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Evaluation of Radioprotective Efficacy of *Drymaria Cordata* Extract on Whole-Body Radiation-Induced Hematological Damage in Mice

Idowu Richard Akomolafe[1], Naven Chetty[1*]

1. School of Chemistry and Physics, Discipline of Physics, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3209, Pietermaritzburg, South Africa

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ABSTRACT

Introduction: Ionising radiation in diagnostic and therapeutic radiology is steadily increasing, with clear significant benefits. However, the issues of unwanted radiation exposure to patients and medical workers, which has a hugely deleterious effect, remain a challenge that requires urgent attention. Thus, this study aimed to evaluate the possible radioprotective potential of *Drymaria cordata* (DC) extract on mice's hematological parameters following exposure to X-ray radiation and investigate its ability to increase the survival rate.

Material and Methods: **S**ixty female mice weighing 38-45g, 10-12 weeks old, were used for this study. The mice were divided into six different groups containing ten mice, sub-divided into irradiated and un-irradiated groups. The animals received 250mg/kg extract of DC by oral gavage for thirteen days in addition to feeding and water *ad libitum*. Mice were irradiated at the Radiotherapy and Oncology Department of Grey's Hospital using a linear accelerator. Blood samples were collected at different time intervals for the hematology test with post-irradiation monitoring for 30 days.

Results: Exposure of mice to 4Gy and 8Gy of X-ray radiation produced significant changes in the mice's erythrocytes, hematocrit, leukocytes and platelets in a dose and time-dependent manner compared with the control (CNT) group. The present study revealed a progressive decrease in all the hematological parameters until 30 days among the irradiated groups. However, animals treated with DC extract before irradiation and animals who received extract only exhibited a significant time-dependent increase in the studied hematological parameters compared to the animals in the CNT group. Furthermore, the pre-treatment of mice with the DC delayed the onset of mortality, thereby increasing the mice's survival rate compared with the irradiated control.

Conclusion: Our findings showed that DC is a potent natural radioprotective agent through its ability to reduce radiation-induced damage in mice's hematopoietic system and increase the survival rate.

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Introduction

In 1895, Wilhelm Conrad Roentgen discovered X-ray radiation that significantly improved human health. It has become a vital tool in the diagnostic and therapeutic process of primary malignant diseases [1]. Notwithstanding the benefit and significant advantages to the medical world, harmful and deleterious effects remain, which cannot be overlooked [2, 3]. The interaction of ionising radiation with cells produces reactive oxygen species and free radicals dangerous to the body. The free radicals cause damage to deoxyribonucleic acid (DNA) through the breakage of both single and double strands and the loss of bases that result in chromosomal aberrations [4]. The search for chemical agents capable of offering protection against ionising radiation began with the report of Patt et al. [5] when they identified the radioprotective potentials of cysteine on rats and mice against the radiation-induced symptoms and death. Their study paved the way for research on radiation protection in human populations. Since then, several studies have been conducted on different compounds to ascertain their radioprotective abilities. The major setback of these synthetic compounds is their toxicity level at the optimum protective dose [2, 6].

Radiotherapy has become an excellent modality in cancerous' cells treatment, with an estimated half of cancer patients benefiting from it [7, 8]. However, radiation in cancerous cells therapy comes with a few challenges, such as exposure to healthy surrounding cells. The existing synthetic radioprotectors have done little in alleviating these challenges due to certain limiting factors [9]. Therefore, researchers' attention has shifted from synthetic compounds to plants,

*Corresponding Author: Tel: +27-332605660; Email: chettyn3@ukzn.ac.za

herbs, and natural products in the last few decades as an alternative to synthetic compounds for radioprotection, reducing radiation side effects [10]. The aim has been to replace the toxic synthetic compounds and make radioprotector drugs affordable, accessible and economically viable to patients and radiation workers worldwide. Therefore, different plants have undergone scientific screening to ascertain their radioprotective efficacy and to deduce their toxicity level. A few examples of plant extracts that have been found to offer protective measures against the radiation-induced damage in mammals include; *Syzygium Cumini,* Mentha arvensis, *Aegle marmelos*, *Amaranthus paniculatus,* Liv-52, *Nardostachys jatamansi,* Green Tea and Grape Seed, *Zingiber officinale,* [9-16]. In light of those mentioned above confirmed radioprotective plants, it is time to turn our attention inwards to other therapeutic plants that can shield us from radiation.

Drymaria cordata (DC) (Linn.) Willd belongs to the family of Caryophyllaceae plants that spread out in various directions. It is a procumbent plant with slender stems, broad and face-to-face leaves. Its leaves and flowers are usually small with tubercle and membranous seeds. It is extensively dispersed in West and Central Africa, Asia and America [17]. A significant criterion for selecting plants and natural products for their pharmacological benefits has been reported in orthodox medicine over a few years. People of various tribes and nationalities have used DC in folklore (traditional) medicine for different purposes. In Nigeria, it is commonly known as “awede-werisa” in Yoruba and Calabar woman's eye in Igbo. It is reported to be used in folklore medicine to treat various diseases such as; convulsions, febrile seizures and sleeping disorders in children. Most studies aimed to determine its potential to cure different ailments, such as treating the respiratory disease in the Democratic Republic of the Congo (Zaire), Rwanda and Tanzania, blurred vision in Tanzania, and cerebral stimulants in Madagascar [18].

Studies have been conducted to verify some of these claims scientifically. For instance, the research undertaken by Mukherjee et al. [19] revealed the antitussive activity of the methanol extract of the plant on a cough model induced by sulfur dioxide gas in mice; their analysis revealed better inhibition of cough after the usage of the plant extract. In the study conducted by Adeyemi et al. [18], they showed that aqueous extract of DC possessed significant anti-inflammatory activity by suppressing either one or a combination of mediators like kinins, prostaglandins, serotonin and histamine. The plant has also been revealed to have anti-inflammatory and antioxidant properties, making it suitable for scavenging free radicals produced indirectly by the action of ionising radiation [20].

Despite the aforementioned medicinal properties of DC and several claims made by folklore medicine practitioners on its capacity to cure certain diseases and ailments, little or no information is documented in literature about its ability to repair radiation-induced damage to hematopoietic cells. Moreover, to the best of our knowledge, no study has been reported on the radioprotective property of the DC plant. Thus, based on the medicinal properties of DC, the present study has been undertaken to determine the possible radioprotective efficacy of DC extract on irradiated mice, emphasising its ability to increase survival and improve hematological parameters.

Materials and Methods

Collection, identification and preparation of Plant Extract

In July, the collection of fresh samples of the DC plant was done from local farmland at the University of Ibadan, Ibadan, and South-west, Nigeria. The identification and authentication of the plant were made by Mr Esinekhuai Donatus- a botanist at the Herbarium, Department of Botany, and University of Ibadan, Nigeria, where voucher specimen number UIH-22933 was documented. The collected samples were washed under running water and dried in an open space for a few days at room temperature. The dried plant samples were pulverised with an electric grinder at the Biomedical Research Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal (UKZN), Pietermaritzburg campus. The pulverisation was provided to give sufficient surface area for maceration. The powdered material with a mass of 433g was macerated in 2.5 litres of absolute ethanol for 72 hours at room temperature. In order to ensure thorough mixing, the macerated solution was shaken intermittently. The ethanolic extract was filtered using Whatman No. 1 filter paper under vacuum filtration. To eliminate all traces of ethanol, the filtrate was evaporated using a rotary evaporator. A 3.5% yield of ethanolic extract was collected, deposited in an airtight container, and kept in a refrigerator at 4°C until needed.

Animal care and handling

For this study, sixty female BALB/c mice weighing 38-45g and 10-12 weeks were used. The animals were raised at the School of Life Sciences' Animal House, University of KwaZulu-Natal Pietermaritzburg campus. The Animal Research Ethics Committee (AREC) of the University of KwaZulu-Natal (UKZN) approved the protocol used for the present research with a protocol reference number AREC/026/019D. Animals received standard food and had access to clean water *ad libitum* throughout the experiment [21]. The experimental animals were treated with care and housed in clean, well-ventilated transparent plastic-type IV cages with wood shavings in a naturally lit animal room. The room temperature (23°C - 25°C) was controlled with 12-hour light and dark cycles. Egg boxes and shredded paper were provided to create behavioural enrichment in the mouse cages. All of our procedures followed the National Institute of Health's recommendations for the treatment of laboratory animals in biomedical research [22].

Table 1. Treatment of animals for *Drymaria cordata* extract

Group code	Treatment
CNT	Control (Sham irradiated and no extract)
DC	Mice treated with 250mg/kg bodyweight only (Sham irradiated)
IR_4Gy	Irradiated (4Gy) mice only
IR_8Gy	Irradiated (8Gy) mice only
DC_4Gy	Irradiated (4Gy) mice treated with 250mg/kg body weight of DCE
DC_8Gy	Irradiated (8Gy) mice treated with 250mg/kg body weight of DCE

DCE: *Drymaria cordata* extract; IR: Ionising radiation; CNT: Control

Experimental design

The sixty female BALB/c mice were randomly distributed into six different treatment groups containing ten mice (Table 1). Animals in the DC, DC_4Gy and DC_8Gy received 250mg/kg body weight extract of DC by oral gavage for thirteen days before radiation exposure. The body mass of animals in each treatment group was recorded before treatment as initial mass. Also, animals were weighed every two days during the treatment periods. The final mass was obtained five days post-irradiation, and the mean value of the mass pre and after treatment was calculated. The effect of DC extract and radiation on the animals' body mass was determined by estimating the mass gain or mass loss.

Irradiation procedure

An hour after the last administration of the DC extract, the mice were taken to the Radiotherapy and Oncology Department, Grey's Hospital Pietermaritzburg, South Africa, for irradiation. The radiation type used was X-ray radiation. The radiation source is a Varian Linear Accelerator (LINAC) (model: Clinac 2100C; Varian Medical Systems, California, United States). The LINAC uses electricity to produce X-rays and beams of electrons usually collimated to treat cancer patients [21]. Ten mice were packed inside a specially constructed transparent plastic cage under a controlled condition, and their movement was restricted during the irradiation process. A total of 40 animals received whole-body X-ray radiation generated from 6MV photons LINAC. The radiation doses of 400cGy and 800cGy were delivered at a dosage rate of 400MU/min under the standard condition of 100 monitor units (M.U.) = 1Gy.

The irradiation was done with a source 85 cm away from the surface and 15 cm deep. The irradiation procedure worked best with a field size of 30 cm by 25 cm. The mice were returned to their cages and returned to the animal house immediately after being irradiated. They were observed daily for signs of radiation-induced sickness and mortality. The rationale for using radiation doses is to evaluate the hematopoietic syndrome (bone marrow syndrome) in the irradiated mice, which usually occur with whole-body irradiation of the dose range between 0.7 and 8Gy [23]. This radiation dose range can produce radiation-induced hematological alterations in humans or animals. This could lead to a decrease in all blood cell counts, survival decreases with increasing dose, and death of stem cells in bone marrow can occur [24]. The reports of Jagetia et al. [11], Krishna and Kumar [12], El-Desouky et al. [15], and Yamamori et al. [25] suggest other studies where similar radiation dose ranges have been used.

Determination of hematological parameters

Three mice in each group (n=3) were sacrificed by cervical dislocation five and fifteen days after irradiation, and blood was collected from the posterior vena cava of the heart using a 23-gauge needle and a 1 ml syringe into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles containing anticoagulant for hematological analysis. Similarly, thirty days after irradiation, the surviving mice in each group were sacrificed, as mentioned above [26]. The hematological parameters analysed include Erythrocyte (red blood cell), Hematocrit (HCT), Leukocyte and Platelet (PLT). The hematocrit was analysed using the microhematocrit method. Simultaneously, the red blood cell detector counts the Erythrocytes, PLTs and Leukocytes via the Hydro-Dynamic Focusing method (direct current detection method) using the Sysmex XE-2100 Automated Hematology Analyser machine (Sysmex America, Inc.). The Hydro-Dynamic Focusing method enhances the accuracy and consistency of blood counts. In addition, because the blood cells travel through the aperture in a straight line, aberrant blood cell pulses are avoided.

Statistical Analysis

The hematological parameters were examined using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, which compared all treatment groups to the control group. The analysis was carried out using the SPSS 20.0® statistical package. The results are shown as means ± SEM (standard error of the mean), and $p<0.05$ was considered a significant value.

Results

Survival Analysis

Table 2 shows the percentage survival analysis of the experimental animals. The first death was recorded in the group (IR_8Gy) on the 8th-day post-irradiation. More death was registered on subsequent days, and by the 25th-day post-irradiation, all the animals in group (IR_8Gy) had died (Table 2).

Table 2. Percentage survival of experimental mice

Group Code	Survival (%)															
Post irradiation Days	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	30
CNT	100	100	70	70	70	70	70	40	40	40	40	40	40	40	40	40
DC	100	100	70	70	70	70	70	40	40	40	40	40	40	40	40	40
IR_4Gy	100	100	70	70	70	70	70	40	40	40	40	30	30	30	30	30
IR_8Gy	100	100	70	70	60	60	60	30	30	30	20	20	0	0	0	0
DC_4Gy	100	100	70	70	70	70	70	40	40	40	40	40	40	40	40	40
DC_8Gy	100	100	70	70	70	70	60	30	30	30	30	20	20	20	20	20

In the pre-treated group (DC_8Gy), there was a delay in mortality in the animals due to treatment with DC. The early death in this group occurred on the 13th-day post-irradiation. It was five days after the early mortality occurred in the IR_8Gy group. Only two mice survived in this group until 30th-days post-irradiation. There was a long delay in mortality for the animals exposed to 4Gy without pre-treatment (IR_4Gy). The first mortality in this group occurred by day 22 post-irradiation, whereas there was no mortality recorded in the pre-treated group (DC-4Gy). All the remaining mice in the groups CNT & DC, after the initial sacrifice on days five & fifteen for haematology analysis, survived until 30-day. The percentage survival of 40% (for group code CNT, DC & DC_4Gy) corresponds to $n = 4$ mice, 30% (for IR_4Gy) corresponds to $n = 3$ mice, 20% (for DC-8Gy) corresponds to $n = 2$ mouse and 0% (for IR_8Gy) corresponds to no mouse survived. The survival analysis result is similar to Adaramoye et al. [26]. They reported the Xylopia aethiopica dried fruit extract on eight weeks of survival of Wistar albino rats after exposure to 5Gy of gamma radiation.

Hematological parameters

Tables 3, 4 and 5 summarise the present investigation results regarding the possible radioprotective efficacy of DC extract in mitigating radiation-induced hematological damage in mice. The exposure of experimental mice to 4Gy and 8Gy of X-ray radiation resulted in a significant decrease in the hematological parameters such as erythrocyte, leukocyte and platelets, except hematocrit, which was not significant ($p>0.05$), in groups IR_4Gy and IR_8Gy compared to that in control (CNT) and extracted only (DC) groups. The decrease in the hematological parameters was radiation dose-dependent. At a higher dose, the reduction in the hematological parameters was more pronounced. The irradiation groups showed a progressive decline in all hematological markers until 30 days in the current investigation. When compared to the CNT, the animals treated with DC extract showed a considerable rise in the examined hematological parameters. Similar improvement was discovered in the mice who received extract only compared with the control group. It increased towards the control level in Group DC_4Gy and DC_8Gy during the 30-day monitoring period. The increase in the hematological parameters in the treated animals showed the DC extract's ability to protect against whole-body X-ray radiation-induced hematological damage in mice.

Table 3. Effect of ethanol extract of *Drymaria cordata* and X-rays radiation on the Erythrocyte, Hematocrit, Leukocyte and Platelet of female mice at 5th-day post-irradiation

Group code	Erythrocyte $(\times 10^{12}/L)$	Hematocrit (L/L)	Leukocyte $(\times 10^{9}/L)$	Platelet $(\times 10^{9}/L)$
CNT	12.13±0.55	0.57±0.05	2.96±0.35	870.67±102.23
DC	10.41±0.13	0.58±0.01	2.46±0.34	738.00±119.82
IR_4Gy	9.41±0.42[a]	0.58±0.02	0.23±0.01[a]	535.25±6.29[a]
IR_8Gy	9.04±0.57[a]	0.54±0.03	0.12±0.02[a]	336.00±45.47[a]
DC_4Gy	10.61±0.30[b]	0.59±0.02	1.84±0.05[b]	548.75±101.02[b]
DC_8Gy	10.98±0.53[b]	0.58±0.03	1.70±0.03[b]	360.50±49.88[b]

Values are given as means ± standard error of the mean ($n = 3$), CNT, control; DC, *Drymaria cordata* extract at 250mg/kg; Gy, Gray (radiation unit); IR, ionising radiation.
L/L: Litre of cells per litre of blood
a: Significantly different from (CNT & DC) at $p<0.05$
b: Significantly different from (IR_4Gy & IR_8Gy) at $p<0.05$

Table 4. Effect of ethanol extract of *Drymaria cordata* and X-rays radiation on the Erythrocyte, Hematocrit, Leukocyte and Platelet of female mice at 15th-day post-irradiation

Group code	Erythrocyte $(\times 10^{12}/L)$	Hematocrit (L/L)	Leukocyte $(\times 10^{9}/L)$	Platelet $(\times 10^{9}/L)$
CNT	12.14±0.15	0.67±0.05	3.02±0.35	880.07±112.23
DC	12.01±0.31	0.71±0.01	2.98±0.34	748.03±109.82
IR_4Gy	8.01±0.12[a]	0.58±0.02	0.21±0.02[a]	405.25±5.39[a]
IR_8Gy	8.21±0.37[a]	0.46±0.03	0.10±0.01[a]	296.00±4.27[a]
DC_4Gy	11.31±0.30[b]	0.67±0.02	2.71±0.04[b]	608.55±7.32[b]
DC_8Gy	11.08±0.53[b]	0.63±0.03	2.60±0.05[b]	540.70±4.28[b]

Values are given as means ± standard error of the mean ($n = 3$), CNT, control; DC, *Drymaria cordata* extract at 250mg/kg; Gy, Gray (radiation unit); IR, ionising radiation.
L/L: Litre of cells per litre of blood
a: Significantly different from (CNT & DC) at p<0.05
b: Significantly different from (IR_4Gy & IR_8Gy) at p<0.05

Table 5. Effect of ethanol extract of *Drymaria cordata* and X-rays radiation on the Erythrocyte, Hematocrit, Leukocyte and Platelet of female mice at 30th-day post-irradiation

Group code	Erythrocyte $(\times 10^{12}/L)$	Hematocrit (L/L)	Leukocyte $(\times 10^{9}/L)$	Platelet $(\times 10^{9}/L)$
CNT*	12.50±0.55	2.35±0.06	3.33±0.53	908±95.32
DC*	11.16±0.31	3.24±0.25	3.09±0.43	905±55.28
IR_4Gy**	7.11±0.12[a]	0.46±0.26	0.14±0.09[a]	381.50±25.93[a]
IR_8Gy****				
DC_4Gy*	11.83±0.30[b]	2.32±0.84[b]	3.29±0.29[b]	805.40±33.53[b]
DC_8Gy***	10.18±0.23[b]	2.16±0.13[b]	2.45±0.54[b]	793.00±3.52[b]

Values are given as means ± standard error of the mean. For the superscript *: $n = 4$, **: $n = 3$, ***: $n = 2$, ****: no animal survived. CNT, control; DC, *Drymaria cordata* extract at 250mg/kg; Gy, Gray (radiation unit); IR, ionising radiation.
L/L: Litre of cells per litre of blood
a: Significantly different from (CNT & DC) at p<0.05
b: Significantly different from (IR_4Gy) at p<0.05

Table 6. Effect of DC extract and X-ray radiation on body mass on day five

Group code	Initial mass (g)	Final mass (g)	mass gain or loss (g)
CNT	41.17±5.22	43.96±7.57	2.79
DC	33.26±1.51	34.29±3.49	1.03
IR_4Gy	37.29±1.20	36.82±3.23	-0.47
IR_8Gy	37.44±0.79	36.89±1.49	-0.55
DC_4Gy	37.73±4.99	36.30±3.21	-1.43
DC_8Gy	42.68±0.29	37.66±8.74	-5.02

Values are given as means ± standard deviation ($n = 10$), CNT, control; DC, *Drymaria cordata* extract; Gy, Gray (radiation unit); IR, ionising radiation; negative (-) sign indicates a decrease in mass; positive (+) sign indicates an increase in mass

Effect of Drymaria cordata extract and X-ray radiation on the body mass of mice

Radiation is generally known to cause significant changes in a living organism's physiological and anatomical structure if exposure occurs. These changes produce biological effects, noticeable in the irradiated animals depending on dose level and vary with time. Biological effects of ionising radiation such as fatigue, facial oedema, loss of appetite, redness of the eye, alopecia (loss of hair) and weight loss were virtually observed in the experimental animals, excluding those in Group CNT and Group DC (mice treated with extract alone). These signs observed display a radiation dose-dependent relationship. The severity of the radiation symptoms increased with a higher dose. Groups (IR_8Gy & DC_8Gy) showed remarkable changes in physical observations during the first seventh day after exposure. The weight gain or loss calculation revealed that the irradiated animals had lost a significant amount of weight due to accruing damaging effect of radiation (Table 6). There was an increase in the average body mass of mice in group CNT (un-irradiated) from (41.17±5.22)g to (43.96+7.57)g up till the day five of their euthanised. Similarly, a slight increase in the mass gain of Group DC mice (animals treated with extract alone) from (33.26±1.51)g to (34.29+3.49)g as compared with mice in the group CNT. However, animals in the remaining groups experienced a reduction in average body mass.

Discussion

Reports have shown that a radioprotective agent's ability to delay mortality thereby increases the survival rate within the thirty-day interval after exposure to whole-body radiation suggests its capacity to modulate the regeneration and recovery of the hemopoietic progenitor and gastrointestinal epithelial cells [13]. Globally research has shown that animal studies with death as a humane endpoint remain the most reliable way of confirming a drug's radioprotective potential. A 30-day survival after exposure to a lethal dose of whole-body ionising radiation concretely reveals the drug's ability to mitigate radiation effects [9]. Though a lesser procedure, another approach can also be to determine the Gastrointestinal (GI) syndrome in mice by evaluating the survival rate up to ten days after exposure to equal doses of whole-body ionising radiation. This method is quite different from the hematopoietic syndrome, which can only be assessed by the 30-day survival of mice [2].

Several effects have been observed in experimental animal studies and epidemiological data. Total body irradiation of different amounts of radiation can affect the body; some of these effects can manifest in hours, days, and years. For example, exposure to a 1 to 2 Gy dose can cause nausea, diarrhoea, vomiting, early skin epilation, fever, anorexia and headache, some of the symptoms at the prodromal stage. If the radiation dose is between 2 to 8 Gy, it causes changes in the hematopoietic cells' blood counts. The gastrointestinal syndrome occurs when the radiation dose is 8-30Gy, whereas doses greater than 30Gy lead to Central Nervous System (CNS) or Cerebrovascular Syndrome [14, 27].

In the present study, mice's exposure to 4Gy and 8Gy caused radiation-induced mortality among the irradiated animals. The death of mice within ten days after irradiation is considered due to injury to the gastrointestinal epithelium. Similarly, research has shown that death between 11 to 30 days after irradiation is due to hemopoietic injury imposed by ionising radiation [2]. This work observed that mice exposed to radiation exhibited radiation-induced sicknesses such as emaciation, diarrhoea, significant weight loss, eyes watering, redness, water and food intake reduction, and lethargy. These radiation symptoms were more pronounced at a higher dose (8Gy). The treatment of mice with the extract of DC delayed the onset of mortality in the irradiated animals.

Moreover, the pre-treatment of mice with DC extract ameliorated the hematopoietic and gastrointestinal tract damage, as revealed by an increase in the 30-day survival studies. The mortality recorded in all the irradiated groups was primarily dependent on the doses of radiation. Generally, the number of survivors improved in the animals pre-treated with the extract of DC before exposure to 4Gy & 8Gy of X-ray radiation. After whole-body irradiation, the survival level observed in the treated groups could be attributed to the extract's ability to scavenge free radicals and regenerate gastrointestinal epithelium and hemopoietic progenitor cells in the red bone marrow [11]. It has been shown that ionising radiation can induce a dose-dependent decrease in circulating hematopoietic cells via a reduction in bone marrow production and apoptosis of mature devised elements of the blood [28]. The improvement in the survival rate of mice pre-treated with the extract of DC before exposure to different doses of radiation revealed the DC plant's effectiveness in arresting deaths from bone marrow and gastrointestinal damage. The result of survival studies obtained from the present investigation agrees with Jagetia et al. [2], who reported that treatment of mice with abana (a herbal preparation) before exposure to 10Gy of γ-radiation delayed the onset of mortality and decreased the symptoms of radiation sickness. In the same vein, the report of Jagetia et al. [11] on the radioprotective effect of bael leaf (Aegle marmelos) showed that treatment of mice with Aegle marmelos extract before exposure to different doses of γ-radiation reduced the symptoms of radiation-induced sickness and increased survival concurs with the present study. DC plant has been reported to possess antioxidant and anti-inflammatory properties capable of scavenging free radicals produced indirectly by the action of ionising radiation [18].

Blood value changes help detect the degree of an organism's radiation exposure. The hematopoietic system, which comprises bone marrow and lymphatic tissues, has been recognised as the most radiosensitive organ in the body [28]. There have been reports of young and dividing blood cells being the most radiosensitive, mature or non-dividing blood cells being the most radioresistant, and the pattern of white blood cells decreasing in response to irradiation in the following order: lymphocytes, thrombocytes, and neutrophils [28]. Due to the high radiosensitivity nature of bone marrow, it becomes easily susceptible to radiation whenever exposure occurs. Studies have shown that exposure to ionising radiation could result in hematopoietic tissue changes and sometimes death [29].

In this study, 4Gy and 8Gy of X-rays radiation released via the whole body irradiation caused significant changes in the hematological parameters, as evident in a substantial reduction in the erythrocyte, leukocytes and platelet counts, whereas a non-significant decrease in the hematocrit counts was observed in the irradiated groups (Table 3, 4 & 5). Furthermore, the erythrocyte, leukocytes and platelets of group IR_4Gy and group IR_8Gy mice (mice exposed to an X-rays radiation dose of 4Gy and 8Gy, respectively) decreased dose-dependent levels when compared with CNT & DC groups at all the different time intervals post-irradiation. However, the number of erythrocytes, leukocytes, and platelet counts significantly increased in the pre-treated groups. The increase in the hematological parameters in the treated animals demonstrated the DC extract's ability in mitigating radiation-induced hematological alterations. Furthermore, it shows that mice's treatment with DC

extract ameliorated radiation's harmful effect on the hematological parameters.

The finding of Shirazi et al. [30] showed a reduction in the number of leukocytes and platelets in the irradiated groups alone. The corresponding increase in the treatment groups is similar to Shirazi et al. [30], who reported the pre-treatment of rats with melatonin (10mg/kg) before exposure to 2Gy and 8Gy statistically increased the leukocyte and lymphocyte counts at 4-hour post-irradiation. Similarly, Lymphocytes are known to be the most radiosensitive among the leukocytes; even at a low (0.25Gy) dose of radiation, they tend to be radiosensitive, while on the contrary, erythrocytes (red blood cells) are fairly radioresistant even up to 30Gy [30, 31]. The radioresistant nature of hematocrit among the hematological parameters is established in this study, where 4Gy and 8Gy did not cause any significant alterations in the peripheral blood.

In this work, irradiation with both sub-lethal and lethal doses of X-ray significantly reduces leukocyte counts among the irradiated groups, indicating the direct killing of lymphocytes. This supports the findings of Saini et al. [32], who reported that exposure of mice to 8 to 10Gy of gamma radiation significantly reduced leukocyte counts. The reduction in leukocyte levels recorded among the irradiated groups may be attributed to the loss of lymphocytes. The lymphocytes are responsible for fighting infectious and help build the body's immune system. A significant reduction in lymphocyte counts could lead to a lymphocytopenia condition.

Similarly, the present study results agree with Waghmare et al. [33], where LIV.52 offered substantial protection against the depletion of leukocytes and increased the recovery rate towards normal by 28-day post-irradiation in mice. However, there was a reduction in the erythrocyte counts of the irradiated groups when compared with the control group. This decrease may be due to defective hematopoiesis and intravascular red cell destruction [32]. Moreover, studies have shown that the reduction in the various blood components is primarily due to radiation's deleterious effect on the blood-forming organs.

The present study revealed that the treatment of mice with the extract of DC at a dose of 250mg/kg body weight (DC group) resulted in a significant reduction in erythrocyte, hematocrit, leukocyte and platelets when compared with the CNT group. These results agree with Adeyemi et al. [18], who reported that the extract (400 and 800mg/kg) reduced the number of migrated leukocytes in the carrageenan-induced pleurisy test. The present study results are also in line with the finding of Eshak and Osama [34], who revealed a significant decrease in the white blood cell, red blood cell, packed cell volume, hemoglobin, and platelet of animals exposed to 4Gy and 6Gy of gamma radiation. Furthermore, AL-Dulamey et al. [35] reported that rats treated with black seed oil exhibited a radioprotective effect by significantly increasing the hematological parameters followed by exposure to 6mGy/h gamma rays for 25 days. AL-Dulamey et al. [35] report revealed the radioprotective effect of black seed oil on hematological parameters after exposure to gamma-ray radiation. The hematological parameters obtained in their study are similar to what is received in the present work. In the same vein, the report of Al-Jawwady et al. [36] demonstrated the effects of gamma-ray radiation on the physiological cases of adult rats.

Moreover, the present investigation corroborates with Gowda et al. [14], where electron beam irradiation caused a significant reduction in the erythrocytes, leukocytes, hemoglobin, packed cell volume and platelet count at 48-hour after irradiation in male rats. The study explained the protective activity of *Nardostchys Jatamansi* by its capacity to modulate the radiation-induced damage to the hematopoietic system [14]. The work of Akomolafe and Chetty [21] on the radioprotective potential of *Costus afer* on hematological parameters concurred with the findings of the present study; they showed that *Costus afer* provided protection on hematological parameters (erythrocyte, leukocyte, hematocrit, lymphocytes, neutrophils, eosinophils and platelet counts) against X-ray radiation. Abdelmageed Marzook et al. [37] gave a similar report on the radioprotective efficacy of *Costus afer* on hematological parameters against gamma radiation; their research agreed with the findings of the present investigation. The precise mechanism of action of the DC is unknown; however, Mukherjee et al. [19] showed the plant exhibited significant antitussive property in experimental mice. Besides, the report of Akindele et al. [17] and Nono et al. [38] revealed that the plant contains analgesic, antipyretic, anxiolytic, and anti-inflammatory properties, which are fundamental components in scavenging free radicals.

Conclusion

The study has demonstrated that DC extract offered protection against radiation-induced hematological damage in mice and reduced mortality, thereby increasing the survival rate. The results of this study support the current usefulness of the plant in treating diverse kinds of ailments. Its ability to scavenge free radicals and reactive oxygen species may explain the elevated levels of hematological markers seen in the treatment groups, thereby protecting mice against ionising radiation as the report from other studies suggested the anti-inflammatory, antioxidant and antitussive properties of the plant. Thus, our findings show that the DC plant is a new natural radioprotector, which may be useful in mitigating radiation-induced hematological disorders before radiation treatment and generally by radiation workers. However, further studies are necessary to determine the plant's active component, its mechanism responsible for the radioprotective effect observed, and its practical applicability in a preclinical trial during radiotherapy of cancer patients.

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