

Methanolic Extracts of *Cochorous olitorous* (L.) and *Adansonia digitata* (L.) Leaves Against Irradiation-Induced Atherosclerosis in Male Wistar Rats

Kabir O. BELLO^{1*}, Sarah O. NWOZO², Johnson OLADELE³,
Kabir K. MUSTAPHA¹, Lukman A. QUADRI¹

¹University of Ilorin, Faculty of Life Sciences, Department of Biochemistry, Nigeria; Karbanion001@yahoo.com; Mustapha.kk@unilorin.ng (*corresponding author)

²University of Ibadan, Faculty of Basic Medical Sciences, Department of Biochemistry, Nigeria; sonwozo@yahoo.com

³Osun State University, College of Pure and Applied Sciences, Department of Biochemistry, Nigeria; oladelejohn2007@gmail.com

Abstract

The present study investigated the anti-atherosclerotic and antioxidative effect of the methanolic extracts (MExt) of *Cochorous olitorous* (CO) and *Adansonia digitata* (AD) leaves on irradiation-induced atherosclerosis in male Wistar rats. Atherosclerosis was induced in male rats by a single dose of 6 gray whole body gamma radiation. MExt of *C. olitorous* and *A. digitata* leaves at 500 and 1,000 mg/kg bwt were administered as treatment for 7 days. Blood serum was analysed for lipid profile, MDA (malondialdehyde) and liver tissue for antioxidants enzymes, whereas the therapeutic potential was compared to the lipids-lowering drug lovastatin at 10 mg/kg/bwt. The phytochemical studies showed the presence of polyphenols, flavonoids, tannins and saponins. Treatment with MExt of CO and AD normalized the elevated MDA level, whereas the activities of superoxide dismutase, catalase and glutathione peroxidase in the treated rats increased. Pronounced changes were observed at 1,000 mg/kg bwt mixture of MExt of CO and AD for 1 weeks and it was more potent than the standard drug. The current study provided strong evidence that MExt of CO and AD might be important in the treatment of atherosclerosis and ROS without any side effects at the studied dosage and duration.

Keywords: antioxidative, atherosclerosis, radiation, lipid peroxidation, lipid profile

Introduction

Virtually, all human are exposed to radiation in one way or the other, either through continuous romance to the several sophisticated technological materials such as phones, laptops, telecommunication gadget, infra-red and bluetooth ear and headphones and the very ironical radiotherapy machines, hence the need for protection against exposure and aftermath effect of radiation (Arora *et al.*, 2000). Most of the ubiquitous technological materials emitted approximately 4-5 gray of gamma radiation, the emitted dose of radiation possesses the potentials of generating and thus increasing the physiological level of free radicals in human system from about 24 ug/mol to about 120 ug/mol (Brioukhanov *et al.*, 2006).

Radiation simply refers to a high energy loop with the ability to producing free radicals upon human exposure, through electron leakages (Rikans and Hornbrock, 1997).

Free radicals mediate the generation of several other unstable-reactive intermediate such as the ROS (reactive oxygen species) and RNS (reactive nitrogen species) family, with the potential of causing tissues and macromolecular damage and damages, the genesis of oxidative stress and oxidative stress-induced disease(s) (Bouffler *et al.*, 2012). Free radicals are constantly formed in the body during normal cellular metabolism: such as during oxidative phosphorylation in the electron transport chains, hypoxanthine and xanthine oxidase catalyzed reaction of nucleic acid metabolism, during the electron cycle of cytochrome P450-NADPH reductase (Belinda *et al.*, 2002). They are unstable electrophilic species with the potential to induced oxidative stress. They attack biological nucleophiles

(membrane proteins, lipids and nucleic acid), damage tissues (liver, kidney and heart) and overwhelmed the system endogenous antioxidant enzymes system to induced oxidative stress, thus, the genesis of various diseases and disorders such as cancer, diabetes, anemia and stroke (Board *et al.*, 2000).

Free radicals attack molecular oxygen, producing a variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive species react with the biomolecules such as lipids, resulting into chain of lipid peroxidation reaction, which have been implicated in pathological aging (Anderson *et al.*, 2001). The formation of lipid peroxy radical (LOO.) and lipid hydroperoxide (LOOH) resulted in destructive processes, which alter the integrity, fluidity, permeability and functions of biomembranes. Thus impairing the activity of membrane-bound enzymes, modifying low density lipoprotein (LDL) to proatherogenic and proinflammatory forms and generates potential toxic products, the major genesis of atherosclerosis disorders (Box *et al.*, 1997).

Atherosclerosis is hallmark by an elevated level of MDA and triglycerides (plaque), this implies that the level of oxidation of low density lipoprotein and chylomicrons by radicals have been exacerbated, thus reducing the activities of high density lipoprotein (Box *et al.*, 1997; Baker *et al.*, 2009). The implication of the changes in lipid metabolism thus far is that more lipid plaques and triglycerides can be transported into the heart and other blood pumping vessels by HDL (Box *et al.*, 1997; Baker *et al.*, 2009). The lipids plaque block blood movement within the vessels, thereby increasing blood pumping pressure from 45.6 mmHg to 85.7 mmHg, which signifies the genesis of hypertension, a major reason why atherosclerosis is also known as a central metabolic disorders (Box *et al.*, 1997; Baker *et al.*, 2009).

Several diseases has been claimed to have been successfully treated with plant materials by traditional medicine practitioners by many authors (Baker *et al.*, 2009). The extract of *Pterocarpus antalinoides* and *Aloe vera* was reported to increase the muscle toning potential. This indicated that such plants may be useful in the management of hypertension (Dapa and Gill, 2002). The reversal of dyslipidemia by use of medicinal plants has been reported: *Terminalia catappa* could be an effective antidyslipidemic agent, inhibiting lipids peroxidation (Cadmak and Horst, 1991).

According to a reviewed work by (Elekwa *et al.*, 2003) *Corchorus olitorius* (Linn) is a leafy vegetable that belongs to the family Tiliaceae. 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. Nutritionally, *C. olitorius* contain 85-87 g H₂O, 0.7 g oil, 5 g carbohydrate, 1.5 g fiber, 250- 266 mg Ca, 4.8 mg Fe, 1.5 mg 300010 vitamin A, 0.1 mg thiamine, 0.3 mg riboflavin, 1.5 mg nicotinamide, and 53-100 mg ascorbic acid per 100 g (Cadenas *et al.*, 1996). The leaf extract of the plant is also employed in folklore medicine in the treatment of gonorrhoea, pain, fever and tumor (Anderson *et al.*, 2001). Leaves also act as blood purifier and the leaf twigs is used against heart troubles while cold leaf infusion is taken to restore appetite and strength (Byrd-Bredbenner *et al.*, 2007).

Adansonia digitata commonly used as traditional plant which are consumed in food or used in the direct treatment of several diseases such as cancer, anemia, diabetes, lipid peroxidation disorders, ischemia reperfusion diseases and inflammatory bowel syndrome in South-western Nigeria (Burkill, 1985). *Adansonia digitata* is a tree found widely throughout Africa due to its ability to sustain life, as well as its many traditional medicinal and nutritional uses (Burkill, 1985; Okpuzor *et al.*, 2008). The baobab trees are an important food, water and shelter source in many African countries (Branda, 2004; Branda *et al.*, 2004). Baobab leaves have been investigated in an attempt to identify the potential bioactive associated with this part of the plant. Only few authors have investigated the vitamin A content of baobab leaves (Borek *et al.*, 1986; 1987; 2001) and found that the simple practice of drying baobab leaves in the shade protects against deterioration of pro vitamin, other authors mention the carotenoid content of baobab leaves.

The aim of this study was to investigate and evaluate the effect of the methanolic leaves extract of *A. digitata* and *C. olitorius* on gamma irradiation-induced atherosclerosis on male Wistar rats.

Materials and Methods

Plants materials

The leaves of *Corchorus olitorius* and *Adansonia digitata* were purchased from a commercial market, Ibadan, Oyo State, Nigeria. The plants were identified and authenticated at University of Ilorin with the voucher number UICO/001/951 and UIAD/002/154 respectively. Five hundred g each of the plants have been blended using manual blender in the laboratory and packed in air-tight container to prevent deterioration.

Plant extraction

The pulverized plant was macerated in 2,000 ml (2 L) of methanol (solvent) contained in a glass cylinder. After 72 hours the solution was filtered with Whatman no. 4 filter paper, the resulting filtrate was then concentrated using rotatory evaporator. The resulting percentage yield of *C. olitorius* was 4.6% and *A. digitata* was 14.8% where then reconstituted into aqueous solution and were administered to the irradiated rat at 500 and 1000 mg/kg body weight respectively.

Animals

Fifty-four adult male albino rats weighing 190-200 g were purchased from the animal house of the Department of Biochemistry, University of Ilorin. The use of experimental animal for this research was consider and approved by the University of Ilorin ethical committee.

Radiation exposure

The animals were exposed to a single dose of 6 gray whole body gamma radiations. The radiation exposure was carried out in the Department of Radiotherapy, College of Medicine, UCH, Ibadan. The animals were kept in an improvised cage to restrict their movement and to ensure

uniform and effective exposure. The animal distributions into groups were as it follows (Table 1):

Table 1. Animal distributions into groups

Control	Non-irradiated, non-treated
IR	Irradiated, non-treated
IR+ E ₁ 500 mg/kg	Irradiated animals treated with 500 mg/kg body weight (RE _{1 500})
IR+ E ₁ 1000 mg/kg	Irradiated animals treated with 1000 mg/kg body weight (RE _{1 1000})
IR+ E ₂ 500 mg/kg	Irradiated animals treated with 500 mg/kg body weight (RE _{2 500})
IR+ E ₂ 1000 mg/kg	Irradiated animals treated with 1000 ml/kg body weight (RE _{2 1000})
IR+E ₁ +E ₂ 500 mg/kg	Irradiated animals treated with 500 ml/kg body weight (R E _{1+ E2 500})
IR+ E ₁ +E ₂ 1000 mg/kg	Irradiated animals treated with 1000 ml/kg body weight (R E _{1+ E2 1000})
IR-lovastatin 10 mg/kg	Irradiated animals treated with reference drug

Where E₁ is *Adansonia digitata* and E₂ is *Cochorous olitorius*

Preparation of tissue homogenates, serum and histology

The procedure described by Yakubu and Akanji (2011) was adopted for the preparation of serum and tissue homogenates. Briefly, the animals were sacrificed under ether anesthesia and 5 mL of the blood were collected and allowed to clot at room temperature for forty-five minutes and then centrifuged at $4,500 \times g$ for 10 min. The serum was then kept frozen for 12 h before being used for the biochemical analyses. The animals were thereafter quickly dissected and the liver removed, cleaned, weighed and stored in ice-cold 0.1 M phosphate buffer solution of pH 7.4. The organ was then homogenized in ice-cold phosphate buffer solution (1:4w/v). The homogenate was centrifuged at $10,000 \times g$ for 15 min and the resulting supernatant stored frozen for 24 h.

Antioxidant assay

Glutathione-S-transferase (GST) assays procedure

The principle is based on the fact that all known glutathione-S-transferase demonstrate a relatively high activity with 1-chloro-2, 4 - dinitrobenzene as the second substrate, consequently, the conventional assay for glutathione-S-transferase activity utilizes 1-chloro-2, 4, - dinitrobenzene as substrate. When this substance is conjugated with reduced glutathione, its absorption maximum shifts to a longer wavelength. The absorption increases at the new wavelength of 340 nm and provides a direct measurement of the enzymatic reaction (Armstrong, 1997).

Glutathione peroxidase (GPx)

In order to determine the glutathione peroxidase 500 ul phosphate buffer was added to 100 ul NaN₃, after which 200 ul GSH was added to the grand mixture, then 100 ul H₂O₂ solution was further added. Added to the entire mixture was 500 ul of the sample, and 600 ul distilled water. The whole mixture will be incubated at 37 °C for 3minutes after which 0.5 ml of TCA was added and thereafter

centrifuge at 3000 rpm for 5minutes. To 1 ml of each of the supernatant, 2 ml of K₂HPO₄ and 1 ml DNTB was added and the absorbance was read at 412 nm against the blank.

Super oxide dismutase (SOD)

The level of SOD was determined by the method of Misra and Fridovich (1972). One ml of sample was diluted in 9 ml of distilled water to make 1: 10 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The absorbance at 480 nm was monitored every 30 seconds for 150 seconds. One unit of SOD activity was provided as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline:

% SOD inhabitation = increase in absorbance for substrate/increase in absorbance of blank \times 100.

Lipid profile assay

Malondialdehyde (MDA)

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) that maybe produced during lipid peroxidation as described by Rice-Evans *et al.* (1986). This method is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde: an end product of lipid peroxidation. On heating in acidic medium, the product is pink-coloured compound known as chromophore which absorbs maximally at 532 nm and which is extractable into organic solvents such as butanol. The results are expressed as the amount of free MDA produced.

Triglycerides

Triglycerides were measured by colorimetric method as described by Friedman and Young (1997). Triglycerides, normally are hydrolysed in the presence of lipase into glycerol and fatty acids, and the product, glycerol, is acted upon by glycerol kinase to produce glycerol-3-phosphate and adenosine diphosphate. The activated glycerol (glycerol-3-phosphate) is further oxidized by glycerol phosphate oxidase to form dihydroxy acetone phosphate and hydrogen peroxide. The hydrogen peroxide formed reacts with 4-aminophenazone and 4-chlorophenol in the presence of peroxidase to give quinineimine, an indicator of triglyceride that can be measured spectrophotometrically at 500 nm.

Trygliceride concentration = absorbance (sample)/absorbance (standard) \times 200 mg/dl.

HDL-cholesterol (High density lipoprotein-cholesterol)

HDL-cholesterol was determined in serum by the method of Jacobs *et al.* (1990). The Randox HDL-cholesterol precipitant Kit was used. Low density lipoproteins (LDL and VLDL) and chylomicron fractions precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions

after centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, was determined.

LDL-cholesterol

LDL-cholesterol was determined by the method of Friedewald *et al.* (1972).

LDL-Cholesterol = total cholesterol – trygliceride – HDL – cholesterol/2 (mg/dl).

Total cholesterol

The level of cholesterol was determined using Randox kits, a method of enzymatic hydrolysis described by Roeschlau *et al.* (1974). The cholesterol was determined after enzymatic hydrolysis and oxidation. Cholesterol ester was hydrolysed to free cholesterol and fatty acids by cholesterol esterase. Free cholesterol was then oxidized into cholesterol-3-one and hydrogen peroxide in a reaction

catalyzed by cholesterol oxidase. The peroxidase catalyzes the reaction between H₂O₂ and 4-amino phenazone and hydroxyl benzoate to produce quinoneimine whose colour intensity is proportional to the total cholesterol present in the sample. The indicator quinoneimine was formed hydrogen-peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Then the amount was mixed and incubated for 10 minutes at 20-25 °C or 5 minutes at 37 °C. Measurements using a sample and A standard against reagent blank within 60 minutes at 500 nm was performed.

Total cholesterol concentration = absorbance (sample)/absorbance (standard) × 200 mg/dl.

Statistical analysis of data

Data obtained was expressed as mean ± standard deviation and analysed using the Analysis of Variance ANOVA, F-ratio and student's *t* test where applicable. Values at P=.05 and P<0.05 were regarded as significant in comparison with appropriate controls.

Table 2. Quantitative phytochemical screening (mean ± standard deviation)

Metabolites	<i>Adansonia digitata</i>	<i>Corchorus olerifolius</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

Table 3. The effects of methanolic extracts of *A. digitata* and *C. olerifolius* on the antioxidants and malonaldehyde status of irradiated male wistar rats

Treatment Group	GST	GPX	CAT	SOD	MDA
Normal	2.95 ± 0.302 ^a	2.67 ± 0.06 ^a	57.88 ± 0.01 ^a	4.59 ± 0.02 ^a	0.230 ± 0.03 ^a
IR	1.20 ± 0.01 ^b	1.08 ± 0.02 ^b	21.79 ± 0.29 ^b	1.89 ± 0.03 ^b	0.845 ± 0.01 ^b
IR(E ₁ 500)	1.89 ± 0.01 ^c	1.63 ± 0.04 ^c	29.82 ± 0.24 ^c	2.56 ± 0.02 ^c	0.789 ± 0.04 ^c
IR(E ₁ 1000)	2.45 ± 0.03 ^d	1.78 ± 0.01 ^d	36.08 ± 0.01 ^d	2.90 ± 0.01 ^d	0.572 ± 0.01 ^d
IR(E ₂ 500)	1.93 ± 0.01 ^c	1.65 ± 0.02 ^c	31.00 ± 0.20 ^c	2.60 ± 0.01 ^c	0.746 ± 0.03 ^c
IR (E ₂ 1000)	2.49 ± 0.01 ^d	1.80 ± 0.01 ^d	38.14 ± 0.07 ^d	2.98 ± 0.01 ^d	0.540 ± 0.05 ^d
IR(E ₁ +E ₂ 500)	2.73 ± 0.01 ^c	2.26 ± 0.01 ^c	44.23 ± 0.04 ^c	3.92 ± 0.01 ^c	0.324 ± 0.06 ^a
IR(E ₁ +E ₂ 1000)	3.04 ± 0.02 ^a	2.73 ± 0.00 ^a	58.77 ± 0.97 ^a	4.71 ± 0.01 ^a	0.221 ± 0.05 ^a

Values are expressed as means ± SEM; n=9. The small letters at the superscript of each values denote level of significance as P<0.05. IR-Irradiated; E₂-*C. olerifolius*; E₁-*A. digitata*; GST-glutathione-S-transferase, GPX-glutathione peroxidase, CAT-catalase, SOD-super oxide dismutase, MDA-Malonaldehyde

Table 4. The effects of methanolic extracts of *A. digitata* and *C. olerifolius* on the lipid profile level of irradiated male wistar rats

Treatment Group	HDL-Chol	LDL-Chol	Total-Chol	Triglycerides
NORMAL	288.74 ± 0.26a	158.39 ± 0.50a	164.89 ± 0.59a	102.08 ± 0.39a
IR	208.75 ± 0.57b	191.09 ± 0.53b	105.63 ± 0.41b	177.81 ± 0.42b
IR(E ₁ 500)	237.00 ± 1.59c	181.36 ± 3.07c	125.96 ± 2.97c	158.57 ± 1.42c
IR(E ₁ 1000)	254.18 ± 0.34d	165.98 ± 0.62d	138.54 ± 0.27d	127.50 ± 0.29d
IR(E ₂ 500)	239.67 ± 1.46c	179.77 ± 1.60c	129.22 ± 0.41c	156.64 ± 0.45c
IR (E ₂ 1000)	258.40 ± 2.07d	164.47 ± 1.80d	140.59 ± 0.71d	123.31 ± 0.68
IR(E ₁ +E ₂ 500)	278.33 ± 0.88e	152.17 ± 2.60a	158.00 ± 1.16e	113.67 ± 0.33
IR(E ₁ +E ₂ 1000)	297.20 ± 1.41a	165.26 ± 3.90a	172.44 ± 3.24a	101.00 ± 0.58
IRVIT.C	235.67 ± 0.67c	178.33 ± 0.33c	129.22 ± 0.41c	139.45 ± 0.55

Values are expressed as means ± SEM; n=9. The small letters at the superscript of each values denote level of significance as P<0.05. IR-Irradiated; E₂-*C. olerifolius*; E₁-*A. digitata*; HDL-Chol-High density lipoprotein cholesterol; LDL-Chol-Low density lipoprotein cholesterol, Total-Chol-total cholesterol.

Results

The results of the phytochemical screening as shown in Table 2 revealed the presence of saponins, polyphenols, flavonoids, alkaloids and tannins. It is notable that the large amount of polyphenols (170.90-*Adansonia digitata* and 330.07-*Corchorus olitorous*), tannins (311.98-*Adansonia digitata* and 289.07-*Corchorus olitorous*) and alkaloids (81.56-*Adansonia digitata* and 68.65-*Corchorus olitorous*) maybe largely responsible for the pharmacological activities of the extract of the two plants.

The result of the antioxidant enzyme status as shown in Table 3, revealed that the combination of the extract from the two plants at 1,000 mg/kg body weight shows better pharmacological activities and induces better the antioxidant enzymes when compared with the negative and positive control group owing to the value of the GST ($3.04 \pm 0.02a$), GPX ($2.73 \pm 0.00a$), SOD ($4.71 \pm 0.01a$) and CAT ($58.77 \pm 0.97a$) obtained from this group. This might partially suggest why the effect of radiation was less pronounced in this group, thus the level of MDA was significantly ($p < 0.05$) reduce among members of this group giving the combination.

Similar trend was also obtained for the lipid profile assay, as the level of LDL-chol, TOT-chol, TRIG was significantly ($p < 0.05$) ameliorated in the group giving combination extract at 1,000 mg/kg body weight (Table 4).

Discussion

The determined secondary metabolites in the present study have been noted by several researchers and review for their pharmacological activities. The pharmacological activities are responsible for their therapeutic potentials.

Lipid peroxidation is a free radical chain reaction and reactive oxygen species can accelerate lipid oxidation (Box and Maccubbin, 1997; Aloha and Johnson, 2013). Cell membranes are phospholipids bilayers with extrinsic proteins and are direct target by free radicals to cause lipid oxidation (Cadmak and Horst, 1991). Malondealdehyde is a product of lipid peroxidation and can react with free amino group of proteins, phospholipids and nucleic acids leading to structural modification. The increase peroxidation product (MDA) is a hallmark of diseases and dysfunction such as atherosclerosis, diabetes, inflammation and liver diseases.

Low density lipoproteins cholesterol (LDL-cholesterol) is complicated lipids structure and an oxidative modification of this LDL-cholesterol has been reported with the development of atherosclerosis and other cardiovascular diseases (Dapa and Gill, 2002). Oxidized cholesterol or fatty acids moieties in the plasmatic LDL can develop (Amstad, 1991; Rikans et al., 2003). LDL facilitate the transport of fats, triglycerides (fat plague) and MDA to the heart, leading to blockage of blood vessel, thereby increasing the heart pressure pump, which is suggesting hypertension. The atherosclerosis is termed as a central metabolic disorder, because of its ability to increase the risk of several other disorders (Achu et al., 2001; Behl et al., 2002; Rikans et al., 2003). However, high density lipoprotein cholesterol (HDL-cholesterol) facilitate the

transport of excess fat, MDA, triglycerides and fat plague out of the heart, in a bid to reduce or even prevent the risk of atherosclerosis and other central metabolic diseases (Behl et al., 2002) the more reason why HDL-cholesterol is called a good cholesterol.

Jubili formula, an herbal preparation made from three medicinal herbs (*Parquetina nigrescens*, *Sorghum bicolor* and *Harungana madagascariensis*) has been successfully used in the treatment of atherosclerosis in humans and also in the lavastatin-induced dyslipidemia in rabbits (Oladiji et al., 2007). Based on the study by Oladiji et al. (2007) it is most likely that the malondealdehyde restoration and antioxidant inducing potentials of Jubili formula may in part be due to the presence of bioactive agents like alkaloids, phenols, tannins, flavonoids and saponins, which are also found in the methanolic extract of *C. olitorous* and *A. digitata* leaves in the present study. This may be suggestive of the MDA, LDL-chol and triglycerides restoration potentials in the irradiated male wistar rat as seen in table 2 and 3 above.

The presence of polyphenols, flavonoids and cucumin in the extract of *Vernonia amygdalina* may be responsible for the induction of superoxide dismutase, catalase, glutathione peroxidase and transferase (Farombi and Yhong, 2001).

In the present study, the presence of a high amount of polyphenol and flavonoids in the methanolic extracts of *C. olitorous* and *A. digitata* maybe suggestive of the extract ability to induce glutathione-s-transferase, glutathion peroxidases, superoxide dismutase and catalase (Table 3). Glutathione-s-transferase, peroxidases, dismutase enzymes have been implicated to scavenge free radicals generated by 6 gray radiations in male wistar rats. All this is possible because the plants contain flavonoids, saponins, polyphenols, alkaloids, tannins which were also presented in the methanolic extracts of the leaves of *C. olitorous* and *A. digitata*. These findings corroborated which findings established by Adaramoye et al. (2001), using methanolic extract of *Occimum grattisum* leaves in crack oil induced-free radical in male Wistar rat.

Conclusions

The combination of the methanolic leaves extract of *A. digitata* and *C. olitorous* can be use as therapy against free radical induced oxidative stress and cellular alteration. This is because the combination of the extract at 1,000 mg/kg dose exact and poses better pharmacological effect than the orthodox medicine and as well better than the individual plant.

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