gamma group, and 7.6 mg/dl in both the X-ray and UV groups (**Table 3**).

Table 3. Effects of the irradiated sorghum seeds on liver function parameters of albino rats

Parameter	Control	X-ray	UV	Gamma
Albumin (mg/dl)	4.8	4.2	4.3	5.0

ALP (u/L)	75.5	230.0	88.0	128.0
ALT (u/L)	141.5	190.7	342.0	129.0
AST (u/L)	279.0	410.7	861.0	253.0
Bilirubin (mg/dl)	0.0	0.0	0.0	0.0
Total Bilirubin (mg/dl)	0.1	0.1	0.0	0.1
Total Protein (mg/dl)	8.2	7.6	7.6	8.8

The hematological tests for Wister rats fed on irradiated sorghums

The white blood cell (WBC) count was 8.4×10^9 /L in the control (C) group, higher than the counts in the gamma (G) group (7.7 x 10^9 /L), X-ray group (X) (7.5 x 10^9 /L), and UV-treated group (UV) (6.3 x 10^9 /L).

For red blood cells (RBC), the control group had $7.14 \times 10^{12}/L$, which was higher than the $6.01 \times 10^{12}/L$ observed in the gamma group, but lower than the values in the X-ray ($8.53 \times 10^{12}/L$) and UV-treated ($7.74 \times 10^{12}/L$) groups. The platelet (PLT) count was $513.5 \times 10^{3}/L$ in the control group, which was higher than the values in the gamma ($407 \times 10^{3}/L$) and UV-treated ($331 \times 10^{3}/L$) groups, but lower than the count in the X-ray group, where it was $665.7 \times 10^{3}/L$ (**Table 4**).

Table 4	 Effects 	of mutant	sorghum	seeds fed	to albino	rats on	their blood	count
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Blood Parameter	Control	X-ray	$\mathbf{U}\mathbf{V}$	Gamma
WBC (x10 ⁹ /L)	8.4	7.5	6.3	7.7
RBC (x10 ¹² /L)	7.14	8.53	7.74	6.01
PLT (x10 ³ /L)	513.5	665.7	331.0	407.0

This study was designed to assess the rate and hematological parameters of male Wistar rats fed with irradiated sorghum (treated with X-ray, gamma-ray, and UV light) in comparison to those fed non-irradiated sorghum.

The kidney plays a critical role in filtering excretory substances from the bloodstream, making it particularly vulnerable to any foreign materials absorbed from the digestive system. These absorbed substances first reach the liver through the portal vein, highlighting the importance of investigating the interrelated functions of the liver, blood, and kidney when examining the effects of potential toxicants on these organs.

Sorghum, as described by Mohammed *et al.* [8], contains 74.68% carbohydrates, 1.71% fibers, 4.24% fat, and 12.25% proteins, along with various vitamins and minerals, all of which were available to the Wistar rats during the study.

The results demonstrated that the rats fed irradiated sorghum showed a reduction in body weight compared to those fed non-irradiated sorghum. This could be because ionizing radiation may alter the chemical structure of essential molecules, such as sugars and amino acids, impacting overall metabolism.

The creatinine levels in the blood of the experimental rats ranged from 0.65 to 0.88 mg/dl, which exceeded the upper limit of 0.6 mg/dl reported by Vigneshwar *et al.* [13].

In terms of urea levels, Yusuf *et al.* [14] reported a normal range of 45.38 ± 2.34 mg/dl in male Wistar rats. The results from this study, however, showed urea levels ranging from 12 to 40.3 mg/dl, which were below this reference range.

Sodium levels in the blood of the male Wistar rats were measured between 144 and 148.4 mmol/L, which aligns with the findings of Vigneshwar *et al.* [13]. Potassium concentrations in the same rats ranged from 4.9 to 5.6 mmol/L, consistent with the range of 4.2-7.8 mmol/L cited by Vigneshwar *et al.* [13].

No significant changes were found in the renal function of rats fed with irradiated sorghum, except urea levels. Vigneshwar *et al.* [13] reported that the typical values for total protein (TP) in male Wistar rats range from 5.1 to 7.6 mg/dl, alkaline phosphatase (ALP) activity from 29 to 35 u/L, alanine aminotransferase (ALT) from 24 to 67 u/L, and aspartate aminotransferase (AST) from 55 to 98 u/L. The rats in this study displayed higher enzyme levels compared to these reference ranges. Additionally, according to Clement *et al.* [15], albumin levels are usually between 3.1 and 3.6 mg/dl and total bilirubin levels range from 0.33 to 1.0 mg/dl in Wistar rats. The results of this study were in agreement with these ranges, with minor differences of less than 1.4 mg/dl for albumin and 0.33 mg/dl for total bilirubin.

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The WBC count in the rats was between 6.3 and 8.4 x 10^9 /L, the RBC count ranged from 6.01 to 8.53 x 10^{12} /L, and the platelet count was between 331 and 665.7 x 10^3 /L. In contrast, Vigneshwar *et al.* [13] reported WBC values of 3.7 to 5.8 x 10^9 /L, RBC counts between 6.1 and 8.5 x 10^{12} /L, and platelets ranging from 315 to 512 x 10^3 /L. The findings of this study showed an increase in white blood cells in the rats fed with irradiated sorghum, except in the group treated with X-rays.

It has been suggested that alterations in the activity of key enzymes, such as AST and ALT, reflect liver damage [16]. El-Naggar *et al.* [17] found a modest rise in these enzymes 7 days after exposure to X-rays.

Sallam [18] observed that gamma-irradiation at a dose of 4 Gy caused alterations in liver function, which were believed to result from direct interactions between gamma rays and cellular membranes, or indirectly through the generation of free radicals. Similarly, Pradeep *et al.* [19] found that exposure to gamma irradiation at doses of 1 Gy, 3 Gy, and 5 Gy led to an increase in AST, ALT, ALP, and GGT levels.

Numerous studies have highlighted the impact of ionizing radiation on renal function, noting that irradiation can induce significant biochemical changes in the affected animals. These changes often result in a continuous reduction in body weight, which is linked to disruptions in nitrogen metabolism. This disturbance typically manifests as a negative nitrogen balance, leading to an elevation in urea levels and an increase in the concentration of amino acids in the blood [20].

Conclusion

This study investigated the impact of a two-month feeding period with irradiated sorghum seeds on the growth, blood parameters, and functions of the kidney and liver in male Wistar rats. The findings provide important insights into weight gain, overall metabolic changes, and potential alterations in liver and renal functions associated with the consumption of irradiated sorghum, with albino rats serving as biological indicators.

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