Prophylactic Effect of Aqueous Extract of Moringa oleifera Lam Leaves against Gamma Radiation Induced Damage on the Haematopoietic Cell Count in Adult Male Wistar Rats


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Abstract: Moringa oleifera plant has been reported to have antioxidant properties which aroused the search for its radioprotective effects. This study is expected to highlight the possible prophylactic role of aqueous extract of Moringa oleifera leaves following exposure to radiation. A total of 90 Wistar rats were divided into six groups of fifteen rats each. These are: group 1 (control without radiation or moringa extract), group 2 (50 mg/kg b. wt. of aqueous extract of M. oleifera), group 3 (100 mg/kg b. wt. of aqueous extract of M. oleifera leaf), group 4 (2.5 Gy of gamma radiation only), group 5 (50 mg/kg b. wt. of M. oleifera + 2.5 Gy of gamma radiation), group 6 (100 mg/kg b. wt. of M. oleifera + 2.5 Gy gamma radiation). Two hours after the final administration on the fifth day, selected rats were exposed to a single dose of 2.5 Gy gamma radiation. The animals were sacrificed at on days 2, 7 and 14 after irradiation. Exposure to the 2.5 Gy showed significant decrease in the WBC, PLT, RBC, HGB, LYM while no significant changes was noted in the MCV throughout the three timelines used for this study. However, pre-treatment with aqueous extract of M. oleifera leaves was observed to reduce these detrimental effects. Thus, aqueous extract of M. oleifera leaves has the prophylactic potency to help reduce radiation damage to the haematopoietic system.

Keywords: prophylactic; aqueous; haematopoietic; Wistar rat
Introduction
Radiation has been used in different fields of study including medical science for the diagnosis and therapy of disease [1]. This usage has also come with health hazards especially to the exposed individuals. This effect is based on the radiation dose and the exposure time which can be either chronic or acute. Acute radiation syndrome has been reported to result from exposure to high dose of radiation over a short period of time [2]. The syndrome include, haematopoietic, gastrointestinal and cerebrovascular syndrome. The damage to the haematopoietic system after acute radiation exposure has been reported to cause reduction in the blood cell count, hemorrhage, anaemia or eventual death in the exposed subjects [3]. The various parts of the tree have medicinal values in the treatment and prevention of different kinds of diseases [4]. It has been used to treat diseases such as inflammation, infectious diseases, cardiovascular, hematological and hepatorenal disorders [5]. It contains antioxidants which help to quench, scavenge and suppress the production of free radicals [5]. Thus, Moringa oleifera has a potential to scavenge the free radicals produced through the radiolysis of the water content of the cell as photons of radiation (X-rays or gamma rays) traverses the body. The aim of the study is to investigate the radioprotective effects of the aqueous extract of Moringa leaves after exposure to a single dose of 2.5 Gy gamma radiations.

Materials and Methods
Experimental animals: Wistar rats with weights ranging from 100-120 g were randomly selected and divided into six groups of fifteen rats each and housed inside well ventilated clean plastic cages under natural light and dark cycles at room temperature adapted for the purpose of this study. Pelletized rat chow feeds and clean water were supplied to the animals ad libitum.

Preparation of aqueous leaf extract: The Moringa oleifera leaves were locally sourced from two different Moringa trees and taken for scientific identification and authentication at the department of Botany, Obafemi Awolowo University, Ile-Ife. The voucher specimen was then deposited at the IFE Herbarium and was given the reference number 17334. Destalked Moringa oleifera leaves were used for this study. The Moringa oleifera leaves still maintained their greenish colour as the time they were obtained. The collected leaves were then air-dried (in the absence of sun) for three weeks after which the dried leaves were grinded with electric blender into powdery form. A total mass of 484.5 g was obtained after grinding. 2.5 L of distilled water was then added to the grinded leaves and shaken intermittently for 24 hours. This was then filtered with a cotton wool in order to obtain the filtrate. The filtrate was then concentrated using a vacuum rotary evaporator operated at a temperature of 45oC.

Dose Administration and Sacrifice: Graded doses of 50 mg/kg and 100 mg/kg of Moringa oleifera leaf extract were orally administered using oral cannula and insulin syringes for five consecutive days before irradiation on the fifth day. The rats were then sacrificed on days 2, 7 and 14 after irradiation.

Source of Radiation: The rats were un-anesthetized and restrained using a well ventilated net-like box and were exposed to whole body radiation from a Gamma Beam X200 Research Irradiator containing a 60CO source. The source to surface distance (SSD) was 80 cm.
and a fixed size of dose rate 19.352 mGy/s was used to deliver a dose of 2.5 Gy gamma radiation at the National Institute for Radiation Protection and Research, University of Ibadan, Ibadan.

Toxicity test: The acute toxicity test for this study was based on standard procedures.

Animal Classification and treatment: The classification was carried out as shown in Table 1.

### Table 1: Animal Classification and Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>1</td>
<td>Rats received animal chow and water only</td>
</tr>
<tr>
<td>2</td>
<td>Un-irradiated rats administered with 50 mg/kg body weight per day for five consecutive days.</td>
</tr>
<tr>
<td>3</td>
<td>Un-irradiated rats administered with 100 mg/kg body weight per day for five consecutive days.</td>
</tr>
<tr>
<td>4</td>
<td>Rats given feeds and water <em>ad libitum</em> and exposed to a single dose of 2.5 Gy of gamma radiation</td>
</tr>
<tr>
<td>5</td>
<td>Rats received food and water <em>ad libitum</em> and aqueous extract of <em>Moringa oleifera</em> 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.</td>
</tr>
<tr>
<td>6</td>
<td>Rats received food and water <em>ad libitum</em> and aqueous extract of <em>Moringa oleifera</em> 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.</td>
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Haematological study: Rats were sacrificed; blood samples were obtained by cardiac puncture and transferred into K3 EDTA bottles for assay for various haematological parameters using H 18 light automatic blood cell counter at the Department of Hematology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ife. The following blood parameters were thus assessed: white blood cell count (WBC), red blood cell count (RBC), mean corpuscular volume (MCV), haemoglobin level (HGB), lymphocyte count (LYM), platelet count (PLT).

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

Physical Observations: The rats were in good health during the period of acclimatization as there was no change in the physical appearance of the rats except that some rats were noticed to eat better than others. The irradiated animals were noticed to be scratching their body for few seconds immediately after removing them from the radiation source. At the end of the experiment i.e. before the rats were sacrificed, the rats were physically observed to be healthy with white hair and pink eyes. No scales or rashes were found on the bodies of the rats at the time of sacrifice.
Blood Cell Count Analysis:

WBC: Two days after irradiation, the WBC count in all the irradiated rats showed a significant decrease when compared with the control and the un-irradiated rats treated with Moringa oleifera leaf extract. Day 7 after irradiation, a significant difference was noticed in the irradiated rats that were not administered the Moringa extract when compared with the control while the improvements noticed in the mean values of the rats pre-treated with Moringa oleifera leaves extract before irradiation was not significant when compared with the control group. There were also decrease in the mean values of the WBC counts of the irradiated rats on day 14 after irradiation but they were not statistically significant (p> 0.05).

RBC: No significant changes (p> 0.05) were observed in the RBC count on day 2 after irradiation. The results from this study showed, that on days 7 and 14 after irradiation, a significant decrease in the RBC count was noted in the rats exposed to the radiation source without Moringa extract pre-treatment when compared with the control group. A significant improvement was thus observed in rats pre-treated with Moringa extract before irradiation.

MCV: Across the three timelines, the results of this study showed no significant changes in the mean corpuscular volume.

HGB: The results of this study showed no significant changes (p> 0.05) in the haemoglobin level on days 2 and 7 after irradiation. However, on day 7 after irradiation, a significant decrease was noted in irradiated rats that were not pre-treated with Moringa oleifera extract. Significant improvement was seen in the rats pre-treated with Moringa oleifera leaves extract before irradiation.

LYM: On days 2 and 14 after irradiation, a significant reduction was observed in the lymphocyte count of the irradiated rats without Moringa when compared with the control. However, significant improvements were noted in the rats pre-treated with the Moringa extract before irradiation. The result also showed no significant changes in the lymphocyte count on day 7 after irradiation.

PLT: Across the three timelines of this study no significant changes were noted in the platelet counts of the irradiated rats when compared with the control group. However, a significant reduction was observed in the irradiated rats without Moringa extract compared with un-irradiated rats treated with aqueous extract of Moringa oleifera leaves.
Discussion

The complete blood cell count plays a very vital role in the provision of relevant information on assessing the health status of an individual [6]. Any abnormal increase or decrease in any of the parameters can be classified as pathologic state which may give different kinds of symptom. The useful application of radiation has been implicated as cytotoxic agents on the haematopoietic precursors which may in turn be detrimental to the exposed individual. Thus, agents capable of ameliorating this detrimental effect are inevitable.

The white blood cells are critical for immune responses, production of haemopoietic growthfactors and have been described as the most sensitive type of the blood cells [7, 8]. Results from this study showed a decrease in the white blood cells and the lymphocytes and are referred to as leukopenia and lymphocytopenia respectively. This may have resulted from the radiation damage to the spleen or the liver. However, improvements were noted in the results of rats that were pre-administered with Moringa oleifera leaf extract before the radiation exposure. This shows a protective effect of Moringa oleifera leaf extract on the white blood cell count following exposure to low dose of gamma radiation.

During the three timelines under study, the results indicated a decrease in the red blood cell count in the animals irradiated with the decrease on the second day of sacrifice showing significant reduction when compared with non-irradiated animals. This reduction may have resulted from the damage to the kidney following radiation exposure resulting in a reduced production of erythropoietin [9, 10]. The results also indicated significant reduction in the haemoglobin level of the experimental rats exposed to 2.5 Gy of radiation when compared with the control rats. This may perhaps be due to impaired biosynthesis of haeme in the bone marrow which may result into reduction in the oxygen supply to different tissues and thus, low energy production [11]. In this study, pre-treated rats with Moringa oleifera leaf aqueous extract showed significantly increased haemoglobin concentration when compared with irradiated rats not given the extract which thus indicates that Moringa oleifera leaf have protective effects on the haemoglobin concentration following radiation exposure.

Although, bleeding was not seen or noticed in the rats during the experimental period, the blood cells responsible for clotting (platelets) were noted to be have decreased following exposure to the 2.5 Gy of gamma radiation when compared with other groups of rats in this study. This is consistent with previous findings by [12] that reported an initial decrease in the platelet count followed by increase after exposure to 5 Gy of radiation from a gamma rays source. They attributed this decrease to the thrombocytopenia and decrease in the number of megakaryocytes in the bone marrow.

Conclusion

In summary, the haematological parameters results as presented in this study showed that low dose of 2.5 Gy of gamma radiation can cause a decrease in the complete blood count and thus, detrimental to the health of exposed subjects. This is in agreement with previous findings [13-17] that this decrease may have resulted due to the high radiosensitivity of haematopoietic tissue and a reduction in the viability of
spleen hematopoietic stem cells. However, the present findings show that aqueous extract of Moringa oleifera leaves is potent as an antioxidant in helping to reduce the effects of the oxidative stress generated through the exposure to gamma radiation. Thus, adequate food supplements of the Moringa oleifera leaves into daily diet should be encouraged especially for patients undergoing radiotherapy, radiodiagnosis or radiation health workers. This extract can be practiced by individuals by rinsing the leaves inside clean/boiled water domestically.

References


