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Radioprotection and Radiomitigating Potential of *Markhamia Tomentosa* Extract Against Gamma Radiation-Induced Damage on Albino Wistar Rats

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ABSTRACT

Introduction: This study evaluated the efficacy of *Markhamia tomentosa* (MT) extract as a potential radiation countermeasure emphasising its radioprotective and radiomitigating properties.

Material and Methods: Forty male albino Wistar rats aged 10-12 weeks were used for the study. Rats were divided into eight groups comprising five animals in each group. The extract was administered for 14 days by oral gavage for both pretreatment and post-treatment. An hour after the last pre-administration, animals received 3 Gy and 6 Gy of gamma radiation by whole-body irradiation (WBI) using ^{60}Co-γ as the radiation source. Rats were euthanised on day 15 for hematological and histological examinations except those in post-treatment groups. Data were analysed by one-way ANOVA and subjected to Tukey's HSD *post hoc* test.

Results: WBI of rats at 3 Gy and 6 Gy significantly reduced the hematological parameters. However, the oral administration of MT extract ameliorated the effect of ionising radiation by considerably improving the hematological parameters leading to high blood counts. Continuous administration of MT extract for additional 14 days showed a more remarkable improvement in the hematological parameters, as evident in the white blood cell, neutrophils and platelet counts. Pre and post-treatment of rats with MT extract decreased changes in the kidney tubules, and the liver showed moderate congestion of hepatic sinusoids in the portal tracts denoting an increased blood flow rate causing healing of the cells.

Conclusion: MT demonstrated its radioprotective and radiomitigating potential in recovering distorted tissues and modulating the effects of gamma radiation-induced damage to blood cells.

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Introduction

Since its discovery, ionising radiation (IR) has greatly benefited medicine, industry, research, agriculture, military, and aviation. Despite the enormous benefits of IR, the harmful effect cannot be overlooked [1, 2]. The interaction of IR with water molecules produces free radicals such as hydroxyl radicals (OH^-), reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) and superoxide (O_2), which can attack macromolecules resulting in cell damage [3]. The damage to deoxyribonucleic acid (DNA) could be a single-strand break (SSB), double strands break (DSB), loss of bases and cross-links of DNA, protein and lipids membrane [2].

Studies have shown that cancer remains the leading cause of death globally, and approximately 10 million deaths occurred in 2020 [4, 5]. The cancer burden increases yearly in both developing and developed countries. Developing countries record high numbers of cancer-associated deaths as a result of a lack of early detection techniques and poor access to treatment [6]. The global cancer incidence has been projected to increase to 28.4 million in 2040, about 47% more than the 2020 report [6]. Radiation therapy remains an excellent modality in cancer treatment [7]. The effectiveness of IR in shrinking tumours and inhibiting their growth make it an indispensable tool in cancer radiotherapy. However, radiation-induced damage to healthy tissues surrounding tumours during cancer radiotherapy has limited the progress of IR utilisation in therapeutic and diagnostic radiology [7]. The limiting factors include the non-availability of safe and non-toxic prophylactic agents. The function of these agents is to protect the healthy cells against the harmful effect of IR while remaining toxic to the tumour. Agents capable of offering this protection are called radiation countermeasures.

Radiation countermeasures are agents given before or after exposure to IR to reduce the detrimental effect of radiation on cells. It has been classified into three: radioprotector, radiomitigator, and radiation therapeutics [8]. Recently, research on radiation countermeasure agents has taken a new

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dimension, with the attention of researchers and scientists shifting to natural plants and plant-based products [9]. Many of these plants have proven to be potentially therapeutic with promising radioprotective properties [9, 10]. These stem from the widespread perception during the last decade that herbal medicine is affordable, easily accessible, less toxic, acceptable and available to various communities [11, 12].

Numerous research works have been done on plants and herbs as potential radioprotectors [2, 3, 7, 9, 10, 13]. For instance, Jagetia et al. [14] studied the radioprotective efficacy of Liv 52 on gamma-irradiated albino mice. Liv 52 is made up of medicinal plants mixed in the right proportion. Liv 52 demonstrated its radioprotective efficacy by reducing gamma radiation's genotoxic and lethal effects in mice [14]. Ansari et al. [10] investigated the radioprotective efficacy of coffee beans, grape seeds and green tea. The research revealed that plants' radioprotective potential and synergetic properties were enhanced when combined since it has been demonstrated that the synergy of all constituents of the plants would bring about the utmost therapeutic potential [15]. The mechanism of actions of melanin, a promising radioprotector, was evaluated by Kunwar et al. [16] on whole-body irradiated mice.

The possible mechanism of radioprotectors of plant origin that have been identified includes scavenging free radicals, stimulating haematopoiesis, activating DNA repairing enzymes, reducing apoptosis, and boosting the immune system [10,17,18]. Other plants showing promising radioprotective properties include *Polyalthia longifolia, Pinus koraiensis, Nigella sativa, Mesua ferrea, and Panax ginseng* [9, 19-22]. Many plants, including *Markhamia tomentosa*, have shown medicinal and therapeutic properties, and it will be worthwhile to examine their efficacy in radiation countermeasures.

Markhamia tomentosa (MT) (Benth.) K. Schum. Ex Engl. belongs to the Bignoniaceae family. It is a shrub or tree that grows up to 15 meters tall in the relic, bordering, transition, and savannah forests of West Tropical Africa, reaching southward to Angola [23, 24]. In the Southwest (Yoruba) part of Nigeria, it is called "*ìrù àáyá*", *ãyá*: a species of monkey; *iru*: Parkia filicoidea. It is commonly known as the "Bell bean tree". In Africa, the plant is known for its therapeutic properties. In Nigeria and Cote d'Ivoire, traditional medicine practitioners claim that MT is beneficial in healing skin ailments, sores, ulcers, and inflammation. In Nigeria, comparative investigations have been conducted on the leaf epidermal properties of eleven species of the Bignoniaceae family [25].

Despite the numerous medicinal benefits of MT reported across Africa, there is no information in the literature on its radioprotective property or its ability to mitigate radiation-induced damage to cells and tissues, to the best of our knowledge. Thus, this paper reports an investigation on the possible efficacy of *Markhamia tomentosa* as a potential radiation countermeasure with emphasis on its radioprotective and radiomitigating properties on male Wistar rats.

Materials and Methods

Collection of plant and preparation

MT was obtained from a local farm in Ibadan, Nigeria. The plant identification was made at the Botany Department, University of Ibadan. The freshly harvested MT was washed under running water and air-dried. The dried sample was ground using a clean electric blender. A 2000 g of the powdered sample was macerated in absolute ethanol for three days at room temperature. After that, it was vacuum filtered using filter paper (Whatman No. 1). The maceration and the extract preparation were done at the Organic Research Laboratory of the Chemistry Department, University of Ibadan, Nigeria. To ensure that the extract was ethanol-free, a rotary evaporator was used to remove all traces of ethanol before placing it in a watertight glass bottle and kept in a refrigerator until the time of administration.

Animals' selection

Forty male albino Wistar rats aged 10-12 weeks were purchased from the Animal Breeding House, Department of Anatomy, University of Ibadan. They were kept in the Experimental Animal House, Zoology Department, the University of Ibadan, for the research period. The animals were housed in a suspended cage with stainless steel top grill. Clean wood shaven was used as a bedding material and placed in a well-ventilated, clean animal house under suitable temperature and humidity conditions. They were provided *ad libitum* with standard commercially formulated pellets diet feed (Ladokun Feeds, Mokola, Ibadan, Nigeria) and tap water in bottles with stainless steel sipper tubes under normal laboratory environmental conditions (12 hours of light and 12 hours of darkness). The animals were handled humanely during the experimental period. The Animal Care and Use Research Ethics Committee, University of Ibadan, Nigeria, authorised the protocol used for this study. Also, our procedure adhered to the acceptable guidelines set by the National Institute of Health [26] on the ethical use of animals in biomedical research.

Acute toxicity test

Ten rats were used in a test to determine the acute toxicity of Markhamia tomentosa extract (MTE). The rats were divided into five groups and were orally administered 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg, and 300 mg/kg body weight of MTE. The administration was done over a period of 14 days [2]. The oral administration of MTE did not cause any toxic effect or death in the experimental rats at the time of observation. Hence, 200 mg/kg was selected as the median dose and used for the further test in the present work.

Experimental design

The experimental animals were divided into eight groups, each with five animals, based on their body mass. The animals were allowed to acclimatise for fourteen days before treatment started. The arrangement of grouping and therapy are presented in Table 1. Based on Adebajo et al. [27] report, the solution of MT was made for this experiment; 0.5 ml of the prepared solution containing 200 mg/kg/day was given orally by gavage for 14 days. All the animals were euthanised on day 15 for hematological and histological examinations except those in groups post-3Gy and post-6Gy, which were post-treated with *Markhamia tomentosa* extract (MTE) for another 14 days and euthanised on the 15th day.

Table 1. Grouping and treatment schedule of the experimental animals

Group code	Treatment
DDW	Rats were pretreated with distilled water for 14 days (Control 1)
MTE	Rats were pretreated with MTE for 14 days (Control 2)
3-Gy	Rats exposed to 3 Gy of gamma radiation only
6-Gy	Rats exposed to 6 Gy of gamma radiation only
Pre-3Gy	Rats were pretreated with MTE for 14 days and irradiated with 3 Gy
Pre-6Gy	Rats were pretreated with MTE for 14 days and irradiated with 6 Gy
Post-3Gy	Rats post-treated with MTE for 14 days after γ-irradiated with 3 Gy
Post-6Gy	Rats post-treated with MTE for 14 days after γ-irradiated with 6 Gy

Note: MTE, *Markhamia tomentosa extract*; DDW, distilled water; Gy, Gray (radiation units).

Irradiation procedures

The cobalt-60 (^{60}Co) Gamma beam X200 irradiator was used for the irradiation. The radiation facility is housed at the National Institute of Radiation Protection and Research (NIRPR) of the Nigerian Nuclear Regulatory Authority, situated at the University of Ibadan campus. Rats were exposed to whole-body irradiation with a single dose of 3 Gy and 6 Gy at a dose rate of 0.9675 Gy/min. At a depth of 5 cm with a field size of 10 cm x 10 cm, the source-to-surface distance was 80 cm, and the percentage depth dose was 78.8%. During irradiation, no parts of the rat's body were shielded. The radiation was delivered whole-body, and the rats were returned to their cages and transported back to the animal house. The radiation doses of 3 and 6 Gy were chosen because these doses would produce sufficient bone marrow (hematopoietic) syndrome in the irradiated rats [28]. Other studies where similar radiation doses range have been used to induce hematological alterations include Akomolafe and Chetty [2], Yi et al. [29], El-Desouky et al. [30], and Jagetia et al. [31].

Determination of hematological parameters

Blood samples were collected twenty-four (24) hours after irradiation for the pretreated groups. In contrast, for the post-treated groups, the collection was made twenty-four (24) hours after the last administration of MTE. After puncturing the retro-orbital sinus, peripheral blood was drawn [32] using heparinised microhematocrit capillary tubes into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles with anticoagulant for hematological analysis and plain sample bottles for serum biochemistry. The haemoglobin (Hb) and haematocrit or packed cell volume (PCV) values were determined by the cyanomethaemoglobin and microhematocrit methods, respectively. The white blood cell count (WBC), Lymphocytes, Monocytes, Neutrophils, Eosinophils, Red blood cell (RBC) and platelet was analysed using the manual analysis method. A drop of each blood sample was placed on a clean, grease-free slide. A thin smear of blood was made on the slide using a coverslip. The smear was allowed to be air-dried and stained with Leishman stain.

Histopathology examinations

The animals' liver and kidneys were obtained after euthanising and fixed in 10 % buffered formalin in labelled 10 ml plain bottles. The tissues were dehydrated in ethanol and then cleaned in xylene before embedded in paraffin wax. A serial section of 4 µm thick was obtained via a rotary microtome on glass slides. The deparaffinised sections were stained routinely with Hematoxylin and Eosin (H and E) and mounted. Photomicrographs of the tissues were obtained using a photographic research microscope (x400)

Body mass and relative organ mass

The body mass of rats in all the groups was weighed and recorded on days 7 and 14 before dosing on the day of administration of MTE and before euthanising on a terminal day. The rationale for the recorded mass was to evaluate the effect of *Markhamia tomentosa* extract on the body mass of the experimental animals. The animals were later euthanised by cervical dislocation. The liver and kidney were surgically removed from the rats, washed in 0.9% normal saline, and weighed. The relative organ mass was then calculated and expressed as a percentage of body mass [2].

Relative organ mass index

$$= \frac{Absolute\ organ\ mass\ (g)}{Body\ mass\ of\ rat\ on\ the\ day\ of\ euthanise\ (g)} \times 100$$

Statistical analysis

Data analyses were performed using the SPSS 20.0® software package. The data were done by One Way ANOVA and subjected to Tukey's HSD *post hoc* test, where the treated data and the MTE group were compared with the DDW group. The mean difference is considered significant at p<0.05, and values were expressed as mean ± SEM (Standard error of the mean).

Results

Physical and biological observations of the experimental animals

There was mortality in animals exposed to post-6Gy treatment, where 4 out of 5 animals in the group died. The

physical appearance observed in the animals includes redness of the eye, loss of appetite, alopecia (loss of hair) and weight loss before death. These observed signs indicate a radiation dose-dependent relationship [33]. Mortality started on day six and continued till day 8, with at least one death recorded daily in post-6Gy treated groups. Exposure to 3 Gy and 6 Gy gamma irradiation led to a considerable loss in average body weight and several degenerative diseases in the hepatocyte of the irradiated groups compared to the control group, as supported by the observation of Savita et al. [34]. Compared to the irradiated-only groups, the combined treatment groups showed that treatment of irradiated groups with extracts of *Markhamia tomentosa* resulted in an increased blood flow of the epithelium cells and replacement of connective tissues.

Effect of Markhamia tomentosa extract on the body mass and relative organ mass

The body mass and relative organ mass of the experimental animals are presented in Table 2. The body mass of the distilling water group increased steadily throughout the experiment. There was a significant difference between all other groups and the Post-3Gy treatment group. There was a slight decrease in the MTE group. The mass gain in the extract-treated groups was lower than in the DDW group. The gamma irradiation groups (3-Gy and 6-Gy) alone showed a decrease in body mass, while the post-treated groups showed an increase in body mass. There were no significant ($p>0.05$) differences in the mass of kidneys and liver of treated and control rats (Table 2). The relative organ (kidney) weight of the treated groups, except for the MTE group, was higher than that of the DDW group, while the relative organ (liver) weight of all the treated groups was higher than that of the DDW group.

Effect of Markhamia tomentosa extract on the hematological parameters of rats after exposure to gamma radiation

Red blood cell (RBC)

Table 3 shows a non-significant difference ($p>0.05$) in the mean of radiation treatment (3-Gy and 6-Gy) compared with the MTE group. However, a significant difference exists between the radiation exposures (3-Gy and 6-Gy) groups in the RBC compared with the DDW. The pretreatment of rats with MTE slightly improved RBC count in the MTE group, Pre-3Gy and Pre-6Gy. Moreover, the pretreatment of animals with MTE significantly ($p<0.05$) increased the RBC of the Pre-6Gy group compared with the 3-Gy and 6-Gy groups.

Table 2. Effect of extract and γ-irradiation on body mass

Treatment Group	Body mass (g)			Relative organ mass	
	Initial mass (g)	Final mass (g)	Mass gain or loss (g)	Liver	Kidneys
DDW	91.86 ± 6.03	99.44 ± 7.02	7.58	3.26 ± 0.38	0.78 ± 0.84
MTE	102.8 ± 8.31	101.06 ± 10.19	-1.74	3.34 ± 0.41	0.60 ± 0.12
3-Gy	102.42 ± 3.14	102.24 ± 2.38	-12.28	4.00 ± 0.25	0.78 ± 0.11
6-Gy	103.48 ± 9.74	106.06 ± 7.95	-13.98	4.48 ± 0.67	0.90 ± 0.12
Pre-3Gy	125.06 ± 8.75	111.08 ± 7.69	-0.18	3.76 ± 0.19	0.84 ± 0.05
Pre-6Gy	110.04 ± 4.21	97.76 ± 4.69	2.58	3.84 ± 0.24	0.86 ± 0.05
Post-3Gy	102.0 ± 3.67	121.7 ± 3.25	41.78	6.08 ± 0.54	1.06 ± 0.21
Post-6Gy	87.96 ± 2.38	129.74 ± 10.15	19.70	5.80*	0.90*

Values are given as means ± SEM, (n = 5, * = 1); DDW, distil water; MTE, *Markhamia tomentosa* extract at 200 mg/kg; Gy, Gray (radiation unit); Negative (-) sign indicates a decrease in mass.

Table 3. Effect of MTE and γ-irradiation on the RBC, PCV, and Hb of Wistar rats

Treatment Group	RBC ($x10^{12}$/L)	PCV (%)	Hb (g/dl^3)
DDW	8.38 ± 0.24^{b}	49.60 ± 1.14^{b}	17.46 ± 0.80^{b}
MTE	7.85 ± 0.53^{ab}	47.80 ± 1.64^{ab}	16.38 ± 1.26^{ab}
Pre-3Gy	7.54 ± 0.52^{ab}	46.00 ± 2.55^{ab}	15.32 ± 1.04^{a}
Pre-6Gy	8.07 ± 0.51^{ab}	48.20 ± 4.15^{ab}	16.06 ± 1.46^{ab}
3-Gy	7.24 ± 0.18^{a}	43.20 ± 1.30^{a}	14.68 ± 0.35^{a}
6-Gy	7.25 ± 0.49^{a}	44.00 ± 3.08^{a}	14.46 ± 1.04^{a}
Post-3Gy	7.62 ± 0.47^{ab}	45.40 ± 3.21^{ab}	15.44 ± 0.91^{ab}
Post-6Gy	8.17^{*}	34.00^{*}	11.80^{*}

Values are given as means ± SEM, (n = 5, * = 1); DDW, distil water; MTE, *Markhamia tomentosa* extract at 200 mg/kg; Gy, Gray (radiation unit); At the 5% ($p<0.05$) level, values in the same column with different superscripts differ significantly.

Packed cell volume (PCV)

Table 3 shows a significant decrease in the PCV caused by the damaging effect of radiation (3-Gy and 6-Gy) compared with the distilled water group (DDW). However, there was a significant improvement in the PCV of animals in the MTE group, Pre-3Gy, Pre-6Gy and Post-3Gy groups compared with both 3-Gy and 6-Gy groups.

Hemoglobin concentration (Hb)

Radiation in 3-Gy and 6-Gy groups caused a significant reduction in hemoglobin concentration compared with the DDW (Table 3). The application of MTE before and after exposure significantly ($p<0.05$) improved the Hb, as shown in the Pre-3Gy, Pre-6Gy and MTE groups. There was a decrease in the Hb of animals in the post-6Gy as with the pretreatment groups.

White blood cell (WBC)

Table 4 shows a white blood cell count. Radiation caused a non-significant reduction ($p>0.05$) in the mean of WBC of the 6-Gy group when compared with the DDW group (distil water). Moreover, a non-significant difference was observed in the WBC of animals in the Pre-3Gy and Pre-6Gy compared with the 3-Gy and 6-Gy. However, a significant difference ($p<0.05$) occurs between Pre-3Gy and 3-Gy compared with the Post-3Gy treatment group.

Differential white blood cell counts

Tables 4 and 5 show neutrophils, lymphocytes, monocytes and eosinophils counts results. There was a decrease in the neutrophils of animals in the 3-Gy and 6-Gy when compared with the DDW. However, no improvement was recorded in the Pre-3Gy group, as the value indicates a non-significance difference compared with the DDW group. The experimental animals experienced an increase in the lymphocyte counts of the 3-Gy and 6-Gy groups. A similar trend was observed in monocytes and eosinophils counts. There was no significant difference in the values recorded in the 3-Gy and 6-Gy compared with the pretreatment and post-treatment.

Platelet counts

Table 5 shows a significant reduction ($p<0.05$) in the platelet counts of the animals in the 3-Gy and 6-Gy groups compared with the DDW and MTE groups. There was no significant improvement in the pretreatment groups. However, there was an increase in the platelet count of the post-treatment groups.

Effect of Markhamia tomentosa extract on histology of rats' liver and kidney after exposure to gamma radiation

The animals in the DDW showed normal cellular features of the liver, i.e., normal parenchyma, well-preserved lobules, closely packed hepatocytes and capsules with no evidence of adhesion and inflammation (Figure 1A). Groups MTE and Pre-3Gy showed multiple foci of moderate thinning of hepatic cords and concurrent sinusoidal dilatation with mild random single-cell hepatocellular necrosis (Figures 1B & 1E). Group Pre-6Gy showed mild random single-cell hepatocellular necrosis foci with moderate Kupffer cell hyperplasia (KCH) (Figure 1F).

Table 4. Effect of MTE and γ-irradiation on the WBC, Neutrophils and Lymphocytes of Wistar rats

Treatment Group	WBC ($x10^3$/L)	Neutrophils ($x10^3$/L)	Lymphocytes ($x10^3$/L)
DDW	4970.00 ± 1736.23^{ab}	35.60 ± 4.93	57.80 ± 4.82
MTE	5260.00 ± 3140.74^{ab}	32.20 ± 7.19	62.80 ± 7.50
3-Gy	3480.00 ± 1834.60^{a}	30.40 ± 4.10	65.80 ± 3.77
6-Gy	4460.00 ± 1953.65^{ab}	29.20 ± 7.89	66.40 ± 7.64
Pre-3Gy	2330.00 ± 1462.70^{a}	35.00 ± 8.92	58.20 ± 10.31
Pre-6Gy	4480.00 ± 1691.74^{ab}	27.40 ± 7.27	68.00 ± 8.57
Post-3Gy	8810.00 ± 2872.37^{b}	36.20 ± 5.89	59.40 ± 5.77
Post-6Gy	3900.00^{*}	32.00^{*}	62.00^{*}

Values are given as means ± SEM, (n = 5, * = 1); DDW, distil water; MTE, *Markhamia tomentosa* extract at 200 mg/kg; Gy, Gray (radiation unit); At the 5% ($p<0.05$) level, values in the same column with different superscripts differ significantly.

Table 5. Effect of MTE and γ-irradiation on the Platelets, Monocytes and Eosinophils of Wistar rat

Treatment Group	Platelets (/L)	Monocytes ($x10^3$/L)	Eosinophils ($x10^3$/L)
DDW	93400 ± 30590.85^{a}	3.60 ± 1.34	3.00 ± 1.22
MTE	102600 ± 60603.63^{a}	2.80 ± 0.45	2.20 ± 0.84
3-Gy	77600 ± 15757.54^{a}	2.20 ± 0.84	1.60 ± 0.89
6-Gy	78800 ± 34622.25^{a}	1.80 ± 0.84	2.60 ± 1.14
Pre-3Gy	53600 ± 14791.90^{a}	3.80 ± 1.79	2.60 ± 1.14
Pre-6Gy	79200 ± 18471.60^{a}	1.80 ± 0.45	2.80 ± 2.05
Post-3Gy	207200 ± 60886.78^{b}	2.20 ± 0.84	2.20 ± 0.84
Post-6Gy	119000^{*}	2.00^{*}	4.00^{*}

Values are given as means ± SEM, (n = 5, * = 1); DDW, distil water; MTE, *Markhamia tomentosa* extract at 200 mg/kg; Gy, Gray (radiation unit); At the 5% ($p<0.05$) level, values in the same column with different superscripts differ significantly.

The effect was better for the post-treatment groups because it minimised the damage caused by radiation. The liver histology of the rats exposed to gamma irradiation at a dose of 3Gy and 6Gy (Groups 3-Gy & 6-Gy) revealed that there are random foci of mild single-cell hepatocellular necrosis with mild congestion of portal vessels as well as the proliferation of bile ducts, bile duct hyperplasia, and severe sloughing off showing huge cells with a large nucleus (Figures 1C & 1D). Rats post-treated with MT extract after 3 Gy of gamma irradiation and rats post-treated with MT extract after 6 Gy of gamma irradiation showed moderate congestion of hepatic sinusoids and multiple foci of moderate vascular change of hepatocytes with a moderate KCH and portal vessels with a mild aggregate of mononuclear cells (MNCs) in the portal tracts denoting an increased blood flow rate causing healing of the cells (Figures 1G & 1H).

Histopathological changes in the kidney sections stained with hematoxylin and eosin (H&E 400X) revealed normal tubular systems and glomerular apparatus. The kidney section of rats treated with distilled water (DDW group) showed no visible lesion in the glomeruli and tubules (Figure 2A). In contrast, the kidney section of rats treated with the extract (MTE group) showed few foci of mild sloughing off of tubular epithelium (Figure 2B). The rats treated with MT extract before the gamma irradiation dose of 3 Gy showed no visible lesions (Figure 2E). Photomicrograph of the kidney section of rats treated with extract prior to gamma irradiation dose of 6 Gy showed multiple foci of moderate sloughing off of tubular epithelial cells with accumulation of intra-luminal tubular eosinophilic casts (Figure 2F). There was less distortion of the glomerular apparatus. The rats exposed to gamma irradiation of 3 Gy dose without treatment showed multiple foci of moderate to marked sloughing off of tubular epithelium with mild congestion of renal interstitial blood vessels (Figure 2C).

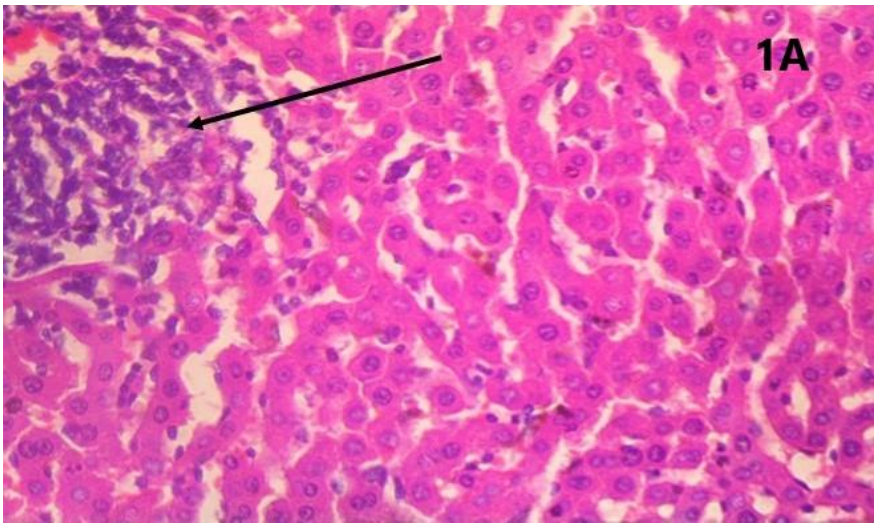

Figure 1A. Photomicrograph of a liver section of DDW rat showing closely packed hepatocytes; no visible lesion with hepatocytes with a few foci of moderate aggregates of mononuclear cells (arrow)

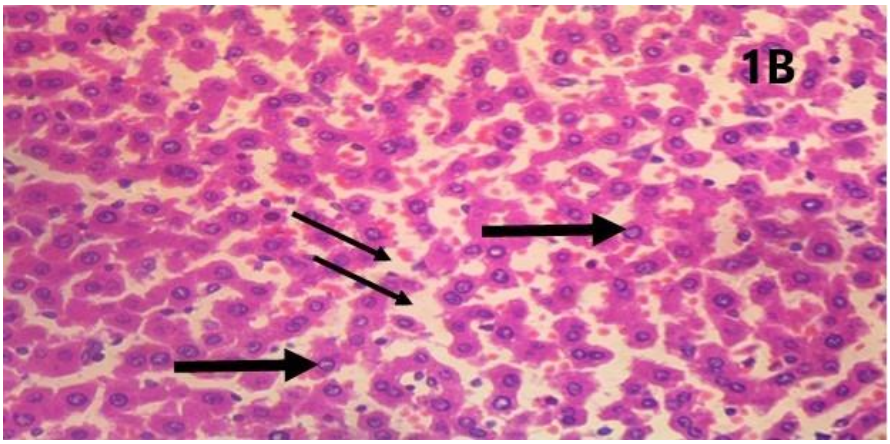

Figure 1B. 1Photomicrograph of a liver section of MTE rat showing there are multiple foci of moderate thinning of hepatic cords and concurrent sinusoidal dilatation [thin arrows] and mild random single-cell hepatocellular necrosis (thick arrow)

In comparison, rats exposed to gamma irradiation of 6 Gy dose without treatment showed few foci of mild cloudy swelling of the epithelial cells of tubules with a severe sloughing off/flattening of the epithelium tubules in the cortico-medullary junction (Figure 2D). The kidney section of rats post-treated with extract after a gamma radiation dose of 3 Gy showed a few foci of sloughing off of tubular epithelial cells and a few foci of aggregates of MNCs in the renal interstitial with moderate congestion of renal interstitial blood vessels (Figure 2G). Similarly, rats treated with extract after gamma radiation exposure of 6 Gy showed a few foci of mild sloughing off of tubular epithelial cells with moderate congestion of renal interstitial blood vessels and glomerular capillary tufts (Figure 2H). Animals in the post-treated groups experienced decreased changes in the kidney tubules with signs of regeneration of the tubules compared to the pretreated groups, which minimises the damage caused by radiation.

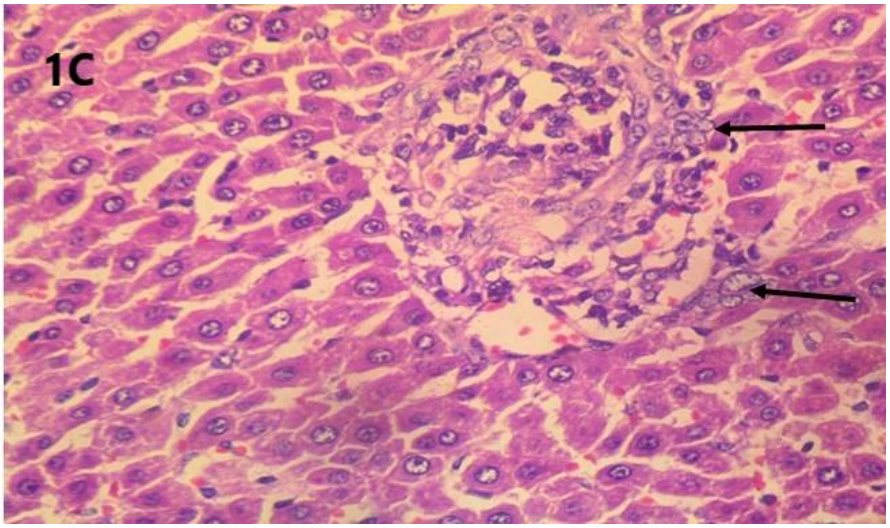

Figure 1C. Photomicrograph of a liver section of a 3-Gy irradiation rat showing there are random foci of moderate single-cell hepatocellular necrosis with a moderate KCH. There are a few foci of moderate aggregates of MNCs in portal tracts and a mild bile duct

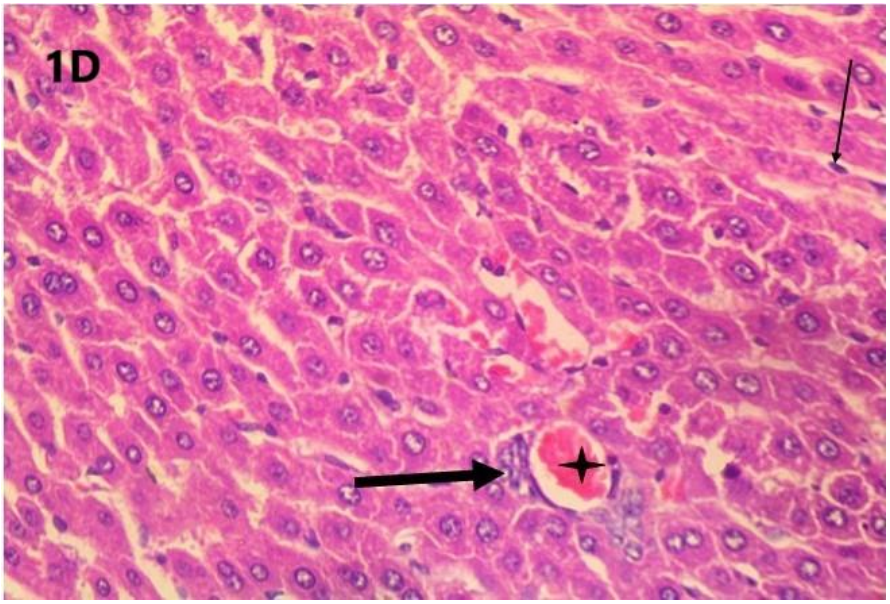

Figure 1D. Photomicrograph of a liver section of 6Gy irradiation rat showing there are random foci of mild single-cell hepatocellular necrosis. There is mild congestion of portal vessels (star) as well as the proliferation of bile ducts (thick arrow) and a moderate

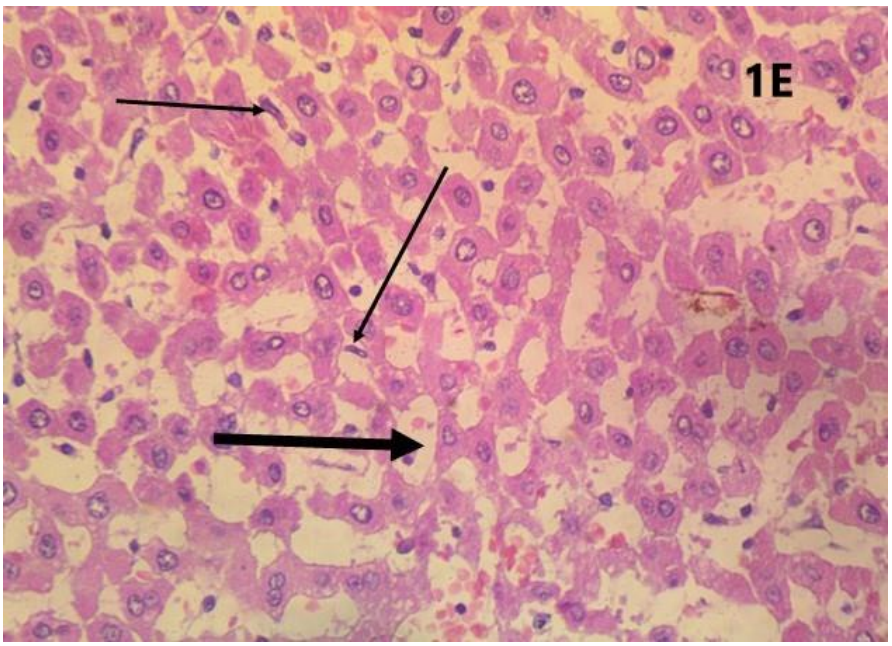

Figure 1E. Photomicrograph of a liver section of a pre-3Gy rat showing multiple foci of moderate thinning of hepatic cords and concurrent sinusoidal dilatation (thick arrow) with a mild random single-cell hepatocellular necrosis and a mild Kupffer cell hyperplasia (t

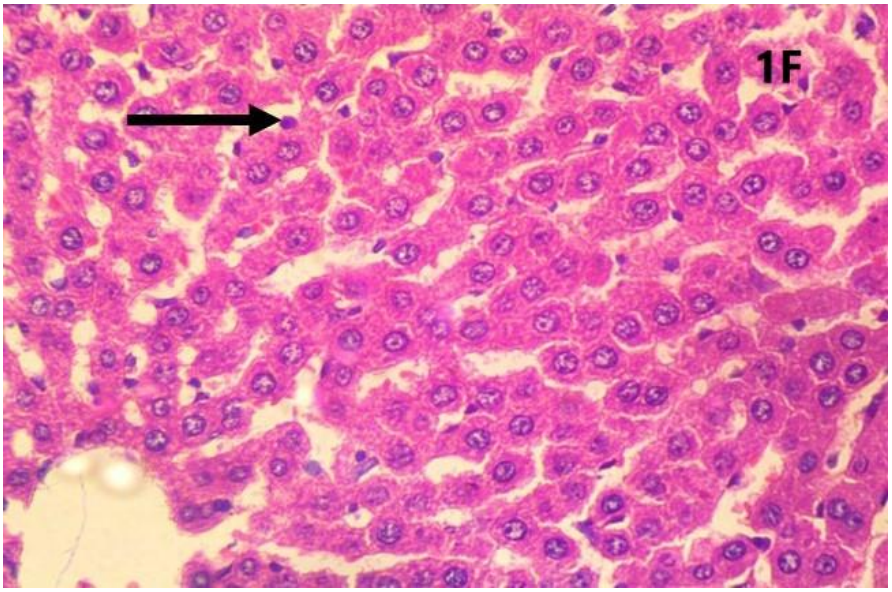

Figure 1F. Photomicrograph of a liver section of a pre- 6Gy rat showing there are foci of mild random single-cell hepatocellular necrosis (thick arrow) and a moderate KCH

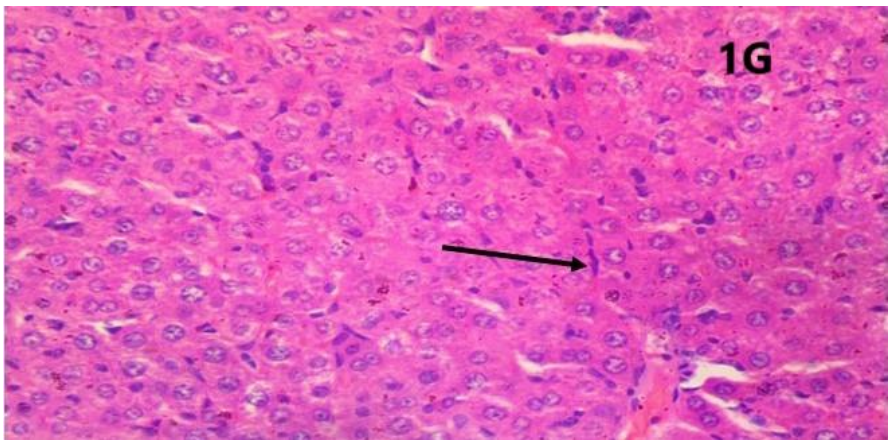

Figure 1G. Photomicrograph of a liver section of a post-3Gy rat showing there is widespread congestion of hepatic sinusoids and portal vessels. There are mild aggregates of MNCs in the portal tracts and a few foci of mild vacuolar change of hepatocytes. There is mode

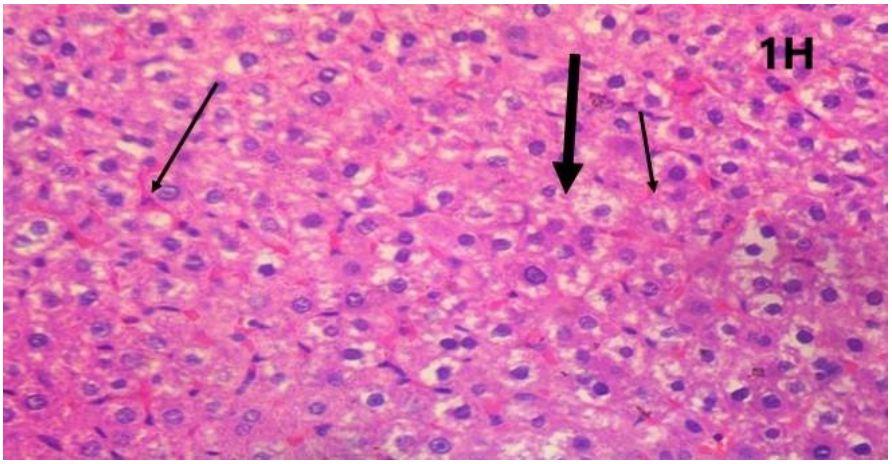

Figure 1H. Photomicrograph of a liver section of a post-6Gy rat showing there are multiple foci of moderate vacuolar change of hepatocytes (thick arrow) and a moderate KCH. There is moderate congestion of hepatic sinusoids (thin arrow)

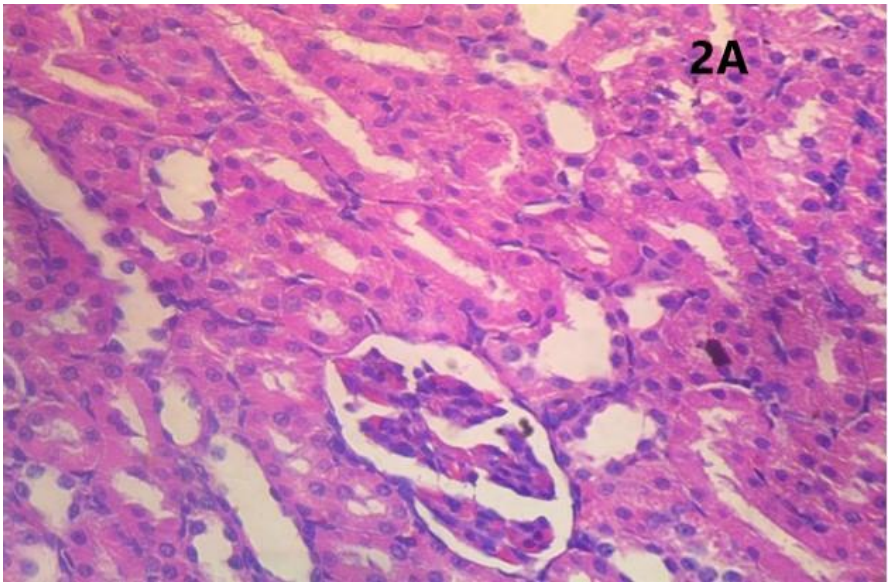

Figure 2A.Photomicrograph of kidney section of DDW rat showing no visible lesion in the glomeruli and tubules

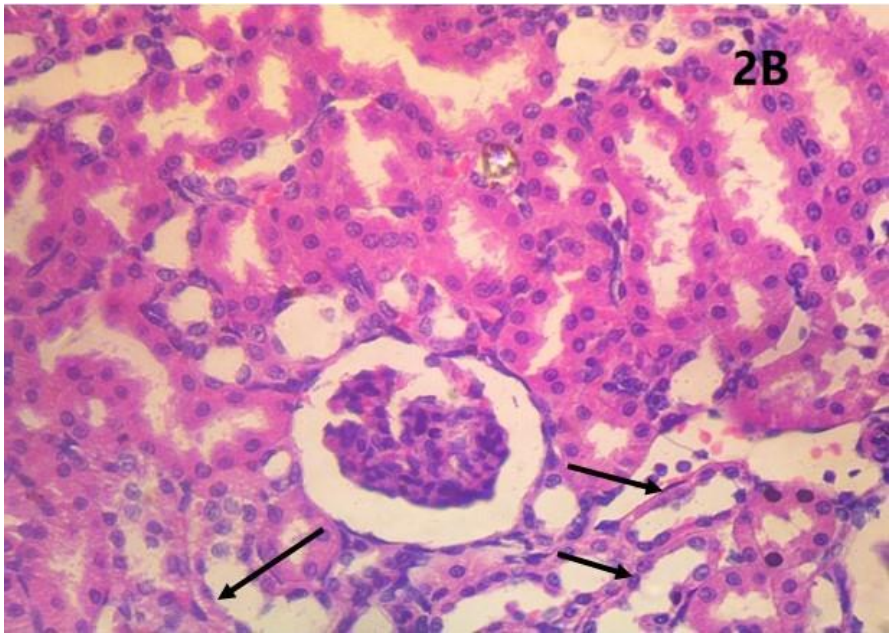

Figure 2B. Photomicrograph of kidney section of MTE rat showing there are few foci of mild sloughing off of tubular epithelium [arrows]

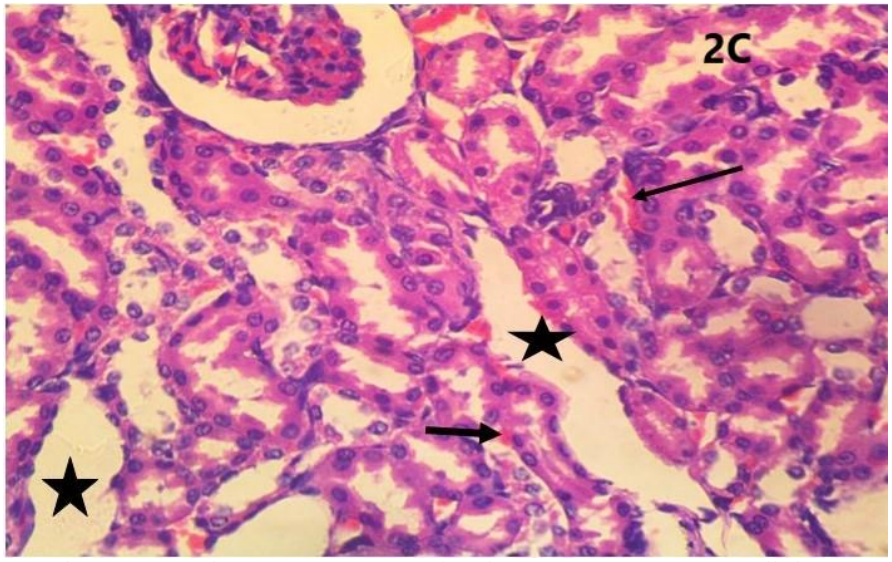

Figure 2C. Photomicrograph of kidney section of 3Gy rat showing multiple foci of moderate to marked sloughing off of tubular epithelium (star). There is mild congestion of renal interstitial blood vessels (thin arrow).

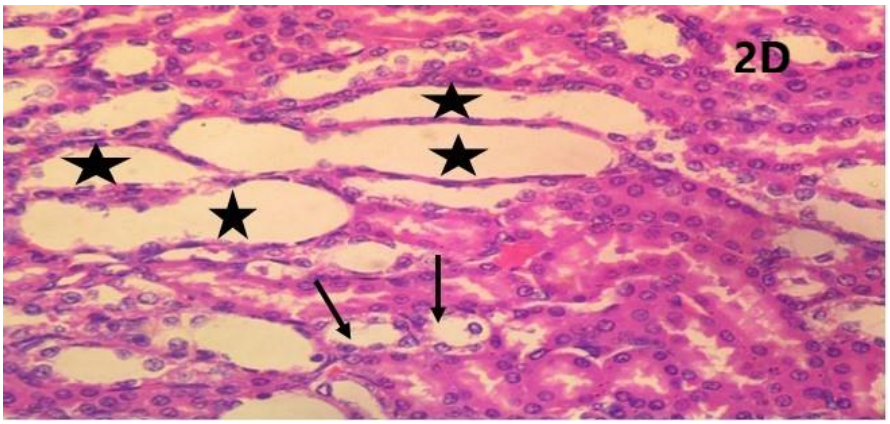

Figure 2D. Photomicrograph of kidney section of 6Gy irradiation rat showing there are a few foci of mild cloudy swelling (thin arrow) of the epithelial cells of tubules. There is severe sloughing off of the epithelium of tubules (stars) in the cortico-medullary

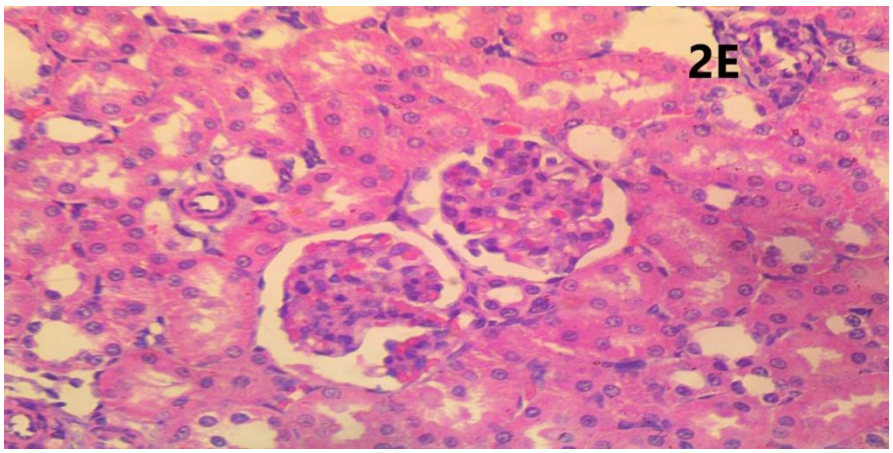

Figure 2E. Photomicrograph of kidney section of pre- 3Gy rat showing no visible lesion.

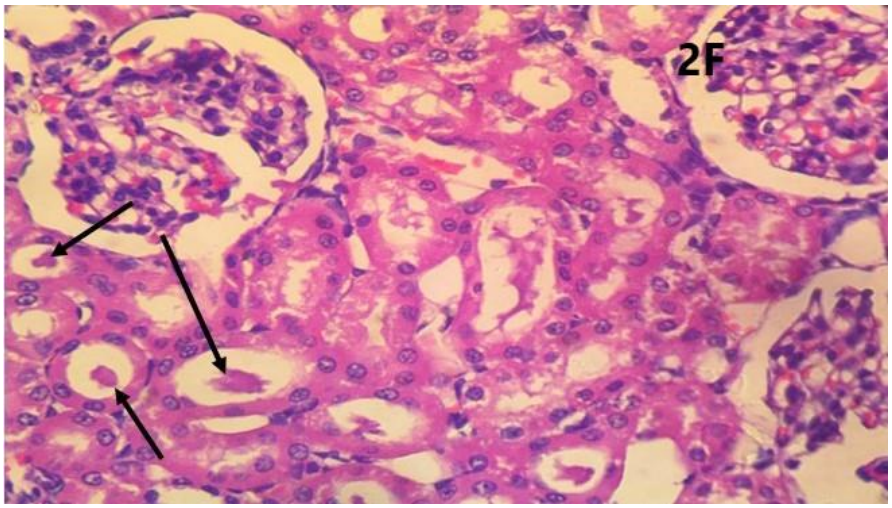

Figure 2F. Photomicrograph of kidney section of pre- 6Gy rat showing multiple foci of moderate sloughing off of tubular epithelial cells with accumulation of intra-luminal tubular eosinophilic casts (thin arrows)

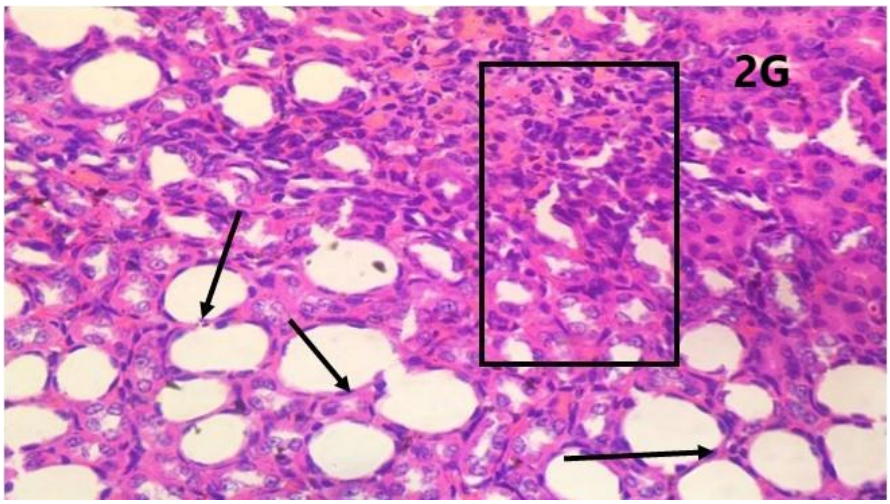

Figure 2G. Photomicrograph of kidney section of post-3Gy rat showing extensive foci of sloughing off of tubular epithelial cells (thin arrows), a few foci of aggregates of MNCs (box) in the renal interstitium. There is moderate congestion of the renal interstitial

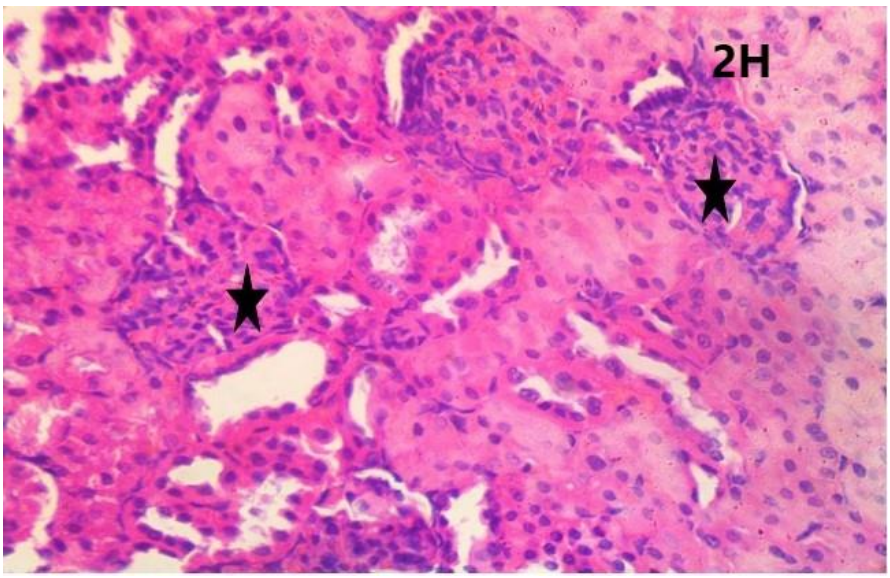

Figure 2H: Photomicrograph of kidney section of post-6Gy rat showing a few foci of mild sloughing off of tubular epithelial cells. There is moderate congestion of renal interstitial blood vessels and glomerular capillary tufts (stars)

Discussion

One of the significant sources of free radicals and ROS produced in the body is exposure to ionising radiation. The interaction of ionising radiation with the human body through the radiolysis of water generates free radicals that cause molecular damage. The extent of molecular damage depends on the radiation's dose (energy), duration of exposure, dose rate and presence of oxygen in tissues and cell cycle [35-37]. Free radicals are extremely reactive due to the existence of unpaired electrons. They attack macromolecules such as DNA and disrupt its replication process leading to cell damage [38]. Significant efforts have been made to develop agents that can scavenge free radicals. These efforts have yielded amifostine as a radioprotector approved by the Food and Drug Administration for use in xerostomia patients undergoing head and neck cancer therapy. One major challenge limiting this drug's use is its toxicity at the optimum protective dose [39, 40]. These limiting factors have motivated research and the development of alternative prophylactic agents with similar features to synthetic compounds. Many research works have shown that plant-based products with antioxidant properties are good scavengers of free radicals [38, 41]. Thus, the search for effective, safe and non-toxic prophylactic agents that can mitigate the harmful effect of ionising radiation and protect healthy cells has received considerable attention as a research priority [7, 9, 35]. In line with the aforementioned, plants and plant-based products have received much attention due to their great medicinal values and therapeutic benefits.

The present paper reports an investigation on the possible potential use of *Markhamia tomentosa* as a radioprotector and radiomitigator against gamma-irradiated male Wistar rats. It was found that 3.0 Gy and 6.0 Gy gamma radiation reduced the packed cell volume (PCV), total red blood cell count (RBC), and hemoglobin (Hb) when compared with Group DDW rats (rats treated with distilled water). Other hematological parameters with a significant difference in mean blood counts include lymphocytes, platelets, neutrophils and eosinophils. The results of the present study appreciably agree with the report of Shaheen and Hassan [42], who reported that gamma-irradiation significantly decreased red blood cell (RBC) count and a non-significant change in haemoglobin levels 24 and 48 hrs after irradiation in male rats. Rana et al. [43] reported a similar finding in chicks exposed to radiation to show the effect on some hematological parameters and its modification by vitamin E. In addition, several studies have demonstrated that a sub-lethal dose of IR can cause a significant change in the hematological parameters of irradiated animals due to the radio-sensitivity nature of bone marrow and the haematopoietic system [9, 33, 36]. This was the pattern observed in the present study. However, the application of *Markhamia tomentosa* ameliorated the effect of IR by significantly improving the hematological parameters leading to high blood counts.

Our results revealed that radiation reduced the mean value of white blood cells (WBC) without any significant improvement in the pretreatment groups. The non-significant improvement may be due to the short 24-hour span of euthanising the animals after irradiation, whereas the reverse was the case in the post-treatment (Post-3Gy) group. The literature has documented that the decrease in granulocyte count requires between 24 to 48 hrs to manifest [36]. It does not reach its climax until three weeks after a moderate dose of whole-body irradiation [36]. The decrease in the WBC from the present investigation is similar to Waghmare et al. [44]. The authors reported a significant decline in the mean value of leucocyte counts during the first 24 hrs after irradiation. The reduction in the average value of WBC may be attributed to the killing

of lymphocytes. Reports have shown that lymphocyte is the most radiosensitive among the haematopoietic cells. It responds to as small as 0.2 to 0.3 Gy of IR. It is believed that a decrease in absolute lymphocyte count is the most feasible and effective laboratory test for assessing IR dose early after exposure [36, 45].

Data from the present investigation revealed that whole-body irradiation at 3 Gy and 6 Gy caused a significant reduction in RBC, PCV, hemoglobin and platelet counts compared to the corresponding values in the pretreatment and post-treatment groups. These results corroborate the findings of Abdel-Aziz et al. [46]. The authors reported that the whole-body gamma irradiation of 6 Gy produced a significant variation in the RBC, total leukocytes, platelets, hematocrit, and hemoglobin values. In addition, the authors revealed that oral gavage of *Spirulina platensis* and *Chlorella vulgaris* at 300 mg/kg body weight one week before and one week post-irradiation alleviated the radiation-induced damage on the hematological parameters mentioned above. Similarly, the present study's results align with the report of Dong et al. [47]. who investigated the radioprotective effect of *Spatholobus suberectus* (JXT) radiation-induced hematopoietic variation. The authors revealed that the exposure of mice to whole-body irradiation of 6 Gy produced hematopoietic syndrome in the gamma-irradiated animals. However, the oral administration of JXT extract for 21 days post-irradiation significantly improved the blood profile with increased blood counts of RBC, platelet, hemoglobin and white cells on day 21. There was a significant reduction in leucopenia and thrombocytopenia in the WBC and platelet counts of post-treatment animals [47].

The post-treatment of rats with the MT extract for 14 days significantly improved the hematological parameters, as evident in the WBC, neutrophils and platelet counts compared with the corresponding values in the pretreatment groups. Leuconeutropenia, a decrease in the white blood cells, occurs in animals exposed to whole-body irradiation, and on day 14, there was an increase in the blood counts. The ability of MT to increase the level of WBC, which is responsible for fighting infections in post-treatment animals, might be due to its scavenging and anti-inflammatory properties, thereby improving the immune system of the post-treated animals. It is estimated that thrombocytopenia takes 5-10 days to manifest after exposure to IR, depending on the radiation dose [36]. The values of platelet counts recorded among the irradiated animals in the present investigation show a non-significant difference compared with the control groups, partly due to the 24 hrs post-irradiation period observed before euthanising the animals. However, on day 14, there was an increase in the platelet counts of post-treatment animals, indicating the plant extract's healing potential. Moreover, the findings of the present investigation corroborate the earlier work of Ibrahim et al. [48] on the toxicity profiles of MT extract, who suggested a significant increase in platelet, WBC, MCV and RBC parameters of rats treated with the MT extract for 28 days. The report of Akomolafe and Chetty [3] on the radioprotective potential of *Drymaria cordata* extract agrees with the findings of the present investigation.

Kidneys are vital organs that play essential roles in the body system, such as regulating blood pressure through fluid balance, stimulating the production of red blood cells and cleaning waste metabolites and electrolytes from the blood [49]. It has been established that whole-body irradiation of pelvic malignancies often leads to radiation-induced kidney injury [49]. Due to the radiosensitivity nature of kidneys, they have been considered dose-limiting organs in radiation therapy for gynaecological cancers, gastrointestinal cancers and lymphomas through the whole-body irradiation process [50]. Even though the latent period for radiation nephropathy takes 6 to 12 months, research has shown that the injury can manifest after a whole-body irradiation dose of 14 Gy split over three days [50]. In the present study, the exposure of rats to whole-body irradiation doses of 3 Gy and 6 Gy produced a few injuries in the kidneys of the experimental animals. The kidneys showed a few foci of mild hazy swelling of tubule epithelial cells in the corticomedullary junction, as well as significant sloughing off and flattening of tubule epithelium. However, the kidney section of rats treated with extract before gamma radiation of 3 Gy revealed no visible lesion. Rats treated with 6 Gy of gamma radiation showed multiple foci of moderate sloughing off of tubular epithelial cells with accumulation of intra-luminal tubular eosinophilic casts. In addition, there was a significant improvement in the kidney section of rats post-treated with the extract of MT after exposure to 3 Gy and 6 Gy of gamma radiation. Rats in the post-treated groups experienced decreased kidney tubular changes with signs of regeneration of the tubules compared to the pretreated groups, which minimises the damage caused by radiation.

Similarly, the histopathological examinations of the liver animals exposed to 3 Gy and 6 Gy revealed radiation-induced liver damage. There were random foci of mild single-cell hepatocellular necrosis with mild congestion of portal vessels as well as the proliferation of bile ducts, bile duct hyperplasia, and severe sloughing off, showing huge cells with a large nucleus. The histological analysis of the liver revealed firm shreds of evidence that the pretreatment and post-treatment groups experienced moderate congestion of hepatic sinusoids and multiple foci of moderate vascular change of hepatocytes with a moderate KCH. The present study's findings concur with Temdie et al. [51], who earlier revealed the hepatoprotective potential of *Markhamia tomentosa* extract on lipopolysaccharide-induced hepatitis in mice. The authors showed that MT extract reduced liver damage produced by lipopolysaccharide [51]; the work of Ibrahim et al. [48] on the sub-acute and chronic toxicity effects of MT in rats showed that the histopathological examinations of

the liver revealed no significant alterations in the treatment and control groups.

Moreover, the pharmacological potentials of medicinal plants have been attributed to the presence of secondary metabolites [52]. Sofidiya et al. [53]. reported that MT contains phenolic, terpenoid and iridoid compounds. The report was further corroborated by Ibrahim et al. [52], who identified four terpenoid compounds containing three tripenoids and one diterpenoid, which were actively present in the MT leaves extract. The presence of these secondary metabolites may be responsible for the pharmacological use of MT in traditional medicine. In addition, the antimicrobial [25, 54] and anti-inflammatory activity [24] of MT leaves extract has been reported; the authors suggested that MT possessed anti-inflammatory activity mediated by histamine. The histological analysis of the rats' hepatocyte showing no lesion or membrane disruption lends credence to this study.

The major limitation of this work was the employment of a single pathologist in examining and interpreting the experimental animals' histopathological slides of kidneys and liver. The results could have been more accurate if multiple pathologists had been used. It is recommended in future work to base the results of histopathological examinations on the interpretation and findings of multiple pathologists.

Conclusion

The present study has demonstrated the radioprotective and radiomitigating properties of *Markhamia tomentosa* on the gamma radiation-induced damage on rats. This research indicated that gamma radiation of two different doses produced significant alterations in the hematological and histopathological parameters of irradiated rats. The damaging effect of radiation on the blood parameters was, however, alleviated by the ethanol extract of *Markhamia tomentosa*, which was evidenced in the increase in the blood counts of the hematological parameters. The antioxidant, anti-inflammatory, free radical scavenging, and healing/repair properties of MT reported in the literature might be responsible for its radioprotective and radiomitigating efficacy. The healing potential of MT was seen in recovering the distorted tissues during pretreatment and post-treatment and modulating the effects of gamma radiation-induced damage to blood cells. Based on the present findings, *Markhamia tomentosa* can serve as a potent natural radioprotector and radiomitigator that would be useful in the radiotherapy of cancer cells and the radiation emergency field.

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