ISSN:2321-1156

International Journal of Innovative Research in Technology & Science(IJIRTS)

PROTECTIVE POTENTIAL OF ORAL ADMINISTRATION OF AQUEOUS EXTRACT OF *MORINGA OLEIFERA* LAM AGAINST GAMMA RADIATION INDUCED DAMAGES ON THE GROWTH PLATES OF MALE WISTAR RATS

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Abstract

This study investigated the radioprotective effects of *Moringa oleifera* aqueous leaf extract on the physis of male Wistar rats. This was with a view to determining the potency of *Moringa oleifera* aqueous leaves extract as radical scavenging agent.

Eight groups of male Wistar rats of 5 rats in each group were used for this study. The rats were grouped based on five days of administration of the *Moringa oleifera* aqueous leaf extract followed by exposure to 2.5 Gy radiation source. Groups 2 and 6, 3 and 7, 4 and 8 were administered with 25, 50 and 100 mg/kg per day of *Moringa oleifera* aqueous leaf extract respectively. The administration was followed by exposure of rats in groups 5, 6, 7 and 8 to a single whole body dose of 2.5 Gy gamma radiation source. Group 1 rats served as the control without exposure to radiation or administration of *Moringa oleifera* leaf extract. The rats were sacrificed after 2, 7 and 14 days post-radiation. Long bones of the rats were thereafter harvested and processed for routine histology and stained with H & E stain.

The results of the irradiated rats showed reduction in the chondrocyte count compared to the un-irradiated rats. Rats pre-administered with the *Moringa oleifera* leaf extract showed improvements in the chondrocyte count. The results also showed disruption in the architecture of the growth plates in the irradiated rats with pre-administered with *Moringa oleifera* aqueous leaf extract rats showing normal growth plate.

This study concluded that *Moringa oleifera* aqueous leaf extract had radioprotective effects on radiation-induced damages on growth plates.

Introduction

Ionizing Radiation is a form of radiation that possesses sufficient energy to eject electrons from the atoms/molecules of the medium through which it traverses (Podgorask, 2006). Such principle has been applied in the clinical practice as diagnostic, therapeutic and palliative tools for various kinds of investigation of human health status and disease control (Syed, 2007; Waghmare*et al.*, 2013). These beneficial effects have also been reported to be associated with different detrimental effects (Hall and Giaccia, 2006; Fazel*et al.*, 2009). The route toward the damaging effects of the ionizing radiation may be direct damage to the essential organelles of the cells, e.g. deoxyribonucleic acid (DNA) or theradiolysis of the water content of the cells which leads to the production of highly reactive free radicals that eventually damage the DNA of the cells (Hall and Giaccia, 2006; Ghoneum*et al.*, 2013). Thus, uncharged photons of ionizing radiation (e.g. X-rays or δ-radiation) are classified as indirectly ionizing radiations that cause radiation hydrolysis in animal cells they traverse (Alpen, 1998). Exposed young and adolescent patients that were treated with radiation for skeletal malignancies such as soft-tissue sarcoma were later discovered to have damages to the physis (Bluemke et al., 1994; Hayashi*et al.*, 2014). Since longitudinal bone growth depends on the growth of the physis, any damage to the normal functioning of the growth plate can thus lead to growth arrest (Van De Graaff, 2001; Ayannuga and Saka, 2012).

Although the cells are capable of repairing damages to its contents, free radicals are said to permanently 'fix' the chemical composition of the cell, thus making it necessary to provide additional agent that will assist the cell in performing its repairing roles. Hence, there is need to reduce the detrimental effects of these ionizing radiations without compromising the beneficial outcomes of the use of the radiations (Devi and Agrawala, 2011). Radical scavenging agents that can serves as radioprotectors are therefore imperative to radiodiagnosis and radiotherapy practice (Donneys*et al.*, 2014).

Since plants supplied with normal nutrients from the soil have been observed to withstand the radiation effects from the sun, they can therefore be utilized to reduce the detrimental effects of ionizing radiation used in clinical practice. Normally, edible plants do not produce toxic substances to the body system and can hence be used as protectors against radiation induced damages.

Moringa oleifera plant has been reported to have antioxidant property which makes the plant suitable for mopping up free radicals produced by indirectly ionizing radiations (Ogbunugafor*et al.*, 2011; Sayeed *et al.*, 2012; Bello *et al.*, 2013). It is well cultivated in the Asia and tropical Africa and grows well in alkaline soils.

The objective of this study is thus to examine the radioprotective effects of the aqueous extract of *Moringa oleifera* leaf extract on the physis of male wistar rats following exposure to gamma radiation.

Materials and Method

Animal Care and Selection: Male wistar rats weighing between 90-100 g were randomly selected into eight different groups. The rats were kept inside clean well-ventilated plastic cages and exposed to natural light and darkness cycle. The rats were given pelletized rat chow and clean water ad libitum.

Extract Preparation: The leaves of *Moringa oleifera* tree was local collected and scientifically identified and authenticated at the department of Botany, Obafemi Awolowo University, Ife. A specimen was deposited and voucher number IFE 17334 was given as the reference number. The leaves were then destalked and, dried for three weeks under the shade. The dried leaves were then pulverized with an electric blender after which a total mass of 484.5 g was obtained. Distilled water of 2.5 L was then added to the powdered leaves inside a large conical flask. The mixture was shaken intermittently with the use of an electric shaker for 24 hours. After thoroughly shake, the mixture was filtered with the use of cotton wool and the filtrate was obtained. The filtrate was then concentrated with the use of a rotary evaporator at a temperature of 45^{o}C and then stored in a glass petri dish.

Extract Administration: The extract was administered to the rats orally with the use of an oral cannula inserted into an insulin syringe. Graded doses of 25, 50 and 100 mg/kg b. wt. of the extract were administered to the rats.

Irradiation: ^{60}Co from a Gamma Beam X200 research irradiator at the National Institute of Radiation Protection and Research, University of Ibadan, Ibadan was used. The source to surface distance of 80 cm, field size of 10 x 10 cm^2, and dose rate of 19.352 mGy/s was set up to deliver a radiation dose of 2.5 Gy whole body radiation to the rats.

Study Design: Table 1 shows the groupings of the rats with their respective extract administrations.

Table 1: Animal Grouping and Treatment

Group	Treatment
1	Rats received animal chow and water only
2	Un-irradiated rats administered with 25 mg/kg body weight per day for five consecutive days.
3	Un-irradiated rats administered with 50 mg/kg body weight per day for five consecutive days.
4	Un-irradiated rats administered with 100 mg/kg body weight per day for five consecutive days.
5	Rats given feeds and water ad libitum and exposed to a single dose of 2.5 Gy of gamma radiation
6	Rats received food and water ad libitum and aqueous extract of *Moringa oleifera* leaves with a dosage of 25 mg/kg per body weight for five consecutive days. They were then exposed to 2.5 Gy of gamma radiation 2 hours after the final administration.
7	Rats received food and water ad libitum and aqueous extract of *Moringa oleifera* leaves with a dosage of 50 mg/kg per body weight for five consecutive days. They were then exposed to 2.5 Gy of gamma radiation 2 hours after the final administration.
8	Rats received food and water ad libitum and aqueous extract of *Moringa oleifera* leaves with a dosage of 100 mg/kg per body weight for five consecutive days. They were then exposed to 2.5 Gy of gamma radiation 2 hours after the final administration.

Sacrifice and Organ Harvest: The rats were humanely sacrificed on days 2, 7 and 14 after irradiation by cervical dislocation. The limbs of the rats were harvested and fixed inside 10% formal saline solution. Bone maceration was carried out by removing the skin and muscles attached to the bones of the harvested limbs. The macerated bones were further fixed in 10% formal saline. The macerated bones were the transferred into tissue cassettes and decalcified using a thoroughly mixed solution that contains EDTA disodium salt (88 g), formaldehyde (160 ml) and distilled water (1440 ml).

Histology and Staining: The decalcified bones were then dehydrated inside increasing concentrations of alcohol, cleared with two changes in xylene and embedded in paraffin using embedding moulds. Sections of 5 µm thick were then made using a rotary microtome. The cut sections were dewaxed in xylene and then rehydrated in decreasing concentrations of alcohol. The sections were then washed in water and stained in haematoxylin for 10 minutes after which they were differentiated in 1% acid alcohol and blued (washed) in running in tap water for 2 minutes. After this, the sections were the counter stained in 1% aqueous eosin

for 3 – 5 minutes. The specimens were then dehydrated rapidly in descending grades of alcohol and then mounted in Distrene Plasticizer Xylene (DPX) using clean glass cover slips.

Microscopic Study: The sections were then examined under a LEICA research microscope (LEICA DM 750, Switzerland) interfaced with digital camera (LEICA ICC 50, manufactured by Leica Microsystems Inc., United States of America). Digital photomicrographs of stained sections were then taken at X40 and X400 magnifications. The photomicrographs of the haematoxylin and eosin stained sections were imported on to the Motic Images Plus Version 2.0 software for chondrocyte counting.

Statistical Analysis: Data were expressed as mean±standard error of mean (SEM). The statistical significance was evaluated by one way analysis of variance (ANOVA) using GraphPad Prism 5 (Version 5.03, GraphPad Inc.) followed by Student Newman-Keuls (SNK) test for multiple comparisons. A value of $p< 0.05$ was considered to indicate a significant difference between groups.

RESULTS

Chondrocyte Population: Cell counts were carried out at the right, left and centre sides if the growth plates. CC1 represents centre number of chondrocyte, CC2 indicates right side chondrocyte count and CC3 represents left side chondrocyte count. All values are given in mean± standard error of mean (SEM).

Chondrocyte Count, day 2 after irradiation: As indicated in table 2, no significant changes were noted in the chondrocyte counts on post-irradiation day 2.

Table 2: Chondrocyte Count of the Growth Plates, day 2 after Irradiation

Groups	CC1	CC2	CC3
1	35.000±2.646	29.000±6.245	23.330±4.910
2	23.670±3.180	29.000±2.309	40.000±2.309
3	41.670±10.350	38.670±3.930	27.000±5.000
4	35.000±7.371	35.000±9.292	27.000±6.658
5	26.670±2.603	30.330±1.453	38.000±6.351
6	39.330±0.882	41.000±0.577	29.670±2.028
7	30.000±2.887	31.000±2.309	21.670±5.487
8	28.670±4.910	25.000±2.309	28.670±1.453

Chondrocyte Count, day 7 after irradiation: Chondrocyte count for second day of sacrifice (day 7 after irradiation) was also carried out for the left side, centre and right side of the growth plates. The chondrocyte count for group 5 rats show significant decrease when compared to the control group rats and the mean value of the group 5 rats showed a decrease in the number of cell counted at the right side of the growth plate when compared to the control group 1 rats. A significant increase was found in groups 6 and 7 rats when compared to the group 5 rats. There was significant increase in group 3 when compared with control group 1. The mean value for the cell count at the centre of the growth plate also shows a significant reduction in number of cell counted in rats in irradiated group 5 when compared with control group 1. Significant increase was also noted in groups 2, 4 and 7 when compared with the group 5. There was significant difference between the mean differences of the left side cell count of groups 1 and 5. Significant increases were noted between groups 2, 4 and 6 rats when compared to irradiated group 5 rats. For the left cell count on day 7 after irradiation, the number of cell count in irradiated group 5 was low compared to groups 1, 2, 3, 4, 6 and 7 groups. Group 8 rats have the lowest cell count at the left side of the growth plate.

Table 3: Chondrocyte Count of the Growth Plates, day 7 after Irradiation

Groups	CC1	CC2	CC3
1	35.670±2.186	29.000±6.245	23.330±4.910
2	44.000±1.732	49.670±0.333[1,4,5]	45.000±2.887[1,3,5]
3	51.670±5.840[1,5]	31.670±0.882[1,2,4,7]	19.330±1.764[4,6]
4	34.670±0.882[3]	36.670±4.910[3,5]	36.670±1.453[3,5,8]
5	14.670±3.180[1,2,4]	9.667±1.333[1,3]	10.670±2.186
6	41.000±0.577[3,5,7]	19.670±2.603[2,4]	36.670±8.950[5,8]
7	29.000±1.732[2,3,5,8]	30.670±1.453[2,5]	25.670±0.882[2]
8	12.670±4.041[1,2,4,6]	11.000±2.309[1,2,3,4,7]	9.333±0.882[2]

Superscript in the data indicates:[1]significantly different from group 1; [2]significantly different from group 2; [3]significantly different from group 3; 4significantly different from group 4; [5]significantly different from group 5; [6]significantly different from group 6; [7]significantly different from group 7; [8]significantly different from group 8.

Chondrocyte Count, day 14 after irradiation: The chondrocyte count obtained on the 14th day after irradiation is shown in table 4 below. Group 5 rats were noted to have significant reduction when compared to the control group 1 rats.

Table 4: Chondrocyte Count of the Growth Plates, day 14 after Irradiation

Groups	CC1	CC2	CC3
1	35.000±2.646	34.000±3.464	24.330±5.812
2	11.330±1.856[1,5,6,7]	12.330±3.283[1,3,4,5,7]	11.000±4.041[3,4,7,8]
3	24.330±3.844[2,7]	46.330±6.173[4]	30.670±6.227
4	27.330±4.256[2,7]	27.000±1.155	33.670±2.186
5	22.000±1.155[1]	33.000±2.309	22.000±1.732
6	25.000±2.310[7]	37.670±0.333[2]	22.000±1.155
7	41.330±0.882[5]	37.000±0.577	39.670±1.202[1,5,6]
8	30.330±0.882[7]	33.000±4.619	27.670±0.333

Superscript in the data indicates:[1]significantly different from group 1; [2]significantly different from group 2; [3]significantly different from group 3; [4]significantly different from group 4; [5]significantly different from group 5; [6]significantly different from group 6; [7]significantly different from group 7; [8]significantly different from group 8.

DISCUSSION

Result of this study showed a significant reduction in the chondrocyte number of the rats when exposed to 2.5 Gy of radiation which is in concordance with the results by Margulies *et al.* (2006) and Hong *et al.* (2014) who, although with a higher radiation dose, reported from an in vitro study of the effects of radiation on chondrocytes. They concluded that radiation adversely altered chondrocyte cell cycle and reduced the proliferation of the chondrocyte. This was found to be as a result of the induction of apoptosis and increased cytotoxicity in the chondrocytes of the rats following irradiation (Margulies *et al.*, 2006). The results from this study conforms with the results of Damron*et al.* (2004) who also concluded that exposure to low dose of radiation can lead to growth arrest and discrepancy in limb length due to the depletion of the chondrocyte number in the epiphyseal plates of irradiated animals. Also, reduction as presented in tables 2-4 suggest that exposure to a low dose of 2.5 Gy from a ^{60}Co radiation source can result in somatic effects such as limb length shortening in individuals or animals through the depletion of the chondrocyte of the epiphyseal plate. From this study, it was noted that treatment with *Moringa oleifera* leaf extract before irradiation showed a significant increase in the chondrocyte number. This increase may be due to the stimulation of endochondral ossification, where an intermediate cartilaginous template is made in the growth plate and then replaced by trabecular bone in the adjacent metaphysis or upregulation of the genes in the chondrocyte of the epiphyseal plate (Damron*et al.*, 2003; Horton *et al.*, 2006; Ayannuga and Shokunbi, 2014). On histological examination, a clear disruption of the growth plate architecture was found in the rats exposed to the ^{60}Co radiation source, as shown in plates 1 and 3, which also conforms to the results of Bakker *et al.* (2003) who reported that the columns of the growth plates were less straight and less parallel to each other as most of the growth plate of the irradiated columns did not extend across the entire growth plate. In plates 1 to 3, pre-administration of *Moringa oleifera* showed improvements in the architecture of the growth plates of the irradiated rats during the three timelines used for this study. This was noted in the photomicrographs of the rats pre-administered with *Moringa oleifera* extract as normal growth plates against mottled and cavitation growth plates observed in the photomicrograph of the irradiated rats without pre-administration of aqueous extract of *Moringa oleifera* leaf.

Conclusion

In conclusion, results from this study indicated that exposure to 2.5 Gy of gamma radiation can cause damage the growth plate of rats. This was noted through the alteration to the epiphyseal plates of the rats used for this study. The implication of such effects are thus noted at the microscopic level which means that an individual exposed to radiation may have suffered body damages although such may physically look healthy. However, *Moringa oleifera* leaf aqueous extract as shown from the results of this study showed the potency to ameliorate these effects as normal growth plates were observed in rats pre-administered with administered with the *Moringa oleifera* extract before exposure to the radiation source.

Acknowledgments

The authors wish to thank the technical staffs of the Nigerain Institute of Radiation Protection and Research for their immense support during the irradiation of the rats at their centre. Our gratitude also go to the academic and non-academic members of staff of the department of Anatomy and Cell Biology, Obafemi Awolowo University, Ife, Nigeria for their assistance and provision of some laboratory material used for the processing if the tissues used in this study.

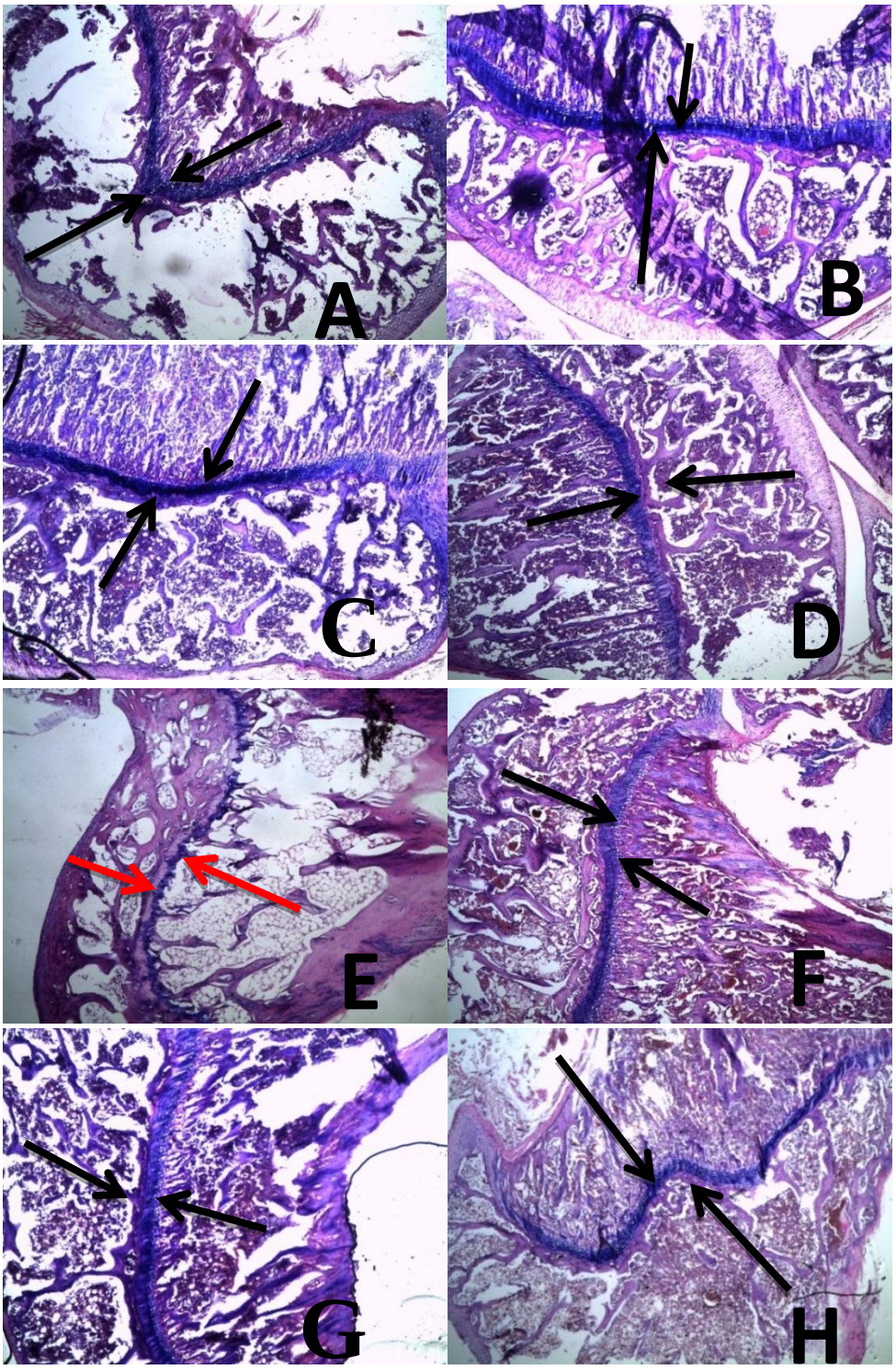

Plate 1: Photomicrographs of the epiphyseal end of representative rats' bone 2 days after exposure to 2.5 Gy of ^{60}Co radiation source, showing apparently normal growth plate (Black arrow) with the exception of E that shows mottled and thin growth plate (Red arrow). A (Control), B (Group 2), C (Group 3), D (Group 4), E (Group 5), F (Group 6), G (Group 7), H (Group 8). H & E. Mag. X40

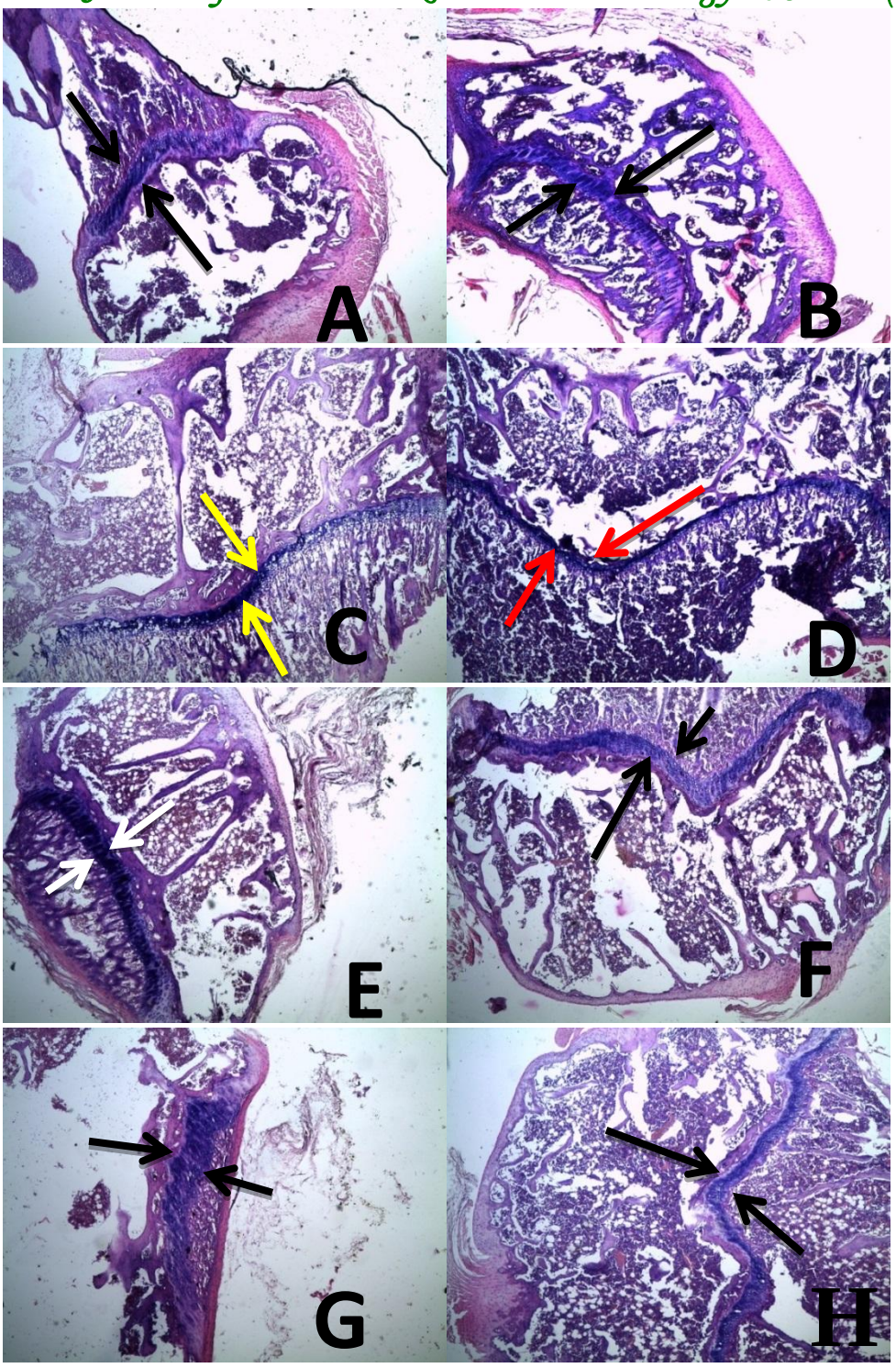

Plate 2: Photomicrographs of the epiphyseal end of representative rats' bone 7 days after exposure to 2.5 Gy of ^{60}Co radiation source, showing apparently normal growth plate (Black arrow) thinned growth plate (Red arrow), intra-growth plate cavitation (Yellow arrow) and growth plate with apparently degenerating chondrocytes (white arrow). A (Control), B (Group 2), C (Group 3), D (Group 4), E (Group 5), F (Group 6), G (Group 7), H (Group 8). H & E. Mag. X40.

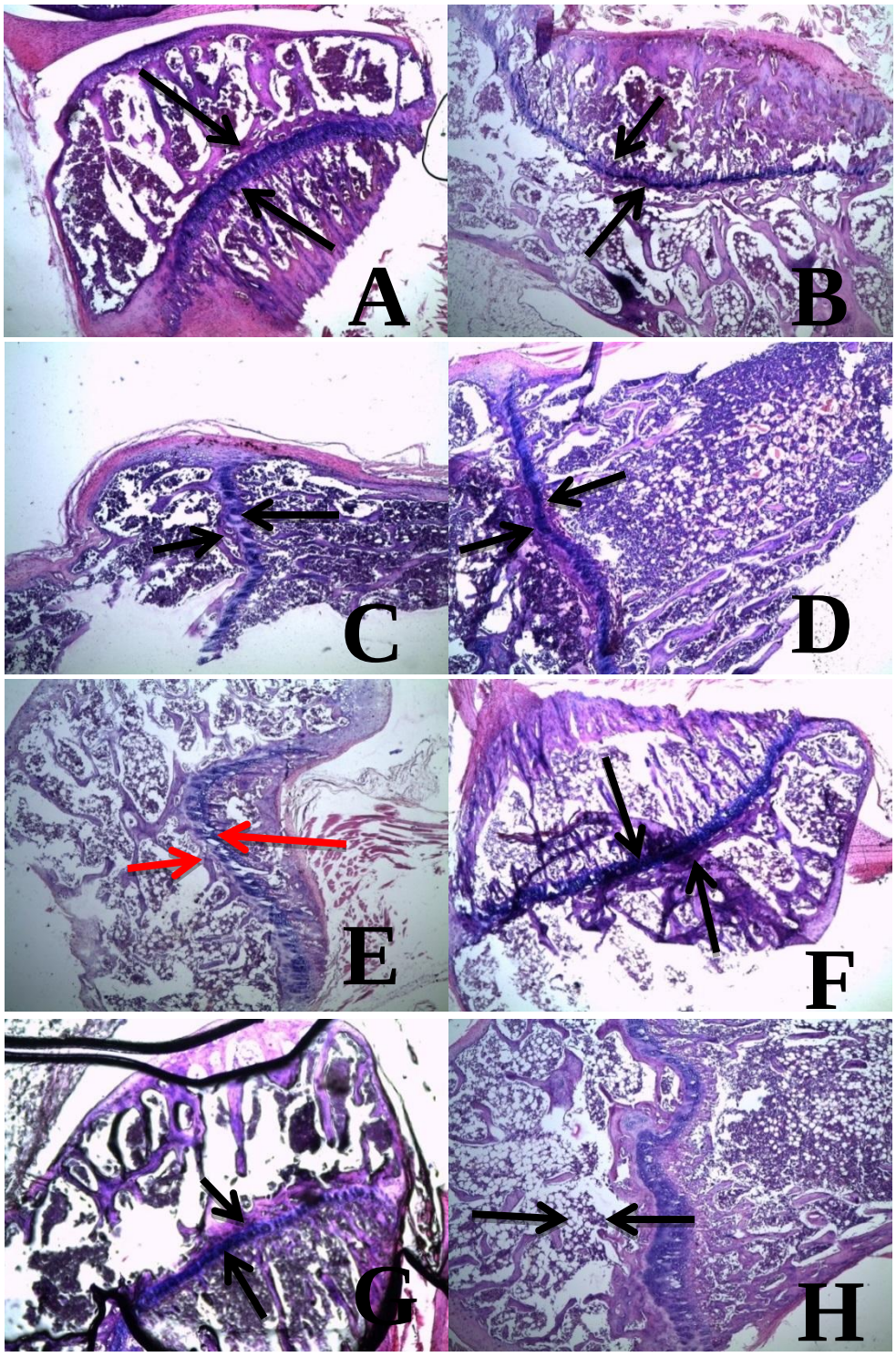

Plate 3: Photomicrographs of the epiphyseal end of representative rats' bone 14 days after exposure to 2.5 Gy of ^{60}Co radiation source, showing apparently normal growth plate (Black arrow), and growth plate with cavitation (Red arrow), A (Control), B (Group 2), C (Group 3), D (Group 4), E (Group 5), F (Group 6), G (Group 7), H (Group 8). H & E. Mag. X40.

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