

Protective Role of *Adansonia digitata* and *Corchorus olitorius* Leaf-Based Diet on Cell Cycle Dysregulation in Gamma-Irradiated Wistar Rats

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Abstract

p53, cyclins D and mdm-2 proteins are key regulators of cell cycle, whose damage can cause abnormal cell cycle progression. This study investigated the protective roles of *Adansonia digitata* and *Corchorus olitorius*-leaf-based diet against gamma-irradiation-induced cell cycle damage in rats. Forty-eight Wistar rats (150 g $\pm$ 20 g) were distributed into 8 groups of 6 animals. Except for Group 1 that was fed with rat chow and water only, all other groups were exposed to gamma-irradiation and fed with different levels of *A. digitata*- and *C. olitorius*-based diets for 14 days after which the rats were sacrificed with blood and liver collected for biochemical analysis. Gamma-irradiation significantly ($p < 0.05$) reduced p53, cyclin D4 and increased mdm-2 concentrations of irradiated rats when compared with the control respectively. Administration of varying doses of leaf-based diets effectively reversed the situation with the most efficient diet being the 10% combination of the two plants, suggesting a synergistic effect. Findings from this study therefore indicate that the leaves of *Adansonia digitata* and *Corchorus olitorius* can be used as oral remedy in the regulation of abnormal cell division occasioned by gamma irradiation.

Keywords: p53, mdm-2, cyclins, gamma-irradiation, cell cycle, dysregulation

1. Introduction

Exposure to Gamma irradiation is widely used for the treatment and diagnosis of diseases [1]. Gamma irradiation is industrially appreciated because it enhances the functions of sophisticated equipment and appliances including sophisticated phones, laptops, CT scanning machines, and telecommunication mast [2,3]. Despite the ease and accessibility offered by these equipment, they are potential health risk in the event of leakages of Gamma rays. These rays have been implicated in the generation of free electrons, which eventually become free radicals, that are capable of increasing reactive oxygen and nitrogen species (RONS) [4,5]. These species induce DNA damage, cellular dysfunction and increased cancer risk [6].

DNA damage is one of the underlying risks for cell cycle dysregulation [7]. When a damaged DNA enters the cycle through G1 phase and transited to synthetic phase (S-phase), the implication is that another defective DNA will be replicated at S-phase. Such replicated DNA moves to G2 and eventually to M-phase. Once this happens, a defective differentiated cell will be produced at G₀ phase. This abnormal cell cycle progression is the genesis of cancer and other inherited diseases [8].

p53 is the guardian of cell cycle such that it locates, arrests and repairs any defective point on the cell cycle. It pauses cell cycle progression until it repairs the defect before it returns the cycle to continue progression [9]. The arrest and repair function of p53 is done in collaboration with cell cycle arrest proteins (INK4 and 5) [10]. The implication is that p53 must be active at all points to be able to arrest and repair any emergency abnormality that might befall the cycle. Unfortunately, increased RONS and free radicals will activate a membrane-bound p53 repressor protein known as mdm-2 [11, 12]. Thus, an increased concentrations of mdm-2 protein represses p53 protein which comprises cell cycle arrest and repair status is compromised leading to a condition known as lithium Fraumeni syndrome [13].

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Normal cell cycle progression is aided by cyclins and cyclin-dependent kinases (CDKs). Cyclins aid progression from G1 to S to G2 and to M phases by interacting with CDKs to enable progression of cell cycle from one phase to the other [14]. When this interaction is prevented, cell cycle progression is halted, thus revealing the importance of cyclins to the survival of cell cycle and cells itself [15]. The tumor suppressor protein p53 and cyclins play crucial roles in maintaining genomic stability such that dysregulation of these proteins by Gamma irradiation may prompt carcinogenesis [16].

Studies have shown that gamma-irradiation leads to p53 suppression [17, 18] and cyclin D dysregulation is the hallmark of abnormal cell cycle progression [19, 20]. A diet rich in antioxidants and essential micronutrients may mitigate radiation-induced cell cycle dysregulation. *Adansonia digitata* (popularly known as *Luru* in Nigeria) and *Corchorus olitorius* (popularly known as *Ewedu* in Nigeria) have been reported to enhance antioxidant defenses [21, 22] and protect against radiation-induced cell cycle alterations [23,24]. However, the protective roles of these plants on p53, cyclin D and mdm-2 damage have not been fully explored. Therefore, the present study investigated the protective role of *Adansonia digitata*- and *Corchorus olitorius*-based diets on cell cycle status of gamma-irradiated rats, focusing on p53, Cyclin D4 and mdm-2 status.

2. Materials and Methods

2.1 Drugs, Chemicals and Assay Kits

p53, mdm-2, cyclins D Enzyme Linked Immunosorbent Assay (ELISA) kits were products of Cayman Laboratory Ltd., USA. All reagents were prepared using all-glass distilled water.

2.2 Collection and identification of plant materials

Fresh leaves of *Adansonia digitata* and *Corchorus olitorius* were purchased from a local market in Ibadan, Nigeria. The plants were authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. Voucher Specimens for *Adansonia digitata* and *Corchorus olitorius* with reference numbers UICH/001/951 and UITH/002/154 respectively were prepared and deposited at the Herbarium accordingly.

2.3 Preparation of *Adansonia digitata* and *Corchorus olitorius*

Fresh leaves of *Adansonia digitata* and *Corchorus olitorius* were washed with clean water, cut into pieces and dried at room temperature (22-24°C) for two weeks. The dried plant samples were pulverized with electric blender (Steelman, K207, H.M and Co. Ltd, China).

2.4 Proximate and phytochemical analyses of *Adansonia digitata* and *Corchorus olitorius*

Proximate analysis of *Adansonia digitata* and *Corchorus olitorius* leaf was carried out using the standard procedures of the Association of Official Analytical Chemists [25]. The proximate analysis of the combination of *Adansonia digitata* and *Corchorus olitorius* leaf was also analyzed. The nutrients analysed include crude protein, crude fat, crude fiber, ash, moisture content and carbohydrates. The phytochemical constituents of *A. digitata* and *C. olitorius* leaves including alkaloids, flavonoids, polyphenols, tannins, terpenoids and steroids were determined using standard methods [26]. All tests were performed in triplicate to ensure accuracy and reliability.

2.5 Formulation of feed

Feed components were combined in the quantities specified in Table 1 with the pulverized leaves of *Adansonia digitata* and *Corchorus olitorius* added at inclusion rates of 5 g and 10 g. Compounded diets were administered to Irradiated ad libitum for a period of 2 weeks.

2.6 Experimental Animals and Ethical Approval

Forty-eight Wistar rats with a mean weigh of 180 g were sourced from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in clean, well-ventilated cages under controlled environmental conditions: temperature (28-30°C), photo period (12 hours light, 12 hours dark), and humidity (45-55%). They were provided with pelletized rat feed (Vital Feed, Grand Cereals, Jos, Nigeria) and clean tap water *ad libitum* throughout the experimental period.

Ethical approval for this study was granted by the University of Ilorin Ethical Committee, with the approval number UERC/ASN/2022/1409, dated September 13th, 2022. The research was conducted in strict adherence to the guidelines on the care and use of laboratory animals, ensuring the highest standards of animal welfare.

Table 1: Composition and Formulation of *Adansonia digitata* and *Corchorus olitorius* leaves diet

Ingredients	Control Diet	<i>A. digitata</i> (5 g)	<i>A. digitata</i> (10 g)	<i>C. olitorius</i> (5 g)	<i>C. olitorius</i> (10 g)	ADCO (5 g)	ADCO (10g)
Starch	512	512	512	512	512	512	512
Soy Beans	250	250	250	250	250	250	250
Soy Oil	40	40	40	40	40	40	40
Maize Husk	40	40	40	40	40	40	40
Vit/Min	50	50	50	50	50	50	50
Sucrose	100	100	100	100	100	100	100
D-Met	4	4	4	4	4	4	4
L-lysine	4	4	4	4	4	4	4
	-	+	++	+	++	+	++
Total	1000	1000	1000	1000	1000	1000	1000

ADCO: combination of *Adansonia digitata* and *Corchorus olitorius* at 5 and 10 g respectively

2.7 Animal Exposure to Irradiation

Rats were exposed to a single dose of 6 grey whole-body gamma radiation using a modified protocol [27]. To ensure uniform radiation exposure, the rats were placed in a specially designed carton cage with ventilation holes, deviating from the traditional radiation exposure coat. The radiation source was a Cobalt 60 Radiotherapy Machine, which operated by moving back and forth over the cage. Each pass was equivalent to 1 grey of radiation, and the rats underwent six passes to achieve the desired dose.

2.8 Animal Groupings and Feeding Experiment

Experimental rats (n=48) were distributed into 8 groups of 6 animals (Table 2). Except Group 1, other groups were exposed to gamma-irradiation. Animals in Group 1 were fed with rat chow and water only while those in Group 2 were not administered leaf-based diet. Animals in Groups 3 and 4 received 5% and 10% *A. digitata*-based diet respectively, while those in Groups 5 and 6 received 5% and 10% of *C. olitorius*-based diet. Animals in Groups 7 and 8 received 5% and 10% combination of both plants respectively.

Table 2: Animal Groupings and feed Administration

Groups	Grouping and feed administering
Group 1: Positive Control	Feed on standard feed and water only
Group 2: Negative Control	Exposed to Irradiation and fed with standard feed
Group 3: IR-5-AD-FB	Exposed to Irradiation and fed on 5 % inclusion of <i>A. digitata</i> -based diet
Group 4: IR-10-AD-FB	Exposed to Irradiation and fed on 10% inclusion of <i>A. digitata</i> -based diet
Group 5: IR-5-CO-FB	Exposed to Irradiation and fed on 5% inclusion of <i>C. olitorius</i> -based diet
Group 6: IR-10-CO-FB	Exposed to Irradiation and fed on 10% inclusion of <i>C. olitorius</i> -based die
Group 7: IR-5-ADCO-FB (1:1)	Exposed to Irradiation and fed on 5% inclusion of <i>A. digitata</i> + <i>C. olitorius</i> -based diet
Group 8: IR-10-ADCO-FB (1:1)	Exposed to Irradiation and fed on 10% inclusion of <i>A. digitata</i> + <i>C. olitorius</i> -based diet

IR- Irradiation

5-AD-FB- 5% inclusion of *A. digitata* Feed-based diet

10-AD-FB- 10 % inclusion of *A. digitata* Feed-based diet

5-CO-FB- 5% inclusion of *C. olitorius* Feed-based diet

10-CO-FB- 10% inclusion of *C. olitorius* Feed-based diet

5-ADCO-FB- 5% inclusion of *A. digitata*+ *C. olitorius* Feed-based diet

10-ADCO-FB- 10% inclusion of *A. digitata*+ *C. olitorius* Feed-based diet

(1:1)- The *A. digitata* was mixed with same quantity of *C. olitorius* in ratio 1 to 1

2.9 Animal Sacrifice and Preparation of Nuclear Extract

At the expiration of 14 days experimental period, the animals were sacrificed by cervical dislocation and blood was collected by jugular puncture. The rats were dissected, and the liver was extracted and gently washed with a 1.14% KCl solution to remove blood stains and other impurities. The liver was weighed and immediately stored in an ice-cold 0.25 M sucrose solution. A portion of the liver (1 g) was cut using a clean scalpel, homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v), and centrifuged at 12,000 *g* for 15 minutes. The supernatant was discarded, and the sediment obtained was treated with DNase inhibitor to prevent DNA degradation and with 80% 5 ml calcol to inactivate and coagulate impurities. The resulting solution was ultra-centrifuged at 4500 *g* for 10 minutes. The sediment obtained was treated with NP40 and Tris-chloride respectively to facilitate the separation of cytoplasmic and nuclear fractions. The resulting solution was cold-centrifuged at 8500 *g* for 10 minutes and the supernatant which contained the rich nuclear extract was used for the determination of p53, mdm-2, and cyclin D concentrations.

2.10 Determination of p53 status in irradiated rats fed with *A. digitata* and *C. olitorius* leaf-based diets

The nuclear extract was immobilized into a 96-well ELISA plate. A specific p53 Primer sequence (p53 short DNA unwind strands) containing a p53 response element, was immobilized into the same 96-wells plate and was mixed gently. The p53 contained in the nuclear extract, binds specifically to the p53 response element immobilized into the 96-well plate. The p53 status was detected by the addition of a specific p53 primary antibody (directed against p53). Furthermore, a secondary antibody conjugated to horseradish peroxidase (HRP) was added, which provided a sensitive colorimetric readout at 450 nm. The absorbance read at 450 nm is equivalent to the number of non-damaged p53 that binds to the p53 primer.

2.11 Determination of mdm-2 status in irradiated rats fed with *A. digitata* and *C. olitorius* leaf-based diets

The nuclear extract was immobilized into a 96-well ELISA plate. A specific mdm-2 primer sequence (mdm-2 short DNA unwind strands) containing mdm-2 response element, was immobilized into the same 96-wells plate and mixed gently. Mdm-2 contained in the nuclear extract, binds specifically to the mdm-2 response element immobilized into the 96-well plate. The mdm-2 status was detected by addition of a specific mdm-2 primary antibody (directed against mdm-2). Furthermore, a secondary antibody conjugated to horseradish peroxidase (HRP) was added, which provided a sensitive colorimetric readout at 450 nm. The absorbance read at 450 nm is equivalent to the number of non-damaged mdm-2 that binds to the mdm-2 primer.

2.12 Determination of cyclin D status in irradiated rats fed with *A. digitata* and *C. olitorius* leaf-based diets

The nuclear extract was immobilized into a 96-well ELISA plate. A specific cyclin D primer sequence (cyclin D short DNA unwind strands) containing cyclin D response element, was immobilized into the same 96-wells plate and mixed gently. Cyclin D contained in the nuclear extract, binds specifically to the cyclin D response element immobilized into the 96-well plate. The cyclin D status was detected by addition of a specific cyclin D primary antibody (directed against cyclin D). Furthermore, a secondary antibody conjugated to horseradish peroxidase (HRP) was added, which provided a sensitive colorimetric readout at 450 nm. The absorbance read at 450 nm is equivalent to the number of non-damaged cyclin D that binds to the cyclin D primer.

2.13 Statistical Analysis

Data are presented as mean $\pm$ SEM of six determinations, unless otherwise specified. Statistical analysis was performed using Duncan's Multiple Range Test, complemented by Student's t-test. All analyses were conducted using the Statistical Package for Social Sciences (SPSS) software, version 21 (SPSS Inc., Chicago, IL, USA). A 95% confidence interval was used to determine statistical significance.

3. Results

3.1 Proximate Composition of *Adansonia digitata* and *Corchorus olitorius* Leaves

The proximate analysis of *Adansonia digitata* and *Corchorus olitorius* leaf yielded various constituents including moisture, fiber, crude protein, fats, total ash, and nitrogen-free extract (Table 3). The results revealed that nitrogen-free extract was the most abundant component in both leaves, followed by other constituents such as moisture content, crude protein, crude fiber, crude fats and non-esterified fat in varying proportions. The proximate composition of the two leaves also compared favourably with the individual plant leaf.

Table 3: Proximate Composition of *A. digitata* and *C. olitorius* leaves

Plant Sample	Moisture Content (%)	Crude Protein (%)	Crude Fiber (%)	Total Ash (%)	Crude Fat (%)	NFE (%)	Total (%)
<i>A. digitata</i>	10.75± 0.05	21.87±0.01	8.80 ±0.09	15.96±0.14	3.84±0.06	38.33±0.61	100
<i>C. olitorius</i>	11.20± 0.11	22.31±0.02	9.40 ±0.10	14.28±0.13	3.72±0.16	39.54±0.05	100
<i>A. digitata</i> + <i>C. olitorius</i>	10.80±0.17	20.46±0.14	10.00±0.21	14.40±0.03	3.31±0.01	41.03±0.02	100

Data are mean ± SEM of three replicates; NFE: Nitrogen Free Extract

3.2 Phytochemical Constituents of *Adansonia digitata* and *Corchorus olitorius*

The phytochemical constituents of *Adansonia digitata* and *Corchorus olitorius* leaves include saponins, alkaloids, polyphenols, tannins, steroids, and terpenoids as summarized in Table 4. Notably, anthraquinone, cardiac glycosides, and phlobatannins were absent. The data revealed that tannins were the most abundant in *Adansonia digitata* (198.98 mg/L), while polyphenols were the most prevalent in *Corchorus olitorius* (203.14 mg/L).

Table 4: Phytochemical Constituents of *A. digitata* and *C. olitorius* Leaves

Metabolites	<i>Adansonia digitata</i> (mg/dl)	<i>Corchorus olitorius</i> (mg/dl)
Alkaloids	78.76 ± 0.43	72.36 ± 2.05
Anthraquinone	ND	ND
Cardiac glycosides	ND	ND
Flavonoids	13.42 ± 1.03	104.81 ± 0.18
Polyphenols	147.90 ± 0.68	203.14 ± 0.32
Phlobatannins	ND	ND
Saponins	16.59 ± 1.85	22.17 ± 0.24
Tannins	198.98 ± 0.14	127.40 ± 0.16
Terpenoids	42.45 ± 0.45	21.25 ± 1.08
Steroids	30.45 ± 0.51	12.59 ± 1.85

Results are mean ± SEM of three replicates; ND: Not Detected

3.3 Effect of *A. digitata* and *C. olitorius* leaf-based diet on p53, mdm-2 and Cyclin D concentrations in irradiated rats

Whole-body gamma radiation (6 Gy) significantly ($p < 0.05$) reduced p53 and cyclin D concentrations in irradiated rats (Figures 1 and 2) and significantly ($p < 0.05$) increased mdm-2 protein concentration (Figure 3) when compared with the non-irradiated controls. However, feeding irradiated rats with diets containing *Adansonia digitata* and *Corchorus olitorius* leaves significantly ($p < 0.05$) increased p53 and cyclin D concentration and reduced mdm-2 respectively. Notably, the most effective treatments were the diets with 5% and 10% inclusion of both leaves, which showed the highest increase in p53 and cyclin D concentration and decreased mdm-2 level when compared with the positive control level.

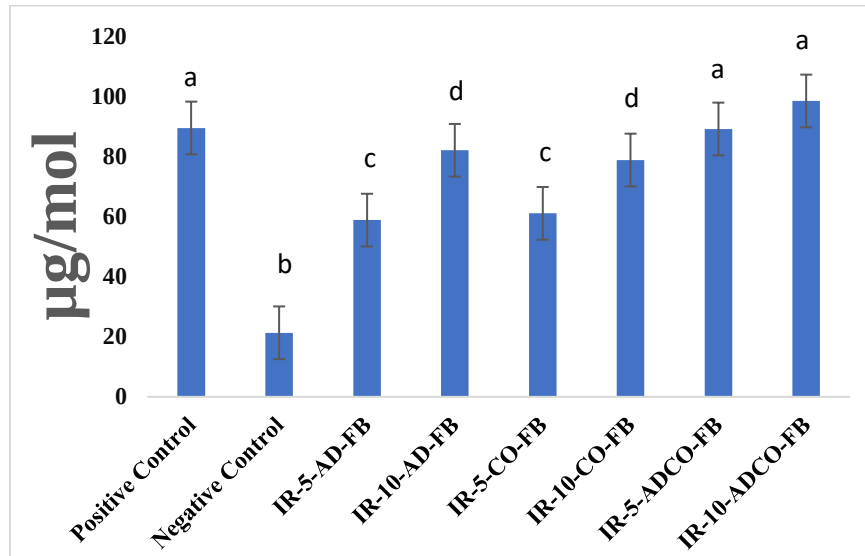


Figure 1: Concentration of p53 in irradiated rats fed with *Adansonia digitata* and *Corchorus olitorius* leaf-based diet. Values are expressed as Means $\pm$ SEM; n=6, (P < 0.05); IR- Irradiation

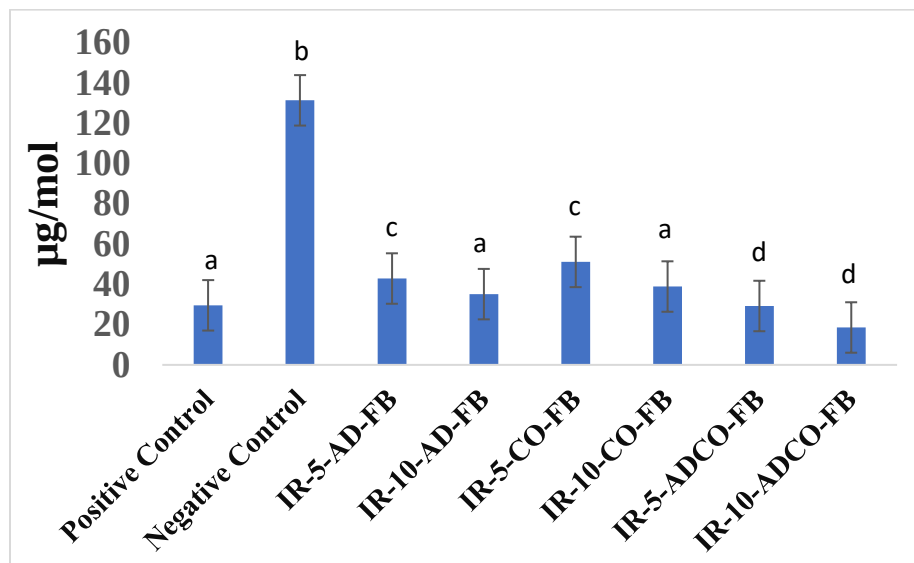


Figure 2: Concentration of mdm-2 of irradiated rats fed with *Adansonia digitata* and *Corchorus olitorius* leaf-based diet. Values are expressed as Means $\pm$ SEM; n=6, (P < 0.05); IR- Irradiation.

5-AD-FB- 5% inclusion of *A. digitata* Feed-based diet
 10-AD-FB- 10 % inclusion of *A. digitata* Feed-based diet
 5-CO-FB- 5% inclusion of *C. olitorius* Feed-based diet
 10-CO-FB- 10% inclusion of *C. olitorius* Feed-based diet
 5-ADCO-FB- 5% inclusion of *A. digitata*+ *C. olitorius* Feed-based diet
 10-ADCO-FB- 10% inclusion of *A. digitata*+ *C. olitorius* Feed-based diet

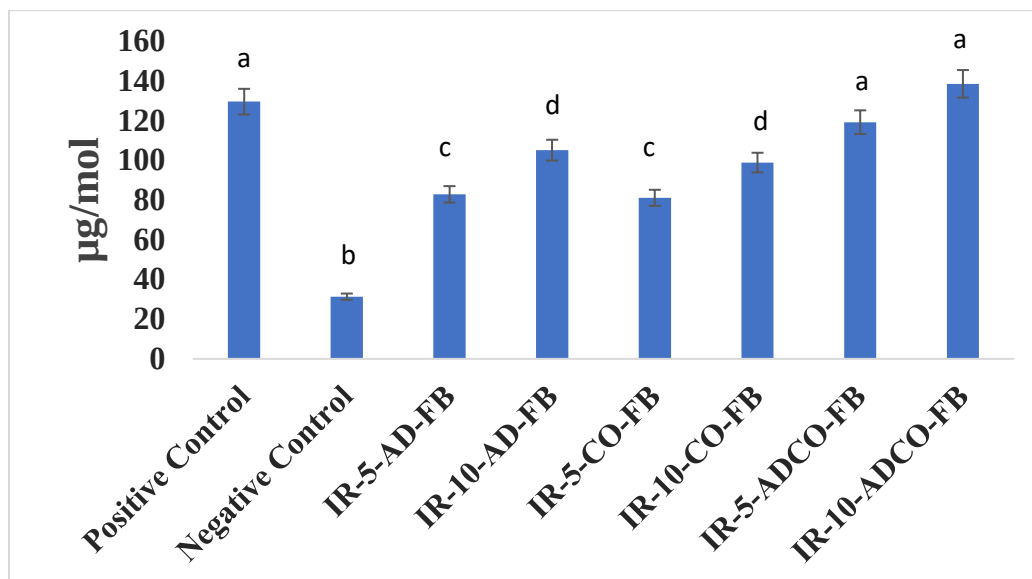


Figure 3: Concentration of cyclin D of irradiated rats fed with *Adansonia digitata* and *Corchorus olitorius* leaf-based diet. Values are expressed as Means $\pm$ SEM; n=6, (P < 0.05); IR- Irradiation.

5-AD-FB- 5% inclusion of *A. digitata* Feed-based diet

10-AD-FB- 10 % inclusion of *A. digitata* Feed-based diet

5-CO-FB- 5% inclusion of *C. olitorius* Feed-based diet

10-CO-FB- 10% inclusion of *C. olitorius* Feed-based diet

5-ADCO-FB- 5% inclusion of *A. digitata*+ *C. olitorius* Feed-based diet

10-ADCO-FB- 10% inclusion of *A. digitata*+ *C. olitorius* Feed-based diet

4. Discussion

The proximate constituents of plants are crucial for their nutritional value and dietary balance [28]. Proteins, for instance, are essential for body building and amino acid synthesis [29]. Carbohydrates, on the other hand, are vital for energy production through glycolysis, generating pyruvate that channeled into the tricarboxylic acid cycle, for more ATP production [30,31]. In the present study, the reasonable quantities of proteins and carbohydrates in the leaves of *Adansonia digitata* and *Corchorus olitorius* may be attributed to the nutritional and dietary balance in the rats fed with the two leaf-based diets. Proximate analysis also revealed crude fat content, which is involved in cholesterol synthesis, a precursor to several hormones. These hormones play significant roles in healthy living [32]. The reasonable amount of crude fats in the leaves of *Adansonia digitata* and *Corchorus olitorius* may be responsible for the healthy living of the rats fed with the leaf-based diets. The crude fat content may have also supported the synthesis of steroids, which are essential repressors of mdm-2 protein production [33]. Dietary fibers, another essential proximate constituent, have been reported to facilitate peristaltic movement of the gastrointestinal tract (GIT), enhancing ease food digestion [34]. Plants with considerable fiber content like *Adansonia digitata* and *Corchorus olitorius* aid GIT movement thereby promoting efficient digestion.

The medicinal properties of plants are attributed to the presence of bioactive constituents such as flavonoids, polyphenols, saponins, alkaloids and tannins and are responsible for their pharmacological effects [35]. The findings of this study suggest that the pharmacological influence of *Adansonia digitata* and *Corchorus olitorius* on cellular parameters and metabolism may be attributed to the presence of flavonoids, polyphenols, tannins, saponins, and alkaloids. The use of secondary metabolites in plants to modulate cellular metabolism, including cell cycle regulation and progression, cell division and hormonal regulation (such as cyclins hormone) has been well-established [36]. Furthermore, plants with medicinal properties have been employed in the prevention and treatment of cellular alterations, highlighting the significance of these bioactive compounds in maintaining cellular homeostasis [35].

Polyphenols have been reported to activate p53 arrest and repair-function as well as down-regulate mdm-2 damaging activities [36]. The activated p53 will arrest abnormality associated with any point (G1, S, G2 and M point) of the cell cycle or any metabolites (cyclins and CDK's) that will aid smooth cell cycle progression [37]. Saponins have been reported to stimulate cyclins (A, D and E) such that the activated cyclins are available to bind to CDK thereby progressing cell cycle [35,36]. In the present study, the significant concentration of polyphenols in the leaves of *Adansonia digitata* and *Corchorus olitorius*-based diet might have activated p53 and down regulate mdm-2 protein of irradiated rats fed with *Adansonia digitata* and *Corchorus olitorius* leaf-based diet. Furthermore, the significant concentration of saponins in the leaves of *Adansonia digitata* and *Corchorus olitorius*-based diets might have activated Cyclin-CDK binding.

Gamma-irradiation has been reported to trigger the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [38]. An increase in ROS/RNS causes DNA damage, cellular dysfunction and a heightened risk of cancer [39]. DNA damage is a key factor in cell cycle dysregulation as it causes damage genetic material to enter the cell cycle [38,39]. When this occurs, the damaged DNA is replicated during the S-phase, leading to the production of defective cells. These aberrant cells can then progress through the G2 and M-phases, ultimately resulting in the creation of differentiated cells with genetic defects during the G0 phase [40]. This aberrant cell cycle progression is a critical step in the development of cancer and other inherited diseases [40].

Cyclins (such as cyclins A, D and E) and cyclin dependent kinases (CDK 2, 4 and 6) have been reported to be damaged by gamma irradiation [41]. Damaged cyclins and CDKs have been linked to an aberrant cell cycle which is the genesis of inherited cell related diseases [42]. When these pathological conditions occur, p53 has been reported to arrest and repair the aberrant cell cycle. However, in the event of irradiation, mdm-2 suppresses the arrest-action of p53, thus compromising the p53 arrest and repair of the cell cycle [42]. In the present study, gamma irradiation (6 Gy) significantly altered the physiology of the cell cycle and its regulation as evidenced by increased mdm-2 levels, decreased p53 levels, and disrupted cyclin D levels. This suggests that irradiation induces cell cycle dysregulation, potentially leading to uncontrolled cell growth and tumorigenesis [41,42]. It has been reported that an increase in mdm-2 levels suppresses p53 arrest and repair function, while its reduction causes inability to regulate cell cycle progression [40]. Disrupted cyclin D levels may also contribute to aberrant cell cycle progression [19,22]. The tumor suppressor gene p53 plays a crucial role in arresting and repairing dysregulated cell cycles and potentially repairing damaged DNA [23,24]. However, p53 is negatively regulated by mdm-2. An increase in free radicals activates mdm-2 protein, leading to p53 down regulation [43]. The reduction in p53 levels was observed in the irradiated-untreated group suggests that radiation induces free radical formation and suppresses p53 by the action of mdm-2. This implies that the increase in free radicals may have suppressed the tumor-suppressing function of p53, potentially contributing to radiation-induced damage. The findings of this study demonstrate that the formulated *Adansonia digitata* and *Corchorus olitorius* diet restored normalcy to p53, mdm-2, and cyclin D levels, indicating a protective effect against radiation-induced cell cycle dysregulation.

Flavonoids inhibit mdm-2 activation by altering p53 repression [27], thus potentially reducing the risk of aberrant cell cycle dysregulation and cell cycle inherited diseases [44]. The reduced mdm-2 levels in irradiated rats fed with *Adansonia digitata* and *Corchorus olitorius* leaf-based diet may be attributed to high polyphenol content. This is consistent with previous studies which reported that *Adansonia digitata* and *Corchorus olitorius* extract restored cellular alterations [45]. Therefore, this study validates previous work on the radioprotective effects of *Adansonia digitata* and *Corchorus olitorius* extracts and demonstrates the potential of dietary interventions in mitigating radiation-induced disorders. However, further research is needed to elucidate the exact mechanisms underlying this radioprotective effect and to explore the potential applications of this finding in the field of radiation oncology. In contrast, irradiated rats experience increased free radical production, leading to enhanced mdm-2 activation [30]. The elevated levels of activated mdm-2 suggest p53 damage, which favors abnormal cell cycle progression and indicates cellular alterations induced by radiation [29]. This implies that radiation-induced free radicals can disrupt normal functioning of p53, leading to uncontrolled cell growth and potential tumor formation [33,34]. The increased level of activated p53 in the group administered *A. digitata* and *C. olitorius* leaf-based diet might be due to the presence of significant ($p < 0.05$) amount of catechin and epicatechin in the the two leaves. It was also observed that the increased was more significant in the combined leaf-diet when compare to others, this is suggestive that the leaves protected the rats against cellular alterations induced by radiation.

5. Conclusion

From this study, it can be concluded that the leaves of *A. digitata* and *C. olitorius* can be used as oral remedy in the treatment of cellular alteration induced by gamma irradiation. This has lend further credence to consumption of the two leaves especially by the locals.

6. References

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