

Radio-protective Potentials of Methanolic/Aqueous Extract of *Adasonia digitata* and *Cochorous olitorious* Leaf Plant on Gamma Irradiated Male Wistar Rats

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Abstract

It is no longer news that technology is eating deep as a cankerworm in the heart of human and it is pertinent to know that 90% of the world-used technological gadget are fully equipped with radiation emitting software, which upon human exposure to, generating free radicals-induce disease and several disorders such as hemorrhage induced anaemia, cancer, ischemia reperfusion diseases, diabetes, atherosclerosis and several others (Haliwell, 2000). Hence the needs to prevent, ameliorate, attenuate and cure the effects of radiation generating disorders. Some groups of rat were exposed to gamma radiation of 6Grey, generating free radicals and inducing several diseases and disorders. *A. digitata* and *C.olitorious* has been established to poses significant ($p<0.05$) amounts of antioxidant phytochemicals (inducers of endogenous antioxidant enzymes) such as Saponin(16.59 ± 1.85 and 22.12 ± 0.24), Tannins(311.98 ± 0.01 and 287.07 ± 0.16), Polyphenols(170.90 ± 0.68 and 330.07 ± 0.32), Alkaloids(81.56 ± 0.56 and 68.65 ± 2.05) and flavonoids(25.38 ± 2.88 and 157.38 ± 0.38) which is suggestive of the free radical scavenging potentials of the two plants extracts. The administration of the plant extract to rats exposed to radiation was able to attenuate and prevent the disorders, implicating the plant extracts to be radioprotective and antioxidative in potentials. The safety evaluation analysis was examined by measuring the serum ALT, AST and ALP level which was not significantly ($p<0.05$) different when compared to the normal control, establishing the Hepatoprotective potential of the plant extracts.

Keywords: Antioxidative, ameliorate, Free Radicals, Radiation, Hepatoprotective, Radioprotective

1.0 INTRODUCTION

Radiation simply refers to a high energy loop which has the potential of producing free radical through electron leakages. Radiation can be excitation (X-ray) or ionization Ultraviolet(UV) radiation), whether the later or the former, the exposure of human body to any of the radiation types (whole or part of the body)resulted to electron leakages, which has the potential to attack molecular oxygen in the aerobic human system, resulting to the formation of superoxide anion, which is the basis for the metabolism of several other ROS/RNS (Hydrogen peroxide, hydroxyl radical, peroxy radical).

The world used technological gadget of the present century are fully equipped with infra-red emitting soft wares, Bluetooth, ionizing radiation and several other radioactive software that serves as sources of high electron leakages, producing free radicals and posed to human several deleterious conditions such as hemorrhage induced anaemia, cancer, ischemia reperfusion diseases, atherosclerosis, diabetes and several other pathological disorders (Haliwell, 2000).

1.1FREE RADICALS

Ionizing radiation initiates the formation of free radicals, with the potential to attacking molecular oxygen, producing a variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS) : Hydroxyl radicals ($\cdot\text{OH}$), Hydrogen peroxide (H_2O_2), Superoxide anion(O_2^-), peroxy radical ($\text{ROO}\cdot$), Nitric oxide ($\text{NO}\cdot$), peroxy Nitrite radicals (OONO) etc. (Sadani and Nadkarni, 1997; Anderson *et al.*, 2001). These reactive species react with the biomolecules such as nucleic acids (DNA and RNA), mostly membrane proteins and lipids, resulting to chain of reaction leading to lipid peroxidation, protein oxidation and Nucleic acid-base breakage ,which has been implicated to pathological aging (Pariharet *et al.*, 2007). Hydroxyl radical ($\cdot\text{OH}$) is considered the most reactive and toxic ROS, due to its high standard one-electron reduction potentials and is potent ability to abstract hydrogen atoms from virtually all molecular substances at a vast rate (aromatic, aliphatic, cyclic , non-cyclic etc) (Rollet-Labelle *et al.*, 1998; Esterbauer *et al.*, 1991; Forman *et al.*, 2008). The formation of lipid peroxy radical ($\text{LOO}\cdot$) and lipid hydroperoxide (LOOH) resulted in destructive processes which alter integrity, fluidity, permeability and functions of biomembranes. This impairs the activity of membrane-bound enzymes, modifies low density lipoprotein (LDL) to proatherogenic and proinflammatory forms and generates potential toxic products (Greenberg *et al.*, 2008). Several pathological conditions, resulting from human exposure to radiations are anemia induced by free radical attack, gastritis, ulcerative colloidosis, Ischemia reperfusion diseases, cancer and atherosclerosis etc. However this review does not preclude the facts that there are other sources of free radicals aside from radiation, they includes: during metabolism in the mitochondria electron transport chain, the

during Xenobiotic metabolism in the endoplasmic reticulum electron transport chain, in the hypoxanthin and xanthin oxidase catalysed reaction that produces hydrogen peroxide (which produces hydroxyl radical), from the oxidation of monogenic amine (epinephrine, nor-epinephrine, dopamine etc) and lastly from cyclooxygenase catalysed reaction, that metabolized arachidonic acid to prostaglandins etc.

1.1.1 FREE RADICAL INDUCED DISORDERS AND THERE MECHANISM OF INDUCTION

1.1.1.1 Biochemistry of Anaemia Induction

Radiation one of the sources of free radicals, forming reactive oxygen species target mostly protein containing sulphur-hydryl group, attack by free radicals on this protein lead to the formation of disulphide linkages which rapidly causes protein aggregation, oligomerization and lastly heinze compound formation (protein compounds that has the potential of attacking other free protein to form aggregated toxic compounds), the protein so formed has changes in structure affecting the function of the protein. A good examples of this protein is heamoglobin: Contain sulphurehydryl group, it is a heamprothetic containing protein, which it uses to transport molecular oxygen in blood with, it is biconcave in structure (Discocyte). An attack by free radicals on heamoglobin lead to formation of disulfide linkages, which disrupts the structure changing it from discocyte to echinocyte, characterized by aggregation, this therefore sensitized the immune system or proteolytic pathways (such as lysosomes, 26s proteasomes) that an hapten (aggregated heamoglobin) is in the system to be hydrolyzed, this lead to loss of iron which is an hallmark of Aneamia.

It's a renowned blood disorder affecting all age groups, although the elderly once, young women of child bearing age and infants are the target age by this disorder, this condition is not a disease but could develop as a result of various diseases (WHO and UNICEF, 2001). There are over 400 types of anaemia, many of which are rare but in all cases there is lower than normal number of circulating red blood cells (Ogbeet *al.*, 2010). The most common form of anaemia is caused by a deficiency of iron which is an essential constituent of haemoglobin. The main cause of iron deficiency anaemia is iron loss due to heavy or persistent bleeding, though menstruation is the most common cause in women of child bearing age, other causes include blood loss from the digestive tract due to disorders such as erosive gastritis, peptic ulcer, stomach ulcer, inflammatory bowel disease, hemorrhoids and blood tumours, all of which are free radical inducing disorders (Medical Dictionary, 2002). Iron deficiency is the most common cause of nutritional anaemia which affects over 600 million people throughout the world particularly in developing countries (Oladijiet *al.*, 2003). The prevalence of anaemia in many populations can also be associated with infectious diseases such as hookworms, ascariis, and schistosomiasis, acute and chronic infections including malaria, cancer, tuberculosis and HIV/ AIDS. For example, *Plasmodium falciparum* malaria induced anaemia contributes significantly to maternal and child mortality, thus prevention and treatment of anaemia in pregnant women and young children are of major importance (Staubli, 2001). Iron deficiency anaemia was considered to be among the important contributing factors to the global burden of diseases (Gadagaet *al.*, 2009). Sickle cell anaemia is one of the diseases ravaging most world population cutting across nations and ethnic divide. According to reports, Africa is believed to be the origin of sickle cell anaemia and those afflicted with the disease are estimated at 200,000 per year. The recurrent and painful symptoms experienced during crises by sickle cell patients are known by various names in different parts of the world with complaints of shortness of breath, heart palpitations, abdominal pains, aches and pains in the muscle (Dapa and Gill, 2002).

1.1.2. BIOCHEMISTRY OF CANCER INDUCTION

Radiation resulted in the production of free radicals, free radicals mediate the formation of reactive oxygen species (O_2 , OH , H_2O_2): hydroxyl radical attack on DNA base causing hydrozylatedbase, not repaired by the cell cycle repair machinery or by apoptosis, it is the genesis of cancer (Breast, ovarian, colon cancer etc). Normal DNA (Non-mutated DNA) transcribe and translate for proteins that are important for normal cell proliferation and division (Cyclin dependent kinases, P53 protein, Ras protein etc), but DNA becomes abnormal or an error DNA (Oncogen and defective tumour suppressor gene) when the base pair has been mutated, which now transcribe and translate for error protein which are called over-expressed protein and it is the basis of Neoplasticism.

Radiation therapy has been used effectively in treating various types of cancers for many decades; it is used for the treatment of primary and metastatic cancers and also to alleviate symptoms associated with cancer (Gokceet *al.*, 2012; Ringborget *al.*, 2001). Radiotherapy is frequently used to achieve local or regional control of malignancies either alone or in combination with other regimens such as chemotherapy or surgery. The primary focus in radiotherapy is to increase double-strand breakage of DNA, so as to kill or to control cancer cells through free radical generation (Hall and Giaccia, 2006; Chitra and Shyamala, 2008; Patel *et al.*, 2001). Radiation damages cells by direct ionization of the atoms that make up the DNA and other cellular targets and by indirect effects through reactive oxygen species. Free radical-mediate excessive oxidative damage to DNA, proteins, lipids, and other macromolecules accompany radiotherapy due to high-energy particles generated by linear accelerator. Radiation, through radiation therapy, uses high-energy radiation to shrink tumors and kill cancer cells (Lawrence *etal.*, 2008). X-rays, gamma rays, and charged particles are types of radiation used for

cancer treatment. The radiation is often delivered by a machine outside the body and this process is known as external-beam radiation therapy (Semelka *et al.*, 2007). The biological effects caused by very high acute radiation exposures are collectively referred to as the acute radiation syndrome and generally, most radiosensitive cells are those ones that are rapidly dividing. The irradiation of noncancerous “normal” tissues during the course of therapeutic radiation can result in a range of side effects which include self-limited acute toxicities, mild chronic symptoms, or severe organ dysfunction. The possibility of developing these complications relates to the volume of an organ irradiated, the radiation dose delivered, fractionation of the delivered dose, the delivery of radiosensitizers and radioprotectors known as modifiers, and individual’s radiosensitivity (Stone *et al.*, 2004; Xavier *et al.*, 2002). But the pathological side effect that ensued after radiotherapy targeted death of cancer cells, the irony is that it also resulted to electron leakages, which mediate the formation of free radicals which alter cellular homeostasis via modifying signal transduction pathways, redox state, and disposition to apoptosis. The cellular changes ideally would enhance the killing of tumor cells while reducing the probability of normal cell death, but the side effect by the formation of reactive oxygen species formed from the free radicals include superoxide anion, hydrogen peroxide and hydroxyl radicals (Joenje, 1989). This free radicals also went ahead to attack nucleic acid of DNA, usually on the base, resulting into mutation, which is the genesis of deregulated proliferation and error in the cell cycle machinery usually at the Gap-1 or S-phase of the cell cycle, the Free radicals also have the potentials to attack tumor suppressor gene and proto-oncogenes (this are genes, implicated to be connected to cell cycle repair machinery, so as to to repair and avert error in cell growth, cell division and differentiation in the cell cycle) (Haliwell, 2001). Mutated tumor suppressor gene (TSG) or overexpressed proto-oncogenes (POG) which lead to loss of function or gain of function of TSG and POG respectively, which has been implicated in several cancerous diseases such as colorectal cancer, prostatic cancer, glioblastoma multistep, breast cancer, liver cancer etc. (Oduola *et al.*, 2002).

1.1.3 BIOCHEMISTRY OF INDUCTION OF ISCHEMIA REPERFUSSION DISEASES

Free radical generated through radiotherapy that has been implicated in altering cellular homeostasis, causes ischemia reperfusion diseases, for instance Nitric oxide radicals (NO[•]) has the potential to behave as a gas and as a free radical; when NO[•] is acting as a free radical, it has the potential of crossing the wall off the intestine to the muscle cells, where its work causes blockage of the blood vessels, resulting into high pressure flow of blood and also impairing proper blood flow which is the genesis of ischemia reperfusion diseases, this free radicals also diffused into the neuronal junction where it causes blockage to transmission of neurotransmitter across the neuronal junction and it is the genesis of weak muscle, blur vision, nausea and other neurodegenerative diseases (Khal, 2006).

1.1.4 MALONDEALDEHYDE (MDA) INDUCED ATHEROSCLEROSIS

It is pertinent to know that the product of lipid oxidation is a very toxic metabolite that has the potential to attack the lipids that produce it and other lipids, it can also attack proteins on membrane and nucleic acid of DNA; resulting into membrane protein oligomerization and aggregation, thereby mediating protein linkages and cross-linking resulting into membrane disruption and it has been implicated to increasing pathological ageing. This product of lipid peroxidation is MDA, it attacked on lipoprotein, resulted into synthesis of VLDL (very low density lipoproteins) which is the genesis of several cardiovascular diseases such as atherosclerosis etc.

1.1 PLANTS AS THERAPEUTICS IN MEDICINE

Although there are various drugs used for the treatment of Free radical induced disorders in Africa, but the affordability of these drugs is a problem to many poor people in the developing countries such as Nigeria. In addition the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of anaemia, cancer, atherosclerosis, ischemia reperfusion and heart diseases, so they depend heavily on plants and herbal products for the treatment of diseases and anaemia. Several diseases has been claimed to have been successfully treated with plant materials by traditional medicine practitioners and many authors (Burkill, 1985; Okpuzoret *et al.*, 2008). The extract of *Pterocarpussantalinoidea* and *Aloe vera* was reported to increase the gelling time of sickle cell blood and inhibits sickling in-vitro. This indicates that such plants may be useful in the management of sickle cell disorder (Okpuzoret *et al.*, 2008). The reversal of sickling by use of medicinal plants has been reported: *Terminaliacatappa* could be an effective antisickling agent, inhibiting osmotically induced haemolysis of human erythrocytes (Mgbemene and Ohiri, 1999). Phytomaterials such as *Annonasenegalensis*, *Cymbopogondensiflorus*, *Brideliaferruginea*, *Ceibapentandra*, *Morindalucida* and *Alchorneacordifolia* have been reported to be useful in the treatment of anaemia (Mpiana *et al.*, 2007). The role of crude aqueous extract of *Zanthoxylummacrophyllar* roots as an antisickling agent was also highlighted and 2-hydroxybenzoic acid was isolated and identified as the antisickling agent obtained from the root of this plant (Elekwaet *et al.*, 2005). An investigation on the aqueous extracts of *Garcinia kola* confirmed that it could be useful in the management of anaemic diseases (Elekwaet *et al.*, 2003).

1.1.1 *Corchorus olitorous*

According to a reviewed work by Hamzah and coworkers in 2001, *Corchorus olitorius* (Linn) is a leafy vegetable that belongs to the family Tiliaceae, and commonly called jute mallow in English and “ewedu” in the

southwestern Nigeria. It is an animal herb with a slender stem and an important green leafy vegetable in many tropical areas including Egypt, Sudan, India, Bangladesh, in tropical Asia such as Philippines and Malaysia, as well as in tropical Africa. The plant is widely grown in the tropics for the viscosity of its leaves. 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 (Brandaet *al.*, 2004). Nutritionally, *C. oleraceus* on an average contains 85-87g H₂O, 0.7g oil, 5g carbohydrate, 1.5g fiber, 250-266mg Ca, 4.8mg Fe, 1.5mg vitamin A, 0.1mg thiamine, 0.3mg riboflavin, 1.5mg nicotinamide, and 53-100mg ascorbic acid per 100g (Branda, 2004). In West African countries including Ghana, Nigeria and Sierra Leone, the vegetable is cultivated for the stem bark, which is used in the production of fiber (Jute), and for its mucilaginous leaves, which are also used as food vegetable (Bryonet *al.*, 2007). The leaf extract of the plant is also employed in folklore medicine in the treatment of gonorrhoea, pain, fever and tumor (Brandaet *al.*, 2007). It is reportedly consumed as a healthy vegetable in Japan because of its rich contents of carotenoids, vitamin B1, B2, C and E, and minerals (Chihandeet *al.*, 1997). Its leaves and roots are eaten as herbal medicine in South East Asia. In some parts of Nigeria, leaves' decoction is used for treating iron deficiency, folic acid deficiency, as well as treatment of anemia. Leaves also act as a blood purifier and the leaf twigs are used against heart troubles (Krivaneket *al.*, 2007) while cold leaf infusion is taken to restore appetite and strength, leaves used for ascites, pains, piles, tumors, gonorrhoea and fever (Dharmendraet *al.*, 2006).

1.1.2 *Adansonia digitata*

Adansonia digitata is a commonly used traditional plant which is consumed in food or used in the direct treatment of several diseases such as cancer, anaemia, diabetes, ischemia reperfusion diseases, and inflammatory bowel syndrome in South-western Nigeria. *Adansonia digitata* is a tree found widely throughout Africa and known locally in African countries as the tree of life due to its ability to sustain life, as well as its many traditional medicinal and nutritional uses (Lewandaet *al.*, 2007). The baobab tree is an important food, water and shelter source in many African countries (Byroet *al.*, 2007). *Adansonia digitata* is commonly called Kukah by the Hausa of Northern Nigeria, konian in Niger, Kenyans: Mwambom, Mali: sira, Senegal: gouli. It is a deciduous tree which has four growth phases and produces a fruit consisting of a yellowish-white pulp which has a floury texture and numerous hard, round seeds, enclosed in a tough shell (Dharmendraet *al.*, 2006). The leaves of the baobab tree are a staple for many populations in Africa, especially the central region of the continent. During the rainy season when the baobab leaves are tender, the leaf is harvested fresh. During the last month of the rainy season, leaves are harvested in great abundance and are dried for domestic use and for marketing during the dry season. The leaves are typically sun-dried and either stored as whole leaves or pounded and sieved into a fine powder (Dharmendraet *al.*, 2006). The powdered leaves are used as a tonic and an antiasthmatic and known to have antihistamine and anti-tension properties. The leaves are also used to treat insect bites, guinea worm and internal pains, dysentery, diseases of the urinary tract, ophthalmia and otitis. In Indian medicine, powdered leaves are similarly used to check excessive perspiration. Baobab leaves are used medicinally as a diaphoretic, an astringent, an expectorant and as a prophylactic against fever. Baobab leaves have been investigated in an attempt to identify the potential bioactive associated with this part of the plant. Certain bioactive compounds may be responsible for the treatment of certain ailments, as well as containing properties that can be beneficial to overall health. Examples of such bioactive compounds include tannins, phlorotannins, terpenoids, glycosides, saponin and terpenoids as well as antioxidants including flavonoids and polyphenols. The chemical profile of the methanolic and aqueous extracts of the leaves of the plant was also investigated. They reported the presence of glycosides, phytosterols, saponins, protein and amino acid, phenolic compounds and tannins, gums, mucilage and flavonoids. Only a few authors have investigated the vitamin A content of baobab leaves (Scheuringet *al.*, 1999) found that the simple practice of drying baobab leaves in the shade protects against deterioration of provitamin, other authors mention the carotenoid content of baobab leaves. Several reviews have revealed a great variation in reported values of nutrient contents of baobab parts, the causes of these variations are not well known, however they made several assumptions: that, the variation may be due to the quality of the sample, the provenance of the sample, the age of the sample, the treatment before analysis, the storage conditions, the processing methods, a probable genetic variation, and the soil structure and its chemical composition. It is a known fact that the consumption of antioxidant-rich foods can contribute to the prevention of oxidation in the human cell, hence of some diseases. In addition to the general chemical composition of baobab pulp and leaves discussed previously, the antioxidant content of extract of *Adansonia digitata* was investigated, which shows that baobab leaves have an antioxidant content of 7.7 $\mu\text{mol/g}$ dw expressed as Trolox equivalents. This result is almost 1000 times lower than composition and nutritional value of baobab foods the one reported by Vertuaniet *al.* (2002), who found that the water-soluble antioxidant capacity of dry baobab leaves was 6.4 mmol Trolox equivalent/g. These antioxidant activities were measured in fresh raw material and the effect of cooking and storage is not well known. Only Tarwadi and Agte (2001) reported the antioxidative activity of was measured as the inhibition of thiobarbituric acid reactive substances (TBARS), superoxide radical scavenging activity (SOSA), and ferrous iron chelating ability (FICA). They reported that there were significant cooking losses for each of the assessed antioxidant parameters. Karumiet *al.* (2003) also reported the gastro protective effect of

Adansonia digitata leaf on ethanol induced ulceration. This study elucidated a significant dose- dependent increase both in preventive ratio and percentage ulcer reduction after pretreatment with *Adansoniadigitata* leaves. Ethanol is an established ulcerogen especially in empty stomach (Agte, 2001). The ulcerogenicity of ethanol is due to intracellular oxidative stress producing mitochondrial permeability, transition and mitochondrial depolarization which results to the death of cells in gastric mucosa. This is because of its congestive inflammation and tissue injury. It is a known fact that flavonoids and antioxidant (Vit A, E and C) present in this plant has protective role on GIT. This view is supported by the fact that gastric mucosa is known to have certain antioxidant activity thereby reducing mucosal damage mediated by free radicals (Dharmendraet *al.*,2006), which in turn attack cell membrane causing a lipid derived free radicals such as conjugated diene and lipid hydroperoxides which are extremely reactive and unstable. This study corroborate with previous report on the anti-ulcerative properties of the aqueous extract of *Adansonia digitata* leaves against ethanol induced ulceration in rats (Dharmendraet *al.*,2006). Although the precise mechanism of action of *A. digitata* is not clear, it was proposed that the gastoprotective role of this vegetable extract may be partly due to its high content of flavonoids and other antioxidant (Krivanek, 2007) which are well known compounds that prevent and combat the formation of reactive oxygen species. Another possible mechanism is the fact that the leaves being an astringent may have precipitated microproteins on the site of ulcer thereby forming an impervious protective pellicle over the lining to prevent absorption of toxic substance and resist the attack of Proteolytic enzymes.

2.0 MATERIALS AND METHODS

2.1 PLANTS MATERIALS

The leaves of *Cochorus oltorous* and *Adansonia digitata* were purchased at bodija market, Ibadan, Oyo State. The plants were identified and authenticated at the federal research institute of Nigeria (FRIN). 500g each of the plants that has been blended or pulverized using manual blender in the laboratory and packed in air-tight container to prevent deterioration till needed for laboratory use.

2.1.1 EXTRACTION OF THE PLANT

500g of the pulverized plant was macerated in 2000ml (2L) of methanol (solvent) contained in a glass cylinder, the solution was left for 72hours (3 days), separation of the plants extract was carried out using whatman no.4 filter paper, the resulting plants extract was then concentrated using rotatory evaporator, the resulting dried extract was then stored in a glass cylinder and covered for laboratory uses. The percentage yield of *C. oltorous* is 4.6%, while that of *A. digitata* extract was calculated to be 14.8 % percentage yields.

2.2 ANIMALS

Ninety adult male albino rats weighing 190-200g were purchased from the Animal house of the Department of Physiology, University of Ibadan. These rats were initially acclimatized to the well-ventilated Animal house of Department of Biochemistry which was regularly cleaned for the period of 2 weeks after purchase. They were housed in rat cages and fed with normal laboratory rat feed bought from Ladokun Feeds, Mokola, Ibadan. Water was also supplied ad libitum.

2.2.1 Irradiation of Animals and Sample Administration

2.2.2 Radiation Exposure

Animals in the irradiated groups were treated with samples, 1 week before radiation exposure and 1 week after radiation exposure. The animals were treated with a single dose of whole body gamma radiation of 6Gray (6Gy), and the unit Gray is the radiation unit equivalent to an energy of one joule/kilogram of target mass (Zubkoveet *al.*, 1995). The last dose of sample administration was 24hours before irradiation whereas the feed was withdrawn 12hours before irradiation. The radiation exposure system was at Radiotherapy Department, College of Medicine, UCH, Ibadan. The animals were kept in an improvised cage to restrict their movement and to ensure uniform and effective exposure.

2.2.3 EXPERIMENTAL DESIGN

Ninety Adult Male Wistar rats of weight ranges between 190-200g, were purchased in the Animal house of Department of Biochemistry, University of Ibadan, and was randomly selected and distributed into fifteen (15) groups of six (6) member each. Below are the designs of the 15 group member.

Table 2.2.3 Extract One (E₁) (*A. digitata* methanolic extract)

Groups	Treatment
(Control	Non-irradiated, non-treated
IR Irradiated, non-treated (R)	
IR + 500mg/kg	Irradiated animals treated with 500mg/kg body weight (RE _{1 500})
IR + 1000mg/kg	Irradiated animals treated with 1000mg/kg body weight (RE _{1 1000})
NR 500mg/kg	Non-irradiated animals treated with 500mg/kg body weight (NRE _{1 500})
NR1000mg/kg	Non-irradiated animals treated with 1000mg/kg body weight (NRE _{1 1000})

Table 2.2.3 Extract Two (E₂) (*C. olitorius* methanolic extract)

Groups	Treatment
Control	Non-irradiated, non-treated
IR	Irradiated, non-treated(R)
IR + 500 mg/kg	Irradiated animals treated with 500mg/kg body weight (RE _{2 500})
IR + 1000mg/kg	Irradiated animals treated with 1000ml/kg body weight (RE _{2 1000})
NR500 mg/kg	Non-irradiated animals treated with 500ml/kg body weight (NRE _{2 500})
NR 1000 mg/kg	Non-irradiated animals treated with 1000ml/kg body weight (NRE _{2 1000})

2.3 PHYTOCHEMICAL SCREENING

The phytochemicals such as flavonoids, tannins, saponins, alkaloids, polyphenols and anthraquinones were identified by chemical method and as modified by Harborne (1996) and Sofowora [1993].

2.4 DETERMINATION OF HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

Whole blood was collected from the eyes by ocular puncture using sterile syringe and needle. The whole blood samples were put in ethylene diamine tetra acetate (EDTA) treated sample tubes. The packed cell volume or the haematocrit and White blood cell count (WBC) was determined by the method of Baker and Silverton (1987), Hemoglobin (Hb) concentration was determined according to Jain (1986), using the cyanomethemoglobin method (1987), while platelets were determined by following the method of Mitruka and Rawnsley (2001). Biochemical assay was carried out as follows: Billirubin content was measured by the method described by Jendrassik and Grof (1987), Total proteins assay was conducted according to Tietz (2002) while serum albumin level was examined by the method described by Grant (2001).

2.5 DETERMINATION OF SERUM ENZYME ASSAY

Alanine transaminase (ALT) and Aspartate transaminase (AST) activities were assayed using the method of Reitman and Franke and Alkaline Phosphatase (ALP) serum level was estimated by the principle of Tietz. All the above biochemical parameter was determined in the plasma using the Randox kits by Cypress diagnostics (Belgium).

2.6 STATISTICAL ANALYSIS OF DATA

Data obtained was expressed as Mean±Standard Deviation and analyzed using the Analysis of Variance 'ANOVA, F-ratio and student's t' test where applicable. Values at $P=0.05$ and $P<0.05$ were regarded as significant in comparison with appropriate controls.

3.0 RESULTS

3.1 Phytochemicals of *Cochorus olitorius* and *Adasonia digitata* Methanolic Leaf Extract shows the presence of Saponin, alkaloids, flavonoids, polyphenols, tannins, terpenoids.

QUALITATIVE PHYTOCHEMICAL SCREENING

Metabolites	<i>Adasonia digitata</i>	<i>Corchorus olitorius</i>
Alkaloids	++	++
Anthraquinone	-	-
Cardiac glycosides	-	+
Flavonoids	++	++
Polyphenols	++	+++
Phlobatanins	-	-
Saponin	++	++
Tannin	+++	++
Terpenoids	+	++
Steroids	+	+

+: Faintly Present ++: Moderately Present +++: Excessively Present -: Absent

3.1.2 QUANTITATIVE PHYTOCHEMICAL SCREENING

Metabolites	<i>Adasonia digitata</i>	<i>Corchorus olitorius</i>
Alkaloids	81.56 ± 0.56	68.65 ± 2.05
Saponin	16.59 ± 1.85	22.17 ± 0.24
Tanins	311.98 ± 0.01	287.07 ± 0.16
Flavonoids	25.38 ± 2.88	157.38 ± 0.38
Polyphenols	170.90 ± 0.68	330.07 ± 0.32

3.2 The result of Methanolic leaf extract of *C. olitorius* and *Adasonia digitata* on some hematological, biochemical parameters and enzyme assay of Wistar rats exposed to radiation are presented in Tables below 500mg/kg body weight and 1000mg/kg body weight of the animals.

Table 3.2.1: The Hematological Analysis of Methanolic Extracts of *A. digitata* and *C. olitorius* on Rats Exposed to Radiation (6Gy).

Treatment Group	PCV (%)	HB (%)	RBC (%)	WBC(10 ⁹ /L)	LYPMS	PLT((10 ⁵ /μL)
NORMAL	36.29±0.36 ^a	12.11±0.21 ^a	7.84±0.06 ^a	8.55±0.04 ^a	47.23±0.39 ^a	134227.00±330.06 ^a
IR	26.97±0.89 ^b	6.40±0.12 ^b	3.76±0.01 ^b	13.51±0.33 ^b	81.52±0.29 ^b	67139.67±204.81 ^b
R(E ₁ 500)	26.70±0.16 ^b	8.82±0.10 ^c	5.37±0.04 ^c	10.16±0.03 ^c	65.48±0.29 ^c	81876.33±1406.43 ^c
R(E ₁ 1000)	32.81±0.42 ^c	10.89±0.26 ^a	8.44±0.26 ^a	11.96±0.04 ^d	69.33±0.33 ^d	97921.33±39.48 ^d
R(E ₂ 500)	27.14±0.32 ^b	9.54±0.03 ^c	5.31±0.05 ^c	10.07±0.07 ^c	65.48±0.29 ^c	81525.00±635.02 ^c
R (E ₂ 1000)	31.99±0.20 ^c	11.07±0.12 ^a	9.07±0.04 ^a	11.05±0.08 ^d	69.20±0.31 ^d	98485.33±257.36 ^d
R(E ₁ +E ₂ 500)	33.51±0.38 ^c	13.00±0.11 ^a	10.99±0.01 ^a	11.99±0.02 ^d	71.74±0.13 ^d	108666.33±605.87 ^e
R(E ₁ +E ₂ 1000)	39.21±0.34 ^a	15.33±0.17 ^a	13.73±0.09 ^d	14.85±0.09	74.99±0.07	129044.67±29.356
RVIT.C	27.40±0.81 ^b	8.20±0.11 ^a	5.34±0.02 ^c	10.38±0.31	64.82±0.24	81709.00±595.84

Values are Expressed as Means ± SEM; n=9, R: Irradiated, E₂: *C. olitorius*; E₁: *A. digitata*. Significant difference of p<0.05

For the Hematological parameters in Table 3.2.1 above, the statistical analysis shows that the methanolic leaf extract of *C. olitorius* and *A. digitata* recorded significant (P=.05) change for PCV, HB, RBC, WBC, LYMPHS and PLT levels for all treatment groups when compared to the negative not treated groups. However a significant (P=.05) decrease was observed for PCV, HB, RBC, WBC, LYMPHS and PLT levels of the negative groups when compared to the Normal control group that was not exposed to radiation.

Table 3.2.2: Therapeutic Potential of Methanolic Extracts of *A. digitata* and *C. olitorius* on Serum AST, ALT, ALP levels (U/L) of rats exposed to radiation (6Gy).

Treatment Group	AST	ALT	ALP
NORMAL	95.39 ± 0.89 ^a	58.23 ± 0.51 ^a	24.68 ± 0.16 ^a
IR	143.33 ± 0.89 ^b	98.00 ± 0.57 ^b	76.49 ± 0.77 ^b
R(E ₁ 500)	126.32 ± 0.36 ^c	72.71 ± 0.16 ^c	62.53 ± 0.24 ^c
R(E ₁ 1000)	101.58 ± 0.27 ^d	53.15 ± 0.45 ^a	46.15 ± 0.57 ^d
R(E ₂ 500)	121.37 ± 0.71 ^c	69.89 ± 0.23 ^c	60.73 ± 0.37 ^c
R (E ₂ 1000)	98.34 ± 1.03 ^a	57.73 ± 0.37 ^d	43.82 ± 0.30 ^d
R(E ₁ +E ₂ 500)	87.26 ± 0.50 ^a	54.45 ± 0.29 ^a	39.07 ± 0.14 ^e
R(E ₁ +E ₂ 1000)	75.89 ± 0.11 ^c	48.67 ± 0.67 ^c	24.30 ± 0.39 ^a
RVIT.C	120.33 ± 0.33 ^c	66.67 ± 0.88 ^c	60.00 ± 0.58 ^c

Values are Expressed as Means ± SEM; n=9, R: Irradiated, E₂: *C. olitorius*; E₁: *A. digitata*. Significant difference of p<0.05

In this work, AST, ALT, and ALP activities were used to determine the therapeutic potentials of *C. olitorius* and *A. digitata* on radiation induced free radical induced toxicity or disorders on male wistar rats and were interpreted as follows, Statistical evaluation revealed that, for AST levels, the extract recorded a significant (P=.05) decrease for all treatment groups; C(126.32±0.36), D (101.58±0.27), E(121.37±0.71), F(98.34±1.03), G(87.26±0.50) H (75.89±0.11) I(120.33±0.33) when compared with negative control B (143.33±0.89). Similar trend were also observed in ALT and ALP levels.

Table 3.2.3: Effects of Methanolic Extracts of *A. digitata* and *C. Olitorous* on liver Protein, Bilirubin and MDA level (mg) of rats after exposure to radiation (6Gy)

Treatment Group	Total Protein	Total Bilirubin	MDA
NORMAL	92.17 ± 0.89 ^a	14.87 ± 0.19 ^a	0.230 ± 0.03 ^a
IR	54.10 ± 0.58 ^b	27.37 ± 0.58 ^b	0.645 ± 0.01 ^b
IR (E ₁ 500)	60.20 ± 0.46 ^c	22.76 ± 0.41 ^c	0.569 ± 0.04 ^c
IR (E ₁ 1000)	68.97 ± 0.23 ^d	20.77 ± 0.16 ^d	0.412 ± 0.01 ^d
IR (E ₂ 500)	60.40 ± 0.30 ^c	23.14 ± 0.60 ^c	0.546 ± 0.03 ^c
IR (E ₂ 1000)	70.14 ± 0.10 ^d	19.62 ± 0.31 ^d	0.400 ± 0.05 ^d
IR (E ₁ +E ₂ 500)	59.85 ± 0.20 ^c	18.02 ± 0.10 ^d	0.284 ± 0.06 ^a
IR (E ₁ +E ₂ 1000)	79.63 ± 0.80 ^c	15.42 ± 0.12 ^a	0.219 ± 0.05 ^a
IR (VIT C)	67.89 ± 3.90 ^d	26.21 ± 0.41 ^b	0.548 ± 0.02 ^c

Values are Expressed as Means ± SEM; n=9, IR: Irradiated, E₂: *C. olitorous*; E₁: *A. digitata* Significant difference of p<0.05

For the biochemical parameters in the Table 3.2.3, statistical analysis showed that the Methanolic leaf extract of *C. olitorous* and *A. digitata* recorded a significant ($P=0.05$) increase for total proteins level for all treatment groups; C (60.20±0.46), D (68.97±0.23), and E (60.40±0.30), F (70.14±0.10), G (59.85±0.20), H (79.63±0.80), I (67.89±3.90) when compared with negative control B (54.10±0.58). But as observed in the bilirubin and the MDA level which reduces upon extract administration when compared with the negative control groups that was exposed to radiation but not treated with the plant extracts.

Table 3.2.4: The Effects of Methanolic Extracts of *A. digitata* and *C. Olitorous* on liver intracellular Antioxidants levels (µmol/mg protein) of rats after exposure to radiation (6Gy).

Treatment Group	GST	GPX	CAT	SOD	GSH
NORMAL	2.95 ± 0.302 ^a	2.67 ± 0.06 ^a	57.88 ± 0.01 ^a	4.59 ± 0.02 ^a	25.70 ± 0.16 ^a
IR	1.20 ± 0.01 ^b	1.08 ± 0.02 ^b	21.79 ± 0.29 ^b	1.89 ± 0.03 ^b	13.92 ± 0.04 ^b
IR(E ₁ 500)	1.89 ± 0.01 ^c	1.63 ± 0.04 ^c	29.82 ± 0.24 ^c	2.36 ± 0.02 ^c	16.98 ± 0.06 ^c
IR(E ₁ 1000)	2.45 ± 0.03 ^d	1.78 ± 0.01 ^d	36.08 ± 0.01 ^d	2.80 ± 0.01 ^d	20.09 ± 0.03 ^d
IR(E ₂ 500)	1.99 ± 0.01 ^c	1.63 ± 0.02 ^c	32.00 ± 0.00 ^c	2.40 ± 0.01 ^c	17.01 ± 0.03 ^c
IR (E ₂ 1000)	2.49 ± 0.01 ^d	1.90 ± 0.01 ^d	38.14 ± 0.07 ^d	2.90 ± 0.01 ^d	20.14 ± 0.02 ^d
IR(E ₁ +E ₂ 500)	2.83 ± 0.01 ^a	1.96 ± 0.01 ^d	44.23 ± 0.04 ^e	3.22 ± 0.01 ^c	22.55 ± 0.22 ^c
IR(E ₁ +E ₂ 1000)	3.64 ± 0.02 ^a	2.63 ± 0.00 ^a	58.77 ± 0.97 ^a	3.91 ± 0.01 ^a	26.91 ± 0.04 ^a
IR(VIT C)	1.97 ± 0.01 ^c	1.60 ± 0.01 ^c	31.56 ± 0.22 ^c	2.37 ± 0.01 ^c	17.78 ± 0.11 ^c

Values are Expressed as Means ± SEM; n=9, IR: Irradiated, E₂: *C. olitorous*; E₁: *A. digitata* Significant difference of p<0.05

For the serum antioxidant assay as seen in 3.2.4, statistical analysis showed that the Methanolic leaf extract of *C. olitorous* and *A. digitata* recorded a significant ($P=0.05$) increase for total antioxidant enzymes level for all treatment groups; GST: C (1.89±0.01), D (2.45±0.03), E (1.99±0.01), F (2.49±0.01), G (2.83±0.01), H (3.64±0.02), I (1.97±0.01) when compared with negative control B (1.20±0.01), this was observed cross-board in the respective enzymes.

Table 3.2.3: The Survival Rate after Exposure to Radiation and treatment with the Extracts

Treatment Group	Initial Numbers of Rats par Groups before exposure	Final Number of Rats per Groups after exposure	Number of Death Recorded after Exposure
NORMAL	6	6	0
IR	6	3	3
IR (E ₁ 500)	6	4	2
IR (E ₁ 1000)	6	5	1
IR (E ₂ 500)	6	4	2
IR (E ₂ 1000)	6	5	2
IR (E ₁ +E ₂ 500)	6	5	1
IR(E ₁ +E ₂ 1000)	6	5	1
IR VIT C	6	3	3

Table 3.2.4: Effects of Methanolic Extracts of *A. digitata* and *C. Oligorous* on Body Weight (g) of rats not expose to radiation (NR) (6Gy)

Treatment Group	Initial b.wt.	Final b. wt.	% Increase in b.wt.
NORMAL	199.42 ± 0.59	209.00 ± 0.33	8.58±0.26
IR	201.22 ± 0.78	143.00 ± 0.58	58.22±.20
NR (E ₁ 500)	200.33 ± 0.33	217.67 ± 0.58	17.34±0.25
NR (E ₁ 1000)	201.67 ± 0.33	223.33 ± 0.58	21.66±0.25
NR (E ₂ 500)	201.00 ± 0.58	217.33 ± 0.58	16.33±0.00
NR (E ₂ 1000)	197.67 ± 0.33	223.33 ± 3.05	25.66±2.72
NR (E ₁ +E ₂ 500)	190.00 ± 0.58	227.00 ± 1.00	37.00±0.42
NR (E ₁ +E ₂ 1000)	197.67 ± 0.33	229.33 ± 0.58	31.66± 0.25

Values are expressed as means ± SEM; n=8, NR: Non-irradiated, E₂: *C. oligorous*; E₁: *A. digitata*
 Significant difference of p<0.05

Table 3.2: Effects of Methanolic extract of *A. digitata* and *C. oligorous* on body weight (g) of rats after exposure to radiation (6Gy)

Treatment Group	Initial b.wt	Final b.wt	Changes b.wt.
NORMAL	199.42 ± 0.59	209 ± 0.00	9.58± 0.59
IR	201.22 ± 0.78	143 ± 0.33	58.22± 0.45
IR (E ₁ 500)	200.33 ± 0.33	166 ± 0.37	34.33±0.04
IR (E ₁ 1000)	201.67 ± 0.33	186 ± 0.58	15.67±0.25
IR (E ₂ 500)	210.00 ± 0.58	169 ± 0.58	41.00±0.00
IR (E ₂ 1000)	197.67 ± 0.33	189 ± 0.58	8.67±0.25
IR (E ₁ +E ₂ 500)	190.00 ± 0.58	194 ± 0.58	4.00±0.00
IR (E ₁ +E ₂ 1000)	197.67± 0.33	208 ± 0.33	11.67±0.00
IR (VIT C)	200.33± 0.33	169 ± 0.33	31.33± 0.00

Values expressed as means ± SEM; n=9, IR: Irradiated; E₂: *C. oligorous*; E₁: *A. digitata*
 Significant difference of p<0.05

The histopathological analysis of rat exposed to radiation and treated with the extracts of *A. digitata* and *C. Oligorous*

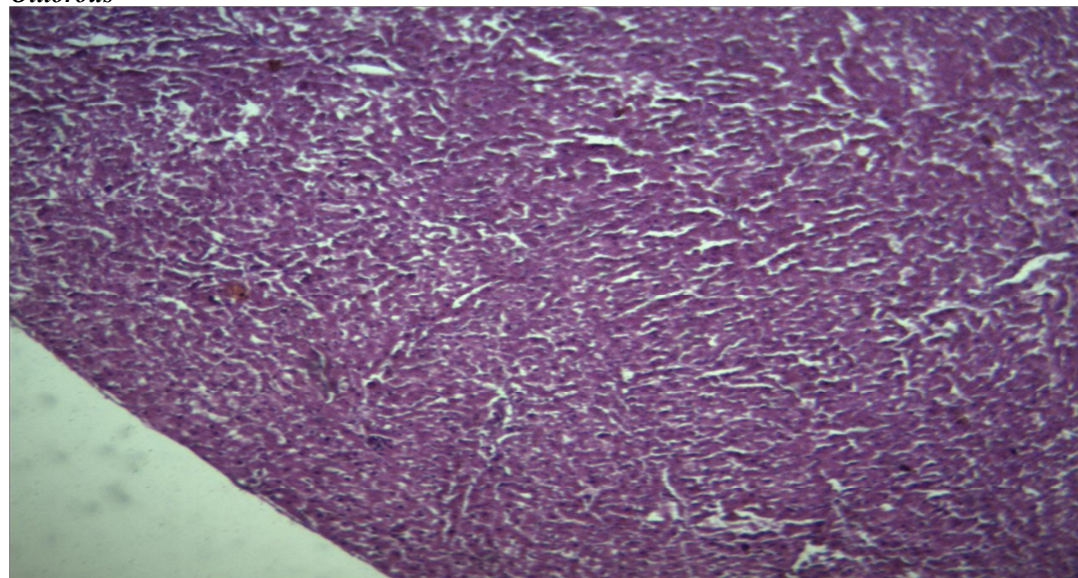


Figure1: Photomicrograph of rats liver histopathological analysis stained with heamatoxylin and eosin: Normal group administered with water and rat chow alone A: No Visible lesion seen. Microscopic Magnification X 400

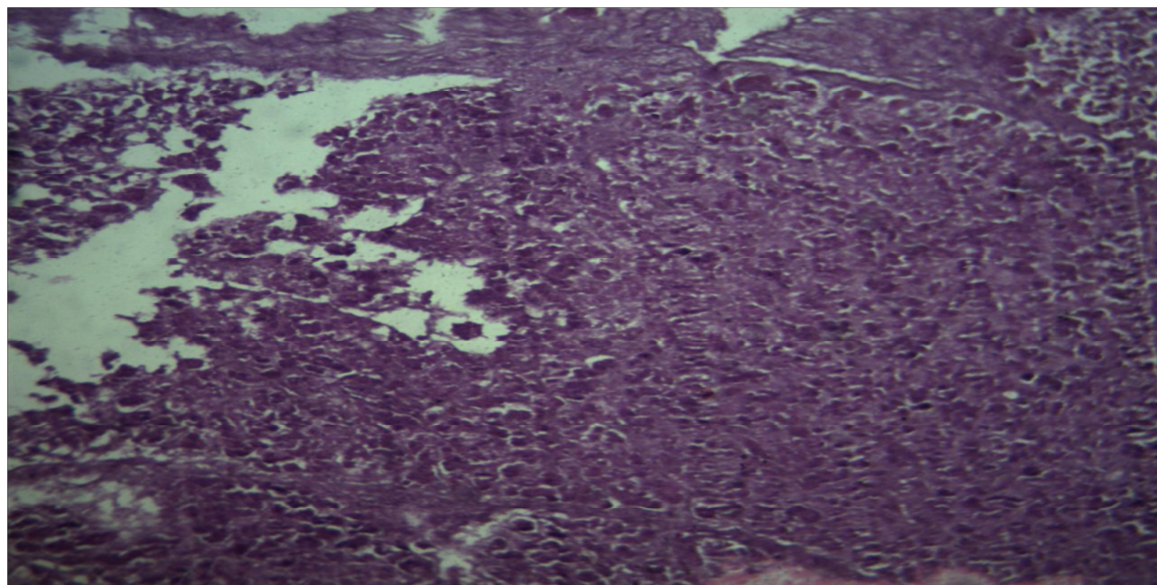


Figure2: Photomicrograph of rats liver histopathological analysis stained with heamatoxylin and eosin: (b) irradiated but non-treated group. There is a severe diffuse hepatic and Macular degeneration

(C)

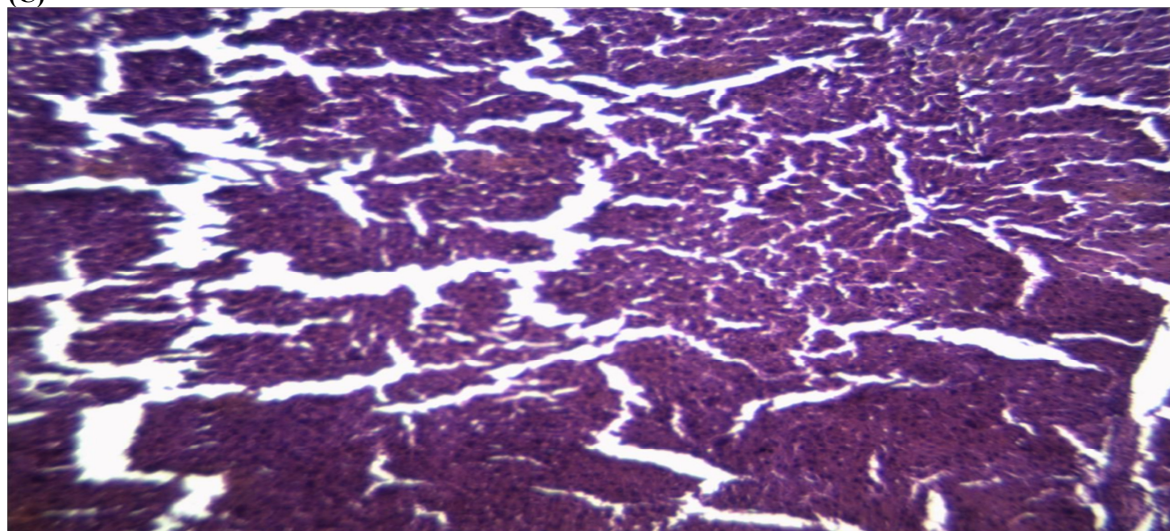


Figure 3: Photomicrograph of rats liver histopathological analysis stained with heamatoxylin and eosin: (c) The effects of the plant extracts on the liver architecture. There was reversal of membrane damage.

4.0 DISCUSSION

The presence of tannins, saponin, polyphenols, flavonoids and alkaloids as revealed in the results of phytochemical analysis in *C. olitorius* and *A. digitata* methanolic leaf extract suggests its usage for various medicinal purposes in folk medicine. Alkaloids are the most efficient therapeutic plant substance. Both natural and synthetic alkaloids are used as basic medicinal agent because of their analgesic, antispasmodic and antibacterial properties (Yakubu *et al.*, 2011). Farombi and Yong Jhur Shur in 2001, established that, the presence of polyphenol in Cucumin is responsible for the induction of several phase and antioxidant enzymes, so also is the presence of very significant ($p < 0.05$) amount of polyphenol in the methanolic extracts of *C. olitorius* and *A. digitata* which is suggestive of the extract ability to induce several of this enzymes (Glutathion-S transferases, Uridyl glucuronosyl transferases, Sulfur transferases, Methyltransferases, Glutathion peroxidases, Heam oxygenases, Superoxide dismutase, Catalase, lactoferine, tranferine, ceruloplasmin, albumin etc.), which have been implicated to scavenge free radicals generated from Radiation, electron transport chain metabolism process, metabolism by Monoamine oxidase etc.

Since it has been established that free radicals generated from exposure to radiation, the free radicals has the potentials to attack iron containing sulfur hydril proteins like heamoglobin (a discocytic, biconcave, quaternary structure protein which has been implicated in oxygen carrying capacity in the blood) resulting to the

formation of a disulfide linkages which disrupt the biconcave structure to an abnormal echinocytic planar structure resulting into an abnormal hemoglobin known as Heinz body protein, which has the potential to recruit several other membrane and cytosolic proteins, which are now tag for phagocytic degradation by phagocytosis or by 26s proteasomes pathway, resulting into iron loss, reduction in the RBC level and packed cell volume, all which is an hallmark of anaemia. The values obtained for PCV in the negative control group shows significant reduction in the PCV, RBC, HB and PLT level in the rat exposed to radiation, when compared to the normal control group, this is suggestive of the effect of free radicals generated from radiation attacked on hemoglobin for destruction resulting into dissociation of the protein (globin) from the heme (iron containing prosthetic group), and the heme is therefore degraded into bilirubin, iron and carbon-monoxide by an heme oxygenase-1 enzyme, therefore reducing iron availability to the blood and resulting into iron deficiency anaemia, but the administration of the methanolic extracts of *C. olitorous* and *A. digitata* on the iron deficient rat was able to compensate for the lost iron and was also able to induce several phase-2 and antioxidant defense enzyme system, which has been implicated to scavenging free radicals and repaired the damaged caused by free radicals on proteins (hemoglobin). Saponin has been established by yakubu *et al* in 2001 to prevent hemoglobin and platelets aggregation and protein oligomerization, via its sedative tonic production, this is because saponin containing herbs have been successfully used in the management of liver inflammation, as tonic, as sedative formula and to promote vitalized blood circulation. The presence of polyphenol in the plant extracts has also been implicated to induce transcription factors that induce antioxidant enzymes which scavenge free radicals. According to table 3.2.1, it shows that upon extracts administration, the PCV, HB, RBC and PLT level that was initially reduced significantly ($P < 0.05$) in the negative control group when compared with positive control group was significantly ($P < 0.05$) increased. The significant ($P > 0.05$) reduction in white blood cell count (WBC) on the treated groups when compared with the negative controls group suggest the presence of bioactive agents like polyphenols, flavonoids and tannins which have been implicated in free radical scavenging. Similar report was given by Oyedeji and Bolarinwa (2013). Iron serves as the core of hemoglobin molecule, which is the oxygen-carrying component of red blood cells. The ability of the red blood to transport oxygen is attributed to the presence of iron in the hemoglobin molecule. A lack or loss of iron, induced by free radical attack on hemoglobin, resulting into hemoglobinolysis and invariable reduction in the hemoglobin and subsequent reduction in red blood cell. Hence, treatment of the radiated groups with the methanolic extracts of *C. olitorous* and *A. digitata* which has been implicated to contain saponins.

Nitric oxide has been physiologically implicated in sexual erection when present in normo-subtoxic concentration. Ischemia reperfusion diseases has been characterized by an excessive production of nitric oxide radicals (NO^\cdot), which is hallmark by the blockage of blood vessels by the excess NO^\cdot (Jain NC, 1986), preventing easy flow of blood through the arterial wall, resulting in high blood pressure which is an hallmark of hypertension (a central metabolic disorders i.e. increasing the risk of other disorders and cardiovascular diseases such as obesity, atherosclerosis, hyper-insulinemia etc.), but upon treatment of the radiated groups (with excessive NO^\cdot) with methanolic/aqueous extracts of *C. olitorous* and *A. digitata* leaves plant was able to scavenge the excess NO^\cdot out of the stressed system, attenuating, ameliorating and preventing the effect of nitric oxide radical and reverting the ischemic effect of NO^\cdot , this is suggestive of the presence of significant ($p < 0.05$) amount of tannins present in the two plant extracts, that has the potential to bind NO^\cdot and convert it to nitrous compound that can be passed out through urine, thereby reducing the excess nitric oxide radical to a sub toxic concentration that is required for physiological process such as in signaling and in the expression of sexual erection, so blood vessel blockage is prevented.

Alkaloids in Cucumin have been established to be an active metabolite, implicating Cucumin as an anticancer agent. It has been utilized in the treatment of cancer via induction and repair of a tumor suppressor protein known as p53 protein (a member of a three family of protein known as p53, p63 and p73 protein family) that has a p21 promoter binding site, the binding of the p21 to the p53 on the promoter site, causes the activation of p21, which it uses to cause cell cycle arrest when abnormal cellular proliferation ensued. The Methanolic extract of *C. olitorous* and *A. digitata* leaves plant was screened and it was confirmed to contain a significant ($P < 0.05$) amount of alkaloids which is suggestive of the two plant extracts potentials to reverse abnormal cell proliferation in rat exposed to radiation inducing free radical that attack nucleic acid base of DNA which is an hallmark of carcinogenesis.

Flavonoids is an essential metabolite of methanolic extracts of *occimum gratissimum*, which has been implicated to preventing the formation of malondialdehyde (MDA), a product of lipid peroxidation, which is a very important biomarkers of toxicity and membrane macromolecular damage, this damage is hallmark by membrane lesions and disruption of the interaction between membrane proteins, lipids and carbohydrates and it is the genesis of several diseases and disorders (CVD, diabetes, cancer, anaemia, atherosclerosis, dyslipidemia ischemia reperfusion diseases etc.). Studies has established the potential of Cucumin, *Occimum gratissimum*, *Vernonia amagdalina* and several other potent plants to induce transcription factors such as Nrf2 (Nuclear factor related factor 2) a member of the basic leucine zipper family of protein, it existed as dimer when inactive),

Ap-1(Activator protein 1) a member of the basic leucine protein), Nf-Kb (Nuclear factor kappa B) a nuclear proteins that existed as trimmer in an inactive state, but becomes dimer when activated) etc. Once this transcription factors are induced they become activated and translated into the nucleus where they bind to the promoter sites of the genes they are activating, such activated genes induces the expression of several phase-2 and antioxidant detoxifying enzymes such as HO-1(Heam Oxygenase 1), SULT(Sulfotransferases), GST(Glutathion-S-Transferases), GPX(Glutathion peroxidases), CAT(Catalase), MET(Methyltransferases), UGT(Uridyl Glucuronosyl transferases) which scavenge free radicals, strengthen the immune system and prevent diseases and disorders that may ensued when humans are subjected to oxidative stress. All this is possible because all the plants contain flavonoids, saponins, polyphenols, alkaloids, tannins which are also present in the methanolic extracts of *C. olitorous* and *A. digitata* leaves plant and this is suggestive of why the plant extracts also induce transcription factors such as NrF2, AP-1, Nf-Kb and others, which then translate to the nucleus to induce the expression of several Phase2 and detoxifying enzymes. This is indicative of reduction or decrease in mortality rate in the rat treated with the plant extracts after exposure to radiation.

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