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INYUVESI YAKWAZULU-NATALI

AN INVESTIGATION INTO THE RADIOPROTECTIVE POTENTIAL OF *COSTUS AFER* AND *DRYMARIA CORDATA* EXTRACTS ON WHOLE-BODY IRRADIATED MICE

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2021

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By

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Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the School of Chemistry and Physics, Discipline of Physics
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Pietermaritzburg Campus
South Africa

2021

ABSTRACT

The need for effective and non-toxic radioprotectors has shifted researchers' attention to plants and natural products as an alternative to synthetic compounds. This study investigated the radioprotective potential of *Costus afer* (CAE*)* and *Drymaria cordata* (DC) extracts on mice's survival, haematological and histopathological parameters following X-ray irradiation.

One hundred and fourteen (54 male & 60 female) mice with total body masses between 38-45g and aged between 10-12 weeks old were used for this study. The mice were divided into twelve groups containing six and ten mice, respectively, for experiments CAE and DC. Animals were further sub-divided into irradiated and un-irradiated groups. The animals in both experiments received 250mg/kg extract of CAE and DC by oral gavage for six days and thirteen days, respectively, in addition to feeding and water *ad libitum*. Exposure of mice to radiation was done at the Radiotherapy and Oncology Department, Grey's Hospital using a linear accelerator. Blood samples were collected at different time intervals for the haematology test. Harvesting of kidney and liver for histopathology examination also occurred. Post-irradiation monitoring then continued for 30 days. Data were analysed by a one-way ANOVA test, followed by Tukey's multiple comparison test.

Our findings revealed that the mice irradiated with 3Gy, 4Gy, 6Gy and 8Gy doses of X-ray radiation experienced a significant reduction in their White Blood Cell, Packed Cell Volume, Haemoglobin, Neutrophils, Lymphocytes, Eosinophils, and Platelet counts when compared with the control group in both experiments. In both experiments, CAE and DC extract offered protection against the radiation-induced haematological alterations by elevating all the blood parameters, except red blood cells and monocyte in the CAE treatment groups. In addition, the pre-treatment of mice with DC delayed the onset of mortality, thereby increasing the mice's survival rate. Histopathological changes in the CAE treatment groups' kidney and liver sections revealed no visible lesion in the pre-treated mice. Hepatocytes seem to be within normal histological limits.

Although it is evident that the CAE and DC extracts protect against radiation-induced haematological damage and increases survival rate, no significant improvement in the histopathological parameters was recorded. Thus, further research is needed to prove the CAE and DC radioprotective potential on histopathological variables.

PREFACE

Idowu Richard Akomolafe undertook the work described in this thesis under the supervision of Professor Naven Chetty in the School of Chemistry and Physics, Discipline of Physics, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg campus, South Africa.

The contents of the thesis, unless otherwise indicated, are my original work and have not been submitted in part, or whole, to any other University for degree purposes. Where use has been made of the work of others, it is duly referenced in the text or bibliography sections.

Signature (Student): [redacted] …… Date: ……29/09/2021……..

Idowu Richard Akomolafe

Signature (Supervisor): … [redacted] …… Date: 04 October 2021….

Professor Naven Chetty

DECLARATION: PLAGIARISM

I, IDOWU RICHARD AKOMOLAFE (Student No. 218076189), declare that:

I. The work reported in this thesis, except where otherwise indicated, is my original research.

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DECLARATION 2-PUBLICATIONS

I, Idowu Richard Akomolafe, declare the contribution details of publications that form part and include research presented in this thesis. The first author (student) carried out the experimental work, data collection, and manuscript preparation under the second author's supervision and guidance (supervisor).

1. Akomolafe IR, Chetty N. Radioprotective potential of *Costus afer* against the radiation-induced haematological and histopathological damage in mice. *Radiat Oncol J* 2021; 39(1):61-71
2. Akomolafe IR, Chetty N. Evaluation of radioprotective efficacy of *Drymaria cordata* extract on whole-body radiation-induced haematological damage in mice. *Iranian Journal of Medical Physics,* in press, doi: 10.22038/ijmp.2021.56512.1946

Signed: [redacted] Idowu Richard Akomolafe

CONFERENCE CONTRIBUTIONS

I, Idowu Richard Akomolafe, declare that the abstract part of this thesis was submitted to the following conferences, but due to the COVID-19 pandemic, the conferences were not held.

1. Akomolafe IR, Chetty N. Radio-protective efficacy of *Costus afer* against the Radiation-Induced Haematological and Histopathological Damage in Mice. The 22nd International Conference on Radiation Biology and Biophysics to be held in Paris, France, during Nov 19-20, 2020.
2. Akomolafe IR, Chetty N. Radio-protective efficacy of *Costus afer* against the Radiation-Induced Haematological and Histopathological Damage in Mice. The 22nd International Conference on Applied Biophysics, Medical Physics and Healthcare Technologies to be held in Toronto, Canada, during Sep 21-22, 2020.

Signed:…… Idowu Richard Akomolafe

DEDICATION

This work is dedicated to my darling wife, Ibukunoluwa Akomolafe, and my precious daughter, Oluwanifemi Akomolafe, for their love, support and perseverance.

ACKNOWLEDGEMENTS

Let me start by giving all glory to God Almighty for the success of this work. This dream would not have been possible without Him. God gives grace to the humble, and He increases their strength to him who has no strength. I am eternally grateful to God for seeing me through this program.

Special thanks go to my amiable supervisor, Prof. Naven Chetty, for his support and guidance during the research. I am indebted to him for believing in my capacity to complete the study despite the challenges encountered at the onset. I want to use this opportunity to appreciate your kind-heartedness and open mind towards seeing your students succeed in their endeavours. I wish you more success in life.

The dream of becoming a PhD holder would not have been possible without the amazing legacy given to me by my parents, Late Mr Lasisi & Mrs Rachel Akomolafe. I owe whatever success I achieve in life to their prayers and support. I must not fail to acknowledge the financial contribution of my uncle-Mr David A. Ogunyebi, who has stood as a Father to me all through my academic journey. I am grateful to you for all God has enabled you to do in my life. I pray for long life, good health and a sound mind for him in Jesus name.

I acknowledge the sacrifice my darling wife, Mrs Ibukunoluwa Akomolafe, made at the onset of this academic pursuit. It would have been challenging to embark on this journey as a young married couple without her backing and support. You occupy a special place in my heart. I appreciate your understanding and commitment to making sure this research is complete in record time. In addition, my gratitude goes to my precious daughter, Oluwanifemi Esther Akomolafe. Thanks for understanding with me whenever I was busy with this work.

My sincere appreciation goes to Daddy and Mummy Kayode Olutimehin for their prayers and support during this research. I am blessed to be one of your sons. I pray that God bless and

keep you in Jesus name. May you live long to enjoy the fruit of your labour in Jesus name. Thank you so much.

I thank my brother Adejuwon Adebayo and his wife for their support and prayers. All my sisters and cousins are well appreciated.

I appreciate Pastor & Mrs S.A Akanji for their prayers and support for my family and me. When I shared the dream with you, you believed God that it would be possible for me, and today it has become a reality. I thank you, sir & ma. I pray that God anoints you more for the work of the ministry and grant you success in your entire endeavour in Jesus name.

A special appreciation goes to Pastor B. Raji for his prayers and guidance during the research.

My sincere appreciation goes to the management and staff of the Department of Radiotherapy and Oncology, Grey's Hospital KwaZulu-Natal, South Africa, for providing the irradiation facility. Special thanks to Mr Mdletshe Nipho for helping in the radiation dosimetry and technical support. My unreserved gratitude goes to Mr Ebrahim Ally of the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg campus, for assisting in the oral gavage and collection of blood samples. The support of the VDX diagnostic service, notably Dr James Hill and Dr Vorster, is well appreciated.

I appreciate all my friends and colleagues in the School of Chemistry & Physics, UKZN, PMB campus, especially the biomedical research group, which assisted me during the program. Special thanks to Dr ‘Bamise Adeleye for his assistance and support during this research. I want to thank notable colleagues- Adedeji Michael, Abdulaziz Yakubu, Edward Chikwelu, Abiola Ilori, Jude Ike and others not listed, for their support and encouragement.

Thanks to you guys, I acknowledge my fellow NRF-TWAS scholars, Ojo Sesan, Raji Abidemi and Ojo Theresa.

This acknowledgement would not be complete without mentioning my pastor's prayers, teaching, and encouragement, Pastor David Olorunda. I appreciate all the Dunamis Faith

Assembly (DFA), RCCG Pietermaritzburg for their prayers and love. My unreserved gratitude goes to Deacon Ayo Adedoyin and his wife for lifting us to church whenever we needed their help. In the same vein, I appreciate Dr & Dr (Mrs) Olarenwaju for their kindness.

This research would not have been possible without the financial support I received from the National Research Foundation (NRF-TWAS).

Funding disclosure

This work is based on the research supported wholly by the National Research Foundation (NRF) of South Africa (Grant Number: 116089). The author acknowledges that opinions, findings and conclusions or recommendations expressed in this publication generated by the NRF-supported research are those of the author and that the NRF accepts no liability whatsoever in this regard.

LIST OF ABBREVIATION AND SYMBOLS

ALARA	As Low As Reasonably Achievable
ALP	Alkaline phosphatase
ALT	Alanine Aminotransferases
ANOVA	Analysis of variance
AREC	Animal Research Ethics Committee
ARS	Acute radiation syndrome
AST	Aspartate Aminotransferases
CAE	*Costus afer* extract
CNS	Central Nervous System
CNT	Control
DC	*Drymaria cordata*
DNA	Deoxyribonucleic acid
DRF	Dose reduction factor
EBRT	External beam radiotherapy
EDTA	Ethylene Diamine Tetra Acetic Acid
FDA	Food and Drug Administration
GI	Gastrointestinal
GPT	Glutathione pyruvate transaminase
GSH	Glutathione
Gy	Gray
H&E	Haematoxylin and Eosin
Hb	Haemoglobin
HCT	Haematocrit
IAEA	International Atomic Energy Agency

IARC	International Agency for Research on Cancer
ICRP	International Commission on Radiological Protection
IJMP	Iranian Journal of Medical Physics
ILO	International Labour Organization
IR	Ionising radiation
JAEA	Japan Atomic Energy Agency
KV	Kolaviron
LD	Lethal dose
LET	Linear energy transfer
LINAC	Linear Accelerator
LPO	Lipid peroxidation
MDA	Malondialdehyde
MU	Monitor units
n	Neutron
NCRP	National Council on Radiation Protection and measurements
NIH	National Institute of Health
ORJ	Radiation Oncology Journal
PLT	Platelet
PVC	Packed cell volume
RBC	Red blood cell
RF	Radiofrequency
RILD	Radiation-induced liver disease
ROS	Reactive oxygen species
RT	Radiotherapy
SEM	Standard error of the mean

SH	Sulfhydryl
SOD	Superoxide dismutase
Sv	Sievert
TBI	Total body irradiation
UKZN	University of KwaZulu-Natal
WBC	White blood cell
WBI	Whole-body irradiation
α	Alpha particle
β	Beta particle
γ	Gamma ray

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CHAPTER ONE

INTRODUCTION

Ionising radiation has been known to be of great benefit to humankind since its discovery. Over the last few decades, the generalised usage of ionising radiation in industries, military, science, healthcare, agriculture, environment, and aviation sectors has increased, leading to excessive exposure to radiation and an increase in the associated cancer risk [1]. Depending on the radiation doses, specific ionising radiation exposure levels can cause irreparable damage to the central nervous systems, gastrointestinal and hematopoietic cells [2].

The interaction of radiation with water can cause excitation and ionisation, leading to water hydrolysis, producing Reactive Oxygen Species (ROS) such as free radicals and hydroxyl ions. These reactive oxygen species are very active due to unpaired electrons. They quickly attack the Deoxyribonucleic acid (DNA) of a cell and cause molecular damage [2, 3]. In a living organism, DNA is a molecule, which carries genetic information. It consists of double strands that wind around each other to form a helix shape through hydrogen bonds between the bases. The molecular damage caused by radiation may be in the form of bond breakage, base loss, single and double-strand breakage [4]. Cells have a repair mechanism, which helps correct the anomaly consequential of a single-strand break. However, if both strands break and are well separated from each other, it can result in a cleavage of chromatin, which in turn can cause cell killing, carcinogenesis and mutation [4]. Thus, double-strand breaks have been considered to be the most harmful radiation-induced DNA damage. Cell mutations are changes that occur to the DNA of cells when the genetic information has been altered.

Cancer is the world's leading cause of death and was accountable for an estimated 10 million deaths in 2020 [5]. About 70% of deaths associated with cancer occur in low and middle-income countries [5]. According to the International Agency for Research on Cancer (IARC),

an estimated 19.3 million new cancer cases (18.1 million precluding non-melanoma skin cancer) and nearly 10.0 million cancer deaths are reported in 2020 [6]. It has been estimated that the global cancer burden is expected to be 28.4 million cases in 2040, about a 47% increase from 2020 [6]. The yearly rise in cancer cases have been attributed to behavioural and dietary risks: low vegetable and fruit intake, high body mass index, lack of physical activity, and alcohol and tobacco use [7]. Moreover, an ageing population and exposure to chemicals, metals and infectious agents are additional factors leading to the high cancer incidence rate [5, 7].

For proper and successful care, a correct cancer diagnosis is necessary, given that every type of cancer needs a particular treatment plan that involves one or more modalities, such as surgery, radiotherapy, chemotherapy, or any combination of therapy. With the increasing number of cancer cases, the need to expand access to radiotherapy treatment for curative or palliative purposes becomes imperative. Reports have shown that nearly 80% of cancer patients undergo radiotherapy during the course of therapy, either as a curative or as a palliative purpose [8]. Radiotherapy is the medical application of ionising radiation to treat cancerous cells, while cytostatic medications are used in chemotherapy [8, 9].

Despite the advances in the treatment planning of radiotherapy on cancer cells, one significant challenge has limited its progress, the radiation toxicity to normal cells [10]. Most cancer cells (tumours) are susceptible to radiation, and they weaken, shrink, and lose the capacity to multiply when irradiated. Therefore, it becomes imperative to strike a balance between protecting normal tissues and eliminating cancer cells. Thus, radiation therapy is expected to achieve minimum damage to normal cells while maximising tumour cell killing [8]. However, this desire can only be fulfilled using drugs to reduce radiation's harmful effects. As reported in the literature, there are several methods to reduce radiation-induced damage to cells during treatment [11]. One such method is reducing the radiation intensity incident on the patient or sensitive organs of the body by using shields [11]. Another way is by using drugs capable of

improving immunity and aiding cell recovery without adverse effects [11]. The substance that can perform this function is called radioprotector [8].

1.2 Radioprotector

Radioprotectors protect cells or tissues against the harmful effect of ionising radiation [8]. They are prophylactic agents given before radiation exposure to lessen the level of cellular or molecular damage. A good radioprotector gives normal tissues a high level of protection, with little to no tumour cell protection and, most importantly, it needs to be non-toxic to normal cells [8]. The action mode is scavenging free radicals and reparation of free radical damage (Figure 1.1) [12]. A good radioprotector's characteristics are that they are easy to access, economically affordable, non-toxic, provide DNA and cell repair assistance, have the free radical capacity to scavenge, have good dose reduction factor, and should also have a reasonably long shelf life for such a product [2]. Intramuscular or oral may be the preferred mode of administration, and an appropriate time window and good stability profile should be reflected [2].

Several studies have been conducted to find very effective compounds as radioprotectors in the laboratory and transfer them for clinical use [2]. Still, these efforts have yielded no or negligible results due to the toxicity and increasing side effects of these compounds [2, 13]. The reason for these compounds' failure in clinical application is their toxicity level and incapability to differentiate between a tumour and normal healthy cells.

The first compound that offered protection against radiation was discovered in 1948, shortly after World War II [4]. The discovery came due to the attack on Hiroshima and Nagasaki by the United States (US) Army [4]. The compound found then was cysteine. Patt et al. [14] carried out pioneering work on cysteine's in vivo radioprotective property; their report revealed cysteine's capacity to protect mice from whole-body exposure to a lethal dose of X-ray [4, 15]. However, the discovery of cysteine as a radioprotector was mitigated by observing that the

drug produced systemic toxicity and revealed that the highest tolerated dose was near the ideal dose for radioprotection [16].

Moreover, the necessity to lower cysteine's toxicity prompted the US Army to launch a different development program in 1959 at the Walter Reed Institute of Research [4]. More than 4,000 substances were synthesised and studied [4, 17]. Part of the discovery was that the compound's toxicity could be significantly minimised if a phosphate group overlaid the sulfhydryl (SH) group [4]. Once the drug is in the cell, the phosphate group is removed, and the SH group begins scavenging for free radicals (Figure 1.1) [4].

Another beneficial compound discovered was WR-2721, otherwise known as amifostine; thus, it remains the most reliable of those synthesised in the Walter Reed series [4, 18]. Amifostine was the only radioprotective drug approved by the United States Food and Drug Administration (FDA) for radiation treatment and marketed under the trade name Ethyol to prevent xerostomia in patients treated for head and necked cancer [18]. Even though amifostine has been shown to offer protection against ototoxicity, nephrotoxicity, and hematologic toxicity in radiotherapy and chemotherapy, there remain the challenges of availability and cost-effectiveness of the drug, which inevitably hampers its wider usage.

Moreover, amifostine's usefulness is greatly hindered by undesirable side effects because of its cumulative toxicity on day-by-day administration with radiation therapy, revealed as sneezing, allergic reactions, somnolence, hypotension, and nausea [2, 9, 16, 18]. Thus, there is an urgent need to find an alternative natural substance with specific characteristics as a synthetic compound that can protect against radiation whilst remaining non-toxic, effective, available, and affordable [15]. The possible natural substance that provides prevention against ionising radiations on biological systems by phytochemicals, plants and herbal extracts is called "Natural Radioprotectants" [2].

1.3 The role of plants and natural products as radioprotectors

The last few decades have witnessed lots of attention shifted to plants, herbs, and natural products as an alternative to synthetic compounds for radioprotection [8]. Some of these plants and herbs have proven effective in treating specific ailments from time immemorial [19]. Moreover, research has shown that natural plants containing antioxidant properties, free radical scavenging, anti-inflammatory and immunomodulatory properties may be helpful in radioprotection [19]. However, it has been established that not all antioxidants based plants can offer radioprotection [20]. The relative abundance, non-toxic nature and proven therapeutic benefits of natural plants naturalise their usage in traditional medicine [15].

The practice of folklore medicine in some parts of the world, especially in Asia, over the past decades has revealed the significant role plants and herbs play in the emergent of new drugs for the therapeutic of human disease [15]; this has demonstrated the contribution of plants and plant products to drug development [15]. The traditional use of plants and plant products to treat various ailments can be traced back to India. Ayurvedic medicine is prevalent in the Indian system, where plant and plant product formulations treat various infections and diseases [16]. After that, multiple plants and herbs have undergone experimental screening to ascertain their radioprotective potential [15]. Researchers from different parts of the world have also delved into this exciting field using an animal model, the most reliable model to test any new radioprotective drug [21]. Animal models are used to investigate the onset and progression of diseases and test experimental treatments before they are administered to humans [22]. Animal models have dealt with many scientific problems, ranging from basic science to creating and evaluating new vaccines and therapies [23]. Observations and testing on animal models have enabled several important achievements in basic science and medical research [23].

There have been many reports in folklore medicine that these plants are used to treat various ailments and illnesses [19]. Most of them serve as medicinal plants edible and do not contain harmful substances to the body system. These plants supplied with regular soil nutrients have withstood electromagnetic radiation such as ultraviolet radiation impact [24]. Therefore, they can be utilised to reduce the detrimental effects of ionising radiation used in clinical practice and protect against radiation-induced damages [24]. There are several advantages of these plants, which include their non-toxicity and established therapeutic benefits.

Moreover, cancer, rheumatoid arthritis, Alzheimer's disease, atherosclerosis, Parkinson's disease, ageing, and various other disorders, as well as inflammatory conditions, have been successfully treated with various plants [19]. As a result, it is plausible to assume that plants may have compounds that can defend against radiation-induced Reactive Oxygen Species (ROS) damage [19]. Studies conducted on animals revealed that selenium compounds and vitamin E could protect against radiation-induced lethality and other effects. Still, they are not as effective as most synthetic protectors [25].

In particular, African countries have vast natural plants and tested, proved, and reliable medicinal resources. Moreover, it is estimated that 70% of the world's population depends on plants and herbs to treat health-related problems [19]. Therefore, it is necessary to divert attention in exploring the prospect of developing scientifically acceptable, economically viable and efficient radioprotective agents for human application from these natural resources [19]. Reports from many researchers, majorly from Asia have revealed the radioprotective potentials of different plants and plant-based products which we shall consider in detail in chapter two.

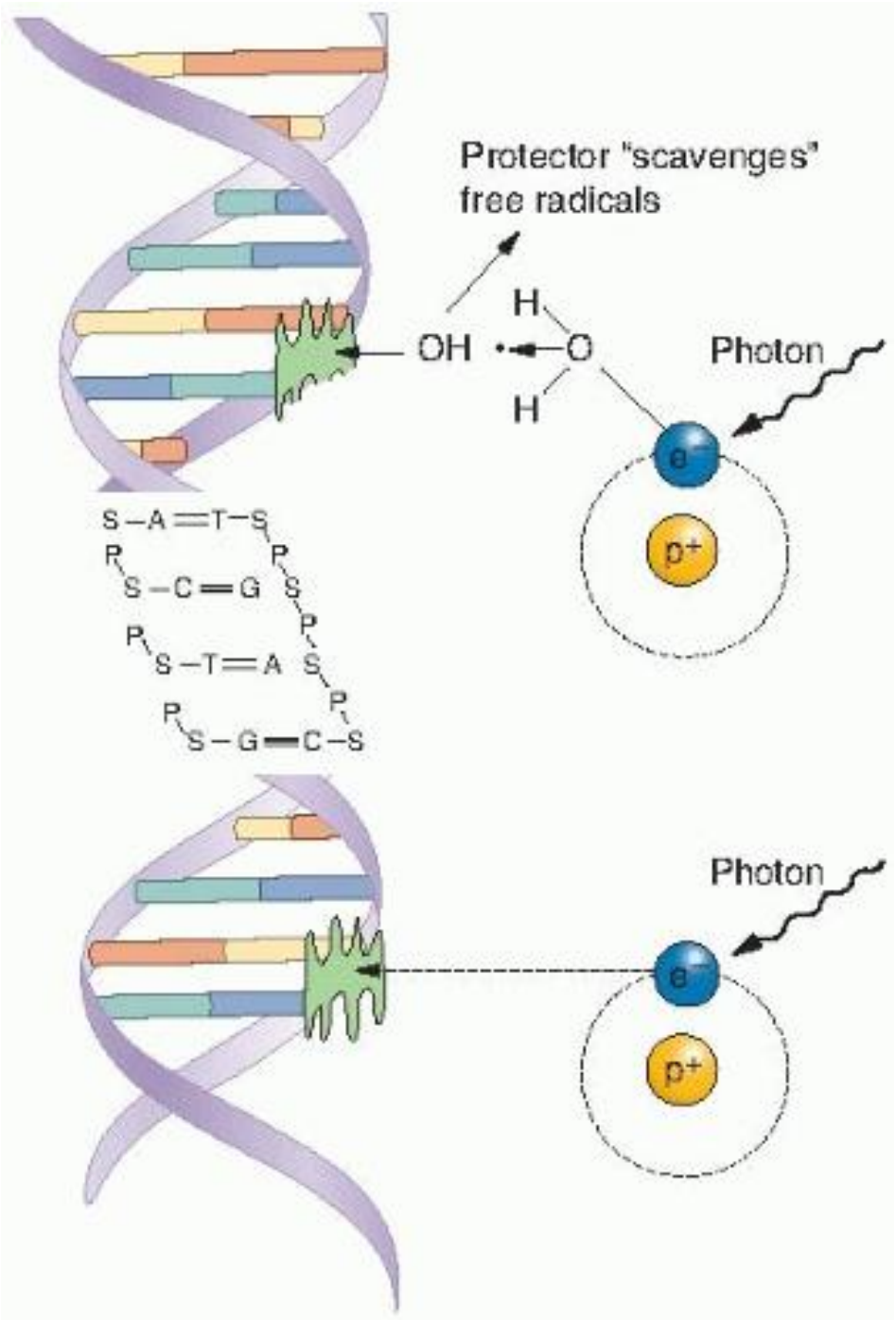

Fig. 1.1: Radioprotectors exert their effect by scavenging free radicals [4].

1.4 Constituent of Radioprotectors

Part of the significant constituents of radioprotectors that made them active against radiation damage are phytochemicals and antioxidant properties [15]. Phytochemicals are compounds present in plants, which help to inhibit pathogens. Research has shown that most of these

phytochemicals protect cells from harm that could lead to cancer. They have exercised their anti-cancer properties by helping avoid the development of possible carcinogens [26]. Radiation protection by phytochemicals has drawn attention due to their ability to scavenge free radicals, inhibit lipid peroxidation and antioxidant status [8]. It is also worth noting that phytochemicals have low toxicity; thus, they might be employed more simply and safely in patients undergoing radiation therapy than other radioprotective substances [8].

Antioxidants are substances, when present in minute amounts, inhibit oxidative damage to a target molecule. They can scavenge free radicals [27]. The physiological role of antioxidants, as this explanation reveals, is to prevent injury to cellular components emanating from a chemical reaction involving free radicals. Antioxidants are also engaged in protecting body cells from the effects of toxins and free radicals that are generated when the body breaks down food or during environmental exposure to smoke, dust and radiation [26]. They can ensure the body waste products, pollutants, toxins and free radicals are flushed out of the body system. Antioxidant compounds scavenge free radicals, such as phenolic acids, polyphenols and flavonoids, so they suppress the oxidative processes that trigger degenerative diseases [27].

Antioxidants help prevent ageing, heart disease, cancer, and age-related macular degeneration by fighting against free radicals in the body. Research has revealed that many of these natural plants contain a tremendous amount of antioxidant properties. For instance, brewer's yeast, which had shown antioxidant benefits, has been recommended for protection against radiation-induced damage [12]. Fruits like apricot combined with wheat germ have been shown to possess antioxidant properties, exhibiting radioprotective attributes [12].

1.5 *Drymaria cordata*

Drymaria cordata (Linn.) Willd belongs to the Caryophyllaceae plant family, which is distributed in different directions. With slender stems, large and face-to-face leaves, it is a procumbent herb. Its leaves and flowers, with tubercular and membranous seeds, are generally

thin [28]. A fresh sample of the *Drymaria cordata* plant is shown in Figure 1.2. The plant is broadly dispersed in the tropics and sub-tropics of West and Central Africa, Asia and America [28, 29]. In folklore (traditional) medicine, people of different tribes and nationalities have used the plant for various purposes. For instance, the plant has been used to treat various diseases such as snakebite, peptic ulcer, skin diseases, headaches or nephritis, sleeping disorders, female infertility, convulsions, and febrile conditions in children [28].

It is commonly known as awede-werisa in Yoruba and Calabar woman's eye in Igbo all in Nigeria. To validate some of these claims, significant experimental experiments had been performed. The majority of these studies aimed to evaluate its ability to treat various illnesses, such as respiratory chest therapy in Zaire, Rwanda and Tanzania; vision problems in Tanzania and cerebral stimulants in Madagascar [29]. It has also been found that the plant is used in Congo, Gabon and Tanzania to treat foot oedema, yaw eruptions and leprosy [29]. In addition, it is used for the treatment of sores in the West Indies and South America [29, 30]. Mexico also used it to treat tumours, and it was used as a powerful herbal medicine in China and South East Asia to treat snakebite [31, 32].

Different studies have been conducted to verify some of these claims scientifically. For instance, Mukherjee et al. [33] reported the antitussive activity of the methanol extract of the plant on a cough model induced by sulfur dioxide gas in mice; their study revealed better inhibition of cough. In the survey conducted by Adeyemi et al. [29], aqueous extract of *Drymaria cordata* possessed significant anti-inflammatory activity by suppressing one or a combination of mediators like kinins, prostaglandins, serotonin and histamine. The plant has also been reported to have anti-inflammatory and antioxidant properties [29]. Mandal and Yonzone [34] said that the plant has medicinal value in West Bengal, India. In addition to the above research, it is imperative to investigate the radioprotective capacity of *Drymaria cordata*. It can be assumed that any plant, which possesses those properties described above, should also

exhibit the characteristics of reducing the harmful effect of radiation on cells. However, this claim cannot be established until scientific research is conducted. To the best of our experience, no study so far has been conducted on the radioprotective property of *Drymaria cordata*.

Fig. 1.2: *Drymaria cordata* plant [35]. Figure adapted from Useful Tropical Plants [35].

1.6 *Costus afer* Ker-Gawl (Costaceae)

Costus afer belongs to the family of Zingiberaceae, otherwise referred to as Costaceae; it is a relatively tall permanent herbaceous, branchless herbal plant with crawling rhizome. A fresh sample of *Costus afer* is shown in Figure 1.3. The plant is grown in the thick forest and

riverbanks of tropical West Africa [36]. *Costus afer* is commonly called ginger lily or bush cane. It has a variety of names such as "Okpete" in the Southeast, "Kakizawa" in the Northern area, "ireke-omode" in the Southwest, "Ogbodou" in the Niger Delta and "Mbritem" in the Southern region all in Nigeria. In Cameroon, it is known as "Monkey sugar cane". It has been revealed that the stem, seeds and rhizomes of *Costus afer* contain numerous bioactive metabolites [37]. Soladoye and Oyesika [38] reported that the *Costus afer* plant is highly regarded for its anti-inflammatory, anti-diabetic, and anti-arthritic features in Southeast and Southwest Nigeria.

This plant has been of great value in traditional medicine to cure ailments such as rheumatism, cough, hepatic disorders, miscarriages, haemorrhoids, inflammation, arthritis, helminthic, epileptic attack, as well as purgative, diuretics, and also had served as a cure for poison [39, 40]. It is also commonly used to treat malaria, cough, inflammation, venereal diseases and skin eruption in various communities in Nigeria. In addition, it is frequently used as a medicinal herb, especially its seeds, stem, leaf, and rhizomes harvested from the wild [36, 41].

The study conducted by Moody and Okwagbe [42] on plant stem extracts of *Costus afer* showed that the plant possessed potent antioxidants in-vitro. The results obtained by Tonkiri et al. [36] revealed that *Costus afer* exhibited high antioxidant and free radical scavenging activities. It signifies that the plant contains a considerable amount of natural antioxidants, which could help prevent various oxidative stressors and reduce drug-induced toxicity.

Several studies have been conducted on *Drymaria cordata* and *Costus afer* about their anti-inflammatory, antioxidant and antitussive activities. However, there have been no comparative studies on the pre-treatment properties of the plants as a radioprotector to the best of our knowledge. As demonstrated in rats, the leaf, stem, and rhizome extract contains an antioxidant property that inhibits lipid peroxidation. However, there is still an unresolved question about

the plants' radioprotective activity because their radioprotective potential on acute radiation syndromes' hematopoietic, gastrointestinal and central nervous systems has not been reported.

Fig. 1.3: *Costus afer* plant [43]. Figure adapted from PROTA4U Record display [43].

1.7 Research Motivation

Chemotherapy and radiation therapy have remained excellent modalities for cancer treatment [9]. However, the use of these modalities has also come with its challenges. A lot of radiation-induced damage is generally associated with normal tissues during radiation treatment [44]. Besides nausea and vomiting, which are common side effects related to the usage of amifostine in radiation therapy, the short time window of radioprotectiveness and its intravenous mode of administration have decremented its performance in medical countermeasures [45]. To overcome these challenges and ensure the protection of normal tissues in radiation therapy,

there is an urgent need to develop novel and effective radioprotectors from plants and natural products, which has gained significant prominence. Over the past few decades, research has continued to find a suitable radioprotective agent, especially natural plants, which can offer protective potential during radiation treatment [44]. The potential radioprotective agents must meet certain characteristics of the existing synthetic compound, and in addition, they must be non-toxic, readily available, less expensive, and highly effective as radioprotectors. These characteristics have been found in some of the natural plants found around us.

Costus afer and *Drymaria cordata* are natural plants used in various communities across Africa and other parts of the world to treat ailments. Multiple claims have been made by folklore medicine about their potential to cure diseases. Moreover, different researchers have also verified some of these claims, such as antioxidants, anti-inflammatory, antitussive etc. However, their radioprotective potentials have not been reported in any literature. Can *Costus afer* and *Drymaria cordata* extracts mitigate the whole-body radiation-induced oxidative damage in mice haematological and histopathological studies? To ascertain the validity of the hypothesis, this study was designed to determine the radioprotective efficacy of *Costus afer,* and *Drymaria cordata* extracts on mice by looking at their mitigating ability on radiation-induced damage on hematologic and histologic parameters.

1.8 Aim and Objectives of the research

This research aims to evaluate the radioprotective effect of *Costus afer* and *Drymaria cordata* extracts on irradiated mice.

The objectives of this study are to:

- Provide information on the radioprotective potential of the methanol extract of *Costus afer* and ethanol extract of *Drymaria cordata* on mice.

- Determine the pre-treatment of the *Costus afer* extract on assessing hepatic and nephritic damage by histopathology of the studied mice's target organ (kidney and liver).
- Determine the types of injury-induced in the kidney and liver of mice exposed to X-ray irradiation by histopathological analysis.
- Determine the efficacy of both *Costus afer,* and *Drymaria cordata* extracts against radiation-induced quantitative variations in haematological parameters of mice.
- Determine the survival rate of mice treated with the *Drymaria cordata* extract after exposure to sources of radiation

1.9 Organization of the thesis

The structural organisation of this thesis is presented in six (6) chapters. Chapter 1 deals with the background of the study, details information on plants and natural products as radioprotectors, radioprotectors currently in use, *Drymaria cordata* plant, *Costus afer* plant, the motivation for the research and the aim and objectives of the study.

Chapter 2 gives the theoretical background (literature review) on ionising radiation, types of ionising radiation, biological effects of ionising radiation, exposure to ionising radiation, acute radiation syndrome (ARS), principles of radiation protection, radiotherapy in cancer treatment, a medical linear accelerator in radiotherapy, whole-body irradiation technique, evaluation of radioprotective efficacy of medicinal plants and herbs, haematology and histopathology. Chapter 3 gives the materials and methods employed in this study. Chapter 4 is in the form of a journal published in the Radiation Oncology Journal (ORJ). The article investigated the possible radioprotective effect of *Costus afer* extract (CAE) on haematological and histopathological parameters of mice. We evaluated the radiation-induced damage to haematological and histopathological parameters after exposure of mice to 3Gy and 6Gy dose

of X-ray radiation. Furthermore, we determined to what extent the *Costus afer* extract protects the irradiated mice's haematological and histopathological parameters.

Chapter 5 is in the form of a journal that has been accepted for publication and waiting for the next issue at the Iranian Journal of Medical Physics (IJMP). The study determined the efficacy of *Drymaria cordata* extract on irradiated mice, emphasising its ability to increase survival rate and improve haematological parameters. Lastly, chapter 6 summarises the crucial findings of the study and provides some directions for future research.

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CHAPTER TWO

LITERATURE REVIEW OF RELATED WORKS

2.1 Radiation and its classification

Radiation is a term that describes the transmission and propagation of energy through space and material mediums [1, 2]. It can exist in two forms: electromagnetic and particulate radiations [1]. Electromagnetic radiation involves energy transmission through space as a mixture of electric and magnetic fields [1]. Examples of electromagnetic radiation include; microwaves, radio waves, infrared, ultraviolet, visible light, x-rays and γ-rays. They have the same velocity, c, ($c = 3 \times 10^8 ms^{-1}$) in a vacuum but with different wavelengths and, therefore, different frequencies [3]. The electromagnetic spectrum that describes the arrangement of electromagnetic radiations according to their wavelength, energy and frequency is shown in Figure 2.1 [4]. Particulate radiation comprises electrons, neutrons, protons, α-particles, β-particles and other atomic and subatomic particles such as quarks and leptons [2]. They are called particles because of a definite rest mass and can occupy a defined position at any instant [2].

Ionisation is a process that leads to the production of positively charged ionised atoms and negatively charged free electrons [5]. For ionisation to occur, sufficient energy called ionisation energy is required to overcome the existing electron binding energy [1]. The ionisation energy of elements such as alkali metals ranges from a few electronvolts (eV). For helium, it is 24.5 eV, and that of water is 12.6 eV [4]. Based on the ability of radiation to ionise matter, two classifications of radiation exist ionising radiation and non-ionising radiation [4]. Radiation with enough energy to liberate one or more orbital electrons from an atom or molecule, thereby leaving it ionised, is called ionising radiation [6]. Most of the aforementioned particulate

radiations are ionising, and their capacity to ionise atoms largely depends on their mass, charge, and velocity [1].

In comparison, non-ionising radiation does not have sufficient energy to remove electrons from an atom [7]. Examples of non-ionising radiation include all the electromagnetic radiations except x-rays and γ-rays.

The diagnostic and therapeutic use of ionising radiation over a few decades is increasing due to its benefit in modern medicine. Even though ionising radiation has many useful applications, including agriculture, industry, military and scientific research, it also has the potential for health hazards if not properly handled or contained [8]. The type of radiation considered in this work is ionising radiation.

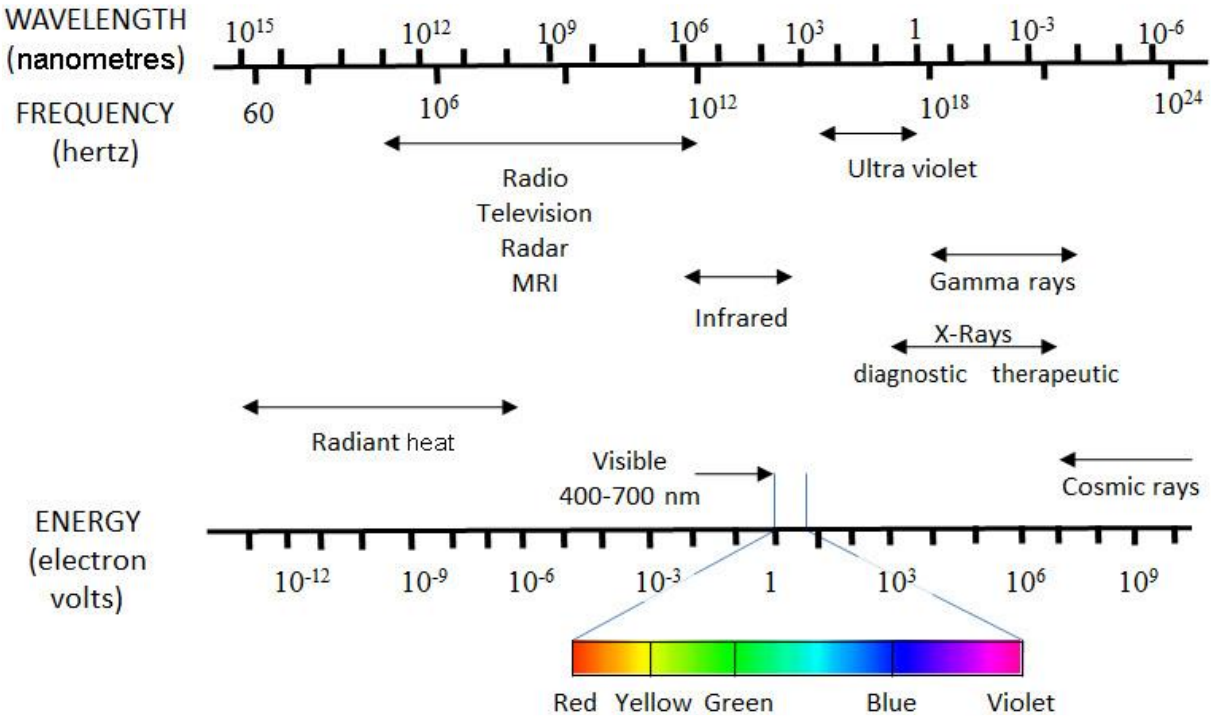

Figure 2.1: The electromagnetic spectrum based on their wavelength, frequency and energy [4].

2.2 Types of Ionising Radiation

Ionising radiation can be separated into five primary types: alpha radiation, beta radiation, neutron radiation, gamma radiation and x-rays (Figure 2.2) [9]. Alpha, beta, and neutron radiation comprise small particles, while x-rays and gamma radiation are high-energy electromagnetic waves [9].

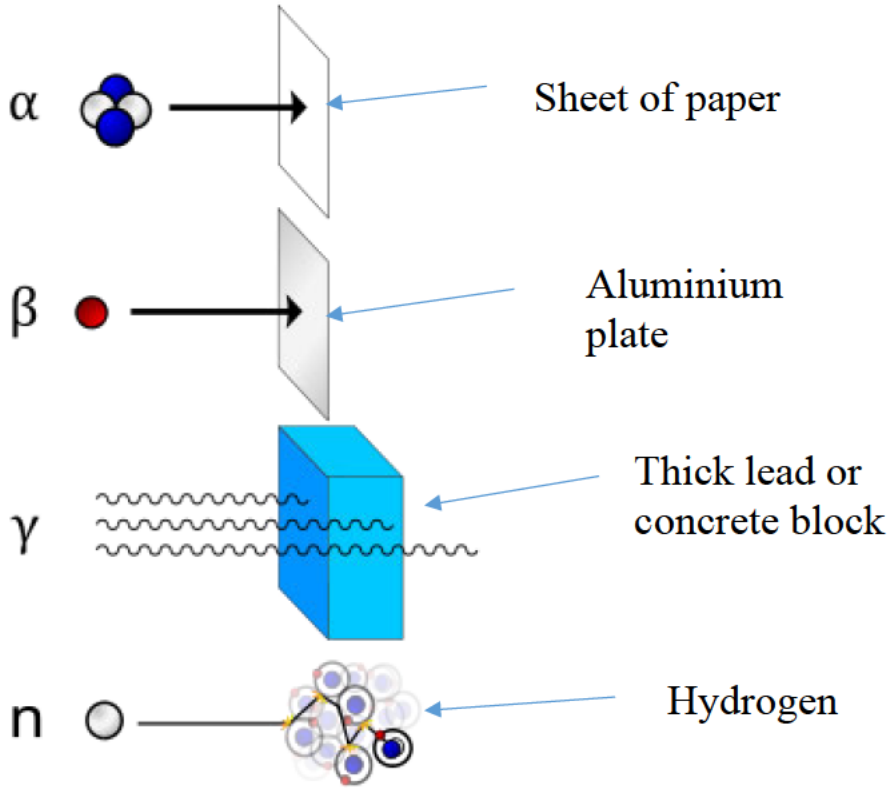

Figure 2.2: Types of ionising radiation and their shielding materials [10].

Figure adapted from Wikipedia contributors [10]

2.2.1 Alpha (α) particle

Alpha particles are nuclei of helium atoms consisting of two protons and two neutrons [3]. They are emitted from the decay of unstable nuclei and naturally occurring radionuclides, such as radium and uranium. The decay of heavy radionuclide by the emission of an α-particle is shown in Fig. 2.3. Alpha particles are heavy and positively charged particles, which do not travel very far in the air and cannot penetrate the skin. However, they pose a radiological hazard to the body through inhalation and ingestion [11]. Generally, alpha particles are known to have

low penetrating power and high ionisation energy. A thin sheet of paper or a few centimetres of air easily stops them [11].

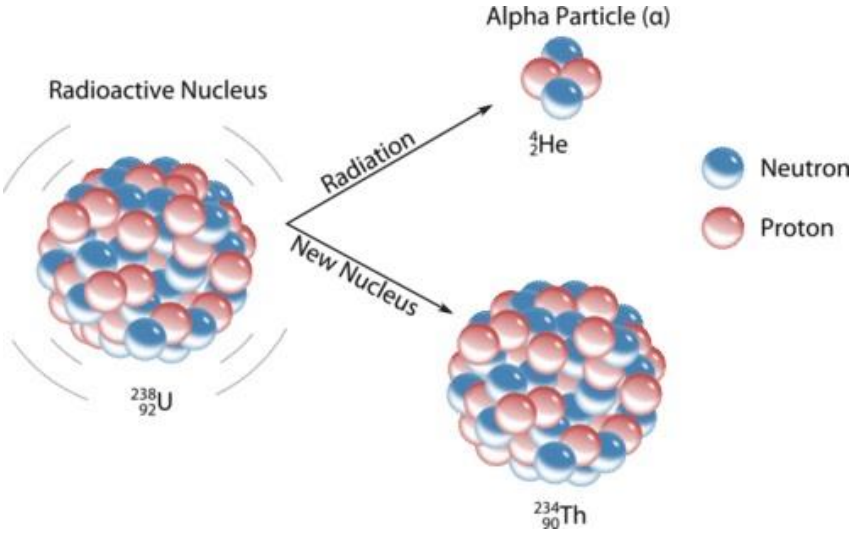

Figure 2.3: Illustration of the spontaneous decay of $^{238}_{92}U$ to $^{234}_{90}Th$ with the emission of α-particle [12].

2.2.2 Beta (β) particle

Beta (β) particles are fast-moving high-energy electrons liberated during radioactive decay. It is usually represented by the symbol ($_{-1}^{0}\boldsymbol{e}$) in a nuclear reaction. They are negatively charged particles that can travel further than alpha particles in the air [11]. Beta particles penetrate the skin but can easily be shielded with a plastic sheet or a thin aluminium plate [11]. Like alpha particles, beta particles can pose a severe risk to human health if inhaled or ingested [9]. The decay reaction where $^{14}_{6}C$ transmutating into a $^{14}_{7}N$ the nucleus is shown in equation 2.1 and Figure 2.4.

$$\boldsymbol{^{14}_{6}C \rightarrow {}^{14}_{7}N + {}_{-1}^{0}e} \qquad \textbf{(2.1)}$$

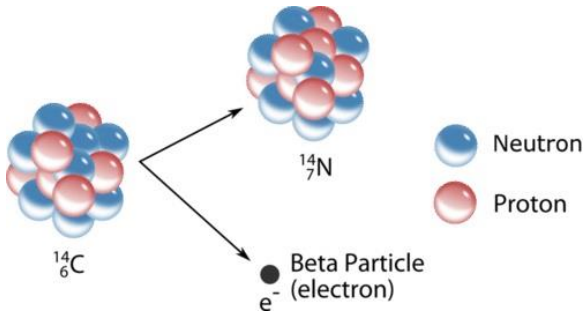

Figure 2.4: Carbon-14 nuclide decay to Nitrogen-14 with the emission of the beta particle [12].

2.2.3 Neutron (n) radiation

Neutrons are usually released through the nuclear reaction process [11] and frequently misinterpret a neutral electrical charge as a zero electrical charge; therefore, they often do not cause ionisation directly in a single-phase or contact with matter. However, by linear energy transfer, fast neutrons can interfere with the protons in hydrogen [5]. This process scatters the materials' nuclei in the target region, causing the hydrogen atoms to be directly ionised. Neutrons have high penetrating power, and they are stopped mainly by using hydrogen-rich material such as water [5, 11].

2.2.4 Gamma (γ) rays and X-rays

Gamma and x-rays belong to the class of electromagnetic radiation with high frequency and low wavelength. Due to their massless nature, they are called photons [11]. They have similar properties but differ in source or origin. Gamma rays originate from an atom's nucleus, whereas x-rays are produced from the bombardment of heavy atoms by fast-moving electrons. Gamma rays usually accompany alpha and beta particles in a radioactive decay process [13]. They are more penetrating than alpha and beta particles. Due to their high penetrating power, gamma and x-rays require high-density materials such as concrete or materials with high atomic weight (lead & steel) for shielding (Figure 2.2) [11]. They are referred to as ionising radiation because their energy is sufficient to eject orbital electrons from the atom, causing ionisation. Due to

their ability to penetrate long enough into the human body and be absorbed by tissue, they have become valuable tools in medicine. This significant feature has given X-ray and gamma radiation essential characteristics in radio diagnosis and therapeutic [3].

2.3 Direct and indirect ionising radiations

There are two classifications of ionising radiation based on ionisation mode, direct ionising and indirect ionising radiation [14]. Direct ionising radiations are made up of charged particles. They interact directly with atoms or molecules through Coulomb force; energy is deposited into the absorber through a Coulomb interaction between the directly ionising particles and orbital electrons of the atoms in the material [3]. This type of ionising radiation has sufficient energy to disrupt the atomic structure of the material they pass through, thereby producing biological and chemical changes [13].

The indirect ionising radiations are uncharged or neutral particles, consisting of electromagnetic radiation (gamma and x-rays) and neutrons. They do not themselves cause chemical and biological damage. Still, they relinquish their energy, as they are absorbed into the substance through which they move, to produce fast-moving charged particles (electrons) that are in turn capable of producing damage [13].

2.4 Interactions of radiation with matter

The interactions of radiation (photon and electron) with matter are stochastic and obey the laws of chance [4]. Photon interactions are expressed in terms of cross-sections for individual interactions and attenuation coefficients for passage through bulk media. A single-photon incident on a slab of material of area A that contains one target of cross-sectional area σ. The probability of the photon interacting with the target will be the ratio of the two areas: σ/A [4].

There are four primary ways indirect ionising radiation (e.g. X-rays) interact with matter: Rayleigh scattering, photoelectric effect, Compton scattering, and pair production [13]. The

first three interactions are very significant in diagnostic radiology, where the energy ranges up to$150\ keV$. In contrast, the pair production is only crucial at higher energy photon, that is, mega electron volt energy (>$1.022\ MeV$) [4].

2.4.1 Rayleigh scattering

Rayleigh scattering, also known as coherent or elastic scattering, occurs when an incident photon interacts with the atomic electrons and be scattered through an angle θ. In this interaction, no energy is lost by the incident photon as it transfers momentum to the atom [4]. The energy of the electron is raised temporarily without necessarily eliminating it from the atom. The electron returns to its former energy level by producing an X-ray photon of equal energy but slightly altered direction. Since the atom cannot experience a notable recoil, the majority of the X-rays are scattered forward by this process [15]. No energy absorption is involved, and most X-rays photons are scattered with a slight angle. The probability of Rayleigh scattering occurring is low, approximately 5% of all scattering processes due to soft tissues' low effective atomic number.

2.4.2 Photoelectric effect

The photoelectric effect occurs when an incident photon (X-rays or γ-rays) interacts with an inner shell electron in the absorbing atom with binding energy similar to but less than the incident photon's energy [15]. The energy of the incident photon is transferred to the electron, which causes the electron to be ejected from its shell (typically the K shell) with a kinetic energy equal to the difference between the incident photon energy hv and the electron shell binding energy E_s [15].

$$E = hv - E_s \qquad (2.2)$$

The vacated electron shell is then filled with an electron from an outer shell with lower binding energy (e.g., the L or M shell), resulting in a characteristics x-ray with an energy equal to the

difference between the source electron shell and the final electron shell's electron binding energies. The photon energy hv must exceed the binding energy of the electron for the photoelectric effect to occur. For diagnostic energy up to 150 keV, photoelectric effect cross-section per atom τ is given by equation (2.3) [4]

$$\tau(hv, Z) = \frac{kZ^n}{(hv)^m} \tag{2.3}$$

Where k is a constant, Z is the atomic number, m is an exponent in the range 2.5-3.5, and n is an exponent in the range 3.6-5.3.

2.4.3 Compton Effect

Compton Effect involves the interaction between the incident photon and the orbital electron with low binding energy compared with the energy of the incident photon [13]. The geometry of the Compton Effect showing the directions of the scattered electron and photon is shown in Figure (2.5). The energy of the incident photon E_0 is partially transferred to the electron at an angle θ leading to its ejection from the atom. The rest of the energy, E_S, is transferred to a scattered x-ray photon with an angle ϕ relative to the incident photon's track [15]. The scattered photon travels through any angle ϕ from 0^o to 180^o And the recoiled electron may be directed forward parallel to the angle θ of the incident photon. The energy of the scattered photon divided by the incident photon's energy is determined by the equation (2.4) for a photon scattering angle because of the physical constraints to conserve energy and momentum.

$$\frac{E_S}{E_0} = \frac{1}{1 + \frac{E_0}{511\,keV}(1 - COS\phi)} \tag{2.4}$$

Mathematically, this equation called the Klein-Nishina equation shows that as the scattering angle increases, the energy of the scattered x-ray photon becomes smaller. At higher incident energies, the effect is magnified [15]. The scattered photon angle and the scattered electron angle are related to equation (2.5).

$$cot\theta = (1+\alpha)\tan\left(\frac{\phi}{2}\right) \quad (2.5)$$

Compton Effect is the primary energy absorption mechanism for X-rays and γ-rays in the intermediate energy between 100 keV to 10 MeV. This is the therapeutic radiation range, and it constitutes the majority of the γ-radiation produced by nuclear explosions [4].

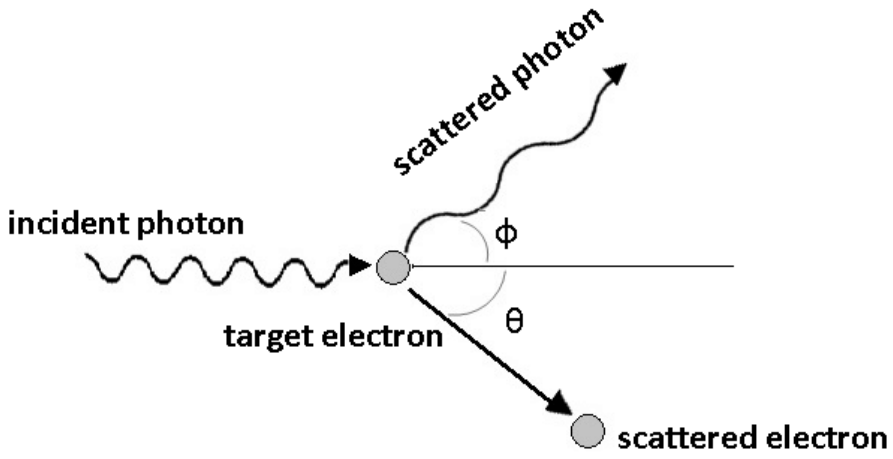

Figure 2.5: Geometry of Compton Effect shows the scattered electron and photon [16].

2.4.4 Pair production

In pair production, the incident photon (X-rays or γ-rays) has energy greater than 1.02 MeV, which is the rest energy of two electrons sufficient to create the pair of electrons. The schematic diagram of the Pair Production process is shown in Figure (2.6). The incident photon with energy hv interacts with atoms of the medium, leading to the production of an electron (e^-)-positron (e^+) pair through the interaction of the Coulomb field presents near the nucleus [13, 15]. The photon energy (1.02 MeV) is distributed equally between the electron-positron pair as kinetic energy; thus, the energy conservation law is obeyed. Equation (2.6) illustrates the pair production process

$$hv = K_{e^+} + K_{e^-} + 2m_0c^2 \quad (2.6)$$

where $2m_0c^2$ is the total rest mass energy of positron and electron, hv is the energy of the incident photon, and $K_{e^+}$and K_{e^-} represent the kinetic energy of positron and electron, respectively.

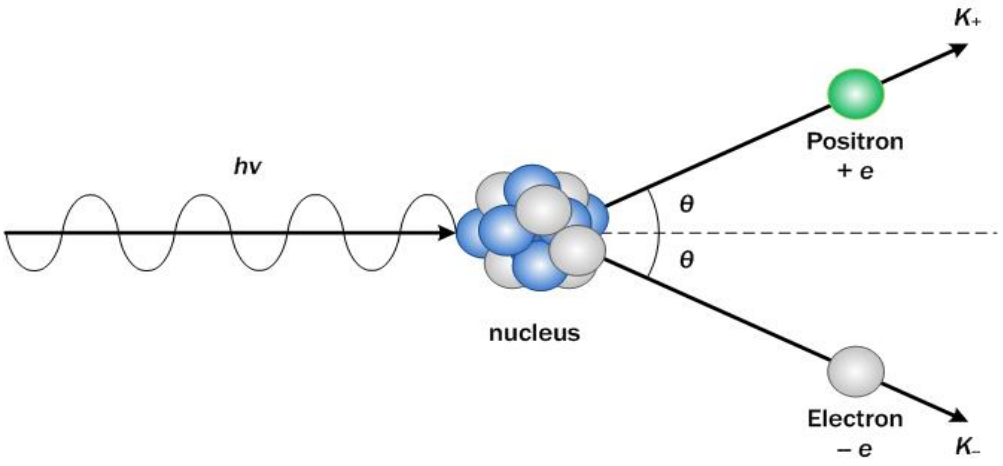

Figure 2.6: Schematic diagram of Pair Production process [17]

When the positron's kinetic energy is expended, it will merge with any available electron to produce annihilation radiation, which is caused by the conversion of the e^+/e^- pair's rest mass energies into oppositely directed 511keV photons [15]. The two photons created are in opposite directions to each other and can initiate further damage as charged particles [13]

2.5 Biological Effects of Ionising Radiation

Ionising radiation can be either beneficial or harmful, depending on its application, duration, location and magnitude of exposure. Ionising radiation deposits energy into the body system, causing the molecules' structure to be distorted. Biological effects occur when electromagnetic radiations such as γ-rays or X-rays are absorbed or scattered by the tissues [13]. The energy deposition may perturb cellular functions or alter nucleotides' ordering in deoxyribonucleic acid (DNA) molecule that carries the cells' genetic information in living organisms. DNA is the main target for radiation's biological effect, including changes in DNA, apoptosis, heritable mutations, and carcinogenesis. The radiation-induced damage to DNA molecules can be in the

form of loss of a base, breakage of strands (single and double strands), and clearage of the hydrogen bond between bases [3].

The biological effects of ionising radiation are primarily the radiation-induced damage to the deoxyribonucleic acid of cells [3]. Ionising radiations exert their biological effects on cells through two primary mechanisms: direct and indirect actions [3].

2.5.1 Direct Action

Direct action occurs when electromagnetic radiation (X-rays or γ-rays), charged or uncharged particles pass through any biological medium; the radiation interacts with the DNA, which is the critical target in the cells [18]. This action can lead to the atom's ionisation, thereby commencing a chain reaction that changes the biological system (Figure 2.7) [3]. The process is dominant; supposing radiations with high linear energy transfer (LET) such as neutrons or α-particles are considered [3]. The linear energy transfer is the energy deposited per unit length when ionising radiation traverses through a medium [3].

Ionising radiation + biological molecules (RH) $\longrightarrow$ $R^* + H^*$

Both R^* and H^* radicals can react with another molecule, e.g. lipids, DNA, proteins.

$R^* + R'H \longrightarrow R'^{\cdot} + RH$

Radicals can produce cross-linking reactions.

$R^* + R^{\cdot} \longrightarrow R^* - R^{\cdot}$

2.5.2 Indirect Action

The second mechanism called indirect action occurs when radiation interacts with a water molecule; since 80% of the mass of living cells is made up of water, the reaction proceeds faster to produce free radicals that attack a cell's DNA [3, 13].

The radiolysis of a water molecule by ionising radiation is illustrated as follows:

Ionising radiation + $H_2O \longrightarrow H_2O^+ + e^-$, where H_2O^+ is an ion radical.

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH^*$$

The primary ion radicals generated have a very short lifetime of the magnitude of 10^{-10}s. They quickly disintegrate to yield free radicals that are uncharged but still have an unpaired electron. The action of secondary electrons on water molecules results in the creation of free radicals. Because of an unpaired orbital electron in their outer shell, free radicals created are reactive. The hydroxyl radical has nine unpaired electrons; thus, it is uncharged, and highly reactive radical travel quickly to reach a critical target in the cell [3].

The chain of events for the indirect action of ionising radiation is described as follows:

Incident x-rays ⟶ Fast electron ⟶ Ion radical ⟶ Free radical

⟶ Chemical changes from the breakage of bonds ⟶ Biologic effects

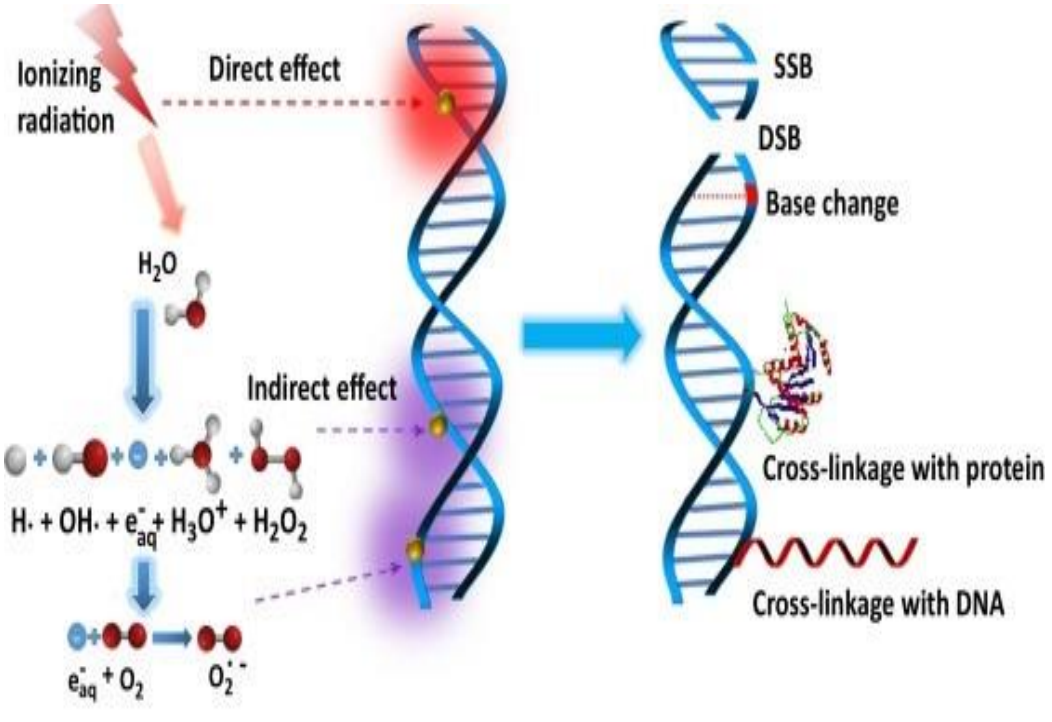

Fig. 2.7: Schematic of the mechanism of direct and indirect effects of ionising radiation [19].

Figure adapted from Wang et al., [19]. Cancer Radiosensitizers. Trends in Pharmacological Sciences.

2.6 Deterministic and Stochastic Effects of Ionizing Radiation

Based on the recommendation by the International Commission on Radiological Protection (ICRP) and the National Council on Radiation Protection and Measurements (NCRP), the biological effects of ionising radiation have been broadly grouped into deterministic and stochastic effects [20].

Deterministic effects occur due to radiation exposure to organs or tissue above a threshold level. The resultant effect cannot occur below a threshold value (Figure 2.8) [20]. Moreover, the severity of the effect is proportional to the dose. The greater the exposure, the more apoptosis is recorded [20]. Apoptosis means the inability of a cell to carry out its reproductive functions, which may occur by necrosis and mitotic catastrophe [13].

Consequently, deterministic effects will only occur if the dose of radiation is significant [1]. This case can only happen when a radiation accident occurs. It is worth noting that the deterministic effects also depend on the duration of exposure and the radiation's type and quality. Examples of deterministic effects include epilation, sterility (temporary or permanent), cataracts of the eyes, skin erythema, nausea, diarrhoea etc. [11].

Stochastic effects are produced by sub-lethal radiation-induced damage to DNA. The probability of causing a stochastic effect is proportional to the radiation dose (Figure 2.8); that is, a single radiation dose may initiate a stochastic effect [1, 11]. The addition of radiation doses to tissue causes more damage to the cell, ultimately increasing the likelihood of stochastic effect [21]. Somatic effects and hereditary or genetic effects are examples of the stochastic effect of ionising radiation. Somatic effects occur when an individual is exposed to a specific dose of radiation. The majority of the affected population during their lifetime feels the effect. Examples of this could manifest as radiation-induced cancers (carcinogenesis) [22]. In contrast, genetic or hereditary effects manifest in the progeny of the exposed individual. For instance,

mutations to an individual's genes because of radiation exposure can constitute abnormality in subsequent descendants' birth [22].

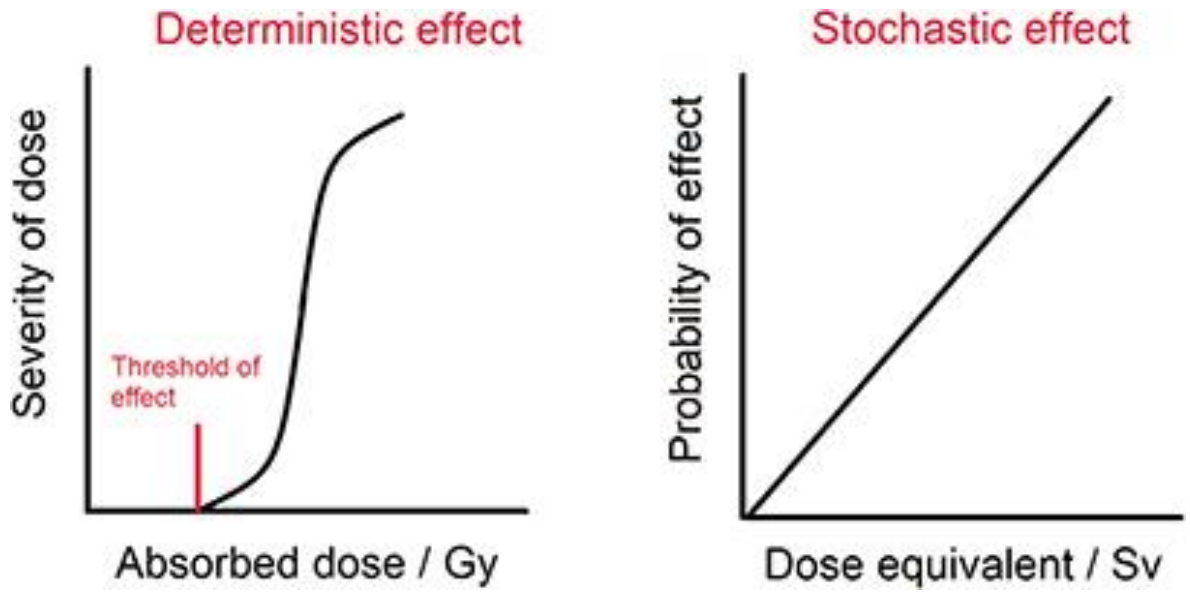

Figure 2.8: Deterministic and Stochastic effects of ionising radiation [23].

Figure adapted from Sperrin and Winder [23].

2.7 Exposure to Ionising Radiation

Living organisms are regularly exposed to ionising radiation from different sources. It could be from natural and artificial sources [13]. The natural source of radiation exposure comes mainly from terrestrial and cosmic radiation. The terrestrial exposure comes from the naturally occurring radionuclides such as potassium, uranium, thorium, and radium present in the earth's crust, air, food and water [13]. Radon, one of the daughter products of uranium during the decay process, emanates from rock and soil, serves as a significant natural radiation source [23]. People's exposure to radon comes through the inhalation or ingestion of radionuclides from food, water, and air [23]. Human activities such as mining produced technologically enhanced naturally occurring radionuclides, contributing to man's exposure to radiation.

Cosmic radiations mainly arise from outer space [20]; radiation comes from the sun and solar flares and is protons and alpha particles. People, particularly at high altitudes, are often exposed to natural radiation from cosmic rays [20]. On average, 80% of a person's annual background radiation dose is due to terrestrial and cosmic radiation sources that occur naturally [24]. Background radiation levels vary geographically due to geological differences. They are not evenly distributed [24]. Besides human activities, which sometimes enhances its manifestation, some places are prone to higher background radiation than others [24].

Moreover, radiation exposure can be over 200 times above the global average in some regions with high background radiation [24]. The human-made source of radiation comes majorly through x-rays and other medical devices [24]. Sources from consumer products, smoking, research institutions, military activities, power generation, the fallout from nuclear explosives testing, nuclear accident, industrial usage of radioactive sources, and radiation exposure to the public are also significant sources of radiation exposure.

Medical exposure remains the most crucial contributor to artificial sources of radiation exposure [24]. It is estimated that 98% of radiation exposure from artificial sources comes from medical use. There are two radiation exposure pathways: Internal Exposure and External Exposure [24]. The illustration of the paths leading to internal and external exposure of man is shown in Figure 2.9. From the Figure, radionuclides in gaseous and liquid effluents from nuclear facilities diffuse into the air, deposit on the soil, travel through water and find their way to the environment. The public receives a certain quantity of these radionuclides via absorption, inhalation and ingestion.

2.7.1. Internal Exposure of Radiation

The inhalation and ingestion of a radionuclide to the body via the bloodstream produced internal exposure to radiation [24]. Alpha and beta particles are good examples of radiation

that constitute internal exposure due to their inability to travel far. The alpha particle is dangerous to body organs such as tissues, lungs and bones. A beta particle is also similar to an alpha particle in its harmful nature. Internal exposure ceases when the radionuclide is removed from the body, either naturally (such as by excreta) or due to treatment [24].

2.7.2 External Exposure of Radiation

Exposure via contamination of the public from nuclear explosives fallout and a nuclear accident is called external exposure. It can also result from irradiation from medical devices such as X-rays machines [24]. External exposure to alpha-emitter will not pose a radiological hazard unless the contaminated material is internalised. Still, external exposure to a beta-emitter may be of great concern due to their ability to penetrate beyond the outer layer of skin, leading to a potential risk for skin damage [11]. Gamma and X-rays rays, highly hazardous to cells because of their strong penetrating power, belong to external radiation exposure [25]. Another important source of radiation exposure is occupational exposure.

2.7.3. Occupational Exposure of Radiation

Occupational exposure is the risk of radiation exposure to personnel in the workplace [24]. Several industries, such as oil and gas, paper industry, agriculture, mining, etc., use ionising radiation. The medical application poses the most significant artificial radiation exposure also falls into this category [24]. Occupational radiation exposure is usually incurred because of negligence in providing safety measures on operational management or inadequate training to the staff on the handling and usage of ionising radiation in the workplace. Based on the recommendation by ICRP, the maximum dose limit for occupational exposure is $20\ mSv/y$, averaged over five years. This means a worker should not expose to more than 100 mSv in five years [26, 27].

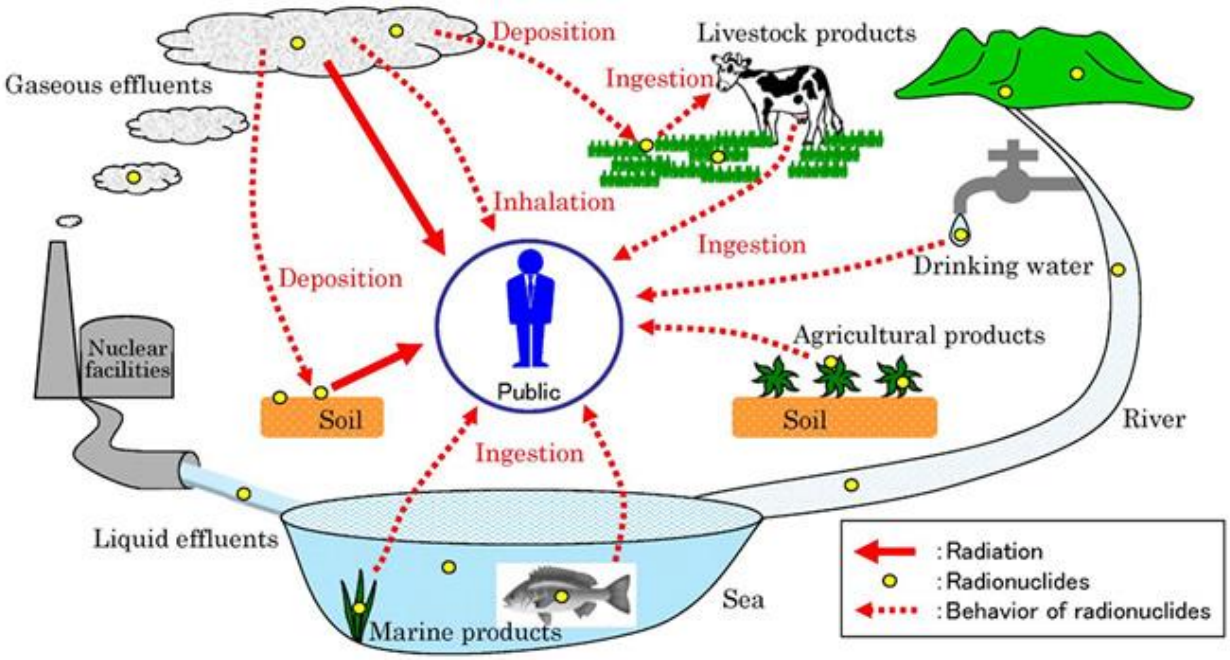

Figure 2.9: Exposure pathways of man due to radionuclides [28]. Adapted from JAEA. Japan Atomic Energy Agency [28].

2.8 Acute Radiation Syndrome

Acute Radiation Syndrome (ARS), also called acute radiation sickness, is described as the collection of signs and symptoms produced due to radiation exposure. The exposure can be a whole-body or a partial body and usually occur in a short time [29]. The dosage of ionising radiation induces ARS is high; greater than 0.7 Gray (Gy) or 70 rads can penetrate the body and enter the internal organs. The radiation source is usually outside the body, and radiation types associated with the acute syndrome are the high penetrating radiations such as gamma rays, X-rays and neutrons [30].

The exposure of the body to ionising radiation leading to ARS can happen accidentally or intentionally. Accidental exposure can create ARS if a nuclear accident results from human error or natural disaster [9]. Accidents from nuclear reactors can result in unintentional exposure. The explosions produced due to the core meltdown of Chernobyl (1986) and Fukushima (2011) nuclear power plants exposed their employees, firefighters and civilians to

a high dose of radiation. More than two hundred people in Chernobyl experienced acute radiation sickness, with over three hundred thousand displaced [9].

Moreover, other events that can lead to accidental exposure are carelessly handling a material that contains radioactive sources, solar flares from cosmic radiation etc. The use of nuclear weapons and other terrorism devices can be described as intentional exposure resulting in acute radiation syndrome. For instance, the Second World War of 1945 in Japan that led to the US forces dropping atomic bombs on Hiroshima and Nagasaki resulted in the death of more 200 000 people and thousands of casualties [9].

Generally, there are four phases of ARS, with each stage has its distinctive signs and symptoms. The four stages include prodromal, latent, manifest illness, recovery, or death, as shown in Figure 2.10 [3]. As shown in Figure 2.10, all the four stages of ARS depend on radiation exposure and time. The prodromal stage comprises signs and symptoms that appear early soon after radiation exposure, and these usually last for minutes up to a few days. The manifestation of these signs and symptoms depends on the radiation dose and exposure duration [31]. It is possible to classify prodromal syndrome's signs and symptoms into two significant groups: neuromuscular and gastrointestinal [3]. The neuromuscular symptoms involve sweating, apathy, headache, fever, and fatigability.

Signs and symptoms of the gastrointestinal syndrome are nausea, diarrhoea, vomiting, anorexia, intestinal cramps, dehydration, fluid loss, and weight loss [3]. For a few hours or even up to a few weeks, the patient usually looks and feels safe at the latent stage. Within 2-3 weeks of exposure, the patient may become asymptomatic.

The period of the latent phase is indirectly proportional to the radiation dose. The latent phase may last for a few hours for high exposures or 2-3 weeks for low exposures [30]. The manifested illness stage is characterised by the symptoms peculiar to the specific syndrome, which may last from hours to months. At this phase, the haematopoietic syndrome grows at a

dose range of 1Gy and 8Gy, even though a slight reduction in the blood cell counts may be possible at a dose below 1Gy. Usually, the presenting illness takes 6 to 8 weeks to appear, and the symptoms produced depend on the radiation dose [31].

The last stage of the ARS is the recovery or death stage. At this phase, the recovery process may last from weeks to two years, and if patients sustain severe injuries from the exposure, it could result in death within several months [30]. Acute radiation syndrome is classified into three major divisions: haematopoietic syndrome, gastrointestinal syndrome and neurovascular or central nervous system syndrome [31].

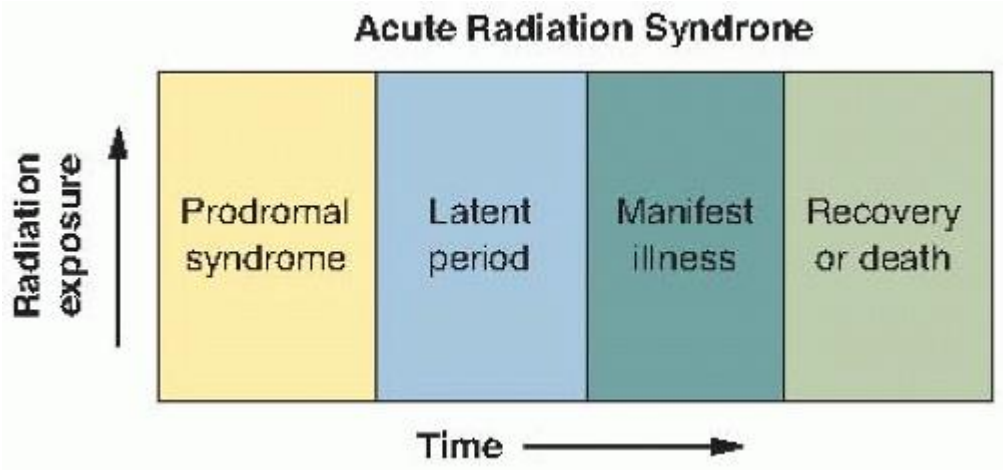

Fig. 2.10. Phases of acute radiation syndrome [3].

2.8.1 Haematopoietic Syndrome

According to Bergonié and Tribondeau law [32], various tissues have different radio-sensitivities; the most susceptible are less-differentiated and actively proliferating cells [11]. The mitotically active and immature cells are more radiosensitive than other cells in the human body [11]. A cell's radiosensitivity is directly proportional to the growth rate and indirectly proportional to the degree of specialisation. The stem cells (germ cells) are more radiosensitive than mature differentiated cells. The haematopoietic cells are the most proliferative cell, and the more significant part of bone marrow cells are immature progenitors [9, 11]. The proliferative cells of the haematopoietic cell are most radiosensitive, and as low as a 0.2Gy

dose can cause radiation-induced alteration [9, 31]. The haematopoietic syndrome also referred to as bone marrow syndrome, is associated with the bone marrow's haematopoietic cells due to acute radiation exposure [11].

Whole-body exposure of 1 to 2Gy dose of radiation is usually characterised by mild symptoms such as non-severe haemorrhage, increased body temperature, fatigue and nausea [33]. As the radiation dose increases above 2Gy, moderate haematopoietic syndrome (Figure 2.11) occurs and is characterised by multiple haemorrhages and mild radiation mucositis [11]. Partial epilation begins between 14-24 days, agranulocytosis starts between 18-28 days and is accompanied by signs of infection [33]. Severe bone marrow syndrome occurs at radiation doses 4Gy to 6Gy. It is characterised by agranulocytosis beginning around 12–24 days with infections and bleeding. This is presented by acute radiation injury of mouth mucositis and skin epilation [33].

Some of these symptoms are due to the manifestation of the depression of blood parameters: leukopenia that often leads to a susceptibility of infection and poor wound healing; thrombocytopenia, leading to purpura, petechiae and haemorrhage; and erythropenia, leading to fatigue, anaemia, and heart failure [9]. As earlier described, blood cells' radiosensitivity varies; the lymphocytes are the most radiosensitive among these blood cells. They are the first blood parameter to be depleted in acute radiation exposure [11]. The depletion of lymphocytes is often proportional to the radiation dose.

Generally, lymphocyte depletion starts 6 to 24 hours after exposure and reaches the lowest point at 3 to 6 days, contingent on the radiation dose [9]. In humans, neutrophils are depleted to their lowest level between 1-2 weeks after a whole-body irradiation dose greater than 2Gy. Lymphocytopenia and neutropenia leave patients immunocompromised and highly vulnerable to infection at the nadir stage [9].

Even though platelets and erythrocytes are relatively radioresistant, their scarcity in the peripheral blood after exposure reflects their precursors' radiosensitivity. Typically, it takes the red blood cell about 120 days to be renewed, and it takes a more extended time and slower rate to be cleared from the circulation. Anaemia due to depletion of mature red blood cells does not occur; other symptoms such as bleeding should be examined [31]. The survival rate of patients with haematopoietic syndrome reduces with increasing radiation dose. If the exposure is more than 3.5Gy, it could lead to death within 60 days due to the bone marrow's destruction, resulting in haemorrhage and infection [30]. The description of ARS as a function of dose and post-irradiation time is shown in Figure (2.11).

2.8.2 Gastrointestinal Syndrome

As shown in Figure (2.11), the full-blown symptoms of the gastrointestinal syndrome generally occur in most mammals when exposure to doses greater than 10Gy of either gamma rays or neutrons, leading to death within 3-10 days [3]. The early symptoms are characterised by apathy, loss of appetite, vomiting, nausea and prolonged diarrhoea. Prolong diarrhoea is an indication that the patient received more than 10Gy, which is generally fatal [3]. Patients with the gastrointestinal syndrome may experience dehydration, emaciation due to weight loss, complete fatigue, bleeding, and multisystem organ failure, culminating in death within a few days [3].

The signs and symptoms of the gastrointestinal syndrome have been attributed to radiation-induced damage to the gastrointestinal tract's epithelial lining. The epithelial cell linings are susceptible to radiation as they slough off and regularly restore [3]. Within a few days of exposure, intestinal mucosa denudation causes watery diarrhoea, vomiting and depletion of electrolytes, gastrointestinal bleeding and perforation. The mucosal barrier breakdown allows the introduction of bacteria into the bloodstream [34].

Bacterial movement from the intestinal tract, shortening of villi, loss of crypts, reduced citrulline levels, abdominal pain, diarrhoea and vomiting are accepted attributes of the gastrointestinal syndrome. The movement of radiation-induced gastrointestinal damage is accompanied by haematopoietic suppression; the haematopoietic syndrome and gastrointestinal syndrome sequelae partially overlap, but they do not evolve together [35].

2.8.3 Central Nervous System or Cardiovascular Syndrome

This syndrome usually occurs at a dose greater than 20Gy, although some authors have claimed that cardiovascular syndrome symptoms can manifest at a dose greater than 10Gy [11]. This syndrome's signs and symptoms generally depend on the radiation dose level and animal species involved in the exposure. At a dose of 100Gy of neutrons, gamma rays or x-rays, the organ systems are damaged, which affect both the haematopoietic and gastrointestinal systems. Death occurs between 24 to 48 hours at this high dose due to cerebrovascular damage, and other systems' symptoms may not appear [3]. Early symptoms associated with this syndrome are severe nausea and vomiting within a few minutes [3].

The symptoms are usually accompanied by loss of coordination of muscular movement, disorientation, ataxia, dementia, delirium, diarrhoea, respiratory distress, coma, convulsive seizures and eventually death [3]. The mechanism of these symptoms may be due to direct nerve injury and indirect capillary circulation impairment. The capillary circulation damage comprises blood-brain barrier integrity and leads to intracerebral haemorrhage and intestinal oedema, resulting in brain herniation, intracranial hypertension and circulatory collapse. Injuries to the body organ due to cardiovascular syndrome are irreversible and severe, and symptoms usually constitute poor prognoses [9].

In retrospect, ARS is a term used to describe signs and symptoms arising from total-body or significant partial-body, which fails in specific organ systems leading to death within a few hours to months after exposure to radiation over a short period [37]. The management and

treatment of patients exhibiting ARS depend primarily on the doses of radiation received. Certain treatment can be prescribed if the radiation doses fall between 1Gy to 10Gy. There is no chance of survival after total-body exposure of above 10Gy doses of radiation [37]. For haematopoietic syndromes, which are signs and symptoms associated with changes in blood counts, complete blood count analysis has been prescribed to monitor the situation. Lymphocytes are known to be the most radiosensitive cell; a decrease in absolute lymphocyte count can serve as evidence of haematopoietic damage [3]. Medical management of ARS includes intravenous glucocorticoids, analgesics, electrolyte and fluid replacement [13].

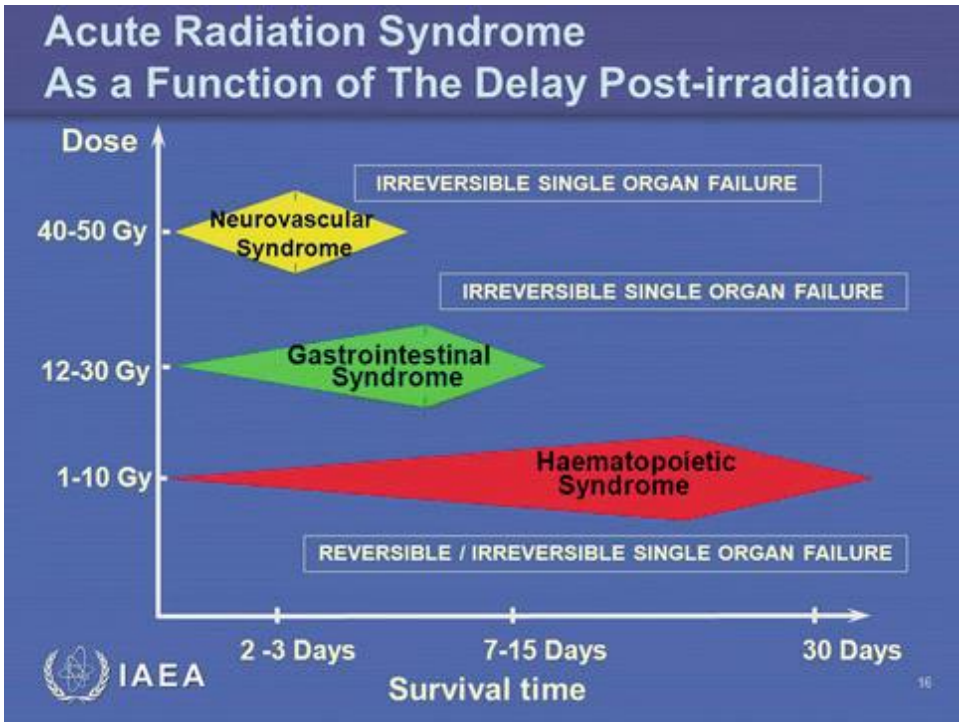

Figure. 2.11. Effects of acute radiation syndrome in different organs (Image courtesy of IAEA) [36].

2.9 Protection from radiation

Ionising radiation is ubiquitous in the environment, and it exists in different forms such as cosmic radiation from outer space, naturally occurring radioactive materials and artificial sources [24]. With the discovery of X-rays by Wilhelm C. Roentgen in 1895, the medical world

has witnessed a tremendous explosion in the wide usage of ionising radiation in diagnosis, imaging, and therapy [38]. Medical application of ionising radiation has also raised public concern about the risk of radiation exposure and how it can be curtailed. The principle of radiation protection can be traced back to the early twentieth century when radiation became a valuable tool in industries, medical diagnosis and the military. It is a technique to protect the radiation workers, public, and medical personnel against ionising radiation's hazardous effects [39].

As reported by International Labour Organization (ILO), the importance of radiation protection is to allow for an appropriate level of protection by preventing the existence of harmful deterministic effects and decreasing the likelihood of stochastic effects like cancer and genetic effects to the human population without excessively hindering the beneficial potential accrue to ionising radiation [39]. The International Commission on Radiological Protection (ICRP) recommended general principles of radiation protection as three main words: justification, optimisation, and dose limit, as detailed in their 26 and 60 reports [40]. These reports were later reviewed, and a new recommendation known as ICRP report 103 for a radiation protection system was issued in 2007 [40]. The latest report guides the basic concepts that can be used to develop effective radiological safety.

Moreover, the new report eliminated the dose limit as part of the radiation protection principle in the medical field since ionising radiation used for medical purposes at an appropriate level of dose is a necessary tool that will produce good than harm. Diagnostic reference levels are often used as a reference value instead of dose limits since medical radiation exposure has special considerations [38]. The principle of justification aims to justify the usage of radiation in treatment. This means that the use of ionising radiation as a form of treatment must be necessary before it can be adopted. Before radiation exposure, the patient and personnel seek to weigh the benefit and dangers of radiation exposure. This principle states the benefit should

outweigh the damages before the exposure can be considered [38]. The decision of radiation exposure should do more good than harm in any radiation treatment.

Based on the ICRP recommendation, there are three levels of justification for radiation protection in medicine [38]. The first and almost general level is that the use of ionising radiation in medicine is accepted as doing good than harm to society. The second justification is that a specified procedure with a specific aim must be defined and justified. This level's sole purpose is to judge if the intending radiological procedure will improve the treatment or provide the patient's necessary information. The third level says that the application should be deemed more good than harm to the exposed patient. Thus, all individual medical exposures must be justified in advance, considering the exposure's specific objectives and the patient's features [40].

The principle of optimisation of protection states that the probability of acquiring exposures, the number of individuals exposed, and the size of the individual doses should all be kept as low as reasonably achievable (ALARA), considering the socio-economic factors. This principle maximises radiation treatment using low doses while useful diagnostic imaging and therapy are not compromised. The process of designing, engineering control and implementation of procedures are critical to minimise radiation exposure through the ALARA method [41]. The third principle-the dose limit means that the dose received by any individual from regular exposure and potential exposure should not exceed the recommended safe limit as prescribed by ICRP [40].

2.9.1 Precautionary Measures of Radiation Protection

There are three necessary measures needed to limit external radiation exposure. Namely: Time, Distance and shielding material [42, 43]

2.9.2 Time

The pragmatic approach in minimising radiation exposure via an external source is to reduce the time spent within the radiation environment or radiation source. The amount of time spent in a radiation field or close to a radiation source significantly impacts the individual's exposure level [39]. It has been discovered that the longer the individual stays in a radiation environment, the greater the level of exposure or dose received [43]. The dose of radiation receives by an individual is a product of dose rate and time, given in equation 2.7. Therefore, one should spend less time in a radioactive environment to avoid damage to the body via external exposure [43].

$$Dose\ (mrem) = Dose\ Rate\ (mremhr^{-1}) \times Time(hr) \tag{2.7}$$

Thus, to limit a person's dose, one can reduce the time spent in the radiation environment. Stay time is the term that describes how long a person stays in a radiation field without exceeding the recommended limit [43]. It is calculated using equation 2.8

$$Stay\ Time = \frac{Limit\ (mrem)}{Dose\ Rate\ (mremhr^{-1})} \tag{2.8}$$

2.9.3 Distance

The external radiation exposure obeys inverse square law, which states that the intensity of the radiation (I) decreases in proportion to the inverse of the distance from the source (d) squared. This means the radiation's intensity decreases as one moves away from the source [43-45]. Inverse Square law is illustrated in equation 2.9 and Figure (2.12). When the distance between the person and the source is doubled, it will reduce the exposure level to one-quarter of its original value. Alpha and beta particles can easily be shielded by creating a reasonable distance between the source and the person at risk [45]. It is imperative always to maintain some distances when working in the vicinity of insufficiently shielded radiation sources.

$I \propto \frac{1}{d^2}$, rewritten we have

$I = K\frac{1}{d^2}$, where K is a proportionality constant of unknown value.

Thus, for an intensity I_1 at distance d_1, and another intensity I_2 at distance d_2:

$$I_1 = \frac{K}{{d_1}^2};\ I_2 = \frac{K}{{d_2}^2}$$

By eliminating K, we have

$$\frac{I_1}{I_2} = \frac{d_2^2}{d_1^2}$$

OR

$$I_1 d_1^2 = I_2 d_2^2 \tag{2.9}$$

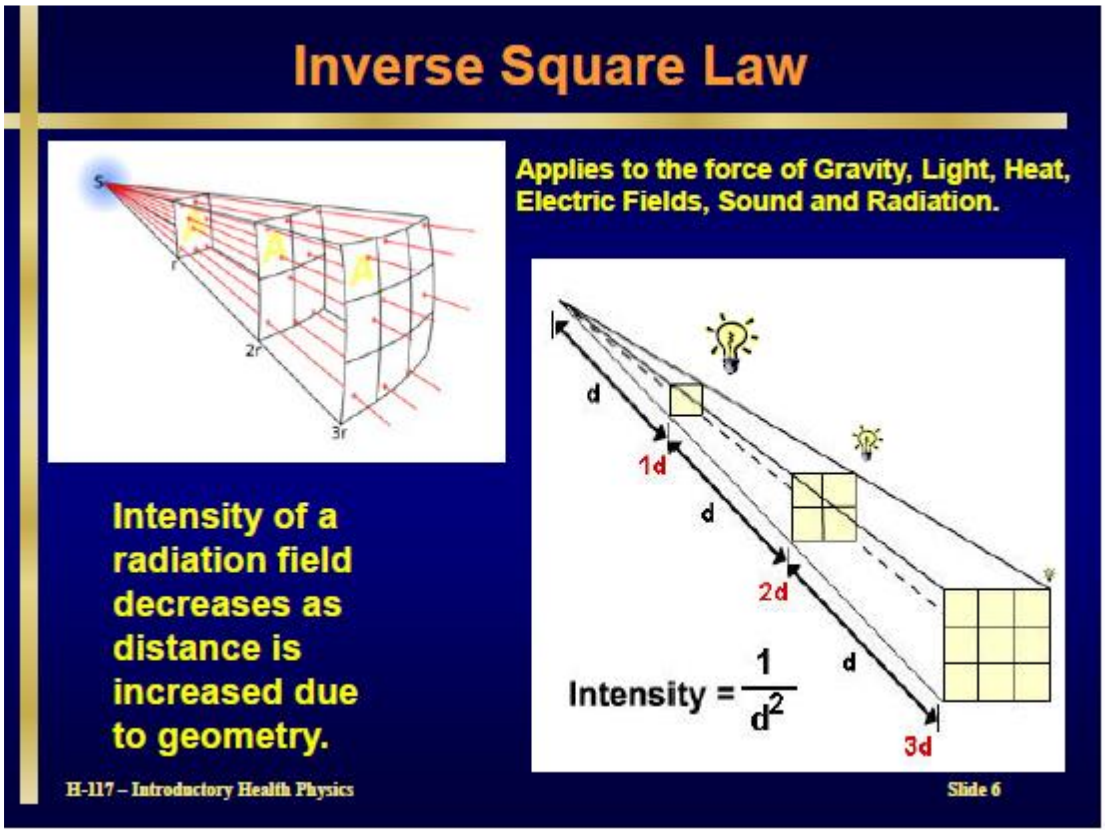

Figure 2.12: Illustration of Inverse Square law [46]

2.9.4 Shielding Material

The type of material used in the shielding process can significantly determine the extent of radiation exposure. As mentioned earlier, different kind of radiation requires additional material to shield it. For instance, gamma rays have high penetrating power and need a thick

lead block or concrete for shielding. Shielding is the process of strategically placing materials of high-density in-between the radiation source [4]. This reduces the exposure level by weakening the penetrating power. This method requires material of a high exponential coefficient for proper shielding to take place. The shielding process may involve some complex calculations using equation 2.10:

$$I = I_o e^{-\mu x} \tag{2.10}$$

where I = transmitted intensity of the radiation when it comes out from the shielding material, I_o is the incident intensity, μ is the attenuation coefficient, and x is the thickness of the shielding material [4]. The attenuation coefficient is a unique factor for every material because different materials have an additional attenuation coefficient value. Other factors determining the shielding material's suitability are radiation, the energy, frequency of emission and source configuration [45].

2.10 Radiotherapy in Cancer Treatment

Radiotherapy (RT), also known as radiation therapy, has become an excellent modality for cancer treatment [44]. More than 40% of cancer patients undergo radiotherapy either as a curative or palliative form of therapy during their illness [47, 48]. There are three main procedures by which radiotherapy can be delivered: external beam radiotherapy (EBRT), brachytherapy and injected radioisotopes [47]. EBRT occurs when the source of ionising radiation is external to the patient. Brachytherapy is a unique form of radiation treatment involving placing a radioactive source directly or near the tumour to kill or shrink it, and injected radioisotopes involve the process of swallowing radioactive liquid or having it injected into the bloodstream [24]. Many radiotherapy procedures are performed as external beam radiotherapy, with brachytherapy used for particular disease sites. Prostate, uterine, cervix, and breast cancers are also treated with brachytherapy [24]. Radiotherapy in cancer treatment aims

to damage the DNA of cancer cells so that they no longer divide and grow and spare or with as little harm as possible to the surrounding healthy cells [24].

2.10.1. Medical Linear Accelerator in Radiotherapy

The operational use of linear accelerator (LINAC) began treating its first patient for cancer at Hammersmith Hospital, with an 8MV machine manufactured by Metropolitan-Vickers in 1953 in London [49]. A year later, in 1954, a 6MV linac was built in Stanford, the USA, which began treatments in 1956. There were seven clinical LINACs in the world at that time. Since then, linear accelerators have increased in popularity to the point that they now number in the thousands [49, 50]. LINAC consists of four main components- a modulator, an electron gun, a radio frequency (RF) power source or photocathode and an accelerator guide (Figures 2.13 & 2.14).

The electron gun house the cathode that generates high-energy electrons via thermionic emission. The electrons produced are then made to pass through radio frequency, where they are accelerated towards a target (anode). A different target can be used depending on the accelerated particles. For instance, for the production of x-rays, electrons are accelerated towards a tungsten target [51]. The x-rays produced are then collimated to form a beam that matches the patient's tumour. The collimated beam comes out of a gantry, which revolves around the patient, as illustrated in Figure (2.13). The patient is positioned on a moveable therapy couch, and lasers are used to ensure the patient is adequately aligned before treatment. The gantry is free to rotate during treatment, and radiation can be delivered to the tumour from different angles [51].

Fig. 2.13: Varian Linear Accelerator (Model: Clinac 2100C) Adapted from DOTMED [52]

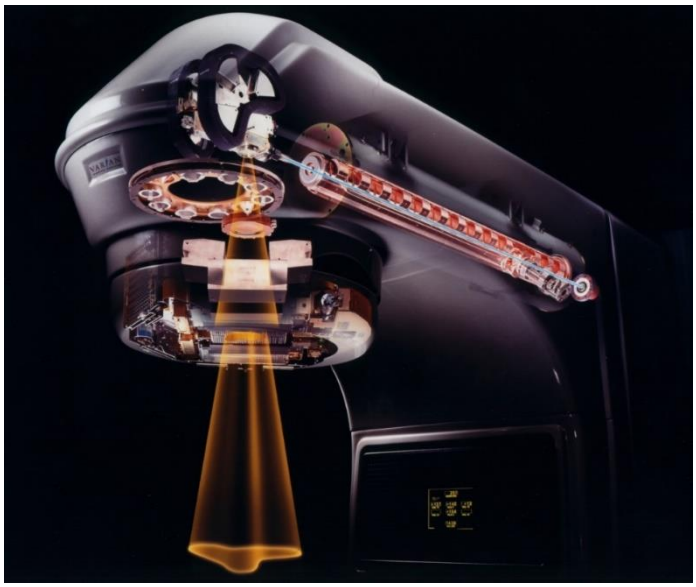

Figure 2.14: Inside a Varian linear accelerator [55]. Adapted from Varian Medical Systems, Inc. [53]

2.10.2. Whole-body irradiation treatment technique

The whole-body irradiation (WBI), also referred to as total body irradiation (TBI), is a technique used in radiotherapy to treat various benign and malignant diseases such as leukaemia, lymphomas and some solid tumours [54]. Whole-body irradiation involves exposing the entire body to radiation. It has become a prevalent form for the treatment of leukaemia before haematopoietic stem cell transplantation. WBI aims to deliver a uniform and

accurate dose to the whole body within ±10% of the recommended dose, including the immune system, circulating malignant cells, and skin [55]. The WBI method should allow precise and well-controlled partial shielding of the brain, eye, kidney and lung. In addition, it should be repeatable, dependable, and comfortable for both patients and staff, and it should be intended to fit readily into the usual treatment environment [55, 56].

2.11 Evaluation of Radioprotective Efficacy of Medicinal Plants and Herbs

The evolution of radioprotectors from natural plants can be traced back to Indian traditional medicine [57]. This traditional medicine known as *Ayurveda* provides a detailed account of various diseases and treatments. The more significant drug and drug conceptualisation used in *Ayurveda* are primarily derived from herbs and plants [57].

Natural plants' efficacy to act as radioprotectors mostly depends on the active components present in such plants, including antioxidants, phytochemicals, immune-stimulants, and compounds capable of destroying disease-causing microorganisms' growth [57, 58]. Natural antioxidants have been discovered to protect against ionising radiation injury by reducing oxidative damage [58].

Detailed experimental work on the antioxidant property and free radicals of natural plants can also supply information concerning the radioprotective efficacy of such plants. The natural plant can inhibit lipid peroxidation and scavenge free radicals to act as a potential radioprotector [59]. Once its scavenging ability has been established, the next thing will be to assess its radioprotective potential *in vitro* using cell survival and chromosome damage. If the test reveals its potential to increase cell survival and decrease radiation-induced damage, it is a potential radioprotector [59].

All the same, globally, research has shown that animal studies with death as a humane endpoint are the most reliable way of confirming a drug's radioprotective potential [60]. This is because 30-day survival after exposure to a lethal dose of whole-body ionising radiation vividly reveals

the ability of the drug to mitigate radiation effect, aid in the recovery of gastrointestinal and haemopoietic cells in the bone marrow, which happen to be the most radiosensitive organs responsible for the maintenance of life [60].

Moreover, the dose reduction factor (DRF) method has shown to be the most dependable technique in determining the 30-day survival in mice and rodents [59]. This technique is achieved by irradiating mice or rats with or without giving the radioprotective drug at an interval of radiation doses (e.g. Lethal dose (LD)) and comparing the endpoint of concern. For instance, the DRF for 30-day survival ($LD_{50/30}$ animal treated with drug divided by $LD_{50/30}$ animal taking as control) measures the protection of the bone marrow system [61]. When there is a significant loss of bone marrow cells, it could lead to death due to infectious, anaemia and haemorrhage.

In addition, a lesser approach can also be to determine the Gastrointestinal (GI) syndrome in mice by evaluating the survival up to ten days after exposure to equal doses of whole-body ionising radiation. The latter method is quite different from a haemopoietic syndrome, which can only be assessed by the 30-day survival of mice [60]. The common illuminating approach to preclinical studies about the radioprotective effects of drugs using the animal model is the intestinal crypt cell assay [62]. Different endpoints exist to determine the efficacy of radioprotectors in clinical practice; the most promising one is to evaluate the protective effect of the drug against head and neck radiotherapy treatment and associated side effects [63].

2.11.1 Plants and Herbs as Radioprotectors

Over the decades, several plants and herbs have been screened for their radioprotective potential, some of which are presented in Table 2.1. The practical way to assess plants and herbs' radioprotective efficacy is to examine the substance's various features. When a plant has anti-inflammatory, antimicrobial, antioxidant, free radical scavenging, immunomodulatory,

etc., such a plant can act as a potential radioprotector. It will be proper to evaluate its radioprotective activity. Several studies have been performed to verify some of these claims.

An experiment conducted by Shobi and Goel [64] on Sprague Dawley male rats using an aqueous extract of *Centella asiatica* revealed that the plant could prohibit radiation-induced body loss though at a given low radiation dose. Further research using this extract at a much higher radiation dose (8Gy) showed the plant's ability to protect mice against radiation-induced weight loss [65].

The life span of rats given an oral gavage of a *Hippohae rhamnoides* fruit juice before and after exposure to ionising radiation increased significantly. There was a restoration in the 11-oxycorticosteroid level in the blood [66]. The various changes observed in the haematological parameters, decrease in the endogenous colony-forming unit and micronuclei formation in mice exposed to gamma radiation showed that the ethanolic extract of berries of *Hippohae rhamnoides* could protect against radiation-induced damage [66].

In the Indian traditional medicine system called *Ayurveda*, the rhizome of ginger has found a prominent role in herbal preparations. It is also commonly used as a spice and flavouring agent worldwide. This plant has been reported by *Ayurveda* medicine to possess various medicinal properties [67]. For instance, folklore medicine claimed the rhizome of *Zingiber officinale*, popularly known as ginger, can be used to treat dyspepsia, pharyngoplasty, vomiting, flatulence, cephalalgia, cough, cardiopathy, inflammation, asthma, otalgia, dropsy, colic, diarrhoea, cholera, nausea and elephantiasis [67].

The radioprotective potential of hydroalcoholic extract of ginger rhizome was also conducted on mice. The study found that the extract of ginger increased the 30-day survival rate of mice. It offered protection against radiation-induced sickness and mortality by scavenging free radicals and improved antioxidant status. It also reduced lipid peroxidation. The treatment of

ginger (ZOE) protected mice against gastrointestinal-related deaths as well as bone-marrow-related deaths. The experiment revealed the dose reduction factor to be 1.2 [67].

Moreover, mint (*Mentha arvensis*) is a common plant-primarily cultivated in India and widely used as food spices, a remedy for a diverse ailment in various homes and a great ingredient in industries [68]. Several claims have been made by Indian traditional medicine that the mint plant possesses an enormous amount of medicinal value. The leaves are mainly used as stimulant, dentrific, febrifuge, antispasmodic, contraceptive and deodorant agents [68]. Among other ailments cured by the mint reported by folklore traditional medicine are diarrhoea, cough, skin diseases, hepatopathy, jaundice, peptic ulcers, halitosis, wounds cuts, fever, and common weakness of the body [68].

Jagetia and Baliga [68] evaluated the radioprotective potential of *Mentha arvensis* as shown in (Table 2.1); their study revealed that the mint extract offered significant protection against radiation-induced sickness and reduced mice's mortality, as demonstrated on the 30-day survival. The protection provided by mint might be the presence of eugenol, flavonoids and terpenes. The free radical scavenging, correctly repair of DNA and antioxidant status predominantly present in *Mentha arvensis* was responsible for the radioprotective capacity observed in the plant [68].

In another related development, Gowda et al. [69] reported the efficacy of *Nardostachys Jatamansi* against radiation-induced haematological damage in rats (Table 2.1). The study indicated that rats were treated with the root extract of *Nardostachys jatamansi* for 15 days before and after exposure to electron beam radiation. The haematological studies on the exposed rats revealed that the root extract of *Nardostachys jatamansi* exhibited a weighty time-dependent elevation in all the blood samples parameters. This extract's mechanism of protection was shown in modulating the radiation-induced damage on the haematological

parameters. The result further indicated a possibility of Nardostachys jatamansi being used as a good blood-booster [69].

In Nigeria, a few works have been carried out on the radioprotective effects of plants and herbs. For instance, Adaramoye et al. [70] reported the protective effects of the extracts of *Vernonia amygdalina*, *Hibiscus sabdariffa* and Vitamin C against radiation-induced liver damage in rats (Table 2.1). The result indicated that the treatment of the extracts of *Vernonia amygdalina*, *Hibiscus sabdariffa* and Vitamin C on rats for four weeks before irradiation significantly reduced serum lipid levels peroxidation. They concluded that the mixture of the three extracts could increase the antioxidant mechanism and particularly protect animals against radiation-induced liver damage [70].

Similarly, Owoeye et al. [71] reported the antioxidant and radioprotective properties of *Vernonia amygdalina* leaf extract against gamma radiation-induced damage in rats' brains. The methanolic extract of *Vernonia amygdalina* was applied to albino rats fourteen days before irradiation. The biochemical assays such as lipid peroxidation (LPO), superoxide dismutase (SOD), glutathione (GSH), protein concentration were carried out; with the treatment of the extract, the study revealed an improvement in the histological changes in the cerebellar layers of the treated animals when compared with the control (untreated) animals. The report also indicated that the extract of *Vernonia amygdalina* demonstrated the antioxidant and radioprotective properties by mitigating the lipid peroxidative response of the brain's tissue to radiation treatment. This activity could be the free radical scavenging ability of the plant extract [71].

Table 2.1: Some plants and herbal formulations with radioprotective property

S/N	Plant Name	Common Name	Dose of Extract Administered	Radiation type and dose	Use in radioprotection	Reference
1	*Abana (a herbal preparation)*	-	20 mg/kg pretreatment on mice for 5 days	γ-radiation, 10Gy	Protected against radiation-induced sickness and mortality in mice	[60]

2	*Aegle marmelos*	Bael	20 mg/kg pretreatment on mice for 5 days	γ-radiation, 6-11Gy	Offered protection against radiation-induced sickness and mortality in mice	[67]
3	*Aloe vera*	Indian aloe	750 mg/kg pretreatment on mice for 15 days	γ-radiation, 8Gy	Offered protection against radiation-induced sickness and mortality in mice	[72]
4	*Black seed oil*	-	400mg/kg pretreatment on rats for 25 days	γ-radiation, 6mGy/h	Reduced oxidative stress on haematological parameters in irradiated rats	[73]
5	*Centella asiatica*	Brahmi	100 mg/kg pretreatment on rats for once	γ-radiation, 2Gy	Protected against radiation damage to DNA and membranes in vivo and in vitro	[74]
6	*Garcinia kola seeds*	-	250 mg/kg pretreatment on rats for 6 weeks and post-treatment for 8 weeks	γ-radiation, 5Gy	Protected against γ-radiation-induced oxidative damage in rats' brain	[75]
7	*Grapeseed*	-	200 mg/kg pretreatment on mice for 1 hour	γ-radiation, 3Gy	Provided radioprotective effect in mouse bone marrow	[76]
8	*Hibiscus sabdariffa*	Red roselle	200, 400 and 800 mg/kg pretreatment on rats for 4 weeks	γ-radiation, 4Gy	Offered protection against radiation-induced liver damage in rats	[70]
9	*Hippophae rhamnoides (a herbal preparation)*	Sea buckthorn	30 mg/kg pretreatment on mice for once	γ-radiation, 10Gy	Offered protection against radiation-induced genomic DNA and mitochondrial damage	[66]
10	*Liv 52 (a herbal formulation)*	-	500 mg/kg pretreatment on mice for 7 days	γ-radiation, 7-12Gy	Reduced genotoxic and lethal effects of gamma radiation-induced in mice	[57]
11	*Mentha arvensis*	Mint	10 mg/kg pretreatment on mice for 5 days	γ-radiation, 6-12Gy	Offered protection against radiation-induced sickness and mortality in mice	[68]
12	*Mentha piperita*	Peppermint	1 g/kg pretreatment on mice for 3 days	γ-radiation, 8Gy	Offered protection against radiation-induced haematopoietic and testicular damage in mice	[77]
13	*Moringa oleifera*	Drumstick	150 mg/kg pretreatment on mice for 5 days	γ-radiation, 4Gy	Protected against radiation-induced oxidative stress in mice	[78]
14	*Nardostachys jatamansi*	-	400 mg/kg pretreatment on mice for 15 days	Electron beam irradiation, 3Gy	Provided protection against radiation-induced damage on haematopoietic system in rats	[69]
15	*Ocimum sanctum*	Tulsi	10 mg/kg pretreatment on mice for 5 days	γ-radiation, 4.5Gy and 11Gy	Offered protection against radiation-induced sickness and mortality in mice	[79, 80]
16	*Piper longum*	Indian Long Pepper	400 mg/kg pretreatment on mice for 5 days	γ-radiation, 6Gy	Offered protection against radiation-induced haematological damage in mice	[81]
17	*Amaranthus paniculatus (Rajgira)*	Amaranth	800 mg/kg pretreatment on mice for 15 days	γ-radiation, 6, 8 and 10Gy	Provided protection against radiation-induced sickness, mortality and maintained LPO and GSH levels in blood and liver.	[82, 83]

18	*Syzygium cumini (jamum)*	-	80 mg/kg pretreatment on mice for 5 days	γ-radiation, 6-11Gy	Offered protection against radiation-induced DNA damage in mice	[84]
19	*Tephrosia purpurea*	-	200mg/kg pretreatment on mice	γ-radiation, 5Gy	Offered protection against radiation-induced haematopoietic damage in mice	[85]
20	*Terminalia chebula*	Black myrobalan	25-200 mg/ml pretreatment on Pbr322 plasmid DNA for a day	γ-radiation, 6Gy	Prevented radiation-induced damage to DNA in lymphocytes	[86]
21	*Tomato Seed Oil*	-	1 ml/kg pretreatment on rats, 3 times/week for 8 weeks	γ-radiation, 6Gy	Protected against radiation-induced oxidative stress and suppressed systemic inflammation	[87]
22	*Tragia involucrata*	-	100 mg/kg pretreatment on mice for 5 days	γ-radiation, 6Gy	Reduced radiation-induced oxidative stress in mice	[88]
23	*Vernonia amygdalina*	Bitter leaf	200, 400 and 800 mg/kg pretreatment on rats for 4 weeks	γ-radiation, 4Gy	Increased antioxidant defence systems and protected against radiation-induced liver damage in rats	[70, 71]
24	*Xylopia aethiopica*	African guinea pepper	250 mg/kg pretreatment on rats for 6 weeks and 8 weeks	γ-radiation, 5Gy	Increased antioxidant defence systems and protected against radiation-induced kidney and liver damage in rats	[89]
25	*Zinger officinale*	Ginger	10 mg/kg pretreatment on mice for 5 days	γ-radiation, 6-12Gy	Offered protection against radiation-induced sickness and mortality in mice	[90]

2.12. Haematology

The clinical study of blood and blood-forming organs in the body is known as Haematology [91]. This study also includes the diagnosis, treatment and control of diseases of the blood, immunologic, vascular system, bone marrow and haemostatics. This study involves analysing and treating animal diseases [91]. Blood belongs to a particular group of fluids that plays an essential function in physiological processes. It has two primary constituents. The first constituent is the blood cells made up of forty-five per cent of the blood, and the second constituent is plasma, which constitutes the remaining fifty-five per cent [92].

Blood has four main cellular components: plasma, red blood cells or erythrocytes, white blood cells or leukocytes, platelets, or thrombocytes (Figure 2.15) [92]. As illustrated in Figure 2.15, blood stem cells (immature cells) are produced in the bone marrow, developing into mature blood cells over time. A blood stem cell can differentiate into a myeloid or lymphoid stem cell.

A myeloid stem cell develops into three types of mature blood cells: white blood cells, red blood cells and platelets. A lymphoid stem cell transforms into a lymphoblast cell made up of the three types of white blood cells: B lymphocytes, T lymphocytes and natural killer cells [92].

The white blood cells are either characterised into granulocytes made up of neutrophils, eosinophils, basophils, or agranulocytes, which are lymphocytes and monocytes [93]. Red blood cells can transport oxygen from the lungs to the tissues and carbon dioxide from the tissues back to the lungs. White blood cells protect the body against infection. Platelets are fragments of cells that ease the creation of blood clots at the site of injury. The formation of platelets at the site of any damage prevents blood leakage [91].

Blood also regulates water balance and body temperature and transporting hormones, beneficial metabolites, absorbed food components, and waste products to the body's many tissues and organs [92]. The production of the cellular components of blood occurs in the bone marrow (haematopoietic) stem cells. This process is referred to as haematopoiesis [91]. The analysis of blood indices has proven to be a valuable approach for analysing farmed animals' health status. These indices provide reliable information on metabolic disorders, deficiencies, and chronic stress status before being present in a clinical setting [94].

Using the haematological technique to identify and treat animal diseases has become essential as it offers sensitivity and often requires a small volume of samples [95]. The acceptable haematology parameters for toxicity studies in experimental animals include erythrocyte count, total leukocyte count, erythrocyte morphology, differential leukocyte count, platelet count, haemoglobin concentration, packed cell volume, mean corpuscular haemoglobin concentration and mean corpuscular volume [95].

2.13 Histopathology

The word histopathology is a combination of three words; 'histo' deals with tissues, 'pathos' is the study of disease, and 'logos' means study. Thus, histopathology is the microscopic study of tissue disease to examine the presence of infections [96]. It is the branch of pathology concerned with the tissue changes characteristics of the disease [96]. The beginning of the 19th century witnessed the theory proposed by Rudolf Virchow- the acclaimed father of modern pathology- where he suggested that injury to the cell is the origin of all disease, and disease is defined as disparities or harmful effects of humour on specific organs [96]. Histopathology is the study of structural and functional disorders that manifest as organ and system diseases. It is a bridging discipline that encompasses both basic science and clinical practice. In addition, it deals with the study of disease-related structural and functional alterations in cells, tissues, and organs [96].

Haematopoietic stem cell differentiation

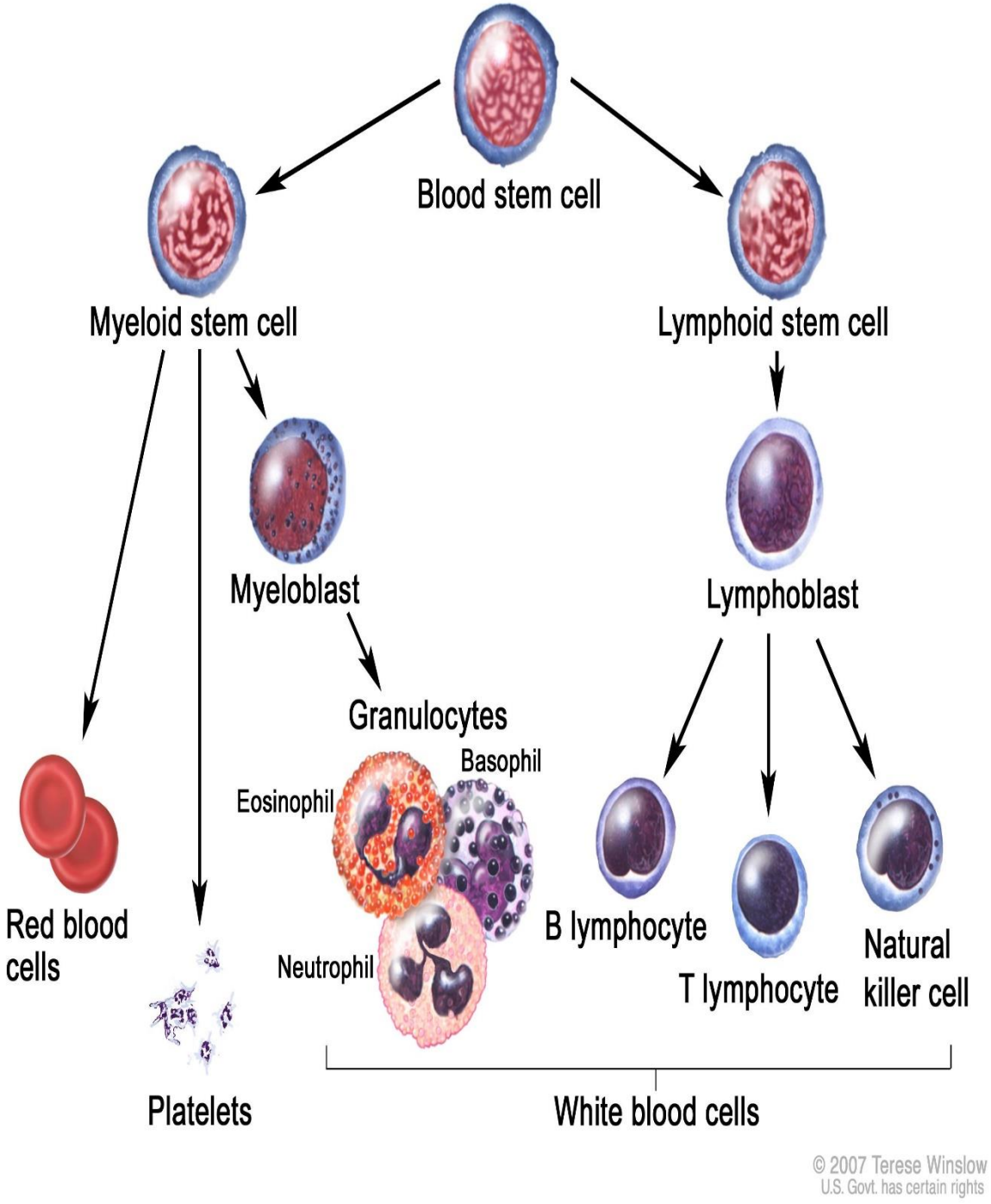

Figure 2.15: Developmental stages of blood stem cells to become mature cells [97].

Figure Adapted from National Cancer Institute [97]

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Plants collection and identification

Drymaria cordata (DC) leaves were harvested at local farmland at the University of Ibadan campus, South-West Nigeria, in July 2019. While the rhizome, stem and leaves of *Costus afer* (CAE) were harvested from uncultivated farmland at Ikole-Ekiti in Ekiti State, South-west, Nigeria, in December of the same year. A Botanist (Mr Esinekhuai Donatus) at the Herbarium, Department of Botany, the University of Ibadan, Nigeria, where voucher specimen number UIH-22932 and UIH-22933 for CAE and DC, respectively, were deposited, made the botanical identification and authentication of the two plants.

3.1.1 Extracts preparation

The collected fresh samples of CAE and DC plants were hand-searched mechanically to ensure they were pest-free. They were also cleaned with tap water and air-dried at room temperature for a few days. After that, the dried plants were pulverised in a mechanical and electric grinder at the Biomedical Research Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal (UKZN) Pietermaritzburg campus. CAE's powder material (638.03g) was macerated in 3.75litres of high quality (99.9%) methanol for 72 hours at room temperature. In addition, 433g of DC was macerated in 2.5litres of absolute ethanol for the same hours at the same physical condition. The macerated solution was shaking intermittently to ensure thorough mixing. The maceration of both plants was done two different times.

The combined extracts in each case were filtered using Whatman No. 1 filter paper under vacuum filtration. The filtrate of CAE and DC was evaporated using a rotary evaporator at the Department of Chemistry's Laboratory, University of KwaZulu-Natal Pietermaritzburg campus to remove all traces of methanol and ethanol. Figure 3.1 shown the rotary evaporator

during the evaporation process. An approximate 4.5% yield of methanol extract of CAE and 3.5% yield of ethanol extract of DC was obtained. The dried extracts were placed separately in an airtight container, stored in the refrigerator at 4°C and protected directly from light until the time of use.

Figure 3.1: Rotary evaporator at the Department of Chemistry's Laboratory, University of KwaZulu-Natal Pietermaritzburg campus removed all traces of methanol and ethanol from CAE and DC, respectively.

3.2 Experimental design

The experimental design involved animal care and selection, administration of extracts and acute toxicity of the extracts, which are discussed in detail as follows:

3.2.1 Animal care and selection

The procedure adopted in this study conformed to the National Institute of Health (NIH) guidelines for laboratory animal care and used in biomedical research [1]. In addition, the Animal Research Ethics Committee (AREC) of the University of KwaZulu-Natal approved the protocol used in the present study with a protocol reference number: AREC/026/019D.

One hundred and fourteen (114) BALB/c mice (male: 54; female: 60) weighing 36-40g; 12 - 13 weeks old were used for this study. The animals were grouped into two batches; fifty-four (54) male mice for the CAE experiment and sixty (60) female mice for the DC study. The animals were inbred at the Animal House of School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg campus. They were kept in the animal house throughout the study and were given free access to a standard diet and clean water *ad libitum.* The experimental animals were humanely handled and kept inside clean, well-ventilated transparent plastic-type IV cages with wood shavings and naturally illuminated animal rooms. Animals were maintained under controlled conditions of temperature (23°C - 25°C) with 12 hours light and dark cycle. Behavioural enrichment in the mouse cages in the form of egg boxes and shredded paper were provided.

The mice were randomised into six (6) different treatment groups for each experiment. The CAE experiment contained nine animals in each group, and DC had ten animals per group. The grouped animals were allowed some days to acclimatise to animal room conditions before treatment commenced. The details of grouping and treatment are presented in Tables 3.1 and 3.2. All animals were examined, and clinical signs were recorded daily before and after dosing during the treatment period. The mass of the animals was recorded prior, during and after treatment.

3.2.2 Administration of extracts

The administration of the extract was performed in two phases. The first phase was for the CAE plant, and the DC extract was administered during the second phase. We had a control group and experimental groups in each stage, as shown in Tables 3.1 and 3.2. Briefly, in the first phase of the experiment (Table 3.1), animals in the group CAE, CAE_3Gy, and CAE_6Gy received 250mg/kg body weight of CAE for six days before radiation exposure, while mice in

group CNT and (IR_3Gy & IR_6Gy) served as unirradiated and irradiated control, respectively.

Similarly, for the second phase of the experiment (Table 3.2), animals in the group DC, DC_4Gy and DC_8Gy received 250mg/kg body weight of DC extract for thirteen days before irradiation. Furthermore, animals in group CNT and (IR_4Gy & IR_8Gy) served as unirradiated and irradiated control, respectively. Animals in both phases received food and clean water *ad libitum* throughout the experiment. The treatment plan and various groups are shown in Table 3.1 and 3.2 for CAE and DC extract. Figure 3.2 shows the animals in the cage during the treatment period.

Table 3.1: Oral administration of CAE on animals

Group code	Treatment
CNT	Control (Un-irradiated)
CAE	Animals treated with 250mg/kg bodyweight only (Un-irradiated)
IR_3Gy	Irradiated (3Gy) animals only
IR_6Gy	Irradiated (6Gy) animals only
CAE-3Gy	Irradiated (3Gy) animals treated with 250mg/kg body weight
CAE-6Gy	Irradiated (6Gy) animals treated with 250mg/kg body weight

CNT, control; CAE, *Costus afer* extract at 250mg/kg; Gy, Gray (radiation unit); IR, ionising radiation.

Table 3.2: Oral administration of DC extract on animals

Group code	Treatment
CNT	Control (Un-irradiated)
DC	Animals treated with 250mg/kg bodyweight only (Un-irradiated)
IR_4Gy	Irradiated (4Gy) animals only
IR_8Gy	Irradiated (8Gy) animals only
DC_4Gy	Irradiated (4Gy) animals treated with 250mg/kg body weight
DC_8Gy	Irradiated (8Gy) animals treated with 250mg/kg body weight

CNT, control; DC, *Drymaria cordata* extract at 250mg/kg; Gy, Gray (radiation unit); IR, ionising radiation.

3.2.3 Acute toxicity study

Twenty male and twenty female mice were used for the toxicity test. In each phase of the experiment, the mice were divided into four groups of five animals in each group. The acute toxicity test of CAE and DC extract was determined over a 14-day observation period. Each extract of CAE and DC was administered by oral gavage at doses of 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg body weight. The mice were observed for 14 days for signs of acute toxicity and death [2]. The oral administration of both extracts did not produce death or toxic effect in the treated groups during the 14 days observation period. The median dose (250mg/kg) for each extract was chosen and used for further studies in this work.

Figure 3.2: Mice at the Animal House, School of Life Sciences, UKZN, Pietermaritzburg campus during the treatment period

3.3 Irradiation Procedure

The exposure of mice to radiation was done at the Department of Radiotherapy and Oncology, Grey's Hospital Pietermaritzburg. The radiation facility used was Linear Accelerator (LINAC), which generates high-energy X-rays and beams of electrons collimated to treat cancer patients. For the first phase of the experiment, the irradiated groups were exposed to whole-body irradiation X-ray radiation doses of $3Gy$ and $6Gy$ at a dose rate of 400MU/min under a standard

condition of 100 monitor units (MU) = 1Gy. The source to a surface distance of 90 cm at a depth of 10 cm was used for the irradiation. The field size of 40 cm by 24 cm was found suitable for the irradiation process. Immediately after the irradiation, the mice were separated into different cages and transferred back to the animal house.

Similarly, for the second phase of the experiment, a total of 40 mice were exposed to 6MV photons from LINAC, with the irradiated groups receiving a whole-body X-ray radiation dose of 4Gy and 8Gy at a dosage rate of 400MU/min under the standard condition of 100 monitor units (MU) = 1Gy. A source to a surface distance of 85 cm at a depth of 15 cm was adopted for the irradiation. The field size of 30 cm by 25 cm was found suitable for the irradiation process. Immediately after the irradiation, the mice were returned to their cages and transferred back to the animal house, where they were monitored every day for the manifestation of radiation-induced illness and mortality. Figures 3.3 & 3.4 represent the setting of the animals' cage on the treatment couch before irradiation.

The rationale for the choice of radiation doses is to evaluate the hematopoietic syndrome (bone marrow syndrome) on the irradiated mice, which usually occur with whole-body irradiation of the dose range between 0.7 and 8Gy [3]. This radiation dose range can produce radiation-induced haematological 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 [4]. The reports of Jagetia et al. [5], Krishna and Kumar [6], El-Desouky et al. [7], Yamamori et al. [8] suggest other studies where similar radiation dose ranges have been used.

Figure 3.3: Setting of the animals' cage on the treatment couch before irradiation.

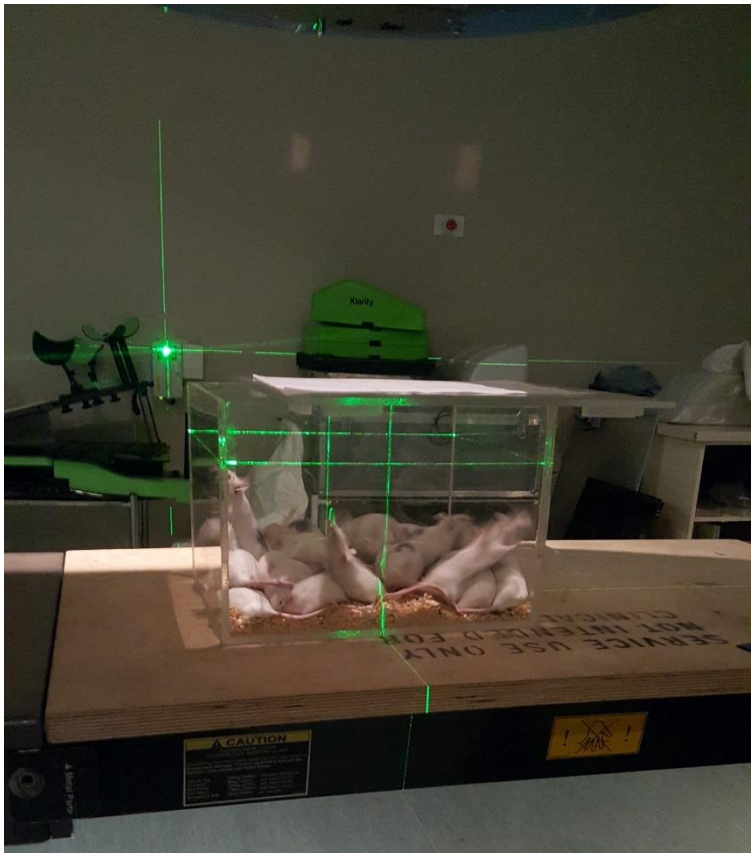

Figure 3.4: Positioning the animals' cage on the treatment couch via the laser light before irradiation.

3.4 Body mass and relative organ mass

The animals' body mass was recorded on the day they were randomised into different groups and every day during the pretreatment process. These served as the initial masses. Two mice from each group were euthanised by cervical dislocation 48 hours after irradiation for the CAE experiment. The mice's visceral organs (kidney and liver) were surgically removed, rinsed in 0.9 % normal saline, blotted with filter paper, weighed. The relative organ mass was calculated and expressed as a percentage of the body mass.

$$Relative\ Organ\ Mass = \frac{Absolute\ organ\ mass\ (g)}{Body\ mass\ of\ mouse\ on\ the\ day\ of\ sacrifice(g)} \times 100$$

3.5 Determination of haematological parameters for CAE

Forty-eight (48) hours after the irradiation, all the animals were euthanised by cervical dislocation, and blood samples were collected from them. A 23-gauge needle was used to collect blood from the heart's Posterior Vena Cava and a 1 ml syringe into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles with anticoagulant for haematological analysis (Figure 3.5). The red blood cell (RBC), packed cell volume (PCV), total white blood cell count (WBC), haemoglobin (Hb), Lymphocytes, Neutrophils, Monocytes, Eosinophils and Platelet count values were determined using the Sysmex XE-2100 Haematology Automated Analyser machine.

3.6 Histopathology Examination for CAE

Shortly after collecting blood samples on 48-hour post-irradiation, two mice were taken from the euthanised animals, and their visceral organs (kidney and liver) were harvested. The mass of the fresh livers and both kidneys were determined (measured in grams), after which they were fixed in 10% buffered formalin. Two hours post immersion in formalin, the tissues were dehydrated in an ascending grade of ethanol, cleared in xylene and embedded in paraffin wax. Serial sections of 4μm thick were obtained on glass slides using a rotary microtome. The

deparaffinised sections were stained routinely with Haematoxylin and Eosin (H and E) and mounted. All sections were examined with a standard light microscope (Olympus) and scanned digitally by an Asperio C52 (Leica). Images and sections were evaluated under 10X, 20X and 40X magnification. Photographs were taken from the digitally scanned slides with Image Scope Software (Leica) and stored as jpeg image files.

3.7 Determination of haematological parameters for DC extract

Five days and fifteen days after irradiation, three mice in each group (n=3) were sacrificed by cervical dislocation and blood 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, which contained anticoagulant for haematological analysis (Figure 3.5). Similarly, thirty days after irradiation, the surviving mice in each group were sacrificed, as mentioned above. The haematological parameters analysed include Erythrocyte (RBC), Haematocrit (HCT), Leukocyte and Platelet (PLT). The haematocrit was analysed using the microhaematocrit method. Simultaneously, the RBC detector counts the Erythrocytes, PLTs and Leukocytes via the Hydro-Dynamic Focusing (DC Detection) using the Sysmex XE-2100 Haematology Automated Analyser machine. The Hydro-Dynamic Focusing method improves blood count accuracy and repeatability. In addition, because the blood cells pass through the aperture in a line, it prevents abnormal blood cell pulses.

3.8 Statistical Analysis

One-way analysis of variance (ANOVA) was employed to examine the haematological parameters, followed by Tukey's multiple comparison test, which compared all treatment groups to the control group. SPSS 20.0® statistical package was used for the analysis. Results are reported as means ± SEM (standard error of the mean), and $p<0.05$ were considered significant values.

Figure 3.5: Collection of blood samples for haematological analysis.

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CHAPTER FOUR

RADIOPROTECTIVE POTENTIAL OF *COSTUS AFER* AGAINST THE RADIATION-INDUCED HEMATOLOGICAL AND HISTOPATHOLOGICAL DAMAGE IN MICE

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This chapter is a published journal article that studied the possible radioprotective efficacy of *Costus afer* extract (CAE) against whole-body radiation-induced haematological and histopathological disorder in mice exposed to double doses of X-ray radiation based on reported folklore medicinal use.[1]

[1] Akomolafe IR, Chetty N. Radioprotective potential of *Costus afer* against the radiation-induced hematological and histopathological damage in mice. Radiat Oncol J 2021:39(1):61-71

4.1 Abstract

Purpose: This study investigated the possible radioprotective effect of *Costus afer* extract (CAE) on haematological and histopathological parameters of mice.

Materials and methods: Fifty-four male mice with a mass between 37-43g, 11-13 weeks old, were used for this study. We divided the mice into six different groups containing nine animals, then further sub-divided into irradiated and un-irradiated groups. Animals received 250mg/kg body weight extract of CAE by oral gavage for six days in addition to feeding and water *ad libitum*. Animals in the irradiated group were exposed to radiation at the Department of Radiotherapy and Oncology, Grey's Hospital, using a linear accelerator. Blood samples were collected at 48-hrs post-irradiation for the haematology test followed by histopathology examination of the kidney and liver.

Results: Our findings revealed that 3Gy and 6Gy doses of X-ray radiation caused a significant reduction in the white blood cell, packed cell volume, haemoglobin, neutrophils, lymphocytes, eosinophils and platelet counts compared with the control group. However, the administration of CAE before irradiation significantly increased the mentioned parameters. There was no increase in red blood cells and monocyte among treated groups compared with the control. Histopathological changes in the kidney and liver sections revealed that no visible lesion in the pretreated mice. Hepatocytes seem to be within normal histological limits.

Conclusions: This study concludes that CAE offered some protection against radiation-induced haematological alterations, but there was no significant improvement in the histopathological parameters. Thus, further studies are needed to validate its radioprotective effect on histopathological variables.

Keywords: Radiation protection, Pathology, Radiotherapy, Linear accelerator, Hematology, Cancer

4.2 Introduction

The usage of ionising radiation for medical purposes has increased significantly over the last few decades, which has increased the cancer risk associated with this increased utilisation of ionising radiation [1]. Radiation exposure can cause measurable injuries to the hematopoietic, gastrointestinal and central nervous systems, contingent on radiation exposure doses [2]. This exposure can also lead to water's radiolysis, which produces reactive oxygen species like free radicals and hydroxyl ions. Due to the presence of unpaired electrons, free radicals are very active. They can damage biological molecules such as deoxyribonucleic acid (DNA), protein and membrane lipids, resulting in biologic and cellular damage [2, 3].

In assessing the degree of radiation exposure to the body, changes in blood values have been established as being an advantage. The haematopoietic system, consisting of bone marrow and lymph tissues, has been described as the body's most radiosensitive organ [4]. Two modes of treatment for cancer are predominant aside from surgery. The first is chemotherapy, which uses cytostatic drugs. Radiotherapy is the other. Radiotherapy is the medical use of ionising radiation in cancerous cells' treatment [5, 6]. However, one significant challenge in cancer cells' radiotherapy is the exposure of surrounding tissues to undesirable radiation doses, leading to biological damage [5]. The need to develop drugs that can reduce the deleterious and harmful effects of radiation and perform reproductive functions becomes vital.

The year 1948 marked the hallmark in the discovery of a compound that offers protection against radiation. The discovery aroused the United States (US) Army's interest, and the compound discovered then was cysteine [7]. Patt et al. [8] were the pioneer researchers to examine the protective effect of amino-acid cysteine in mice and rats exposed to lethal radiation doses. The report revealed cysteine's potential to enhance mice and rats' survival against radiation-induced lethality [9]. However, it was discovered that cysteine, as a radioprotector, posed severe challenges, as it was toxic and caused nausea and vomiting at the level of the dose

required for protection [10]. The need to reduce the toxicity level led to a further development program initiated in 1959 by the US Army and conducted at the Walter Reed Institute of Research. During this time, more than 4,000 compounds were synthesised and tested. One of the active compounds discovered during the same study was WR-2721, also known as amifostine [6]. To date, it remains the most reliable of those synthesised in the Walter Reed series [11], and amifostine is the only radioprotective drug approved by the United States Food and Drug Administration (FDA) for use in radiation treatment [6]. Although amifostine was the only radioprotector drug approved by FDA against radiation for thwarting xerostomia in patients treated for head and necked cancer, there remains its cumulative toxicity on daily administration with radiotherapy, which was revealed in sneezing, allergic reactions, somnolence, hypotension, and nausea [6, 11].

Thus, an urgent need to find an alternative natural substance with similar characteristics to the synthetic compound that can offer protection against radiation while remaining non-toxic, effective, available and affordable. A few of the plant extract which has been found to provide a protective measure against the radiation-induced damage in mammals include *Mentha arvensis, Syzygium cumini,* Liv-52, *Nardostachys jatamansi, Ocimum sanctum,* Aegle marmelos (L.), *Zinger officinale, Tragia involucrate, grape seed, nanocurcumin* [7, 12-16].

Costus afer belongs to the family of Zingiberaceae, otherwise called Costaceae; it is a relatively tall permanent herbaceous, branchless herbal plant with crawling rhizome. It is predominantly grown in the thick forest and riverbanks of tropical West Africa [17]. *Costus afer* is often called a bush cane or ginger lily and has a variety of names in Nigeria, such as "Okpete" in the Southeast, "Kakizawa" in the Northern area, "ireke-omode" in the Southwest, "Ogbodou" in the Niger Delta and "Mbritem" in the Southern region [17]. In Cameroon, it is referred to as "Monkey sugar cane" [17]. It has been reported that the stem, seeds and rhizomes of *Costus*

afer contain numerous bioactive metabolites [18]. A report from Soladoye and Oyesika [19] on *Costus afer* indicates that the plant is highly regarded for anti-inflammatory, anti-diabetic, and anti-arthritic features in the Southeast and Southwest region. It is widely used as a medicinal herb, most notably its seeds, stem, leaf, and rhizomes harvested from the wild [17, 20].

The present study aimed to investigate the possible radioprotective efficacy of the *Costus afer* plant against whole-body radiation-induced haematological and histopathological disorder in mice exposed to double doses of X-ray radiation based on reported folklore medicine use.

4.3 Materials and Methods

4.3.1 Plant collection, identification and extract preparation

The rhizome, stem and leaves of *Costus afer* were harvested from uncultivated farmland at Ikole-Ekiti in Ekiti State, South-west, Nigeria, in December 2019. A Botanist (Mr Esinekhuai Donatus) at the Herbarium, Department of the Botany, University of Ibadan, Nigeria, where voucher specimen number UIH-22932 was deposited, made the botanical identification and authentication of the plant. The leaf, stem and rhizome were hand-searched mechanically to ensure they were pest-free. They were also rinsed with tap water and air-dried for a few days at room temperature. After that, they were pulverised at the Biomedical Research Laboratory, School of Chemistry & Physics, University of KwaZulu-Natal (UKZN), Pietermaritzburg campus, with an electric grinder to provide enough surface area for maceration to occur. The powder material (638.03g) of *Costus afer* was macerated in 3.75 L of high quality (99.9% pure) methanol for 72 hours at room temperature. The macerated solution was shaken intermittently to ensure thorough mixing. The maceration of each plant was carried out twice. The combined extract was filtered using a Whatman No. 1 filter paper under vacuum filtration. The obtained filtrate was concentrated and evaporated using a rotary evaporator to remove all traces of

methanol. An approximate 4.5% yield of the extract obtained was placed in an airtight container and stored in a refrigerator at 4^{o}C until the time of use.

4.3.2 Animal care and selection

Fifty-four male BALB/c mice of mass between 37-43g, 11 -13 weeks old, were used for this study. The animals were inbred at the Animal House of the School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg campus. The University of KwaZulu-Natal Animal Research Ethics Committee (UKZN, AREC) approved the research protocol used in this study with a protocol reference number AREC/026/019D. Moreover, all our procedures conformed to the National Institute of Health guidelines for laboratory animal care and were used in biomedical research [21]. Throughout the study, they were kept in the animal house, maintained under a strictly controlled temperature of between 23°C - 25°C, with 12-hour light and dark cycle and were given free access to a standard diet and clean water *ad libitum.* The experimental animals were humanely handled and kept inside clean, well-ventilated transparent plastic-type IV cages with wood shavings and naturally illuminated animal rooms. Behavioural enrichment in the mouse cages in the form of egg boxes and shredded paper were provided. The mice were allowed some days to acclimatise to animal room conditions before treatment commenced. All animals were examined, and clinical signs were recorded daily before and after dosing during the treatment period. The mass of the animals was also recorded.

4.3.3 Acute toxicity study

Twenty male mice were used for the toxicity test. The mice were divided into four groups of five animals in each group. The acute toxicity test of *Costus afer* extract was determined over a 14-day observation period. CAE was administered by oral gavage at doses of 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg body weight. The mice were observed for 14 days for signs of acute toxicity and death [22]. CAE's oral administration to mice did not produce death

or toxic effect in the treated groups during the 14 days observation period. The median dose (250mg/kg) was chosen and used for further studies in the present work.

4.3.4 Administration of extracts

Fifty-four male BALB/c mice were used for the experiment. The mice were randomly divided into six groups, with nine animals in each group. The grouping and treatment of animals are presented in Table 4.1. Animals in the group code CAE, CAE-3Gy and CAE-6Gy received 250mg/kg body weight of extract of *Costus afer* by oral gavage for six days before radiation exposure.

Table 4.1: Treatment of animals for *Costus afer* extract

Group code	Treatment
CNT	Control (Un-irradiated)
CAE	Animals treated with 250mg/kg bodyweight only (Un-irradiated)
IR_3Gy	Irradiated (3Gy) animals only
IR_6Gy	Irradiated (6Gy) animals only
CAE-3Gy	Irradiated (3Gy) animals treated with 250mg/kg body weight
CAE-6Gy	Irradiated (6Gy) animals treated with 250mg/kg body weight

CNT, control; CAE, *Costus afer* extract; IR, ionizing radiation.

4.3.5 Procedure for Irradiation

An hour after the last administration of the extract, the mice were exposed to X-ray radiation at the Department of Radiotherapy and Oncology, Grey's Hospital Pietermaritzburg, South Africa. A Linear Accelerator (LINAC) manufactured by Varian (model: Clinac 2100C) serves

as the radiation source. The LINAC uses electricity to produce energy beams of X-rays and beams of electrons usually collimated to treat cancer patients. Nine animals were packed inside a specially designed transparent plastic cage, and their movement was restrained during the irradiation process. A total of 36 mice (excluding animals in group CNT & CAE) were exposed to 6-MV photons from LINAC, and the irradiated groups were exposed to whole-body low energy X-ray radiation dose of 300cGy and 600cGy at a dose rate of 400MU/min under a standard condition of 100 monitor units (MU) = 1Gy. A source to the surface distance of 90 cm at a depth of 10 cm was used for the irradiation, while a field size of 40 cm by 24 cm was found suitable for the irradiation process. After the radiation exposure, the mice were put into their cages and transferred back to the animal house.

4.3.6 Body mass and relative organ mass

The animals' body mass was recorded on the day they were randomised into different groups and every day during the pretreatment process. These served as the initial masses. Two mice from each group were euthanised by cervical dislocation 48 hours after irradiation, the visceral organs (kidney and liver) of the mice were surgically removed, rinsed in 0.9 % normal saline, blotted with filter paper, weighed, and the relative organ mass was calculated and expressed as a percentage of the body mass.

$$Relative\ Organ\ Mass = \frac{Absolute\ organ\ mass\ (g)}{Body\ mass\ of\ mouse\ on\ the\ day\ of\ sacrifice(g)} \times 100$$

4.3.7 Determination of haematological parameters

Forty-eight hours after the irradiation, all the animals were euthanised by cervical dislocation, and blood samples were collected from them. The blood collection was done from the posterior vena cava of the heart using a 23-gauge needle and a 1-ml syringe into ethylenediaminetetraacetic acid (EDTA) bottles with anticoagulant for haematological

analysis. The packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), total white blood cell count (WBC), neutrophils, lymphocytes, monocytes, eosinophils, platelet count, values were determined using the Sysmex XE-2100 Haematology Automated Analyser machine.

4.3.8 Histopathology examination

Shortly after collecting blood samples on 48-hour post-irradiation, two mice were taken from the euthanised animals, and their visceral organs (kidney and liver) were harvested. The mass of the fresh livers and both kidneys were determined (measured in grams), after which they were fixed in 10% buffered formalin. Two hours post immersion in formalin, the tissues were dehydrated in an ascending grade of ethanol, cleared in xylene and embedded in paraffin wax. Serial sections of 4-μm thick were obtained on glass slides using a rotary microtome. The deparaffinised sections were stained routinely with hematoxylin and eosin (H&E) and mounted. All sections were examined with a standard light microscope (Olympus, Tokyo, Japan) and scanned digitally by an Asperio C52 (Leica Biosystems, Heidelberg, Germany). Images and sections were evaluated under x10, x20, and x40 magnification. Images were taken from the digitally scanned slides with Image Scope Software (Leica) and stored as jpeg image files.

4.3.9 Statistical analysis

The haematological parameters were analysed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test in which all the treatment groups were compared with the control group. SPSS 20.0® statistical package was used for the analysis. Results are reported as means ± SEM (standard error of the mean), and $p<0.05$ were considered significant values.

4.4 Results

4.4.1 Effect of extract on relative organ mass

Exposure of animals to radiation can cause a reduction in food intake, which may lower the immune system, thereby causing changes in the bodyweight of the exposed living animals. Moreover, these changes depend on the types of radiation, the dose of radiation and post-irradiation time (latent period). In this investigation, the mean relative organ mass of the kidneys in the control group (CNT) was 1.13±0.04 as compared with the mean relative organ mass in the irradiated groups (IR_3Gy and IR_6Gy) of 0.76±0.01 and 0.93±0.04, respectively. Our results revealed that radiation significantly ($p<0.05$) decreased the relative organ mass of the kidney of the irradiated groups when compared with the un-irradiated group (control). However, there was no significant alteration in the pretreatment groups' relative organ mass (CAE_3Gy and CAE_6Gy) compared with the irradiated groups only (Table 4.2). The mean relative organ mass in the treatment groups (CAE_3Gy and CAE_6Gy) were 0.80±0.07 and 0.89±0.05, respectively. The relative organ mass of the irradiated groups' kidneys decreased compared with the control, but there was no significant improvement in the pretreatment groups. The levels of radiation-induced damage observed in the pretreatment groups' kidneys did not cause noticeable changes in their relative organ mass, probably due to the short period between exposure and the time the organs were harvested. Similar results were observed in the liver analysis. The mean relative organ mass of the liver in the control group was 6.71±0.08. At the same time, the mean relative organ mass for the irradiated groups was 5.50±0.04 and 4.88±0.05 for group IR_3Gy and IR_6Gy, respectively. There was no significant increase in the relative organ mass of the pretreatment groups.

Table 4.2: Effect of extract on the relative organ mass

Code	Kidneys	Liver
CNT	1.13±0.04	6.71±0.08
CAE	0.89±0.05	5.10±0.18
IR_3Gy	0.76±0.01	5.50±0.04
IR_6Gy	0.93±0.04	4.88±0.05
CAE_3Gy	0.80±0.07	5.46±0.04
CAE_6Gy	0.89±0.05	4.19±0.32

Values are given as means ± SD, $(n = 2)$, CNT, control; CAE, *Costus afer* extract; IR, ionizing radiation.

4.4.2 Effect of extract on haematological parameters

Changes in blood parameters are generally known to be an asset in determining the extent of radiation exposure. The effects of CAE on haematological parameters of both irradiated and un-irradiated mice are discussed in the following subheadings.

4.4.2.1 Red blood cell

Table 4.3 shows a significant (P<0.05) decrease in the mean value of RBCs of the irradiated groups (IR_3Gy & IR_6Gy) when compared with the control (CNT). Moreover, there was a significant difference (P<0.05) in the erythrocyte value of the CAE group when compared with the control (CNT). However, the pretreatment of mice in groups (CAE_3Gy and CAE_6Gy) with the extract did not improve the blood parameter, as there was no significant increase in the red blood count of the pretreatment groups.

4.4.2.2 Packed cell volume

Table 4.3 shows a significant ($P<0.05$) reduction of the mean of PCV of mice in groups (IR_3Gy and IR_6Gy) when compared with the control (CNT) at 48-hour post-irradiation. Also, a significant reduction ($P<0.05$) in PCV of mice in the CAE group was observed compared with the control (CNT). The mean of PCV of mice in the group IR_6Gy was seen to be slightly less than the mean value of group IR_3Gy; this shows the damaging effect of ionising radiation at a higher dose. In the pretreatment groups (CAE_3Gy and CAE_6Gy), the administration of the extract before exposure ameliorated the disorder caused by X-ray radiation by significantly increasing the mean of PCV when compared with groups (IR_3Gy and IR_6Gy).

4.4.2.3 Haemoglobin

There was a slight reduction in the mean value of Hb of mice in the irradiated groups (IR_3Gy and IR_6Gy) when compared with the control (CNT), and a significant decrease ($p<0.05$) in the Hb of mice in the CAE group was recorded when compared with the control (CNT) (Table 4.3). However, the treatment of mice with the extract before exposure seemed to have a slight increase in the mean of Hb of the group CAE_3Gy and group CAE_6Gy at a significant level ($p<0.05$) when compared with the irradiated groups (IR_3Gy and IR_6Gy).

4.4.2.4 White blood cell

Ionising radiation caused a significant reduction ($p<0.05$) in the mean of WBC in groups IR_3Gy and IR_6Gy when compared to the control (CNT). Similarly, the mice that received extract only (CAE group) showed a significant reduction ($P<0.05$) in their WBC when compared with the control (CNT). The alterations in the WBC of mice among the treatment groups (CAE_3Gy and CAE_6Gy) were significantly increased compared with the control (CNT) (Table 4.3).

4.4.2.5 Neutrophils count

Table 4.3 shows a significant reduction ($p<0.05$) in the mean of the irradiated groups' mean neutrophils compared with the control (CNT). Moreover, a significant reduction ($p<0.05$) in the neutrophil of mice in the CAE group was observed when compared with the control (CNT). The treatment of mice with CAE did not statistically increase the mean of neutrophil in the group CAE_3Gy, whereas there was a significant improvement in the group CAE_6Gy compared with group IR_6Gy.

4.4.2.6 Lymphocytes count

In Table 4.4, ionizing radiation caused a significant decrease ($p<0.05$) of the mean of lymphocyte count in the irradiated groups compared with the control (CNT). Similarly, a significant reduction ($p<0.05$) in the lymphocyte of mice in the CAE group was discovered when compared with the control (CNT). However, the treatment of mice with the extract significantly improved the lymphocyte count in the group CAE_6Gy. There was no significant improvement in the lymphocyte count for group CAE_3Gy relative to group IR_3Gy.

4.4.2.7 Monocytes

Table 4.4 shows a non-significant ($p>0.05$) reduction in the monocyte count of both the irradiated groups alone compared with the control (CNT). Whereas, a slight significant increase ($p<0.05$) in the monocyte of mice in the CAE group was observed when compared with the control (CNT). The CAE administration did not offer protection against the damaging effect of X-ray radiation on the pretreatment groups' monocyte count, as these groups show a non-significant increase in their mean value compared with groups IR_3Gy and IR_6Gy.

4.4.2.8 Eosinophils

Table 4.4 shows a slight reduction in the mean eosinophils count of the groups IR_3Gy and IR_6Gy caused by radiation, which was significant compared with the control (CNT). Also, a

significant reduction ($p<0.05$) in the eosinophil of mice in the CAE group when compared with the control (CNT) was discovered. The slight increase in pretreatment group CAE_6Gy was significant ($P<0.05$) compared with group IR_6Gy, that of group CAE_3Gy was not significant relative to group IR_3Gy.

4.4.2.9 Platelet

The significant reduction of platelet count caused by ionizing radiation is shown in Table 4.4. The irradiated groups (IR_3Gy and IR_6Gy) revealed a statistically significant decrease ($P<0.05$) in the platelet count compared with the control (CNT). Similarly, the mice in the CAE group had a significant reduction ($P<0.05$) in their platelet count when compared with the control (CNT). There was an improvement in the pretreatment groups (CAE_3Gy and CAE_6Gy), as evidence in an increase in platelet count compared with the irradiated groups (IR_3Gy and IR_6Gy).

Table 4.3: Effect of methanol extract of *Costus afer* and X-ray radiation on the RBC, PCV, haemoglobin, WBC and neutrophils of mice

Group code	RBC ($\times 10^{12}/L$)	PCV(L)	Hb (g/dL)	WBC ($\times 10^{9}/L$)	Neutrophils ($\times 10^{9}/L$)
CNT	11.05±0.58	57.20±2.06	17.46±0.12	9.22±0.10	7.63±0.31
CAE	9.94±0.35*	47.47±3.70*	16.38±0.22*	7.60±0.21*	5.16±0.41*
IR_3Gy	9.41±0.38*	33.96±0.22*	13.60±0.13*	2.54±0.11*	5.43±0.37*
IR_6Gy	8.99±0.47*	33.23±1.09*	12.20±0.21*	1.26±0.13*	2.74±0.43*
CAE_3Gy	9.69±0.58	41.10±1.09**	14.64±0.27**	3.38±0.33**	5.20±0.35
CAE_6Gy	9.61±0.18	41.83±0.82**	14.56±0.39**	2.95±0.38**	6.38±1.04**

Values are given as means ± standard error of the mean ($n = 9$). CNT, control; CAE, C*ostus afer* extract; IR, ionizing radiation; RBC, red blood cell; PCV, packed cell volume; WBC, white blood cell. *p<0.05 versus CNT, **P<0.05 versus IR_3Gy & IR_6Gy. Values along the same column with different superscripts are significantly different at the 5% ($p < 0.05$) level.

Table 4.4: Effect of methanol extract of *Costus afer* and X-ray radiation on lymphocytes, monocytes, eosinophils and platelet of mice

Group code	Lymphocytes ($\times 10^9/L$)	Monocytes ($\times 10^9/L$)	Eosinophils ($\times 10^9/L$)	Platelet ($\times 10^9/L$)
CNT	4.94±0.13	2.53±0.03	3.60±0.15	3401.00±45.36
CAE	3.66±0.37*	3.52±0.04	2.80±0.21*	1355.80±254.74*
IR_3Gy	3.58±0.21*	2.18±0.02	2.20±0.03*	551.60±5.91*
IR_6Gy	1.29±0.10*	1.84±0.04*	1.60±0.13*	340.40±35.49*
CAE_3Gy	2.01±0.24	2.10±0.07	2.60±0.05	1272.00±197.43**
CAE_6Gy	2.33±0.19**	1.80±0.03	2.80±0.10**	471.20±40.13**

Values are given as means ± standard error of the mean ($n = 9$). CNT, control; CAE, *Costus afer* extract; IR, ionizing radiation. . *p<0.05 versus CNT; **P<0.05 versus IR_3Gy & IR_6Gy Values along the same column with different superscripts are significantly different at 5% ($p < 0.05$) level

4.4.3 Effect of extract on histology kidney and liver of mice after exposure to X-ray radiation

The histopathological examination of kidney and liver sections stained with H&E showed that X-ray radiation-induced changes in mice's kidney and liver's renal architecture in a dose-

dependent manner. Fig. 4.1 presents the pathological analysis results of the kidney in various groups studied. Group CNT mice (control group) showed that sections from both left and right kidneys examined the renal architecture seem intact with a normal cortex and medulla in which normal convoluted tubules and tubular epithelial cells, glomeruli, blood vessels and stromal tissues are seen (Fig. 4.1A). Meanwhile, the kidney section of mice pretreated with the extract alone (group CAE) showed a normal cortex and few foci of mild sloughing off tubular epithelial cells (Fig. 4.1B). The mice exposed to 3Gy and 6Gy of X-ray radiation, group IR_3Gy and IR_6Gy, respectively, showed few foci of mild cloudy swelling of the epithelial cells tubules moderate flattening of epithelial cells in the cortex-medullary junction (Fig. 4.1C, 4.1D). The mice in the groups (CAE_3Gy and CAE_6Gy) pretreated with extract before exposure to X-ray radiation of 3Gy and 6Gy showed no visible lesion. The renal architecture seems intact with a normal cortex and medulla in which normal convoluted tubules and tubular epithelial cells, glomeruli, blood vessels and stromal tissues are seen (Fig. 4.1E, 4.1F).

The pathological analysis of the liver of mice in the group CNT (control) and group CAE (mice received extract only) revealed that a normal hepatic architecture is evident with a typical ratio of portal triads and hepatic lobules. No congestion is seen in the hepatic sinusoids. Hepatocytes seem to be within normal histological limits and have no evidence of adhesion and inflammation (Fig. 4.2A, 4.2B). The liver histology of the mice exposed to 3Gy and 6Gy showed random foci of mild single-cell hepatocellular necrosis. Moderate congestion is seen in the hepatic sinusoids (Fig. 4.2C, 4.2D). Group CAE_3Gy mice (mice pretreated with extract followed by 3Gy) showed foci of mild random single hepatocellular necrosis. Hepatocytes seem to be within normal histological limits. The mice in the group CAE_6Gy (mice pretreated with extract followed by 6Gy) showed a normal hepatic architecture that is evident with a typical ratio of portal triads and hepatic lobules. Mild congestion is seen in the hepatic sinusoids. Hepatocytes seem to be within normal histological limits (Fig. 4.2E, 4.2F).

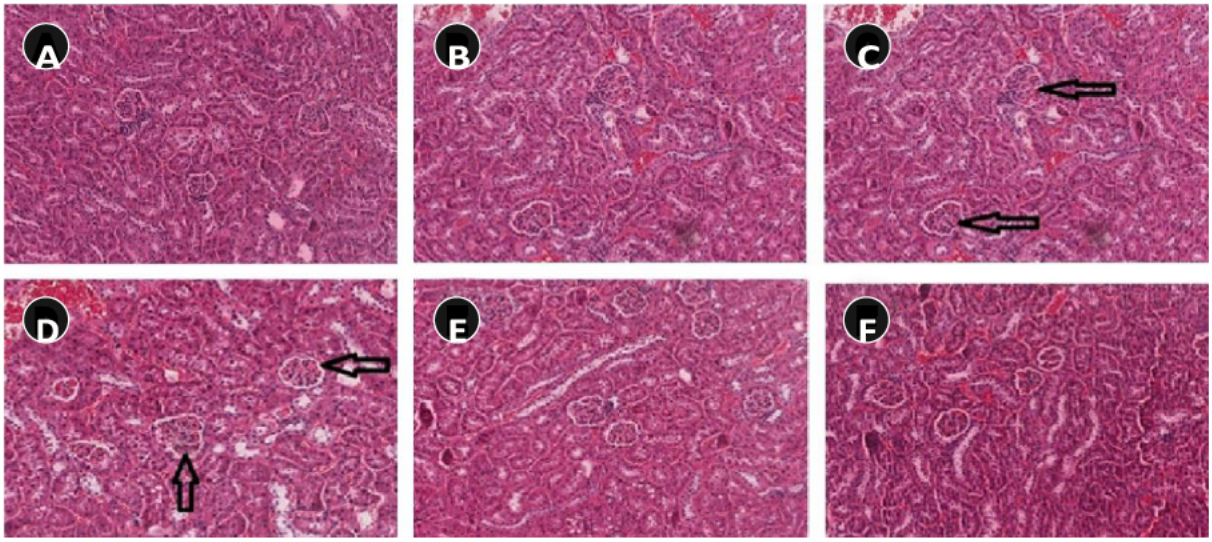

Fig. 4. 1. Effects of *Costus afer* and X-ray radiation on the histological parameters of mice: the pathological analysis results of the kidney. (A) Light micrograph of the kidney section of the control mice (group CNT), the renal architecture seem intact with a normal cortex (H&E, ×400). (B) Light micrograph of the kidney section of mice treated with *Costus afer* extract (group CAE) showing a normal cortex and few foci of mild sloughing off tubular epithelial cells (H&E, ×400). (C) Light micrograph of the kidney section of mice exposed to 3 Gy of X-ray showing a few foci of mild cloudy swelling of the epithelial cells (arrows) (H&E, ×400). (D) Light micrograph of the kidney section of mice exposed to 6 Gy of X-ray showing a few foci of moderate cloudy swelling of the epithelial cells (arrows) (H&E, ×400). (E) Light micrograph of the kidney section of mice treated with CAE & exposed to 3 Gy of X-ray showing no visible lesion (H&E, ×400). (F) Light micrograph of the kidney section of mice treated with CAE & exposed to 6 Gy of X-ray showing no visible lesion (H&E, ×400). CNT, control; CAE, *Costus afer* extract; H&E, hematoxylin and eosin staining.

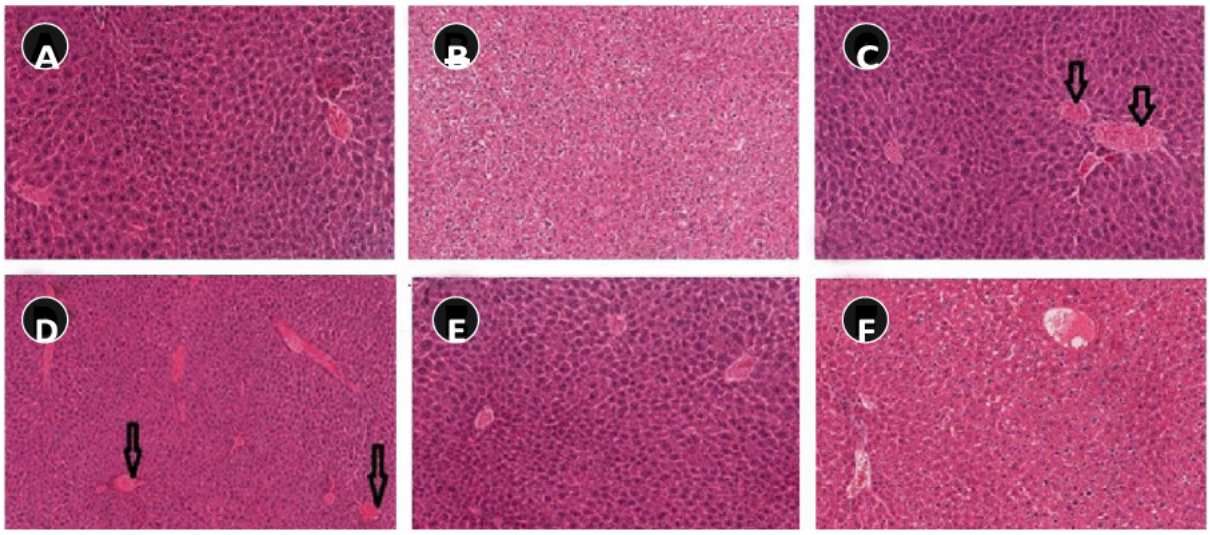

Fig. 4.2. Effects of *Costus afer* and X-ray radiation on the histological parameters of mice: the pathological analysis results of the liver. (A) Light micrograph of the liver section of the control mice, a normal hepatic architecture is evident with a normal ratio of portal triads and hepatic lobules (H&E, ×400). (B) Light micrograph of the liver section of mice treated with CAE, no congestion is seen in the hepatic sinusoids (H&E, ×400). (C) Light micrograph of the liver section of mice exposed to 3 Gy of X-ray showing random foci of mild hepatocellular necrosis (arrows) (H&E, ×400). (D) Light micrograph of the liver section of mice exposed to 6 Gy of X-ray showing random foci of mild hepatocellular necrosis (arrows) (H&E, ×400). (E) Light micrograph of the liver section of mice treated with CAE & exposed to 3 Gy of X-ray showing no visible lesion (H&E, ×400). (F) Light micrograph of the liver section of mice treated with CAE & exposed to 6 Gy of X-ray showing no visible lesion (H&E, ×400). CNT, control; CAE, *Costus afer* extract; H&E, hematoxylin and eosin staining.

4.5 Discussion

Exposure of humans to ionizing radiation can cause the production of free radicals and reactive oxygen species (ROS), which are capable of damaging the DNA, protein and membrane lipids, thus resulting in biological and cellular damages [23]. Since the significant component of the human body is primarily made up of water, there is a high probability of radiation interacting

with the water molecules and, in the process, cause radiolysis of water, which produces free radicals. The interaction of radiation with water leads to the breakage of bonds holding the water molecules together and thus producing fragments such as hydrogen and hydroxyls. These fragments are highly mobile due to unpaired electrons and can combine to form toxic substances [24]. Reports from experimental and clinical studies have revealed that kidneys are relatively more radiosensitive organs than other organs. The development of radiation-induced injuries sustain by the kidney may take months to years before manifesting [25]. Radiation-induced liver disease (RILD) is a dose-preventing intricacy of the liver exposed to radiation, and the therapy alternative for RILD is restricted [26]. Even in acute cases, it causes the liver's inability to carry out its metabolic functions, leading to death. Ingold et al. [27] reported the first description of radiation-induced liver disease as a significant complication associated with the liver's disease radiotherapy. Even though this disease's latent period takes 4-8 weeks post-radiation treatment, an investigation has revealed that the disease has a potency of manifesting as early as two weeks or as late as seven months post-irradiation therapy [28, 29]. Even though the clinical application of ionizing radiation in radiotherapy and other medical areas is widely accepted and has accrued colossal success, the damage to the healthy surrounding tissues has limited its usage. Thus, there is an urgent need to develop drugs from plants and herbs capable of scavenging free radicals, thus protecting the normal cells during radiotherapy and reducing radiation's harmful effect in an emergency radiation accident. This is the motivation for this work.

In the present study, we assessed *Costus afer* extract's ability in mitigating radiation-induced haematological and histopathological disorder in mice. The results showed that X-ray radiation caused a significant alteration in the haematological parameters, as evident in the degree of blood counts. Our findings revealed that 3Gy and 6Gy doses of X-ray radiation caused a substantial reduction in the PCV, Hb, WBC, lymphocytes and platelet counts compared with

those in control (CNT) and extract only (CAE) groups. The pretreatment of mice with the extract of *Costus afer* improved the listed haematological variables with a significant increase in their mean values. The reduction in the named haematological variables among the irradiated groups is an indication that whole-body irradiation is mostly observed in the proliferating bone marrow progenitor cell. The decrease in the number of white blood cells and lymphocytes in the irradiated groups and the corresponding increase in the treatment groups are comparable to Shirazi et al. [26]. They reported that pretreatment with melatonin in rats (10mg/kg) before exposure to 2Gy and 8Gy statistically increased the number of WBC and lymphocytes at 4 hours post-irradiation.

Moreover, gastrointestinal and haemopoietic cells in the bone marrow, which happen to be the most radiosensitive organs, are essential for maintaining life, and any injury to these cells can damage normal physiological activities [11]. The present study results concur with the findings of Gowda et al. [13], who reported that electron beam radiation caused a significant reduction in the Hb, erythrocytes, leukocytes, PCV, and the platelet count 48-hour post-irradiation in male rats. Similarly, the results of the present study are consistent with the findings of Eshak and Osama [30], who showed a substantial decrease in WBC, RBC, PCV, Hb and platelet exposure of 4 Gy and 6 Gy of gamma radiation to animals. In the present study, X-ray radiation caused a significant reduction in the mean of PCV and Hb. Our result is also in total agreement with Udem and Ezeasor's [31] findings, who reported that *Costus afer* extract caused a significant reduction in the Hb, RBC, and PCV compared with the control group. A non-significant decrease in the mean of RBC was observed in the mice exposed to both radiation doses. This may be attributed to the erythrocyte's relatively radioresistant nature compared with other blood's cellular components [4]. The observed decrease in the mean value of erythrocytes in this study might be due to mature RBC damage. In addition, it may be due to hemolysis and decreased erythrocyte production [32]. The present study revealed that the given radiation

doses significantly lowered the number of neutrophils in the experimental animals, giving rise to a condition known as neutropenia. Neutrophils are primarily present in the WBC.

The pretreatment of mice with the CAE partially ameliorated the damage. WBC assist the body in fighting infectious and destroying harmful bacteria that spread into the body. Neutrophils are the most crucial protector present in the WBC that fights against infection. Report from literature revealed the radioprotective and antioxidant properties of *Costus afer*. For instance, the plant is used to cure ailments or conditions such as rheumatism, cough, hepatic disorders, miscarriages, haemorrhoids, inflammation, arthritis, helminthic, epileptic attack, as well as purgative, diuretics. It has also been tested as a cure for poison [33, 34]. Okugbo and Oriakhi [35] reported that *Costus afer* could serve as free radical scavengers, acting perhaps as critical antioxidants, which could treat the disease that results from oxidative damage.

The inhibition of lipid peroxidation is another biomarker in determining the radioprotective property of the plant. The study conducted by Moody and Okwagbe [36] on plant stem extracts of *Costus afer* shows that the plant possesses potent antioxidants *In-vitro*. The results obtained by Tonkiri et al. [17] show that *Costus afer* exhibits high antioxidant and free radical scavenging activities. The plant is a significant source of natural antioxidants, which may be of great value in hindering the progress of various oxidative stresses and modulation of drug-induced toxicity. The present study results correlate with the report of Abdelmageed Marzook et al. [37], who earlier worked on the radioprotective efficacy of *Costus* in protecting haematological parameters. They revealed that *Costus speciosus* offered protection on haematological parameters (RBC, hematocrit, WBC, and reticulocytes) against gamma radiation. The findings of Anyasor et al. [33] revealed that the aqueous fraction of leaves and stem bark of *Costus afer* exhibited a high degree of inhibition of lipid peroxidation. In another related development, Anyasor et al. [18] reported that *Costus afer* contained anti-oxidative properties, the plant could serve as bioactive and antioxidants compounds for nutrition and

therapeutic purposes. Other studies have shown that *Costus afer* possess antioxidant, anti-inflammatory, anti-cancer, hepatoprotective and could stimulate total lymphocytes proliferation [33, 38]. Research has shown that free radicals can cause oxidative stress resulting in cellular and biological damage [39].

Moreover, antioxidants have been shown to offer resistance against oxidative stress by scavenging free radicals. The report of Atere et al. [40] revealed that the antioxidant activity of *Costus afer* might be responsible for its medicinal potentials. Furthermore, their information deduced that polyphenols, flavonoids and other antioxidants compounds account for their ability to scavenge free radicals [40].

Kidneys are critical organs in the body that play basal functions in both health and disease conditions. Due to the kidneys' relative radiosensitivity nature, the organs are prone to damage through radiation effects [41]. The exposure of kidneys to radiation during radiotherapy has raised serious concern over the practical applicability of ionising radiation for therapeutic and diagnostic purposes. The degree of radiation damage on the kidney largely depends on the volume and dose of the incident radiation dose [25, 42]. Reports in many clinical and experimental studies have shown that the liver is also one of the most commonly injured organs during radiotherapy for cancers of the abdominal region [43]. In the present investigation, whole-body exposure of mice to 3 Gy and 6 Gy radiation, the histological examination of the kidney revealed a few foci of mild cloudy swelling of the epithelial cells of tubules with a severe flattening of epithelial cells in the cortex-medullary junction. The administration of extract before irradiation showed no significant improvement in the histological examination of the kidney.

Similarly, the liver histology of the mice exposed to 3 Gy and 6 Gy radiation showed random foci of mild single-cell hepatocellular necrosis, whereas, in the pretreatment groups (group

CAE_3Gy and CAE_6Gy); hepatocytes seem to be within normal histological limits. This may be due to the time interval between irradiation and the harvesting of organs. Our findings contradict the findings of Tonkiri et al. [17], who reported *Costus afer* extract's protective ability and showed that the extract could act as a potent hepatoprotective agent against alcohol-induced liver cirrhosis. The contradiction in the results of the present investigation may be because a short time frame between exposure and harvesting of organs was observed. It could also be that the extract was administered over a few days, and lower radiation doses (3 Gy and 6 Gy) were used, which were insignificant when compared with radiation dose tolerance of kidney and liver tissue. The six days administration of *Costus afer* adopted in the present study is negligible compared with the six weeks employed in the report of Tonkiri et al. [17]. The administration of the extract over a few days may be one factor for its non-significant effect in the histology analysis. However, the present findings correlate with the Udem and Ezeasor [31] report, where the acute and subchronic toxicity of *Costus afer* was performed in mice. Their results showed no significant lesions in the kidney, liver, heart of the experimental, and control mice were recorded as revealed in the histopathological analysis. The report of their findings is in agreement with the result obtained from the present study.

Similarly, the present study agrees with the report of Ezejiofor et al. [44] on the activity of *Costus afer* against hyperglycaemic induced hepatotoxic and histopathological changes; their report showed no histological changes in the harvested organs. However, the haematological analysis revealed that rats treated with the extract of *Costus afer* had a significant increase in WBC, RBC, Hb, and platelet count compared with the control group. Ezejiofor et al. [44] also saw a significant increase in the lymphocyte level.

In conclusion, evidence from the present study indicates the possible potential of *Costus afer* to mitigate radiation-induced haematological alterations. Even though CAE's mechanism of

action exerts its protective effect is unknown, it may be due to its ability to scavenge free radicals and reactive oxygen species. As reports from literature show, the plant exhibited antioxidant properties, capable of neutralising the toxic peroxides and hydroxyl ions formed from the hydrolysis of water molecules after radiation exposure. The various compounds such as polyphenols, flavonoids and other antioxidants present in CAE may also protect hematopoietic cells in mice against radiation-induced damage, leading to increased blood counts in the haematological parameters. The histology examination revealed no visible lesion associated with irradiated and treated mice's kidneys and liver. Further studies are warranted to validate the histopathology analysis; we propose varying parameters such as an increase in radiation dose, latent period and quantity (dose) of the extract can help authenticate the radiation-induced disorder to the histopathological parameters. The results of the haematology analysis from the present investigation support local claims of the therapeutic uses of *Costus afer* in the treatment of various kinds of ailments in folklore medicine; thus, *Costus afer* plant may be a potent radioprotector in the treatment of cancerous cells and for general use in case of a radiation emergency.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

The authors wish to express their sincere appreciation to the management and staff of the Department of Radiotherapy and Oncology, Grey's Hospital KwaZulu-Natal, South Africa, for providing the irradiation facility. Special thanks to Mr Mdletshe Nipho for helping in the radiation dosimetry and technical support. We also extend gratitude to Mr Ebrahim Ally of the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg campus, for assisting in the oral gavage and collection of blood samples.

This work is based on the research supported wholly to the first author by the National Research Foundation (NRF) of South Africa (Grant Number: 116089). The author acknowledges that opinions, findings and conclusions or recommendations expressed in this publication generated by the NRF-supported research are those of the author and that the NRF accepts no liability whatsoever in this regard.

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CHAPTER FIVE

EVALUATION OF RADIOPROTECTIVE EFFICACY OF *DRYMARIA CORDATA* EXTRACT ON WHOLE-BODY RADIATION-INDUCED HAEMATOLOGICAL DAMAGE IN MICE

Running title: Radioprotective efficacy of *Drymaria cordata*

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This chapter is an accepted manuscript that determined the possible radioprotective efficacy of *Drymaria cordata* extract on irradiated mice, emphasising its ability to increase survival and improve haematological parameters.[2]

[2] Akomolafe IR, Chetty N. Evaluation of radioprotective efficacy of *Drymaria cordata* extract on whole-body radiation-induced haematological damage in mice. *Iranian Journal of Medical Physics, in press,* doi: 10.22038/ijmp.2021.56512.1946

5.1 Abstract

Introduction: This study aimed to evaluate the radioprotective potential of *Drymaria cordata* (DC) extract on mice's haematological parameters following exposure to X-rays radiation.

Materials and Methods: Sixty 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 haematology 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, haematocrit, 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 haematological 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 haematological 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 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 haematopoietic system and increase the survival rate.

Keywords: Radiation protection; radiotherapy; linear accelerator; haematology; Cancer drug; X-ray.

5.2 Introduction

The discovery of X-ray radiation by Roentgen in 1895 has 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, there remain harmful and deleterious effects, which cannot be overlooked [2, 3]. The interaction of ionising radiation with cells produces free radicals and reactive oxygen species 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 started with the report of Patt et al. [5] when they discovered 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. Ever since then, several studies have been conducted on different compounds to ascertain their radioprotective abilities. The major setback on these synthetic compounds is their toxicity level at the optimum protective dose [2, 6].

Radiotherapy has become an excellent modality in the treatment of cancerous cells, 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 the exposure of 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, herbs, and natural products in the last few decades as an alternative to synthetic compounds for radioprotection, thereby reducing the radiation side effect [10]. The aim has been to replace the toxic synthetic compounds and make radioprotector drugs affordable, accessible and economically viable to both 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. It is commonly known as awede-werisa in Yoruba and Calabar woman's eye in Igbo, in Nigeria. It is reported to be used in folklore medicine to treat various diseases such as; convulsions, febrile seizure and sleeping disorders in children. Most studies aimed to determine its potential to cure different ailments, such as treating the respiratory disease in D.R. 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 usages 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 haematological parameters.

5.3 Materials and methods

5.3.1 Collection, identification and preparation of Plant Extract

The collection of fresh samples of the DC plant was done from local farmland at the University of Ibadan, Ibadan, South-west, Nigeria, in July. A botanist (Mr Esinekhuai Donatus) at the Herbarium, Department of Botany, University of Ibadan, Nigeria, where voucher specimen number UIH-22933 was deposited, performed plant identification and authentication. The collected samples were washed under running water and air-dried for a few days at room temperature. The dried plant samples were then pulverised with an electric grinder at the Biomedical Research Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal (UKZN), Pietermaritzburg campus provide enough surface area for maceration to take place. The powdered material with mass 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. The resulted filtrate was evaporated using a rotary evaporator to

remove all the traces of ethanol. An approximate 3.5% yield of ethanolic extract was collected, placed in an airtight container and stored in a refrigerator at 4^0C until the time of use.

5.3.2 Animal care and handling

Sixty female BALB/c mice weighing 38-45g, 10-12 weeks were used for this study. The animals were raised at the School of Life Sciences' Animal House, University of KwaZulu-Natal, Pietermaritzburg campus. The research protocol used in this study was approved by the University of KwaZulu-Natal Animal Research Ethics Committee (UKZN, AREC) with a protocol reference number AREC/026/019D. Animals received food and 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 given as 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].

5.3.3 Experimental design

The sixty female BALB/c mice were randomly distributed into six different treatment groups containing ten mice (Table 5.1). 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 obtained five days post-irradiation, the mean value of the mass pre and after treatment periods, was calculated. The effect of DC extract and radiation on the animals' body mass was determined by estimating the mass gain.

Table 5.1: Treatment of animals for *Drymaria cordata* extract

Group code	Treatment
CNT	Control (Un-irradiated)
DC	Animals treated with 250mg/kg bodyweight only (Un-irradiated)
IR_4Gy	Irradiated (4Gy) animals only
IR_8Gy	Irradiated (8Gy) animals only
DC_4Gy	Irradiated (4Gy) animals treated with 250mg/kg body weight
DC_8Gy	Irradiated (8Gy) animals treated with 250mg/kg body weight

5.3.4 Irradiation procedure

The mice were exposed to X-ray radiation an hour after the last administration of the extract at the Radiotherapy and Oncology Department, Grey's Hospital Pietermaritzburg, South Africa. The radiation source is a Varian Linear Accelerator (LINAC) (model: Clinac 2100C). The LINAC uses electricity to produce X-rays and beams of electrons usually collimated to treat cancer patients [21]. Ten animals were packed inside a specially designed transparent plastic cage under a controlled condition, and their movement was restrained during the irradiation process. A total of 40 mice were exposed to 6MV photons from LINAC. The irradiated groups received a whole-body X-ray radiation dose of 400cGy and 800cGy at a dosage rate of 400MU/min under the standard condition of 100 monitor units (M.U.) = 1Gy. A source to a surface distance of 85 cm at a depth of 15 cm was adopted for the irradiation. The field size of 30 cm by 25 cm was found suitable for the irradiation process. Immediately after the irradiation, the mice were returned to their cages and transferred back to the animal house, where they were monitored every day for the manifestation of radiation-induced illness and mortality. The rationale for the choice of radiation doses is to evaluate the hematopoietic syndrome (bone marrow syndrome) on 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 haematological 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], Yamamori et al. [25] suggest other studies where similar radiation dose ranges have been used.

5.3.5 Determination of haematological parameters

Five and fifteen days after irradiation, three mice in each group (n=3) were sacrificed by cervical dislocation and blood 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, which contained anticoagulant for haematological analysis. Similarly, thirty days after irradiation, the surviving mice in each group were sacrificed, as mentioned above [26]. The haematological parameters analysed include Erythrocyte (RBC), Haematocrit (HCT), Leukocyte and Platelet (PLT). The haematocrit was analysed using the microhaematocrit method. Simultaneously, the RBC detector counts the Erythrocytes, PLTs and Leukocytes via the Hydro-Dynamic Focusing (D.C. Detection) using the Sysmex XE-2100 Haematology Automated Analyser machine. The Hydro-Dynamic Focusing method improves blood count accuracy and repeatability. In addition, because the blood cells pass through the aperture in a line, it prevents the generation of abnormal blood cell pulses.

5.3.6 Statistical Analysis

One-way analysis of variance (ANOVA) was employed to examine the haematological parameters, followed by Tukey's multiple comparison test, which compared all treatment groups to the control group. SPSS 20.0® statistical package was used for the analysis. Results

are reported as means ± SEM (standard error of the mean), and $p<0.05$ were considered significant values.

5.4 Results

5.4.1 Survival Analysis

Table 5.2 shows the percentage survival analysis of the experimental mice. The first mortality was recorded in the group (IR_8Gy) on the 8th-day post-irradiation. More death was registered in subsequent days, and by 25th-day post-irradiation, all the animals in group (IR_8Gy) died (Table 5.2). 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 sacrificed 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 the finding of Adaramoye et al. [26]. They reported the dried fruit extract of Xylopia aethiopica on eight weeks of survival of Wistar albino rats after exposure to 5Gy of gamma radiation.

Table 5.2: 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

5.4.2 Haematological parameters

Tables 5.3, 5.4 and 5.5 summarise the present investigation results regarding the possible radioprotective efficacy of DC extract in mitigating radiation-induced haematological damage in mice. The exposure of experimental mice to 4Gy and 8Gy of X-ray radiation resulted in a significant decrease in the haematological parameters such as erythrocyte, leukocyte and platelets, except haematocrit that 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 haematological parameters was radiation dose-dependent. At a higher dose, the reduction in the haematological parameters was more pronounced. The present study revealed a progressive decrease in all the haematological parameters until 30 days among the irradiated groups. However, the animals treated with DC extract exhibited a significant increase in the studied haematological parameters compared to the CNT. 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 at the 30-day monitoring period. The increase in the haematological parameters in the treated animals showed the DC extract's ability to protect against whole-body X-ray radiation-induced haematological damage in mice.

Table 5.3: Effect of ethanol extract of *Drymaria cordata* and X-rays radiation on the Erythrocyte, Haematocrit, Leukocyte and Platelet of female mice at 5th-day post-irradiation

Group code	Erythrocyte ($\times 10^{12}/l$)	Haematocrit (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.

a: Significantly different from (CNT & DC) at $p<0.05$

b: Significantly different from (IR_4Gy & IR_8Gy) at $p<0.05$

Table 5.4: Effect of ethanol extract of *Drymaria cordata* and X-rays radiation on the Erythrocyte, Haematocrit, Leukocyte and Platelet of female mice at 15th-day post-irradiation

Group code	Erythrocyte ($\times 10^{12}/l$)	Haematocrit (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.

a: Significantly different from (CNT & DC) at p<0.05

b: Significantly different from (IR_4Gy & IR_8Gy) at p<0.05

Table 5.5: Effect of ethanol extract of *Drymaria cordata* and X-rays radiation on the Erythrocyte, Haematocrit, Leukocyte and Platelet of female mice at 30th-day post-irradiation

Group code	Erythrocyte ($\times 10^{12}/l$)	Haematocrit (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.

a: Significantly different from (CNT & DC) at $p<0.05$

b: Significantly different from (IR_4Gy) at $p<0.05$

5.4.3 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 5.6). 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.

Table 5.6: Effect of DC extract and X-ray radiation on body mass at day five

Group code	Initial mass (g)	Final mass (g)	mass gain/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.

5.5 Discussion

Reports have shown that a radioprotective agent's ability to delay mortality within the period of the 30-day interval after whole-body irradiation suggests its capacity to modulate the recovery and regeneration of the haemopoietic 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. Whole-body irradiation of different amounts of radiation can impact the body differently; some of these effects can manifest in hours, days, and years. For example, exposure to 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 post-irradiation is considered due to damage to the gastrointestinal epithelium. Similarly, research has shown that death between 11 to 30 days after irradiation is due to haemopoietic damage imposed by ionising radiation [2]. This work observed that mice exposed to radiation exhibited radiation-induced sicknesses such as emaciation, diarrhoea, significant weight loss, watering of eyes, redness of eyes, 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 observed 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. The survival level observed in the treated groups after whole-body irradiation could be attributed to the extract’s ability to scavenge free radicals and regenerate gastrointestinal epithelium and haemopoietic progenitor cells in the red bone marrow [11]. It has been shown that ionising radiation can induce a dose-

dependent decrease in circulating haematopoietic 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 the finding of Jagetia et al. [2], where they reported that treatment of mice with abana (a herbal preparation) before exposure to 10Gy of γ-radiation delayed the onset of mortality and reduced 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].

Changes in blood values have been identified as an asset in determining the extent of radiation exposure to the organism. The haematopoietic system, which comprises bone marrow and lymphatic tissues, has been recognised as the most radiosensitive organ in the body [28]. Reports of immature and dividing blood cells being the most radiosensitive, the mature or non-dividing cells being radioresistant, and the pattern of white blood cells decreasing in response to irradiation occurring in the sequence: lymphocytes, thrombocytes, neutrophils have been documented [28]. Due to the high radiosensitivity nature of bone marrow, it becomes easily susceptible to radiation exposure. 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 haematological parameters, as evident in a substantial reduction in

the erythrocyte, leukocytes and platelet counts, whereas a non-significant decrease in the haematocrit counts was observed in the irradiated groups (Table 5.3, 5.4 & 5.5). 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 level when compared with CNT & DC groups at all the different time intervals post-irradiation. However, in the pre-treated groups, the number of erythrocytes, leukocytes and platelet counts significantly increased. The increase in the haematological parameters in the treated animals demonstrated the DC extract's ability in mitigating radiation-induced haematological alterations. Furthermore, it shows that mice's treatment with DC extract ameliorated radiation's harmful effect on the haematological parameters.

The reduction in the number of leukocytes and platelets in the irradiated groups alone and the corresponding increase in the treatment groups is also similar to the finding of Shirazi et al. [30], who reported the pre-treatment of rats with melatonin (10mg/kg) prior 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 haematocrit among the haematological 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 brings about a significant reduction in 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 the level of leukocyte counts. The reduction in leukocyte level recorded among the irradiated groups may be attributed to the loss of lymphocytes. The lymphocytes are responsible for fighting infectious and help in building

the body's immune system. A significant reduction in lymphocyte counts could lead to a lymphocytopenia condition.

Similarly, the present study results agree with the finding of 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 haematopoiesis and intravascular red cells 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, haematocrit, leukocyte and platelets when compared with the CNT group. These results agree with those obtained by 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, haemoglobin, and platelet of animals exposed to 4Gy and 6Gy of gamma radiation. Furthermore, AL-Dulamey et al. [35] evaluated the radioprotective effect of black seed oil on haematological parameters after exposure to gamma-ray radiation. The report showed that rats treated with black seed oil exhibited a radioprotective effect by significantly increasing the haematological parameters followed by exposure to 6mGy/h gamma rays for 25 days [35]. The results of the haematological 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 the report of Gowda et al. [14], where electron beam irradiation caused a significant reduction in the erythrocytes, leukocytes, haemoglobin, 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 on the haematopoietic system [14]. The work of Akomolafe and Chetty [21] on the radioprotective potential of *Costus afer* on haematological parameters concur with the findings of the present study; they showed that *Costus afer* provided protection on haematological parameters (erythrocyte, leukocyte, haematocrit, 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 haematological 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, the result of Mukherjee et al. [19] showed the plant exhibited significant antitussive properties 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.

5.6 Conclusion

The study has demonstrated that DC extract offered protection against radiation-induced haematological damage in mice and reduced mortality, thereby increased the survival rate. The results of this study support the current usefulness of the plant in treating diverse kinds of ailments. The elevation levels observed in the haematological parameters among the treatment groups may be partly due to its ability to scavenge free radicals and reactive oxygen species, 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

against radiation-induced haematological disorder 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.

Acknowledgements

The authors wish to express their sincere appreciation to the management and staff of the Department of Radiotherapy and Oncology, Grey's Hospital KwaZulu-Natal, South Africa, for providing the irradiation facility. Special thanks to Mr Mdletshe Nipho for helping in the radiation dosimetry and technical support. We also extend gratitude to Mr Ebrahim Ally of the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg campus, for assisting in the oral gavage and collection of blood samples.

Funding: This work is based on the research grant supported wholly to the first author by the National Research Foundation of South Africa DST-NRF-TWAS (Grant Number: 116089). The author acknowledges that opinions, findings and conclusions or recommendations expressed in this publication generated by the NRF-supported research are those of the author and that the NRF accepts no liability whatsoever in this regard.

Competing Interest: The authors declare no competing interests

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CHAPTER SIX

CONCLUSION AND FUTURE WORK

6.1 Conclusion

The search for potential drugs from natural plant and plant-based products, which can protect the immune system from the harmful effect of radiation without side effects, has continued over a few decades. Moreover, the development of novel, non-toxic and effective natural radioprotectors has received much attention from researchers globally. It has been reported that plants with free-radical scavenging, antioxidant, anti-inflammatory and immunomodulatory properties have a protective effect on radiation-induced damage to the human population [1].

Despite the medicinal properties exhibited by *Costus afer (*CAE) and *Drymaria cordata* (DC) plants as established in the literature, little is known about their ability to protect cells against radiation-induced damage. Thus, this study was conducted to evaluate the radioprotective potential of these plants against radiation-induced haematological, histopathological and survival analyses and scientifically validate several claims made by folklore medicine about their potential to cure certain diseases and ailments. This formed the motivation for this study.

Evidence from the present investigation indicates the possible potential of CAE and DC to mitigate radiation-induced haematological alterations. In addition, the study has demonstrated that DC extract offered protection against radiation-induced mortality in mice, thereby increased the survival rate. The protective effect of CAE on histopathological parameters could not be established as the histology examination revealed no visible lesion associated with irradiated and treated mice's kidneys and liver. The results of this study on the haematological and survival studies support the current use of plants in treating diverse kinds of ailments.

Despite the evidence from the present study on CAE and DC that suggests their ability to repair radiation-induced damage to the haematopoietic system, the mechanism of action through which they exercise their radioprotective properties remain unknown. A report from the present

investigation suggests the significant elevation levels in the haematological parameters among the treatment groups may be partly due to the ability of the plants to scavenge free radicals and reactive oxygen species, thereby protecting mice against ionising radiation. Moreover, according to studies in the literature, the plants possessed anti-inflammatory, antioxidant and antitussive properties capable of neutralising the harmful peroxides and hydroxyl ions produced by the hydrolysis of water molecules following radiation exposure.

The results of the haematology analysis and survival studies from the present investigation support local claims of the therapeutic uses of CAE and DC in treating various kinds of ailments in folklore medicine. Thus, CAE and DC plants may be a new natural radioprotector, which may help mitigate against radiation-induced haematological disorder, apply before radiotherapy of cancerous cells and for general use by radiation workers.

6.2 Future work

The prospect of developing a natural radioprotector is very high considering the importance of natural plant and plant-based products in treating diverse kinds of human ailments and diseases from time immemorial. Humanity is blessed with an enormous heritage of vast medicinal plants, some of which have tested and proven therapeutic benefits.

Based on the outcome of our findings, further studies are necessary to validate the radioprotective efficacy of CAE on histopathological parameters. Our future work will focus on how to vary some of the research parameters (radiation dose, latent period and quantity (dose) of the extract) adopted in the present study. Furthermore, the radioprotective potential of the two plants can be enhanced if they are combined in the right proportion since research has shown that the synergy of all constituents of the plants will bring about the utmost therapeutic efficacy [2]. In addition, further research will focus on identifying the active

components present in the plants through research collaboration and establish the mechanism of action responsible for their radioprotective properties.

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