Entomology and Applied Science Letters
Volume 11, Issue 3, Page No: 9-14
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Available Online at: www.easletters.com

ISSN No: 2349-2864

Biological Assessment of Irradiated Sorghum (*Sorghum bicolor* (L.) Moench) Seeds Fed on Wistar Rats (Males)

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ABSTRACT

Sorghum, the world's fourth major cereal in terms of production, is a staple food crop of millions of poor in the semi-arid tropics of the world. The objective of this study was to investigate the growth rate and some hematological parameters of Wistar rats (males) fed on irradiated (by X-ray, Gamma-ray, and UV light) sorghum (*Sorghum bicolor*), for two months. Sorghum seeds were brought from the local market, cleaned manually, divided into four groups, and put in clean Petri dishes. Three groups were treated with X-ray, gamma ray, and UV light, while the last one was taken as control. The growth rate was measured as initial and terminal weights (g) of the experimental rats. After 60 days of feeding on these sorghum, blood samples from these rats were collected from the retrorbital sinus. The blood count and renal and liver functions were determined. The results showed that feeding on irradiated sorghum reduced the growth rate compared to control. Control-sorghum-fed rats showed a higher growth rate (3.57 g/day) followed by UV-sorghum-fed rats (3.4 g/day), X-ray-sorghum fed rats (3.33 g/day) and at last Gamma-sorghum fed rats (3.25 g/day). The irradiated sorghum altered some of the blood count and renal and liver function parameters. Foods that are exposed to direct or indirect irradiation should be checked for their safety before being used for human food or animal feeds.

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

HOW TO CITE THIS ARTICLE: AdamIbrahim N, Kehail MAA. Biological Assessment of Irradiated Sorghum (*Sorghum bicolor* (L.) Moench) Seeds Fed on Wistar Rats (Males). Entomol Appl Sci Lett. 2024;11(3):9-14. https://doi.org/10.51847/DeFmonAZXu

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Received: 26/06/2024
Accepted: 10/09/2024

INTRODUCTION

The Wister rats (Rodentia, Muridae: *Ratus norvgicus domestica*) are scientific research rats that are bred widely as an animal model for psychological or biomedical studies, as well as in pharmacological or cancer research [1]. It is the white color or albino rat, and it is involved and reflected in the scientific papers more than house mice or rate [2]. Ionizing radiation (Gamma, X-rays, and UV) consists of electromagnetic waves that have sufficient energy to ionize atoms or molecules, unlike visible light, laser, infrared, microwaves, and radio waves, which are considered non-ionizing radiation [3]. Ionizing radiation is usually used in fields of medicine (imaging and radiotherapy) and nuclear power (nuclear reactors or nuclear weapons). Exposure to ionizing radiation may lead to cell damage. In high acute doses, it may result in radiation burn, but in low sustained doses, it can cause cancer [4], or mutation of somatic or reproductive cells [5]. The DNA molecule is always susceptible to ionizing radiation. Radiation may cause DNA damage, which may be extended to cause tissue damage and biological system disruption [6]. Sorghum (Poaceae: *Sorghum bicolor*) is a genus of flowering, monocot grass grown as cereals for human and animal consumption [7]. It contains 74.68 % carbohydrates, 1.71% fibers, 4.24% fat, and 12.25% proteins, in addition to Vitamins (B1, B2, B3, B5, B6, B9, C, and E) and minerals (Ca, Cu, Fe, Mg, Mn, P, K, Si, Na, and Zn). Its grain is edible and can be eaten raw when young, but it must be boiled or milled into flour when becomes mature and dry [8, 9]. Hematology is the science concerned with the study of diagnosis, prevention, and treatment of blood-related diseases. It includes blood cells, hemoglobin, bone marrow, blood vessels, spleen, and coagulation agents. The laboratory analysis of blood involved renal function (e.g., cretin, urea, Na, and K), liver function (e.g., albumin, bilirubin, total bilirubin, total protein, ALP, ALT, GGT, and AST), and complete blood count (e.g., WBC, RBC, and platelets) among other tests [10-12].

The objective of this study was to investigate the growth rate and some hematological parameters of Wistar rats (males) fed on irradiated (by X-ray, Gamma-ray, and UV light) sorghum (*Sorghum bicolor*), for two months.

MATERIALS AND METHODS

Materials

Sorghum seeds (*Sorghum bicolor*) were the raw material used in this study, cleaned manually, and were divided into four groups in clean Petri dishes. One group was treated with X-ray (at 100 eV) using an X-ray machine, and the second group was treated with gamma-ray (at 200 cGY) using a Co-60 device, at the National Cancer Institute, Department of Radiation, University of Gezira, Sudan. The third group was put under UV light for 30 minutes, at the Food Microbiology Laboratory, Faculty of Engineering and Technology, University of Gezira, while the last group was used as a control (C). The sorghum seeds of each group were ground separately to prepare the sorghum cake required for feeding Wistar rats during this experiment.

The physiological aspects of the Albino rats fed on irradiated sorghum

The experimental Albino rats

Twelve healthy Wister Albino rats (4-week-old males) were purchased from the Biosafety Center Laboratory, Khartoum State. These animals were weighed and distributed randomly in four groups according to their feed. The weight of each Albino rat was measured twice during the study period; before and after experimental under standard conditions. The experimental rats were reared in clean separate polypropylene cages. Each rat was fed on 12 g/day of wheat bran and the flour of each irradiated sorghum seed (at a ratio of 1:1). Each feeding component was mixed with a considerable amount of water to make a compact paste, which was then cut into small pieces, compressed and let to dry separately. Each sorghum cake piece was prepared at 12 g. No other food or additives were added throughout the experiment period (60 days). This experiment was approved by the ethical committee, Faculty of Scientific Research, University of Gezira (1523-October, 2018).

Sampling blood for hematological analysis

At the end of the experimental period, blood samples of the Albino rats were collected from the retro-orbital sinus (a system of dilated venous channels at the back of the orbit). The rats were fasted overnight before collection of the blood on the morning of the next day. Wister rats were not anesthetized before sampling blood. A microhematocrit tube was along the inner corner of the eye (medial canthus) beside the eyeball.

About 2.5 ml of blood was poured into a clear container containing the anticoagulant EDTA to avoid clotting. The Sysmex KX 21N model was used for counting blood cells. The collected blood samples were put on the hematology mixer machine to determine the blood cells: white blood cells (WBC), red blood corpuscles (RBC), and platelets. The plasma of each of the collected blood was separated after centrifugation at 2500 rpm for 5 minutes. Renal function parameters were creatin, urea, Na^+, and K^+. These tests in addition to the liver function parameters: Total protein, Albumin, Bilirubin, Total bilirubin, Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanin aminotransferase (ALT)] were run at the Blood Bank Laboratory, Wad Medani Teaching Hospital.

Statistical analysis

The Least Significant Difference (LSD) was used to analyze the initial and terminal weight of the experimental Wistar rats used for this study (growth rate). For the hematological parameters results, the standard limits recorded for Wister rats were usually used to evaluate the normal level or situation (as was usually done in the clinical tests) to reflect the effect of the irradiated sorghum samples on the rats fed on them.

RESULTS AND DISCUSSION

Wistar rat body weight

The mean initial (before the study) weight (g) of the four-week-old Wistar rats was 81.2 ± 3.41 g in the control group, 82.2 ± 2.73 g in the X-ray treated group, 78.3 ± 4.52 g in UV-treated group, and 80.2 ± 2.84 g in the Gamma treated group. The LSD showed that these rats were significantly similar in their weights **(Table 1)**.

After 60 days (the experimental period) the terminal weights of the experimental Wistar rats were 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 showed that the control group differed significantly in its weight than the other groups. Control-sorghum-fed rats showed a higher growth rate (3.57 g/day) followed by UV-sorghum-fed rats (3.4 g/day), X-ray-sorghum fed rats (3.33 g/day) and at last Gamma-sorghum fed rats (3.25 g/day).

Table 1. Mean ± 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 renal function

Cretin was 0.70 mg/dl in the blood of control-sorghum-fed rats C, as same as X-ray irradiated sorghum-fed rats X, while it was 0.65 mg/dl in Gamma irradiated sorghum-fed rats G, but it was 0.88 mg/dl in the blood of UV irradiated sorghum fed rats UV. Urea was 24.5 mg/dl in C-fed rats, which was less than those of X-fed rats (40.3 mg/dl) and in G-fed rats (36 mg/dl), but it was 12 mg/dl in the blood of UV-fed rats.

Sodium (Na) was 145.0 m mol/L in the blood of C-fed rats, as same as X-fed rats, while it was 148.4 m mol/L in G-fed rats, but it was 144.0 m mol/L in the blood of UV-fed rats. Potassium (K) was 5.3 m mol/L in the blood of C-fed rats, as same as UV-fed rats, while it was 5.6 m mol/L in X-fed rats, and it was 4.9 m mol/L in the blood of G-fed rats **(Table 2)**.

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

Parameter	Control	X-ray	UV	Gamma
Cretin (mg/dl)	0.70	0.70	0.88	0.65
Urea (mg/dl)	24.5	40.3	12.0	36.0
Na (m mol/L)	145.0	145.0	144.0	148.4
K (m mol/L)	5.3	5.6	5.3	4.9

The liver function

As shown in **Table 3**, Albumin was 4.8 mg/dl in the blood of C-fed rats, which was more than that of X-fed rats (4.2 mg/dl), and it was 4.3 mg/dl in UV-fed rats, but it was 5.0 mg/dl in the blood of G-fed rats. ALP was 75.5 u/L in C-fed rats, and it was 88 u/L in the UV-fed rats, but it was farther within G (128 u/L) and in X (230 u/L) fed rats.

ALT was 141.5 u/L in the blood of C-fed rats, which was more than that of G-fed rats (128 u/L), and it was 190.7 u/L in (X) fed rats, but it was 342 u/L in the blood of UV fed rats. AST was 279 u/L in C-fed rats, and it was 253 u/L in the G-fed rats, but it was farther within X (410.7 u/L) and in UV (861 u/L) fed rats.

Bilirubin was 0 mg/dl within the blood of all samples. Total bilirubin was 0.1 mg/dl in the blood of C, X, and G-fed rats, while it was 0 in UV-fed rats. Total protein was 8.2 mg/dl in C-fed rats, and it was 8.8 u/L in the G-fed rats, but it was similar within X and UV (7.6 mg/dl) fed rats.

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

Blood cell count

White blood cells (WBC) were 8.4 ($x10^9$/L) in the blood of the C-fed rats, but they were less than that in the blood of G (7.7 x 10^9/L), X (7.5 x 10^9/L), and UV (6.3 x 10^9/L) fed rats. Red blood corpuscle (RBC) was 7.14 ($x10^{12}$/L) in the blood of the C-fed rats, but it was less than that in the blood of G (6.01 x 10^{12}/L), while it was more within the blood of X (8.53 x 10^{12}/L), and UV (7.74 x 10^{12}/L) fed rats. Platelets (PLT) was 513.5 ($x10^3$/L) in the blood of the C-fed rats, but it was less than that in the blood of G (407 x 10^3/L) and UV (331 x 10^3/L), but it was more in the blood of X (665.7 x 10^3/L) fed rats **(Table 4)**.

Table 4. Effects of mutant sorghum seeds fed to albino rats on their blood count

Blood Parameter	Control	X-ray	UV	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 aimed to evaluate the growth rate and hematological parameters of male Wistar rats that fed on irradiated (X-ray, Gamma-ray, and Ultra Violet treated) sorghum (*Sorghum bicolor*) seeds compared to a non-irradiated sorghum group.

Because the circulatory system takes the blood from the whole body to the kidney through the renal artery to get rid of the excretory materials, the kidney is then highly exposed to strange substances passed through the intestinal absorption. The absorbed materials were first visited the liver through the portal vein. Hence, blood, liver, and kidney were the important triangles to investigate the toxicants that alter their composition and functions.

According to Mohammed *et al*. [8], sorghum contains 74.68% carbohydrates, 1.71% fibers, 4.24% fat, and 12.25% proteins, in addition to some vitamins and mineral elements. These contents are expected to be fed by Wister rats during this study.

According to the obtained results, it was obvious that feeding on irradiated sorghum led to decreased body weight of the experimental Wister rats than those fed on non-irradiated sorghum. This may be attributed to the fact that ionizing radiation may alter the chemical molecules (e.g., sugars and amino acids).

Cretin which was detected in the range of (0.65 to 0.88 mg/dl) in the blood of the experimental-fed male rats exceeded the maximum limit reported by Vigneshwar *et al*. [13], which ranged between 0.3 – 0.6 mg/dl.

Abubakar *et al*. [14], reported that, urea was 45.38 ± 2.34 mg/dl in male Wister rat. The obtained results reported a range of 12 – 40.3 mg/dl, which were below the above minimal limits.

Na which was detected in the limits of 144 – 148.4 m mol/L in the blood of the experimental male Wister rats, was within the limits detected by Vigneshwar *et al*. [13], Also, K in the blood of the tested rat was detected at the range of 4.9 – 5.6 m mol/L, which were within the range of 4.2 – 7.8 m mol/L reported by Vigneshwar *et al*. [13]. It was clear that the irradiated sorghum did not produce any significant alteration in the renal functions, except the urea level, among the tested Wister rats. According to Vigneshwar *et al*. [13], TP in the blood of male Wister rats was 5.1 – 7.6 mg/dl, ALP was 29 – 35 u/L, ALT was 24 – 67 u/L, and AST was 55 – 98 u/L. The tested Wister rats in this study scored higher levels of ATP, ALP, ALT, and AST in terms of unit/L than the above-mentioned limits. Also, according to Clement *et al*. [15], Albumin was 3.1 – 3.6 mg/dl, and total bilirubin was 0.33 – 1.0 mg/dl in Wister rats. The obtained values in this study did not differ from the mentioned limits (the difference did not exceed 1.4 mg/dl for albumin and 0.33 mg/dl for total bilirubin).

White blood cell (WBC) count was 6.3 – 8.4 x 10^9 L, RBS was 6.01 – 8.53 x 10^{12} L, while platelets were 331 – 665.7 x 10^3 L in the blood of the tested Wister rats. Vigneshwar *et al*. [13] found that WBC was 3.7 – 5.8 x 10^9 L, RBS was 6.1 – 8.5 x 10^{12} L, and platelets were 315 – 512 x 10^3 L. It was clear that the irradiated sorghum seed cake increased the white blood counts in albino rats after 60 days of feeding, except X-ray X-ray-treated sorghum.

Several studies postulated that the effect of irradiation on the activity of vital enzymes (e.g. AST and ALT) reflects the degree of liver injury [16]. El-Naggar *et al*. [17] observed a mild increase in the levels of both enzymes on the 7th day post X-irradiation.

Sallam [18] reported liver function alteration induced by gamma-irradiation (at 4 Gy) which was attributed to the interaction of cellular membranes with gamma rays directly or indirectly through an action of free radicals. Pradeep *et al.* [19] also, reported that rats exposed to gamma irradiation (1Gy, 3Gy, 5 Gy) showed an increase in AST, ALT, ALP, and GGT.

Many authors reported that ionizing radiation greatly affected renal function, and they explained that irradiation leads to biochemical changes in the irradiated animals, which may suffer from a continuous loss in body weight which could be attributed to disturbances in nitrogen metabolism usually noticed as negative nitrogen balance which lead to an increase in the urea level, and amino acid contents in blood [20].

CONCLUSION

The present study evaluated the effect of feeding on the irradiated sorghum seeds for two months on the growth, blood, renal, and liver functions (hematological parameters) in male Wistar rats. The obtained results offered valuable information on gaining weight in addition to the whole situation of metabolism and expected alterations in renal and liver function when feeding on irradiated sorghum using albino rats as a bio-indicator.

ACKNOWLEDGMENTS: Thanks, are to the Biosafety Center Laboratory, Khartoum State, the ethical committee, the Faculty of Scientific Research, the University of Gezira, and the Blood Bank Laboratory, Wad Medani Teaching Hospital.

CONFLICT OF INTEREST: None.

FINANCIAL SUPPORT: None.

ETHICS STATEMENT: The study was conducted by the Declaration of Helsinki and was approved by the Ethics Committee of Scientific Research College, University of Gezira, under code (1523-October 2018). We used animals to try to assess fed sorghum (*Sorghum bicolor*) irradiated (by X-ray, Gamma-ray, and UV light).

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