

## Protective and Ameliorative Effects of *Adansonia digitata* and *Corchorus olitorius* Leaves-based Diet on Gamma-irradiation induced Genotoxicity and p53 Damage in Cellular System of Rats

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### ABSTRACT

Exposure to gamma irradiation ( $\gamma$ -IR) causes cellular alterations (CA) in animals. Extract of *Adansonia digitata* and *Corchorus olitorius* leaves (ADCOL) have been shown to protect against  $\gamma$ -IR-induced CA in rats. Traditionally, the leaves are consumed as part of diets, however, there is paucity of scientific information on the use of ADCOL based-diet (BD) against CA. Thus, the study investigated the effects of ADCOL BD in cellular system of irradiated rats. The objectives of the study were to determine the secondary metabolites in ADCOL, induce cellular alterations in rats and evaluate the protective, ameliorative and toxicity of ADCOL BD on rats. A total of 72 rats ( $186 \pm 4.90$ g) were used in the 2 phases of this study. In the ameliorative phase, 36 rats were assigned into 6 groups of 6 each; Group I were fed with rat chow and distilled water only, all other groups were irradiated, such that, Groups II, III, IV, V & VI were fed rat chow only, 80% inclusion of ADBD, 80% inclusion of COBD, 80% inclusion of combined ADCOLBD and Vitamin-C-BD respectively. Similar design was adopted for protective phase, except that, the rats were initially fed the inclusion diet before irradiation. Micronuclei status were viewed by automated electron microscope, p53 level was determined by enzyme linked immunosorbent assay and antioxidant status of rat were determined by standard methods. Data were analysed using Duncan multiple range test at  $p < 0.05$ . The findings in this study, were that; secondary metabolites constituents of *A. digitata* and *C. olitorius* were alkaloids (78.76 and 72.36g/dl) terpenoids (42.45 and 21.25 g/dl) flavonoids (13.42 and 104.81g/dl) polyphenols and (147.90 and 203.14 g/dl) respectively;  $\gamma$ -irradiation significantly ( $p < 0.05$ ) increased mean number of micronuclei and decreased p53, superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) level in rats; 80% inclusion of *A. digitata* and *C. olitorius* leaf-BD significantly ( $p < 0.05$ ) reduced mean number of micronuclei and increased p53, SOD, CAT and GSH level in irradiated rats. The study concluded, that *A. digitata* and *C. olitorius* protected cellular damage induced by radiation. Thus, recommending their use against cellular damage.

**Keywords:** Chromosomal aberration, micronuclei DNA, *Adansonia digitata*, *Corchorus olitorius*,  $\gamma$ -irradiation, p53 and cellular alterations.

### Introduction

It is important to know, that of the estimated 7 billion people in the world, 6 billion have access to one or more technological gadget(s) that emits

radiation (Mathew *et al.*, 2014). The prevalence of radiation-induced cellular alterations is projected to rise from 42 to 61 % in developed countries and 22

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to 41 % in developing countries like Nigeria by the year 2025 (WHO, 2014).

4608 Radiation emitting gadget have ushered in ease and accessibility to humans, they still remain risk to human health, as was coined out from the memorandum of understanding between a telecommunication company and a land owner, the statement revealed that “Before telecommunication mast is mounted in this property, you’ll be paid #10-20 million, we are giving you this money for you to relocate, because it’s dangerous to your health if you continue to stay here for the next 5-10 years (MOU signed by MTN and land owner in 2017)”. But people don’t even read this as it relates to their health.

A recent work by Yhabum *et al.* (2018) explain how radiations from phone placed together with raw egg rapped in a polytene bag cooked the egg. He then infers such effect to how radiation from phones (placed in the pocket) could cause changes to the structure-functions of the human scrotal after upon, long period of exposure. About 50% of Nigeria population are exposed to radiation via phones; 25% via both phones and telecommunication mast; 5% through other means (radiotherapy) and only 20% are virtually safe (age 1-8) (WHO, 2015).

Human exposure to radiations from any of the above equipment results in electron leakage, capable of inducing free radical production above the subtoxic concentrations (24 ug/mol) in humans, usually to pathological level (120 ug/mol) (Karumi & Ghaxul, 2000). Increased free radical level from 24 to 120 ug/mol in human system, is the contributory factor to oxidative stress and cellular alterations (Haliwell *et al.*, 2009). Free radical level of 120 µg/mol caused dysregulated bone marrow (micronucleus component) thus altering DNA status, which eventually induced chromosomal aberration (Karumi and Ghaxul, 2000).

Micronuclei DNA formation, formed from chromosomal damage, that are ROS/RNS-induced, are capable of increasing free radical above the

subtoxic concentration, this condition result to micro nuclei DNA formation. The extent of micronuclei formation, is a function of the extent of the chromosomal aberration, as both measure chromosomal alterations (Dearfield *et al.*, 1991; Auletta *et al.*, 1993). Micronucleus status are used to detect chromosomal breakage and loss following rats exposure to radiation (Dearfield *et al.*, 1991). The assay is conducted in the bone marrow or less frequently, the peripheral blood erythrocytes of experimental animal.

The human cells are continuously attacked by varieties of genotoxic substances such as radiation, capable of causing DNA damages and chromosomal aberrations (Gertner *et al.*, 2000). Most DNA repairs have come with errors that are capable of causing mutations and chromosomal aberrations. These changes usually lead to loss of functions of tumor suppressor genes, gain of functions of oncogenes and the genesis of cancer development (Marteijn *et al.*, 2014). p53 is a central tumor suppressor, guarding the genome by orchestrating a variety of DNA-damage-response (DDR) mechanisms (Derry *et al.*, 2001). p53 induces apoptotic responses to compromised cells and plays prominent roles in facilitating DNA repair through cell cycle arrest and mediate its repair and restoring genomic stability (Schumacher, 2005a).

All these revealed the multitasking quality of p53 in providing protection from cancer development by maintaining genome stability. Hence the name guardian of all genome (Hofmann *et al.*, 2002). In the absence of this “guardian of the genome,” cells are adequately not protected from mutations and genomic aberrations.

Several synthetic agents have been used, either to scavenge or stabilises free radicals in experimental rats, for example synthetic vitamin E protects the integrity of acetyl choline receptors in normal neurons and prevents toxicity and apoptosis induced-ROS in male rabbits (Kennedy *et al.*, 2001; Behl and Moosmann, 2002). Synthetically produced vitamin C, stabilize reactive intermediate as well as

neutralized free radical in albino rat (Adaramoye *et al.*, 2001). Most of these synthetic drugs haven't come with adverse side effect, as well as high cost, even when the money is available, they may not be accessible.

Plant and plant products such as vegetables and fruits, contain secondary metabolites (flavonoids, polyphenols, tannins, saponins, glycosides, vitamins) that are cheap, accessible and safe. They mediate and scavenge free radicals and stabilises reactive species intermediate. Thus, the aim of this study were to determine the protective and ameliorative effects of the leaves based-diet of *A. digitata* and *C. olitorius* on the cellular system of rats exposed to gamma irradiation

### ***Corchorus olitorius***

*Corchorus olitorius* (Linn) is a leafy vegetable that belongs to the family Tiliaceae, it is known as Jute mallow in English and Ewedu in Yoruba. It is an annual herb with a slender stem and an important green leafy vegetable in many tropical areas including Nigeria, Egypt, Sudan, India, Bangladesh, in tropical Asia such as Philippine and Malaysia. The leaves (either fresh or dried) are cooked into a thick viscous soup or added to stew or soup and are rich sources of vitamins and minerals (Branda *et al.*, 2004) with various therapeutic uses in herbal medicine. The leaves extracts have been reported to possess hypotensive, anti-diabetic, anti-malarial, hepatoprotective, anti-microbial, anti-inflammatory, analgesics, anti-diarrhea, anti-viral, anti-carcinogenic and anti-mutagenic properties (Thyagarajan *et al.*, 1988; Odetola & Akojenu, 2000; Sripanidkulchai *et al.*, 2002; Adeneye *et al.*, 2006).

### ***Adansonia digitata***

*A. digitata* is a member of the baobabs family and commonly known as Kukah in Hausa, luru in Yoruba and tree of life in English. It's a deciduous tree with four growth phases and produces fruits consisting of a yellow-white pulp, with a floury texture and numerous hard-round seeds, enclosed in

a tough shell (Dharmendra *et al.*, 2006). The leaf of the baobab tree is food for many populations in Africa, especially the central region of the continent. *Adansonia digitata* is a commonly used traditional plant which are consumed in food or used in the direct treatment of several diseases such as anaemia, diabetes, lipid peroxidation disorders, ischemia reperfusion diseases and inflammatory bowel syndrome in South-west Nigeria (Lewanda *et al.*, 2007). The powdered leaves are used as a tonic, an anti-asthmatic, anti-histamine and anti-tension properties. The leaves are also used to treat insect bites, guinea worm, internal pains, dysentery and urinary tract, ophthalmia and otitis. Baobab leaves are used medicinally as a diaphoretic, astringent, expectorant and as a prophylactic against fever (Scheuring *et al.*, 1999). The carotenoid content of baobab leaves has also been established to contribute to the prevention of oxidation in the human cell and lots of other diseases.

## **Materials and Methods**

### ***Plant Materials and Authentication***

Fresh leaves of *A. digitata* and *C. olitorius* were purchased from 'Ipata' Market, Ilorin, Kwara State, Nigeria. The plants were identified and authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Kwara State. Voucher Specimen Number (UICH/001/951 and UITH/002/154) were deposited in the Herbarium respectively.

### ***Experimental Animals***

A total of seventy-two (72) healthy Wistar rats (198 ± 5.00g) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in clean cages and adequately-ventilated house condition. They were allowed free access to rat pellets (Vital Feed, Grand Cereals, Jos, Nigeria) and clean tap water. The cages were cleaned daily and the study was conducted following the guidelines on the care and use of laboratory animals. Ethical

approval was obtained from the University of Ilorin Ethical Committee, University of Ilorin.

4610 **Drugs, Chemicals and Assay Kits**

The drugs, chemicals and fetal bovine system (FBS) used in the study were commercially obtained from Monobind International, Lake Forest, USA. ELISA kits were products of Cayman Laboratory LTD., USA. All other chemicals will be of analytical grades and prepared in all-glass distilled water.

**Collection and Preparation of Plant Extracts**

Fresh leaves of *Adansonia digitata* and *Corchorus olitorius* were washed with clean water, chopped into pieces and dried at room temperature for two weeks. The dried plant samples were pulverized with grinding machine (Steelman K207, H.M and co Ltd, China). A known quantity (50 g) of pulverized *Adansonia digitata* and *Corchorus olitorius* leaves were extracted in 200 ml Methanol (98.8 %) for 72 hours. The extracts were filtered using white clean Muslin clothe. The filtrates were lyophilized using Rotary evaporator (RE, k25, Longfin, Australia).

**Proximate Composition of *Adansonia digitata* and *Corchorus olitorius* leaves**

Proximate analysis of *Adansonia digitata* and *Corchorus olitorius* leaves were carried out using the standard procedures of the Association of Official Analytical Chemists (1990).

**Secondary Metabolite Constituents of *Adansonia digitata* and *Corchorus olitorius***

Qualitative and quantitative secondary metabolites screening of *A. digitata* and *C. olitorius* leaves were determined, by using the methods of Ajayi *et al.* (2010); Harborne (1993) and Sofowora (1993). All determinations were done in triplicates.

**Feed Formulation**

**Table 1:** Feed Constituents and source

Feed Constituents	Sources
Corn starch: was prepared using yellow Corn and its source of starch	Was obtained from ‘Oja-Tuntun’ Market, Ilorin, Kwara State, Nigeria
Vitamins-mineral mix: Source of essential vitamins	Product Ahirma, India
D-methionine and L-Lysine: Source of amino acid	Product Ahirma, India
Maize husk: source of cellulose Soybean oil: source fat and oil	Was purchased from Olufadi Market, Ilorin and was milled accordingly.
Pulverized leaf of <i>Corchorus olitorius</i>	At 80 % inclusion
Pulverized leaf of <i>Adansonia digitata</i>	At 80 % inclusion

**Animal Exposure to Radiation**

Wistar rats were exposed to 6 grey whole-body gamma radiations by the method described by (Kamani *et al.* (2008) and modified by Nwozo and Bello (2014). The exposure was carried out in the Department of Radiotherapy, College of Medicine, University of Ibadan, Ibadan, Nigeria.

**Data Analysis**

Data were expressed as mean ± SEM of 6 determinations, except otherwise stated. All results were statistically analysed using Duncan Multiple Range Test and complemented with Student’s t-test. Statistical Package for Social Sciences, version 21 (SPSS Inc., Chicago, IL, USA) were used for statistical analyses. Statistical significance was set at 95% confidence interval (Mahajan, 1997).

**Table 2:** Formulation of *Adansonia digitata* and *Corchorus olitorius* Leaf-based diets

Ingredients	Control Diet	<i>A. digitata</i> based-diet (40%)	<i>A. digitata</i> based-diet (80%)	<i>C. olitorius</i> based-diet (40%)	<i>C. olitorius</i> based-diet (80%)	ADCO based-diet (40%)	ADCO based-diet (80%)
Corn Starch	512	307.20	102.40	307.20	102.40	307.20	102.40
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 Mix	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-lysin	4	4	4	4	4	4	4
<i>A. digitata</i>	-	204.80	409.60	-	-	104.20	204.20
<i>C. olitorius</i>	-	-	-	204.80	409.60	104.20	204.20
<b>Total</b>	<b>1000</b>	<b>1000</b>	<b>1000</b>	<b>1000</b>	<b>1000</b>	<b>1000</b>	<b>1000</b>

ADCO: *A. digitata* + *C. olitorius*; LBDAC (Leaf based-diet of *A. digitata* and *C. olitorius*); FB: Feed based; AD: *A. digitata*; CO: *C. olitorius*; IR: Irradiation

**Table 3:** Grouping of Rat on *C. olitorius* and *A. digitata* leaf based-diet before exposure to Radiation (Protective Study)

Animal grouping	Treatments
Control (NIR)	Non-irradiated, fed with normal
Negative (IR)	Irradiated, fed with normal fed
80-FB-AD-IR	Pre-fed with 80% inclusion <i>A. digitata</i> based-diet before irradiated
80-FB-CO-IR	Pre-fed with 80% inclusion of <i>C. olitorius</i> based-diet before irradiated
80-FB-ADCO-IR	Pre-fed with 80% inclusion <i>A. digitata</i> + <i>C. olitorius</i> based-diet before irradiated
VIT-C-IR	Pre-fed with formulated diet of Vitamin C (2mg/ml) before irradiated

ADCO: *A. digitata* + *C. olitorius*; LBDAC (Leaf based-diet of *A. digitata* and *C. olitorius*); FB: Feed based; AD: *A. digitata*; CO: *C. olitorius*; IR: Irradiation

**Table 4:** Grouping of Rat on *C. olitorius* and *A. digitata* leaf based-diet after exposure to Radiation (Ameliorative)

Animal grouping	Treatments
Control (NIR)	Non-irradiated, fed with normal
Negative (IR)	Irradiated, fed with normal fed
IR -80-FBAD	Irradiated, then fed with 80% inclusion <i>A. digitata</i> based-diet
IR-80-FBCO	Irradiated, then fed with 80% inclusion <i>C. olitorius</i> based-diet
IR-80-FB-ADCO	Irradiated, then fed with 80% inclusion <i>A. digitata</i> + <i>C. olitorius</i> based-diet
IR-VIT-C	Irradiated, then fed with vitamin-C based-diet

ADCO: *A. digitata* + *C. olitorius*; LBDAC (Leaf based-diet of *A. digitata* and *C. olitorius*); FB: Feed based; AD: *A. digitata*; CO: *C. olitorius*; IR: Irradiation

**Table 5:** Assays and parameters determined on irradiated rats fed with *A. digitata* and *C. olerius* leaf based-diet

Assays	Procedure and References
Micro-nuclei assay (Cell Cycle Status)	Odunola and Owumi (2015)
p53 assay	Cayman <i>et al.</i> (2003)
Alanine amino transferase, Aspartate transaminase, Alanine phosphatase	Reitman and Frankel (1960b); Reitman and Frankel (1960b); Caboli <i>et al.</i> (1965b)
Superoxide dismutase,	Haliwel <i>et al.</i> (2001);
Catalase,	Yhung-shur (1980);
Glutathione-s-transferase,	Abolaji (2015);
Glutathione peroxidase	Lazekhs <i>et al.</i> (1982b); Beutler <i>et al.</i> (1963)

**Result**

The secondary metabolites constituents of *Adansonia digitata* and *Corchorus olerius* leaves revealed the presence of saponins, alkaloids, polyphenols, tannins, steroids and terpenoids respectively at varying concentration as shown in Table 6. Anthraquinone, cardiac glycosides and phlobatannins were absent.

**The effects of the administration of *A. digitata* and *C. olerius* Leaves-based diet on p53 of rat exposed to irradiation**

The figures (Fig 1a and b) below shows the protective effects of *A. digitata* and *C. olerius* Leaves-based diet on p53 status of rat exposed to 6 grey gamma irradiation. Such that the most effective (protective or ameliorative) are the combination of *A. digitata* and *C. olerius* Leaves-based diet at 80

mg/kg body weight Such that Figure 1a is the protective and 1b is the ameliorative.

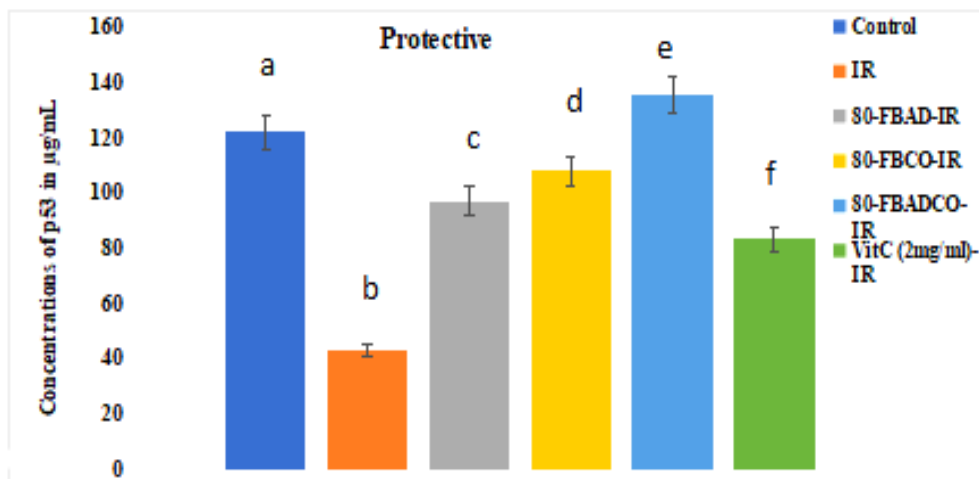
**The effects of the administration of *A. digitata* and *C. olerius* Leaves-based diet on micronuclei DNA status of rat exposed to irradiation**

The figures (Fig 2a and b) below shows the protective and ameliorative effects of *A. digitata* and *C. olerius* Leaves-based diet on micronuclei status of rat exposed to 6 grey gamma irradiation. Such that the most effective (protective or ameliorative) are the Leaves-based diet at 80 mg/kg body weight.

**Table 6:** Secondary Metabolite Constituents of Methanol Leaves Extract of *A. digitata* and *C. olerius*

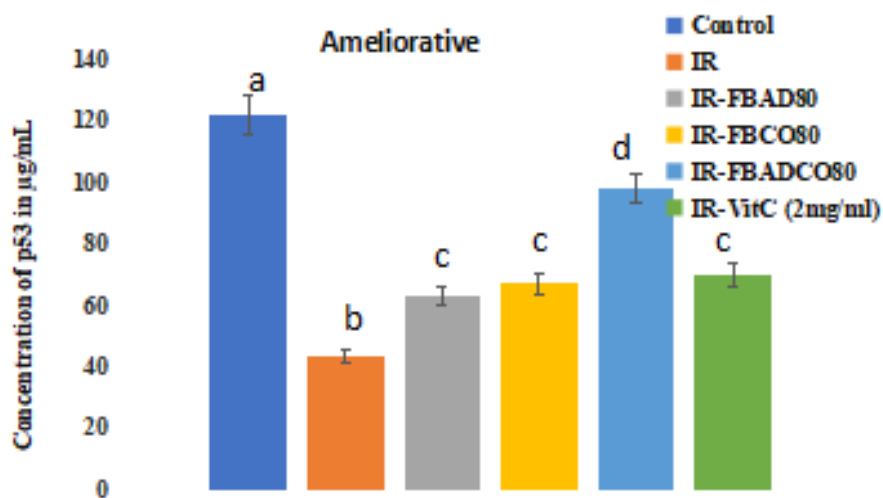
Metabolites	<i>Adansonia digitata</i> g/dL	<i>Corchorus olerius</i>
Alkaloids	78.76 ± 0.43	72.36 ± 2.05
Anthraquinone	ND	ND
Cardiac glycosides	ND	ND
Flavonoids	13.42 ± 1.03	104.81 ± 0.18
Polyphenols	147.90 ± 0.68	203.14 ± 0.32
Phlobatannins	ND	ND
Saponins	16.59 ± 1.85	22.17 ± 0.24
Tannins	198.98 ± 0.14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

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**Figure 1a:** p53 status of  $\gamma$ -irradiated Rats administered on *A. digitata* and *C. olitorius* Leaves-based diet.

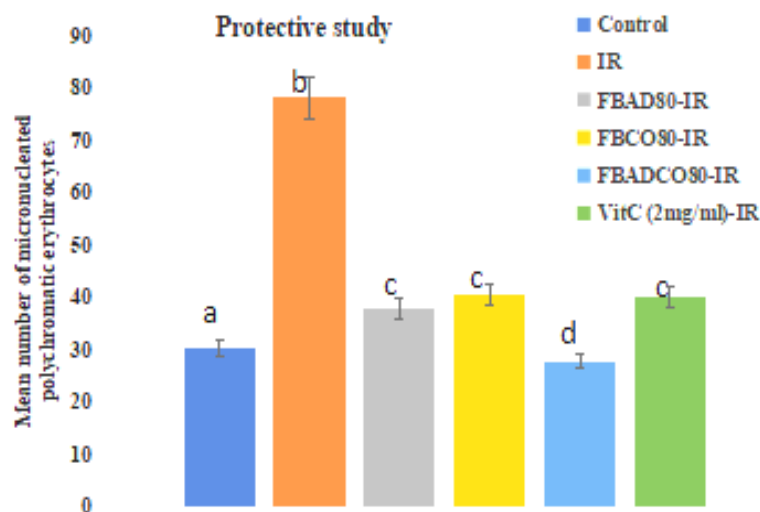
Means  $\pm$  SEM; n=6, (P < 0.05); 80-FBAD-IR= fed on 80 % inclusion feed-based *Adansonia* before exposure to irradiation; 80-FBCO-IR = fed on 80 % inclusion feed-based *Corchorus* before exposure to irradiation; 80-FBADCO-IR= fed on 80 % inclusion feed-based *Adansonia* + *Corchorus* before exposure to irradiation.



**Figure 1b:** p53 status of  $\gamma$ -irradiated Rats administered on *A. digitata* and *C. olitorius* Leaves-based diet

Means  $\pm$  SEM; n=6, (P < 0.05); IR-FBAD80%= irradiation-feed-based *Adansonia* at 80 % inclusion  
 IR- FBCO80%= irradiation-feed-based *Corchorus* at 80 % IR-FBADCO80 = inclusion irradiation-feed-based  
*Adansonia* + *Corchorus* at 80 % inclusion

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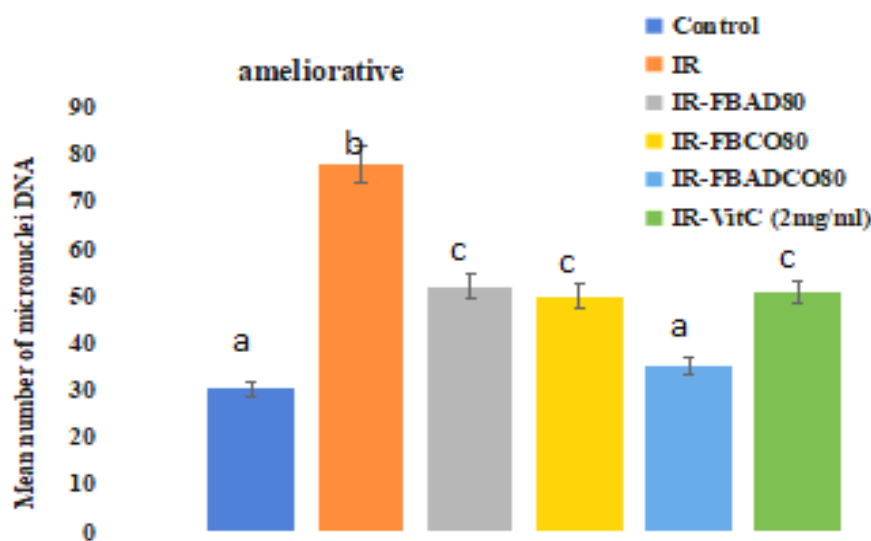
**Figure 2a:** Micronuclei DNA status of  $\gamma$ -irradiated Rats Administered on *A. digitata* and *C. olitorius* Leaves-based diet

Values are expressed as Means  $\pm$  SEM; n=6, (P < 0.05);

80-FBAD-IR= fed on 80 % inclusion feed-based Adansonia before exposure to irradiation-

80-FBCO-IR = fed on 80 % inclusion feed-based Corchorus before exposure to irradiation

80-FBADCO-IR= fed on 80 % inclusion feed-based Adansonia + Corchorus before exposure to irradiation.



**Figure 2b:** Micro-nucleated Polychromatin Erythrocyte of  $\gamma$ -irradiated Rats administered on *A. digitata* and *C. olitorius* Leaves-based diet

Values are expressed as Means  $\pm$  SEM; n=6, (P < 0.05); IR-FBAD80%= irradiation-feed-based Adansonia at

80 % inclusion; IR- FBCO80%= irradiation-feed-based Corchorus at 80 %; IR-FBADCO80 = inclusion

irradiation-feed-based Adansonia + Corchorus at 80 % inclusion.



**Table 7a:** Effects of *Adansonia digitata* and *Corchorus olitorius* Leaves Based-diet on Selected Antioxidant Parameter of Irradiated Rats (Protective)

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Groups	SOD	CAT	GST μg/dL	GPx	GSH	MDA
Control	14.12±0.21a	24.04±0.11a	19.02±0.04a	13.12±0.15a	11.23±0.52a	18.23±0.22a
IR	4.56±0.11b	12.04±0.12b	9.34±0.07b	5.87±0.18b	5.34±0.61b	87.23±0.14b
80-FBAD-IR	11.23±0.15c	18.12±0.10c	16.86±0.03c	11.45±0.11c	9.89±0.18c	24.05±0.12c
80-FBCO-IR	11.00±0.12c	18.24±0.12c	17.11±0.05c	11.56±0.15c	10.01±0.18c	23.13±0.11c
80-FBADCO-IR	15.87±0.14d	25.01±0.15a	19.85±0.06d	14.65±0.18d	13.05±0.18d	16.23±0.17d
FB-Vit.C-IR (2mg/ml)	11.34 ±0.15c	16.10±0.12d	15.01±0.04e	10.11±0.18e	8.90±0.18e	31.12±0.18e

Means ± SEM; n=6, (P < 0.05);

80-FBAD-IR= fed on 80 % inclusion feed-based *Adansonia* before exposure to irradiation-

80-FBCO-IR = fed on 80 % inclusion feed-based *Corchorus* before exposure to irradiation

80-FBADCO-IR= fed on 80 % inclusion feed-based *Adansonia* + *Corchorus* before exposure to irradiation

**Table 7b:** Effects of *Adansonia digitata* and *Corchorus olitorius* Leaves Based-diet on Selected Antioxidant Parameter of Irradiated Rats (ameliorative)

Groups	SOD	CAT	GST μg/dL	GPx	GSH	MDA
Control	14.12±0.21a	24.04±0.11a	19.02±0.04a	13.12±0.15a	11.23±0.52a	18.23±0.22a
IR	4.56±0.11b	12.04±0.12b	9.34±0.07b	5.87±0.18b	5.34±0.61b	87.23±0.14b
IR-FBAD80	7.45±0.10c	16.02±0.09c	15.34±0.05c	8.01±0.21c	8.04±0.53c	34.32±0.19c
IR-FBCO80	8.00±0.11c	15.87±0.20c	16.01±0.02c	7.89±0.19c	8.49±0.41c	34.12±0.15c
IR-FBADCO80	11.34±0.11d	21.12±0.10d	18.12±0.14a	12.89±0.21a	9.02±0.31d	21.02±0.16d
IR-Vit.C (2mg/ml)	6.34±0.12a	13.23±0.21a	14.12±0.07d	7.56±0.18c	7.64±0.21c	40.12±0.17a

Means ± SEM; n=6, (P < 0.05);

IR-FBAD80%= irradiation-feed-based *Adansonia* at 80 % inclusion

IR- FBCO80%= irradiation-feed-based *Corchorus* at 80 %

IR-FBADCO80 = inclusion irradiation-feed-based *Adansonia* + *Corchorus* at 80 % inclusion

## Discussion

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Exposure of rats to 6 grey gamma radiation altered cellular system, by altering cellular parameters associated with physiological functioning of such cellular system (Haliwell, 2001). An increase in free radical level, above physiological concentration (from 20-120ug/mol) is an underlying mechanism through which radiation altered cellular parameters (Simões, 2009). Radiation altered chromosomal structure to aberrant structure, thus increasing micronuclei DNA formation, this is hallmark of abnormal cell cycle. Increased free radical concentration to 120 ug/mol altered p53 structure, leading to Li-Fraumeni syndrome (Hayes, 2010). The significant ( $p<0.05$ ) increase in the level of micronuclei DNA, Nf-kB, ALT, AST and ALP of rat exposed to 6 grey gamma radiation as seen in figure 4.1a, b and 4.2a, b above, is an indication of altered cellular system in rat. Gamma radiation-induced-cellular alteration was further corroborated by the significant ( $p<0.05$ ) reduction in SOD, CAT, GSH, GST and GPx of irradiated rats as seen in table 1a and b. It was also observed that the reduction was more significant in the protective group than the ameliorative, this is suggestive that the leaves protected the rats against cellular alterations induced by radiation.

The medicinal quality of plants is associated with the bioactive constituents therein the plants. (Baskurt *et al.*, 2009). The pharmacological influence of plants on cellular parameters and metabolisms, have strongly been alluded to the presence of flavonoids, polyphenols, tannins, saponins (Benzeggouta *et al.*, 2017), thus the presence of significant ( $p<0.05$ ) amounts of flavonoids, polyphenols, tannins and saponins (as shown in table 4.1a) in the methanolic extract of *A. digitata* and *C. olitorius* leaves maybe responsible for the significant protection and reversal of the altered cellular system of rats exposed to gamma irradiation as shown in figure

4.1, 4.2 and table 4.1 and 4.2. The use of secondary metabolites in plants to regulate cellular metabolism (glucose uptake, enzyme activities, regulation of cell division, hormonal regulation) and offer pharmacological protection have long been validated (Olanlokun, 2013). Many plants with medicinal properties have been used in the treatment and protection of cells from cellular alterations. Flavonoids have been implicated in the prevention of malondialdehyde formation in rat's cellular system, by altering chain of lipid peroxidation processes (Finkel, 2012). Malondialdehyde is a product of lipids peroxidation, which have been implicated in various cardiovascular diseases such as hypertension, ischemia reperfusion diseases, atherosclerosis etc. (Kuete, 2010). The significant ( $p<0.05$ ) reduction in the level of MDA in rat fed on *A. digitata* and *C. olitorius* leaves based-diet, maybe responsible to the significant ( $p<0.05$ ) amounts of flavonoids in both leaves plant as shown in table 4.1b and c. It was also observed that the reduction was more significant in the protective group than the ameliorative, this is suggestive that the leaves protected the rats against cellular alterations induced by radiation.

The mean numbers of micro nucleated DNA formed, is suggestive of the extent of damages that befall the DNA of rats exposed to radiation as shown in figure 4.2a and 4.2b, it also suggests the level of Philadelphia chromosomes (aberrant chromosomes or polychromatic micronuclei DNA) produced and how dysregulated the cell cycle have become (Odunola and Owumi, 2008). As observed in the irradiated not treated groups (rats), there was significant ( $p<0.05$ ) increased in the number of micronuclei DNA formed, suggestive that radiation-induced damages on the chromosomes of rats exposed to radiation as shown in figure 4.1 and 4.2 above. Flavonoids scavenges free radicals, it induced antioxidant enzymes that are capable of mopping-up free radicals (Rushmore, 1991). The significant

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( $p < 0.05$ ) amounts of flavonoids in the *A. digitata* and *C. olitorius* leaves based-diet maybe responsible for the significant ( $p < 0.05$ ) reversal in the level of micronuclei DNA in the groups fed with *A. digitata* and *C. olitorius* leaves based-diet (as shown in figure 4.1 and 2) maybe due to the presence of flavonoids and polyphenols. It was also observed that the reduction was more significant in the protective group than the ameliorative, this is suggestive that the leaves protected the rats against cellular alterations induced by radiation.

Catechin (polyphenols) offer protection to the bone-marrow, by protecting the proteins (cyclin and cyclin dependent kinases) responsible for the physiological progression of the cell cycle. Cyclins and cyclin dependent kinases are responsible for cell cycle progression from phase to the other (Saitoh, 1998). Thus, the significant amount ( $p < 0.05$ ) of polyphenols in the *A. digitata* and *C. olitorius* leaves based-diet maybe responsible for the reversal and protection of the bone marrow in the group fed on the leaves based-diet as shown in figure 4.1 and 2. It was also observed that the reduction was more significant in the protective group than the ameliorative, this is suggestive that the leaves protected the rats against cellular alterations induced by radiation.

Flavonoids mediate p53 arrest during abnormal cell cycle progression (usually at the M-phase) (Walters, 2009). The presence of significant amount ( $p < 0.05$ ) of flavonoids in the leaves of *A. digitata* and *C. olitorius* might be responsible for the arrest and restoration of dysregulated cell cycle (at the M-phase) in the group administered with *A. digitata* and *C. olitorius* leaves-based diet as shown in figure 4.1a and b. It was also observed that the reduction was more significant in the protective group than the ameliorative, this is suggestive that the leaves protected the rats against cellular alterations induced by radiation.

p53 is a tumour suppressor gene responsible for the arrest and repair of dysregulated cell cycle and possibly repair damage DNA (Kensler, 2010). p53 are repressed by mdm-2. Increased free radicals is responsible for the activation of mdm-2 protein (implicated in p53 down-regulation) (Kensler *et al.*, 2010). As observed in the irradiated not treated group in all the figure above, there was significant ( $p > 0.05$ ) reduction in the level of p53, suggesting that radiation-induced free radical have deactivating effects on p53 level as shown in figure 4.1a and b.

Farombi and Yhong-sur (2001) validated that catechin and epicatechin mediates the activation of p53 and downregulates mdm-2 productions; Catechins are flavonoids responsible for the arrest and repair of dysregulated cell cycle of rats exposed to castor oil. Catechin work by upregulating the activation of p53 and downregulating mdm-2 activities in castor oil induced cell cycle dysregulations (Farombi, 2002). The upregulated p53 is responsible for the cell cycle arrest and repair. In irradiated rat as shown in figure 4.1a and b.

The decreased number of micronuclei DNA formation observed in *A. digitata* and *C. olitorius* leaves-based diet administered group might be due to the presence of significant ( $p < 0.05$ ) amount of catechin and epicatechin in *A. digitata* and *C. olitorius* leaves. Catechin and epicatechin mediated the arrest of abnormal cell cycle progression and repair of damage DNA through arrest and repair mechanism of p53 (Farombi and Yhong-sur, 2001). The increased number of activated p53 in the group administered *A. digitata* and *C. olitorius* leaves-based diet might be due to the presence of significant ( $p < 0.05$ ) amount of catechin and epicatechin in the two leaves. It was also observed that the increase was more significant in the protective group than the ameliorative, this is suggestive that the leaves protected the rats against cellular alterations induced by radiation.

As observed in the irradiated not treated group, there were significant ( $p>0.05$ ) reduction in the level of antioxidant system parameter (superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase and reduced glutathione) of irradiated rats.

An imbalance in the free radical/antioxidant system have been implicated in the genesis of various cellular alterations and diseased state of rat; Farombi and Yhong-sur (2001) have implicated the depletion of GST and Gpx in rat cellular system to increased free radicals generated by STZ. Farombi (2002) revealed that epigallocatechin gallate scavenge ROS and RNS produced by administration of aspartame to mice. Thus, the significant ( $p>0.05$ ) reduction in the antioxidant capacity of rat in this research, maybe due to free radical produced by exposure of the rats to irradiation.

Epigallocatechin have been implicated in the activation of Nrf-2; Nrf-2 mediated the over induction and expression of antioxidant enzymes responsible for the scavenging of free radicals in rats exposed to oral anthracene (Olanlokun and Akomolafe, 2013). Quercetin have been implicated in expression and induction of Gpx. Gpx is responsible for the stabilization and scavenge of ROS/RNS generated by benzo(a)pyrene in rabbits (Rushmore, Morton and Pickett, 1991). The significant ( $p<0.05$ ) increase in SOD, CAT, GST, Gpx and GSH in rats administered with *A. digitata* and *C. olitorius* leaves-based diets maybe due to the presence of significant ( $p<0.05$ ) amount of catechin, quercetin and epigallocatechin in the leaves of *A. digitata* and *C. olitorius*. They might also be responsible for the increase in the induction of the antioxidant enzymes of the administered rats in this study.

### Conclusion

From the findings above, its therefore logical to conclude that, *Adansonia digitata* and *Corchorus*

*olitorius* offer synergistic protection against  $\gamma$ -irradiation-induced-Genotoxicity and p53 Damage, thus can be explore as oral remedy in the treatment of radiation-induced disorders.

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### Conflict of Interest

There is/are no conflict of interest on this work be it at the level of research or manuscript submission.

### References

- Adesokan, A.A., Akanji, M.A., Yakubu, M.T. (2003). Antimicrobial potential of aqueous extract of *Enantia chlorantha* stem bark. *Africa Journal of Biotechnology*. 6(22):2502–2505.
- Atata, F.R., Alhassan, S., Ajewole, S.M. (2003). Effect of stem bark extracts of *Enantia chlorantha* on some clinical isolates. *Biochemistri Journal*. 15 (2):84–92.
- Aarts, J.D., Banga, H.C., van-Houwelingen, R.M., Heethaar, and J.J. Sixma, (1986). Increased red blood cell deformability due to isosuprine administration decreases platelet adherence in a perfusion chamber: A double-blind cross-over study in patients with intermittent claudication; *National Immunology Journal*. 11:136–40.
- Ajila, C.M. and Prasada U.J.S. (2008). Protection against hydrogen peroxide

4619

- induced oxidative damage in rat erythrocytes by *Mangifer indica* (L.) peel extract, *Food and Chemical Toxicology*. 46(303–309).
- Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemical pathology*. 470–475.
- Balaban, R.S., Nemoto, S., Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell biology journal*.120:483–95.
- Baldino, N., Gabriele, M., Migliori, B. and de Cindio (2010). The influence of formulation and cooling rate on the rheological properties of chocolate. *European Food Research and Technology*. 231 (821–828).
- Baskurt, O.K., Boynard, M., Connes, P., Cooke, B.M., Forconi, S.F. and Liao, M.R. (2009). International expert panel for standardization of hemorheological methods. *New guidelines for hemorheological laboratory techniques*.75–97..
- Baskurt, O.K. and Meiselman, H.J. (2003). Blood rheology and hemodynamics. *SemiThromb Hemost* (29) 453–450.
- Bossy-Wetzel, E., Schwarzenbacher, R., Lipton, S.A. (2004). Molecular pathways to neurodegeneration. *National Medical Journal*. 10(Suppl.): S2–9.
- Buccolo, G. and David, H. (1973): Quantitative determination of serum triglycerides by the use of enzymes. *Journal of Clinical Chemistry*.19-476.
- Burtis, C.A., Ashwood, E.R., Bruns, D.E., Rifai, N. and Warnick, G.R. (2006). Measurement of lipids, lipoproteins and apolipoproteins in albino rats. *Tietz Textbook of Clinical Chemistry and Molecular Diagnosis*, Elsevier Saunders, Missouri. eds., pp.938–952.
- Carr, R. and Cokelet, G.R. (1981). Rheology of suspensions of normal and hardened erythrocytes and their mixtures. *Journal of Rheology*.67–82.
- Chan, K., Han, X.D., Kan, Y.W. (2001). An important function of Nrf-2 in combating oxidative stress: detoxification of acetaminophen. *Proc. Natl. Acad. Sci. USA*. 98:4611-16.
- Chen, H., Yunpeng, W., Shaoxi, C. and Mian, L.A. (1996). Research on rheological properties of erythrocyte of lung cancer patients. *Journal Clinical Hemorheology*.135-141.
- Christopher, M.M. and Lee, S.E. (1994). Red cell morphologic alterations in cats with hepatic disease. *Journal of Veterinary Clinical Pathology*. 7–12.
- Cooper, R.A., Leslie, M.H., Knight, D. and Detweiler, D.K. (2003). Red cell cholesterol enrichment and spur cell anaemia in dogs fed a cholesterol-enriched atherogenic diet. *Journal of Lipid Restitution*. 1082–1089.
- Djeussi, E., Noumedem, A.K., Seukep, J.A., Fankam, A.G., Voukeng, K.I., Tankeo, S.B., Voukeng, K.I. and Tankeo, S.B. (2013). Antibacterial activities of selected edible plants extracts against multidrug-resistant gram-negative bacteria. *Journal of Complementary and Alternative Medicine*. 13:164. doi: 10.1186/1472-6882-13-164.
- Ebelle-Etame, R.M., Kenfack, C.L., Voukeng, I., Tchientcheu, R., Assam-Assam, J.P. et al. (2018). Effect of fractioning on antibacterial activity of *Enantia chlorantha* Oliver (*Annonaceae*) methanol

4620

- extract and mode of action. Evid Based Complement, *Alternat Med.* 23:15, pg 2345-2367.
- Ejele, A.E., Akpan, I.O., Ogukwe, C.E., Onyeocha, V.O., Ukiwe, L.N. (2012). Bioassay-guided isolation and partial characterization of an antisickling compound from *Enantia chlorantha*. *Int J Biosci Biochem Bioinforma.* 2(7):149–154.
- Farombi, E.O. and Yhong-sur J.S. (2001). Effects of Free radical on the physiological roles of p53 in mice. *J. Biol. Chem.* 266:4556–61.
- Farombi E.O. (2002). The modulatory effect of catechin and epicatechin in rat administered on castor oil. *J. Biol. Chem.* 106:162–61.
- Fasola, T.R., Adeyemo, F.A., Adeniji, J.A., Okonko, I.O. (2011). Antiviral potentials of *Enantia chlorantha* extracts on yellow fever virus. *Journal of National Sciences.* 9(9):99–105.
- Favreau, L.V. and Pickett, C.B. (1991). Transcriptional regulation of the rat NAD(P)H: quinone reductase gene; Identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. *J. Biol. Chem.* 266:4556–61.
- Finkel, T. (2011): Signal transduction by reactive oxygen species. *Journal of Cell Biology.* 194:7–15.
- Finkel, T. (2012). Signal transduction by mitochondrial oxidants. *Journal of Biological Chemistry.* 287:4434–40.
- Friling, R.S., Bensimon, A., Tichauer, Y., Daniel, V. (1990). Xenobiotic-inducible expression of murine glutathione S-transferase and subunit gene is controlled by an electrophile-responsive element. *Proc. Natl. Acad. Sci. USA.* 87:6258–62.
- Gordon, M.C., Newman, J.D. (2005). Biodiversity: a continuing source of novel drug leads. *Pure Appl Chem.* 77(1):7–24. doi: 10.1351/pac200577010007.
- Gurib-Fakim, A. (2005): Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol Asp Med.* 27:1–93. doi: 10.1016/j.mam.2005.07.008.
- Haliwell, H.Y. (2001). Radiation Induced-Free radical production in rat brain. *Journal of Biomedical Sciences and Engineering.* 6:877–884. doi: 10.4236/jbise.2013.69107.
- Harbone, J.B. (1973): *Phytochemical methods.* New York: Chapman & Hall.
- Hayes, J.D., McMahon, M., Chowdhry, S., Dinkova-Kostova, A.T. (2010). Cancer chemoprevention mechanisms mediated through the Keap1-Nrf2 pathway. *Journal of Antioxidant and Redox Signal.* 13:1713–48.
- Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K. *et al.* (1996). Nrf-1 and Nrf-2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H: quinone oxidoreductase 1 gene. *Proc National Academy of Sciences. USA.* 93:14960–65.
- Institute of Radiation and Environmental Studies (IRES) (2007). Radiation and Free Radical production in diseased condition. *Africa Journal of Biotechnology.* 6(22):2502–2505.
- Institute of Radiation and Environmental Studies (IRES) (2016). Africa the world dumping ground for radiation emitting equipment's and the death trap. *Africa Journal of Biotechnology.* 6(22):2502–2505.

- Janssen-Heininger, Y.M., Mossman, B.T., Heintz, N.H., Forman, H.J., Kalyanaraman, B. (2008). Redox-based regulation of signal transduction: principles, pitfalls, and promises. *Free Radic. Biol. Med.*:1–17.
- Karumi G.H. (2002): Humans and the use of Information technological in the 21<sup>st</sup> century. *Journal of Complementary and Alternative Medicine*.15:25. doi:10.1186/s12906-015-0541-7.
- Kensler, T.W., Wakabayashi, N., Biswal, S. (2007). Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Journal of Pharmacology and Toxicology*. 47:89–116.
- Kensler, T.W., Wakabayashi, N. (2010): Nrf2: Friend or foe for chemoprevention? *Carcinogenesis*. 31:90–99.
- Klaassen, C.D., Reisman, S.A. (2010). Nrf-2 the rescue effects of the antioxidative/electrophilic response on the liver. *Journal of Toxicology and Applied Pharmacology*. 244:57–65.
- Kuete, V., Wansi, J.D., Mbaveng, A.T., Kana, M.M., Tadjong, A.T., Beng, V.P. *et al.* (2008). Antimicrobial activity of the methanolic extract and compounds from *Teclea afzelii*. *South Africa Journal of Botanical study*.74:572–576. doi:
- Kuete, V. (2010). Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Med*. 76:1479–1491. doi: 10.1055/s-0030-1250027. 10.1016/j.sajb.2008.02.004
- Lee, S.R., Kwon, K.S., Kim, S.R., Rhee, S.G. (1998): Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *Journal of Biol. Chem*. 273:15366–72.
- Mathew H.Y., Jhart D.F., Kabur Q.W., Yuri, D.F., Aspouty, G.B., Hoigy, F.P. *et al.* (2014). Population, civilization and radiation effect. *Journal of Biological Chemistry*. 265:14648–53.
- MaQ, G. (2008). Xenobiotic-activated receptors from transcription to drug metabolism to disease. *Chem. Res. Toxicol*. 21:1651–71.
- MaQ, G. (2010). Transcriptional responses to oxidative stress: pathological and toxicological implications. *Pharmacology J. Ther*; 125:376–93.
- MaQ, G., He, X. (2012). Molecular basis of electrophilic and oxidative defence: promises and perils of Nrf2. *Journal of Pharmacology. Rev*. 64:1055–81.
- Mentouri-Voukeng, I.K., Nganou, K.B., Sandjo, L.P., Celik, I., Beng, P.V., Tane, P. *et al.* (2017). Antibacterial activities of the methanol extract, fractions and compounds from *Elaeophorbium drupifera* (Thonn.) Stapf. (Euphorbiaceae) *Complementary Alternative Med*. 17:28.
- Moi, P., Chan, K., Asunis, I., Cao, A., Kan, Y.W. (1994). Isolation of NF-E2-related factor 2(Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the  $\beta$ -globin locus control region. *National Academy of Sciences, USA*. 91:9926–30.
- Motohashi, H., Yamamoto, M. (2004). Nrf2-Keap1 defines a physiologically important stress response mechanism. *Trends Mol. Med*.10:549–57.
- Njateng, G.S., Zhizhi, D., Gatsing, D., Donfack, A., Talla, M., Wabo, H. *et al.* (2015). Antifungal properties of a new terpenoid saponin and other compounds from the stem bark of *Polyscias fulva* Hiern (Araliaceae). *Journal of Complementary and Alternative*

- Medicine*.15:25. doi: 10.1186/s12906-015-0541-7.
- 4622 Nigam, S. and Schewe, T. (2000). Phospholipid A<sub>2</sub> and lipid peroxidation, *Biochem Biophys. Acta*1488. 167–181.
- Nyong, E.E., Odeniyi, M.A., and Moody, J.O. (2015). *In vitro* and *in vivo* antimicrobial evaluation of alkaloidal extracts of *Enantia chlorantha* stem bark and their formulated ointments. *Acta Pharma* 72(1):14–52.
- Odunola, O.O. and Owumi L.G. (2008). Genotoxicity: The effects of lead in car battery on the mean number of micronucleated DNA of mice. *J. Biol. Chem.* 126:1354–61.
- Olanlokun, J.O., Akomolafe, S.F. (2013). Antioxidant potentials of various solvent extracts from stem bark of *Enantia chlorantha*. *Journal of Biomedical Sciences and Engineering*. 6:877–884. doi: 10.4236/jbise.2013.69107.
- O’neill, J. (2014). Antimicrobial resistance: tackling a crisis for the health and wealth of nations. The review on antimicrobial resistance. 14p. *J. Biol. Chem.* 266:4556–61.
- Paiva-Martins, F., Fernandes, J., Rocha, S., Nascimento, H., Vitorino, R and F. Amado, *et al.* (2009). Effects of olive oil polyphenols on erythrocyte oxidative damage. *Journal of Molecular Nutrition Food Research*. 53, 609–616.
- Rao, M.A (1999). *Rheology of Fluid and Semisolid Foods, principles and Applications*. An Aspen Publication. Gaithersburg ed., Maryland, 119–123.
- Rushmore, T.H., Pickett, C.B. (1990). Transcriptional regulation of the rat glutathione-S-transferase Ya subunit gene. Characterization of a xenobiotic-responsive element controlling inducible expression by phenolic antioxidants. *Journal of Biological Chemistry*. 265:14648–53.
- Rushmore, T.H., Morton, M.R., Pickett, C.B. (1991). The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J. Biol. Chem.* 266:11632–39.
- Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K. *et al.* (1998). Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *Embryology Journal*. 17:2596–606.
- Shull, R.M., Bunch, S.E., Maribei, J. and Spaulding, G.L. (1978). Spur cell anaemia in a dog. *Journal of America Veterinary Medicine*. 173, 978–982.
- Simões, M., Bennett, R.N., Rosa, E.A. (2009). Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. *Nat Prod Rep*. 26:746–757. doi: 10.1039/b821648.
- Spengler, M.I., Rasia, M, Palma, S. and Allemandi, D. (2011). Effects of fatty ester derivatives on erythrocyte membrane lipoperoxidation. *Journal of Clinical Haematology*. 47, 163–168.
- Sundaresan, M., Yu, Z.X., Ferrans, V.J., Irani, K., Finkel, T. (1995). Requirement for generation of H<sub>2</sub>O<sub>2</sub> for platelet-derived growth factor signal transduction. *Journal of Science*. 270:296–99.
- Taguchi, K., Motohashi, H., Yamamoto, M. (2011). Molecular mechanisms of the Keap1-Nrf-2 pathway in stress response and cancer evolution. *Genes Cells*. 16:123–40.
- Talay, P., Dinkova-Kostova, A.T., Holtzclaw, W.D. (2003). Importance of phase 2 gene regulation in protection against electrophile and reactive oxygen toxicity and



- carcinogenesis. *Journal of Advanced Enzyme Regulation.* 43:121–34.
- 4623 Ulibarrena, C., Vecino, A. and Cesar, J.M. (1994). Red cell lipid abnormalities in acquired acanthocytosis are extended to platelets. *Journal of Haematology.* (87)-614–616.
- Venugopal, R., Jaiswal, A.K., Nguyen, T., Sherratt, P.J., Pickett, C.B. (2003). Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annual Review Journal of Pharmacology and Toxicology.* 43:233–60.
- Walters, D.M., Cho, and H.Y., Kleeberger, S.R. (2009). Oxidative stress and antioxidants in the pathogenesis of pulmonary fibrosis: a potential role for Nrf-2. *Jnr. Biol. Chem.* 266:4556–61.
- Watternberg, L.W. (1972). Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. *Journal of National Cancer Institute.* 48:1425–30.
- World Health Organization (2017). Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis WHO/EMP/IAU/2017.12.
- Yhabum G.M., Hart, M.P., Siot, T.W., Oout, U.W., Dou, R.Q. and Qoiut, A.C., *et al.* (2018). Effect of radiation on Fertility and infertility. *J. Biol. Chem.* 266:11632–39.
- Zhou, R., Tardivel, A., Thorens, B., Choi, I., Tschopp, J. (2010). Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *National Immunology Journal.* 11:136–40.