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Original Research Article

Hepatoprotective Effect of N-Hexane Extract of *Alchornea laxiflora* against Methylcholanthrene-Induced Cancer in Wistar Rat

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Abstract

Alchornea laxiflora is a shrub belonging to the family of Euphobiaceae and is widely distributed in tropical Africa. The present study evaluated the *in vitro* antioxidant activities, *in vivo* antioxidant activities, anti-toxicity potentials of *Alchornea laxiflora* root extract, possible hepatoprotective effect against methylcholanthrene-induced cancer and the hepatoprotection compared to cyclophosphamide using adult *Wistar* rats. N-hexane root extract of *Alchornea laxiflora* demonstrated appreciable *in vitro* antioxidant capacity and radical scavenging ability compared with reference standards. Oral administration of the extract at varying doses (50, 100, and 200mg/kg body weight) was shown to significantly decrease ($p<0.05$) the effect of liver damage on methylcholanthrene-induced (50mg/kg) cancer when compared to the group administered cyclophosphamide (20mg/kg body weight) by reducing the activities of aspartate aminotransferase, alanine aminotransferase and alkaline Phosphatase (AST, ALT and ALP) in the serum. In the 3-Methylcholanthrene administered groups (50 mg/kg), there was significant increase ($p<0.05$) in Malondialdehyde (MDA) level as well as a decrease in reduced glutathione (GSH) content when compared to the positive control. Animals administered with *Alchornea laxiflora* (50, 100, 200mg/kg) showed a significant increase in GSH content and significant decrease in malondialdehyde (MDA) level relative to the animals administered cyclophosphamide. The result from the histological assay showed that there was no observable lesion in groups administered *Alchornea laxiflora* when compared to the negative control which showed plates of hepatocellular atrophy. These findings suggest that *Alchornea laxiflora* root has potent antioxidant activity which may be responsible for some of its reported anticancer activities.

Keywords: hepatoprotection, Cyclophosphamide, Methylcholanthrene, *Alchornea laxiflora,* Malondialdehyde, Antioxidant.

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INTRODUCTION

Background of study

Indigenous herbal medicines are the first-line treatment for most third-world countries (Mahomoodally, 2013). According to the World Health Organization (WHO), about 80% of the world population employs herbal medicine for their primary health care using plant extracts (Nabatanzi *et al.,* 2020). Various factors encourage herbal medicines, such as acceptability, poverty, cost-effectiveness, accessibility, and unavailability of modern health facilities (Hossain *et al.,* 2014). However, there are concerns about the toxic effects of certain botanical drugs if used unchecked and irrationally (Okaiyeto and Oguntibeju, 2021).

Globally, 28,187 plant species have been recorded as constituting medicinal use in 416 families of angiosperm plants. Euphorbiaceae is among the top three families with a significantly higher proportion of medicinal plants (Phumthum *et al.,* 2019). The Euphorbiaceae, sometimes known as the spurge family, includes monoecious or dioecious trees, shrubs, and plants. The common names of *Alchornea laxiflora* are “Low yield bead string/Venda bead string/three veined bead string,” derived from the shape of its open inflorescences. It has several vernacular names depending on the cultural and ethnic diversity in Africa (Magwede *et al.,* 2019). In the Ekiti state of Nigeria, it is also known as Canestiks and Arithmetic stick (Olanipekun and Aladetimiro 2017). *A. laxiflora* has profound effects on Africa's natural and cultural features in addition to its medical applications. *A. laxiflora* has a wide range of folk medicine uses, including emmenagogue, dental hygiene promotion, toothache

relief, and sickle cell disease management, in addition to being antidiabetic, anti-inflammatory, antioxidant, anti-infectious, antianemic, and antifungal. Similarly, the whole plant has been reported for the treatment of malaria, pile, dysentery, eczema, cough, and high fever in the Ekiti state of Nigeria (Jayeoba *et al.,* 2012; Adeniran, 2015).

Polycyclic aromatic hydrocarbons (PAHs) are one of the most persistent organic contaminants and are widely distributed in our environment, including in the air, soils, water bodies, as well as the various tissues of animals or plants (Wang *et al.,* 2018). They are very alarming pollutants because of their association with carcinogenicity, teratogenicity and genotoxicity (Kim *et al.,* 2013). 3-Methylcholanthrene (3-MC), a class of potential carcinogens, is a member of a large family of PAHs, which was determined based on different approaches, such as generating cholesterol by pyrolysis of phytosterols during different cooking processes, or by pyrolysis of organic material from other combustion processes (Rhon Calderon *et al.,* 2020).

The liver is the main organ for metabolism and elimination of drugs (Singh *et al.,* 2012). Liver is the body's biggest reticulo-endothelial cell network, as well as an important function in the host's ability to fight against infection. Exposure to various xenobiotics and environmental contaminants is a major contributor to many illnesses (Guillouzo *et al.,* 2007). Excessive exposure to toxins, alcohol, chemotherapy, viruses, and protozoan infections are the main causes of liver impairment (Fisher *et al.,* 2015). Liver damage can also be caused by drugs, particularly anti-tubercular drugs, general anesthetics, paracetamol and some anti-cancer drugs. Toxic hepatitis is the most severe adverse reaction to antituberculosis drugs, it usually initiates in the first few weeks of treatment along with liver necrosis, which may evolve to encephalopathy and death (Ringehan *et al.,* 2017).

Cyclophosphamide is a type of nitrogen mustard drug which exerts its effects through the alkylation of DNA. The drug is not cell-cycle phase-specific and metabolizes to an active form capable of inhibiting protein synthesis through DNA and RNA crosslinking (Mills *et al.,* 2019). The majority of the antineoplastic effects of cyclophosphamide are due to the phosphoramide mustard formed from the metabolism of the drug by liver enzymes like cytochrome P-450. Hepatic enzymes first convert cyclophosphamide to hydroxycyclophosphamide and then subsequently metabolized to aldophosphamide. Aldophosphamide is cleaved to the active alkylating agent phosphoramide mustard and acrolein (Emadi *et al.,* 2009).

MATERIALS AND METHODS

Materials

Experimental animals

An ethical approval from UAREC University of Medical Sciences, Ondo was received for this work. In this study, a total of 30 male Wistar rats were used. The University of Medical Sciences' Animal Unit in Ondo State provided the rat. The weight of the rats was between 90-120g on procurement. The animals were housed at the Animal Unit in the University of Medical Sciences, Ondo State, where they were kept under standard and favourable laboratory conditions. The rats were allowed to acclimatize for one (1) week before the commencement of the experiment. They were given rat feeds and water ad libitum.

Chemicals and solvents used

N-hexane and 3-methylcholanthrene and were gotten from Pascal Scientific Limited, Akure, Nigeria. All other chemicals/reagents used in this research work were of analytical grade.

Collection of roots

The roots were uprooted in a farm in Adeyemi College of Education, Ondo State, Nigeria. The roots were authenticated by the Department of Plant Biotechnology, University of Medical Sciences, Ondo. Nigeria.

Methods

Extraction of oil from *Alchornea laxiflora* roots

The extraction technique used in this work was modelled after that described by Sankeshwari *et al.,* (2018). *Alchornea laxiflora* roots were uprooted, cut into little pieces, and allowed to air dry for two weeks at room temperature. The air-dried roots were milled into a powder and air dried again. The ground sample (50 g) was weighed and put in the thimble. The thimble was placed in the extraction chamber of the soxhlet extractor. The solvent (n-hexane) was measured to 500ml and poured into separate round bottom flasks. The Soxhlet extractor, round bottom flask, and condenser were then attached to the apparatus with the aid of clamps and a stand and set on a heating mantle. After heating the solvent, extraction was done while in reflux. After the extraction process was completed, the extract (oil) was collected in a round-bottom flask and put in a rotary evaporator to evaporate the solvent and leave solidified oil. The extraction was done in a lab at the University of Medical Sciences, Ondo, Ondo state, Department of Biochemistry.

Experimental design

In this study, the rats procured were divided into 4 groups, which are;

Group A: Rats were fed with rat pellet and water only (Positive control)

Group B: Rats were administered toxicant only (3-methylchorlarnthrene) (50mg/kg). The toxicant was administered intraperitoneally (Negative control).

Group C: Rats were pre-treated with n-hexane extract of *Alchornea laxiflora* root for 7days (C1-50mg/kg, C2-100mg/kg, C3-200mg/kg) and the toxicant 3-methylcholanthrene (50 mg/kg) was administered intraperitoneally on the 8th day and then sacrificed after 24 hours.
Group D: Rats were pre-treated with cyclophosphamide for 2 days (10mg/kg) and the toxicant 3-methylcholanthrene (50 mg/kg) was administered intraperitoneally on the 3rd day and then sacrificed after 24 hours.

Preparation and induction of 3-methylcholanthrene

The toxicant used in this study was 3-methylcholanthrene in powdered form. 3-methylcholanthrene was mixed with paraffin oil. The animals were induced intraperitoneally with a single dose per body weight each for 24 hours before sacrifice.

Euthanization of animals and collection of samples

The rats were weighed and sacrificed by cervical dislocation. To reveal the internal organs, the abdominal cavity was opened up using a midline abdominal incision. A portion of the liver was preserved in 10% formalin for histological investigation after the liver had been removed and blotted in 10% KCl. The remaining liver tissue was then put into simple tubes with 10 ml of regular saline and tris buffer and kept in the freezer until analysis.

Preparation of homogenates

2.0g of the liver tissue stored in normal saline was homogenized in 10ml of tris buffer. The homogenized samples were then centrifuged at 10000g for about 10minutes after which the supernatant was collected and used for subsequent analysis of antioxidant enzymes.

***In vitro* antioxidant assay**

Estimation of diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

The free radical scavenging capacity of the root extracts against 1,1–diphenyl–2–picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.,* (1995). 0.5 ml of 0.3mM DPPH solution in methanol was added to 2ml of various concentrations (0.01 – 0.2 mg/ml) of the extracts. The reaction tubes were shaken and incubated for 15 min at room temperature in the dark; absorbance read at 517 nm. All tests were performed in triplicates. Ascorbic acid was used as standard control, with similar concentrations as the test samples prepared. A blank containing 0.5ml of 0.3mM DPPH and 2ml methanol was prepared and treated as the test samples. The radical scavenging activity was calculated using the following formula:
DPPH radical scavenging activity (%) = $[(A_0-A_1)/(A_0)] \times 100$

Where A_0 is the absorbance of DPPH radical + methanol; A_1 is the absorbance of DPPH radical + sample extract or standard. The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

Ferric reducing antioxidant power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain (1996). To 1.5 ml of freshly prepared FRAP solution was added to 1ml of the extracts (1mg/ml). The reaction mixtures were incubated at 37^0C for 30 min and the absorbance at 593nm was measured. $FeSO_4$ was used for the calibration curve and Ascorbic acid served as the positive control. FRAP values (expressed as mg Fe(II)/g of the extract) for the extracts were then extrapolated from the standard curve.

Hydroxyl radical scavenging activity

In 1.5 mL of each diluted extract, 60 µL of $FeCl_3$ (1 mM), 90 µL of 1,10- Phenanthroline (1 mM), 2.4 ml of 0.2 M phosphate buffer, pH 7.8 and 150 µL of H_2O_2 (0.17 M) were added respectively. The mixture was then homogenized and incubated at room temperature for 5 min. The absorbance was read at 560 nm against the blank. The percentage of the radical scavenging activity of each extract was calculated from the equation below:
Percentage of radical scavenging activity =

$$[(\text{OD control} - \text{OD sample})/\text{OD control}] \times 100.$$

The extract concentration providing 50% inhibition (IC_{50}) was calculated and obtained by interpolation from linear regression analysis.

Biochemical enzyme analysis

The serum collected was used to assay for the liver enzyme activity.

Estimation of Alanine aminotransferase activity

The Alanine transaminase activity was carried out as described in the kit instruction leaflet (Product code: BXC0212).

Procedure

To 100µL of sample, 500µL of R1 buffer was added. This buffer contains phosphate buffer (100 mmol/L, pH 7.4), L-alanine (200 mmol/L), and alpha-oxoglutarate (2.0 mmol/L). At 37^0C, the mixture was incubated for 30 minutes. The reaction mixture was mixed with 0.5 ml of R2 Dye Reagent containing 48 2,4-Dinitrophenyl Hydrazine (2.0 mmol/L) and let to stand for 20 minutes at 20-250C. After adding 5 ml of sodium hydroxide solution containing 4.0 mol/L, the absorbance was measured at 546 nm after 5 minutes in comparison to the sample blank.

Estimation of Aspartate aminotransferase activity

The Aspartate transaminase activity was carried out as described in the kit instruction leaflet (Product code: BXC0202).

Procedure

To 100µL of sample, 500µL of R1 AST buffer was added. This buffer contains phosphate buffer (100 mmol/L, pH 7.4), laspartate (200 mmol/L), and alpha-oxoglutarate (2.0 mmol/L). 30 minutes were spent heating the mixture to 37^{0}C. The reaction mixture was mixed with 0.5 ml of R2 Dye Reagent containing 2,4-dinitrophenyhydrazine (2.0 mmol/L) and let to stand for 20 minutes at 20-25^{0}C. After adding 5 ml of sodium hydroxide (4.0 mol/L), the absorbance was measured at 546 nm after 5 minutes against the reagent blank.

Estimation of alkaline phosphate activity

The Alkaline phosphate activity was carried out as described in the kit instruction leaflet.

***In vivo* antioxidant enzyme assay**

Estimation of reduced glutathione (GSH) level

The level of reduced glutathione (GSH) was calculated using the method developed by Beutler *et al.,* 1963.

Procedure

0.4 ml of sample was added to 0.4 ml of precipitating solution which was vortexed and centrifuged at 4000 rpm for 5 minutes. Thereafter, 0.5 ml of the supernatant was added to 1.5 ml of Ellman's reagent. The absorbance of the reaction mixture was read at 412 nm against a reagent blank.

Assessment of lipid peroxidation

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Varshney and Kale (1990).

Procedure

An aliquot of 0.4 ml of the test sample was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml of 30% TCA was added. Then 0.5 ml of 0.75% TBAS was added and placed in a water bath for 45 minutes at 80oC. This was then cooled in ice to room temperature and centrifuged at 3000 rpm for 10 minutes. The clear supernatant was collected, and absorbance measured against a reference blank of distilled water at 532 nm.

The MDA level was calculated using an extinction coefficient of 0.156 µM-1cm-1 (Adam-Vizi and Seregi, 1982).

Lipid peroxidation (nmole MDA/mg protein = Absorbance × volume of mixture E532nm × volume of sample × mg protein/ml

Histopathology

Immediately after dissection, the sections of the liver were placed in a tissue cassette and fixed in 10% buffered formalin for 24 h after which they were processed using standard histopathological methods. The processed tissues were then embedded in paraffin Sections of 5 µm thickness were cut on a rotary microtone and stained with haematoxylin and eosin for microscopic assessment (Avwioro, 2010)

Statistical analysis

Data analysis was performed using GraphPad Prism Software version 8.0.1 (GraphPad Software Inc). All data are reported as means ± standard deviation (SD). Groups were analysed using one way analysis of variance (ANOVA) and significant differences in mean values were evaluated using Tukey's multiple range test and p value < 0.05 was regarded as significance.

RESULTS

***In vitro* antioxidant assay**

Ferric reducing antioxidant power (FRAP)

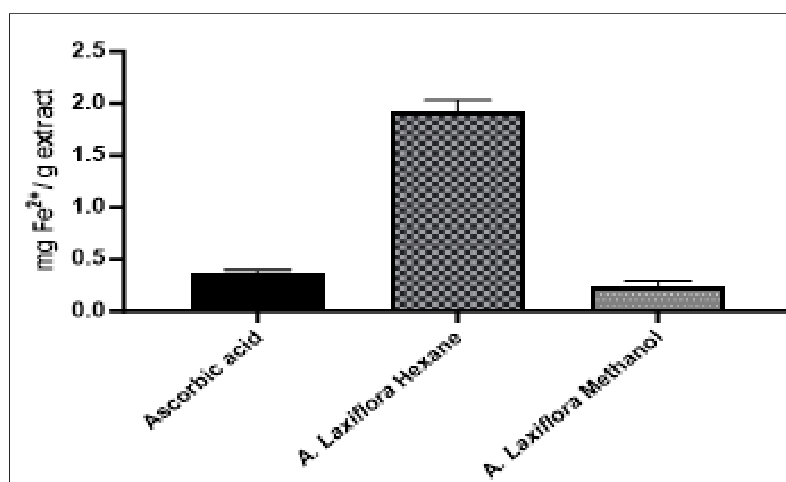

Figure 1: The hexane extract of *Alchornea laxiflora* root was significantly higher ($p < 0.05$) than the methanol extract of *Alchornea laxiflora* root extract was significantly lower ($p < 0.05$), the methanol extract had no significant difference with that of Ascorbic acid standard. Values are expressed as mean ± SD (n = 3).

3.1.2 DPPH radical scavenging ability

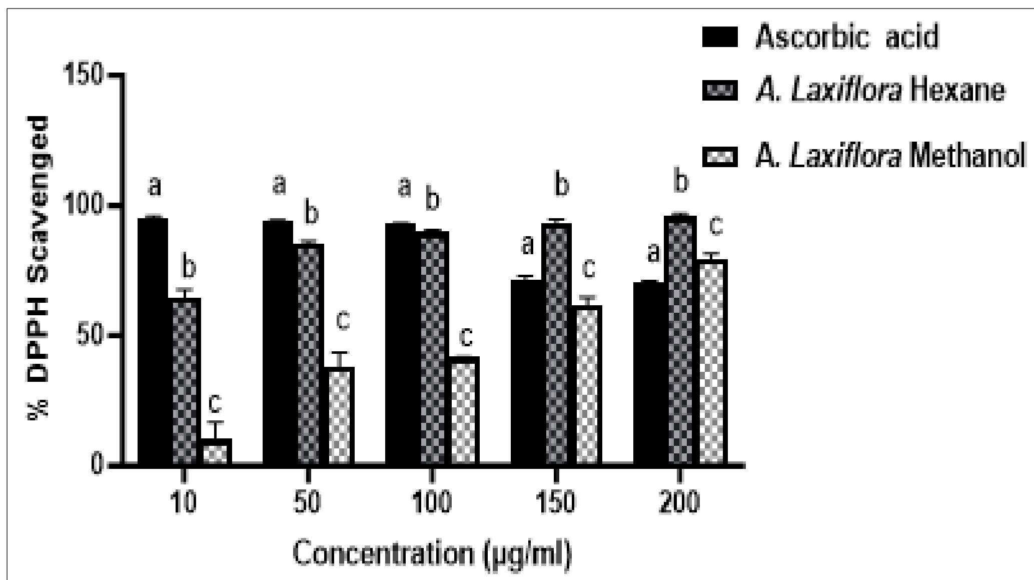

Figure 2: DPPH radical scavenging activity of hexane extract and methanol of *Alchornea laxiflora* root. Different alphabets on grouped bars show that values for Ascorbic acid, hexane extract and methanol extract are significantly different from each other at p< 0.05. Values are expressed as mean ± SD (n = 3)

3.1.3 Hydroxyl radical

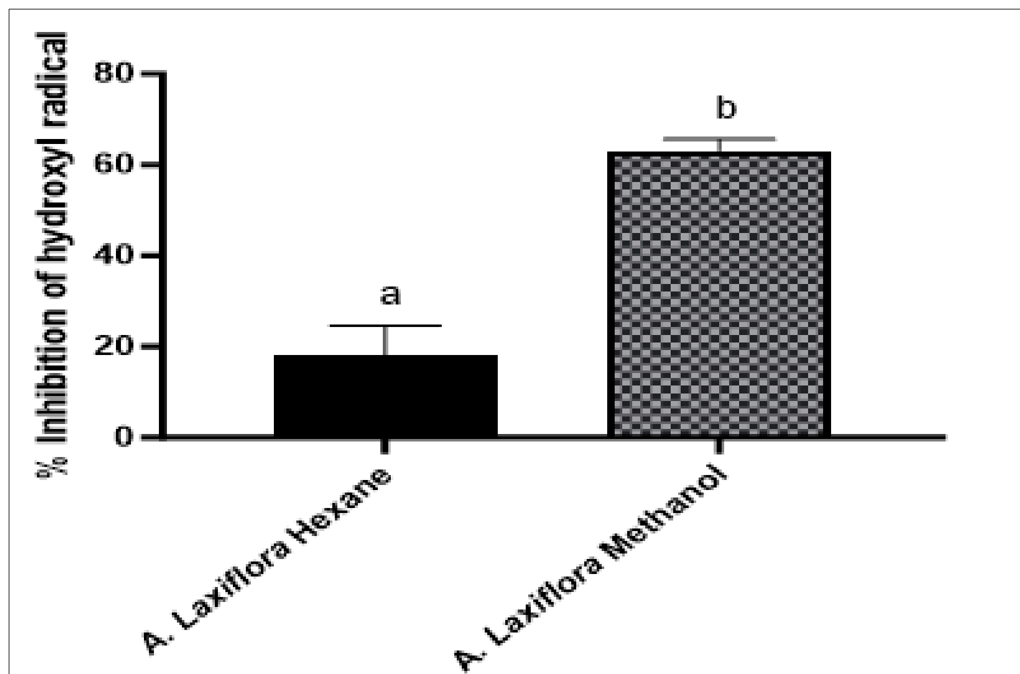

Figure 3: Hydroxyl radical scavenging action of hexane and methanol root extract of *Alchornea laxiflora*. Values are expressed as mean ± SD (n = 3)

Biochemical enzyme assay
Aspartate aminotransferase

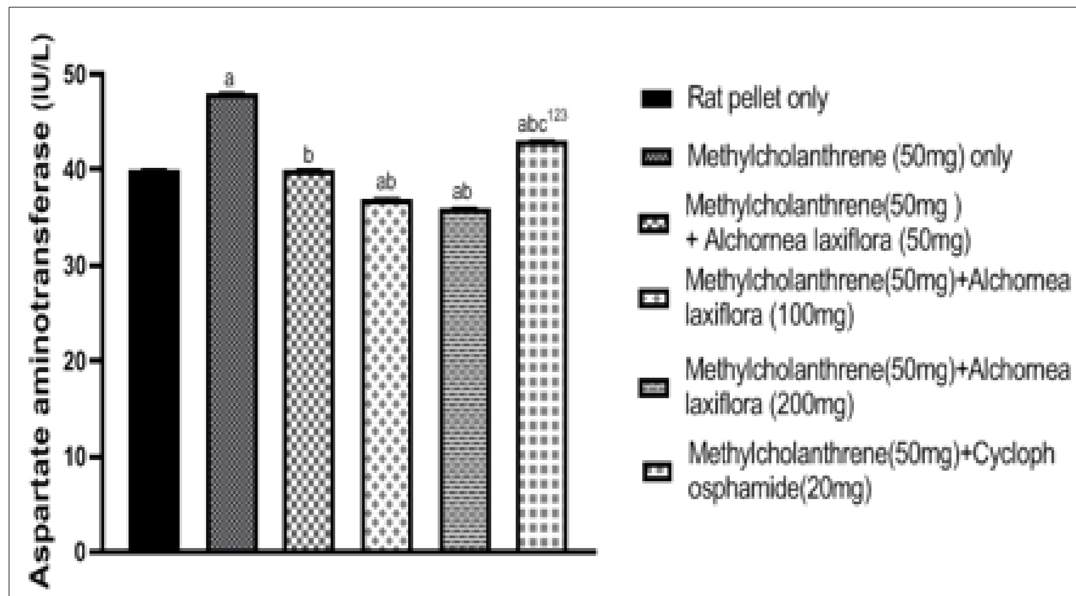

Figure 4: Effect of feeding *Alchornea laxiflora* and cyclophosphamide on serum Aspartate aminotransferase (AST) activity. There was a significant increase ($p<0.05$) in the serum AST activity in untreated group (b) compared to control group (a). A significant decrease ($p<0.05$) was also observed in the serum AST activity in the pre-treated groups (c) relative to the cyclophosphamide group (d)

3.2.2 Alanine aminotransferase

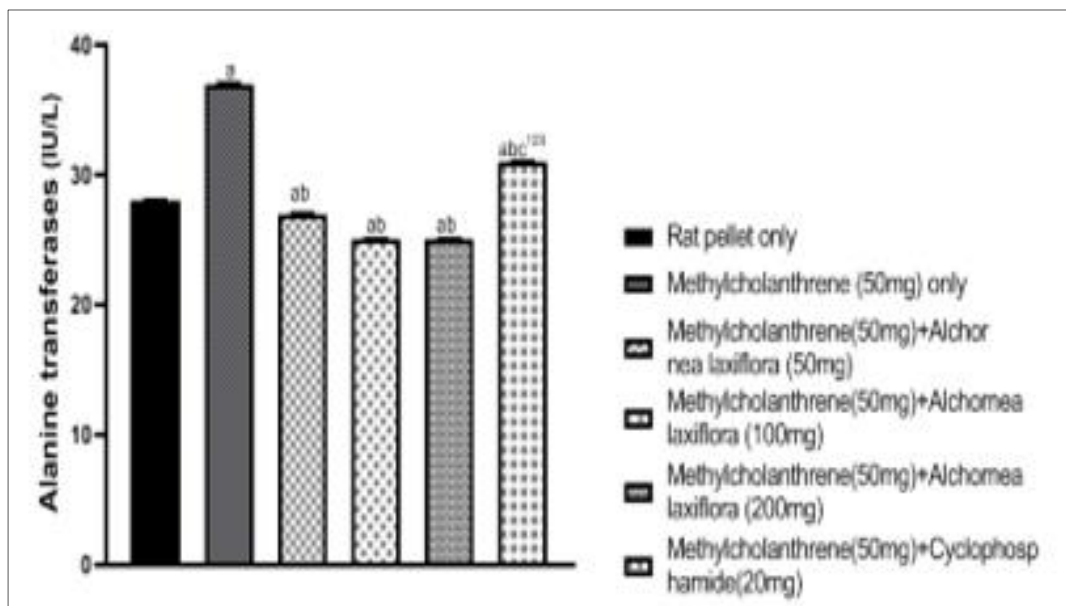

Figure 5: Effect of feeding *Alchornea laxiflora* and cyclophosphamide on serum Alanine aminotransferase (ALT) activity. There was a significant increase ($p<0.05$) in the serum ALT activity in untreated group (b) compared to control group (a). A significant decrease ($p<0.05$) was also observed in the serum ALT activity in the pre-treated groups (c) compared to the cyclophosphamide group (d)

3.2.3 Alkaline phosphatase

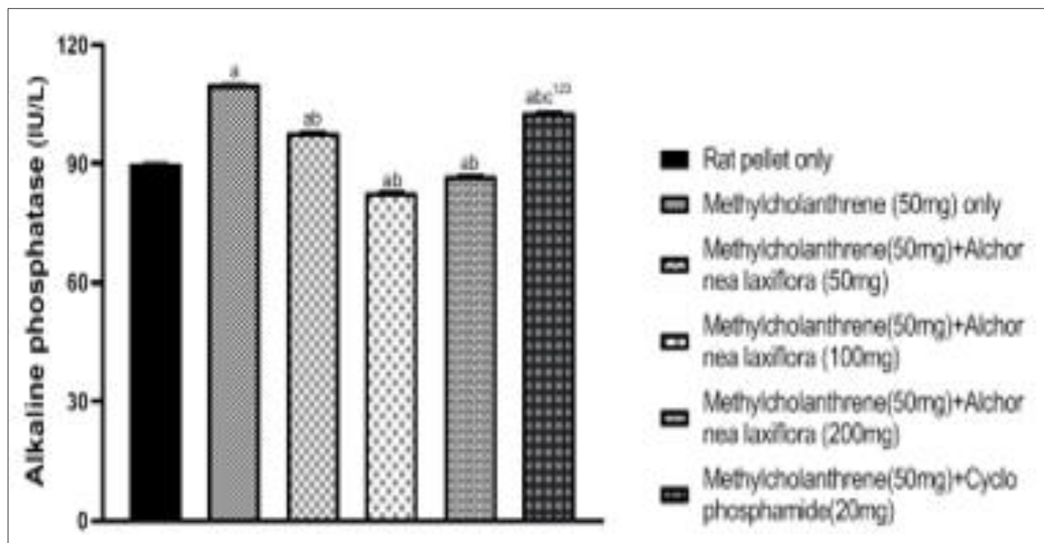

Figure 6: Effect of feeding *Alchornea laxiflora* and cyclophosphamide on serum Alkaline phosphatase (ALP) activity. There was a significant increase (p<0.05) in the serum ALP activity in untreated group (b) compared to control group (a). A significant decrease (p<0.05) was also observed in the serum ALP activity in the pre-treated groups (c) compared to the cyclophosphamide group (d)

3.3 *In vivo* Antioxidant enzyme

3.3.1 Glutathione

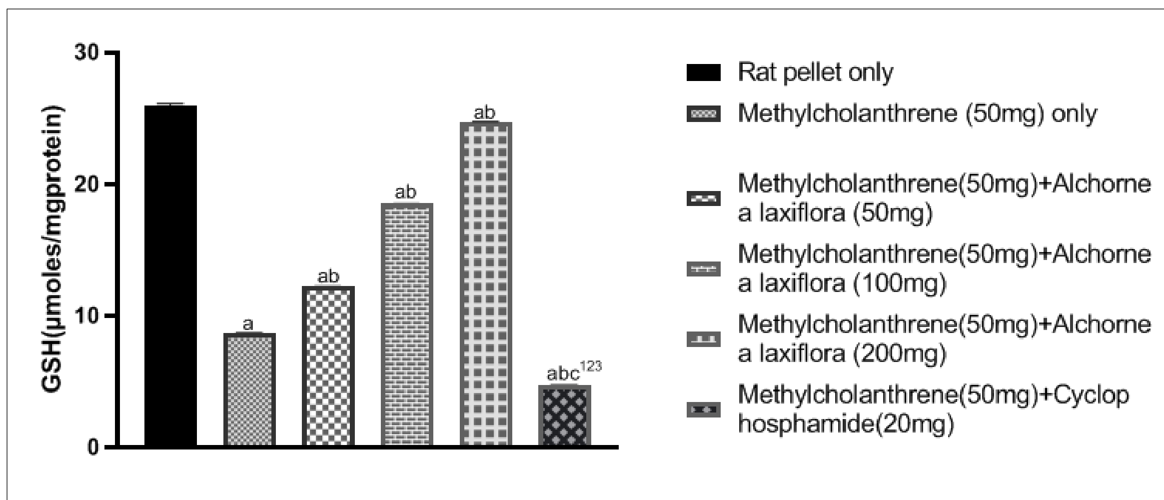

Figure 7: Effect of feeding *Alchornea laxiflora* and cyclophosphamide on reduced glutathione (GSH) content. There was a significant decrease (p<0.05) in the GSH level in untreated group (b) compared to control group (a). A significant increase (p<0.05) was also observed in the GSH level in the pre-treated groups (c) compared to the cyclophosphamide group (d).

3.3.2 Malondialdehyde

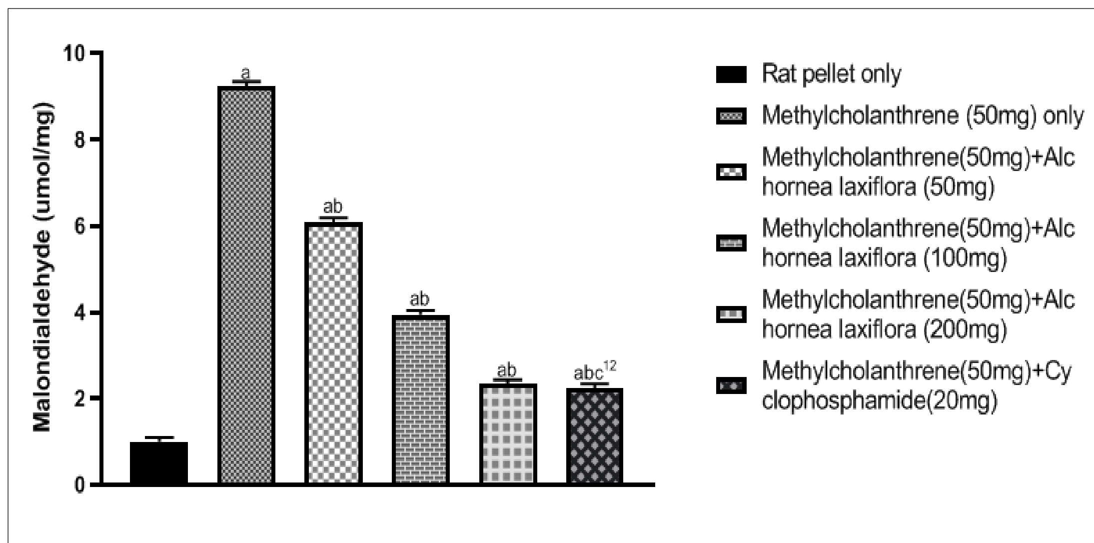

Figure 8: Effect of feeding *Alchornea laxiflora* and cyclophosphamide on malondialdehyde level. There was a significant increase ($p<0.05$) in the MDA level in untreated group (b) compared to control group (a). A significant decrease ($p<0.05$) was also observed in the MDA level in the pre-treated groups (c) compared to the cyclophosphamide group (d)

3.4 Histopathology

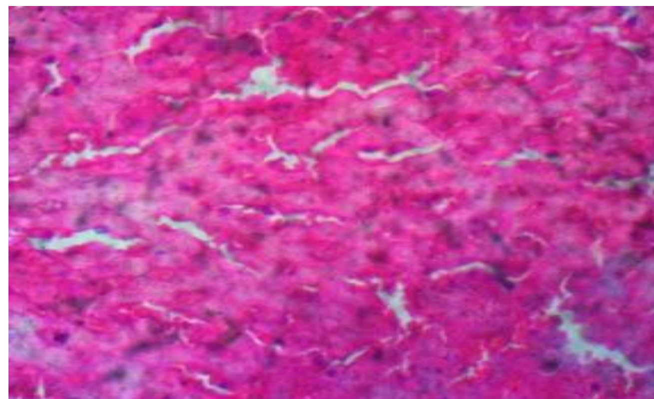

Figure 9: Photomicrograph of a liver section of control group (fed water and rat pellet only) stained by Haematoxylin and Eosin. The photomicrograph shows there is no observable lesion

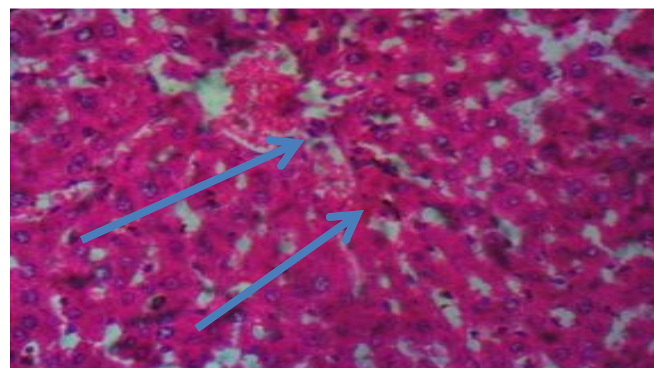

Figure 10: The photomicrograph of a liver section of group administered methylcholanthrene only (negative control) to induce hepatic damage stained by Haematoxylin and Eosin. The photomicrograph shows plates of hepatocellular atrophy and Kupffer cell hyperplasia (blue arrows).

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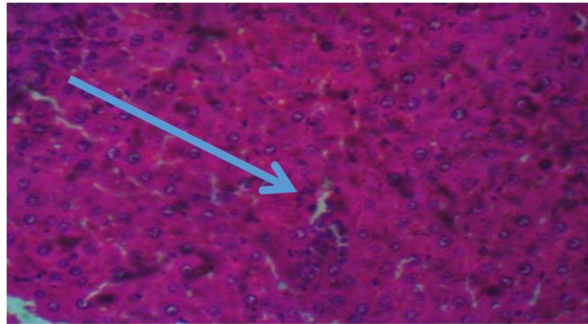

Figure 11: The photomicrograph of a liver section of group fed with 50mg n-hexane extract of *Alchornea laxiflora* and methylcholanthrene stained by Hematoxylin and Eosin. The photomicrograph shows that there is foci of vasculitis and portal inflammation (blue arrow)

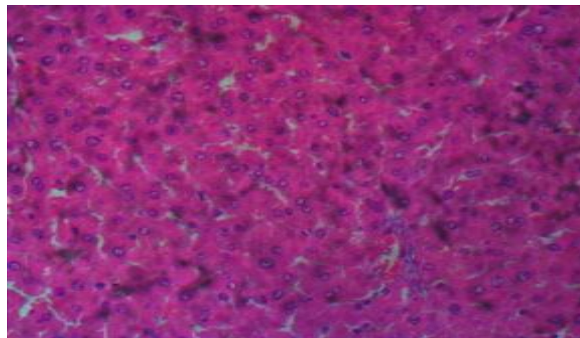

Figure 12: The photomicrograph of a liver section of group fed with 100mg n-hexane extract of *Alchornea laxiflora* and methylcholanthrene stained by Hematoxylin and Eosin. The photomicrograph shows that there is no observable lesion

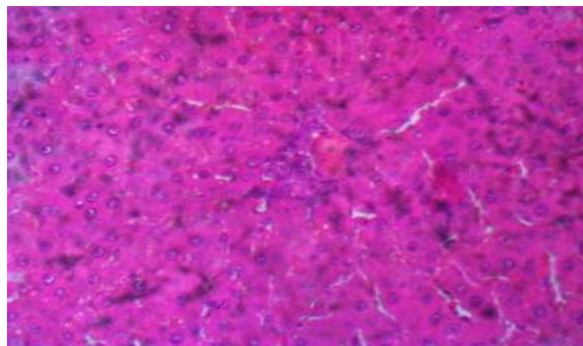

Figure 13: The photomicrograph of a liver section of group fed with 200mg n-hexane extract of *Alchornea laxiflora* and methylcholanthrene stained by Hematoxylin and Eosin. The photomicrograph shows that there is no observable lesion.

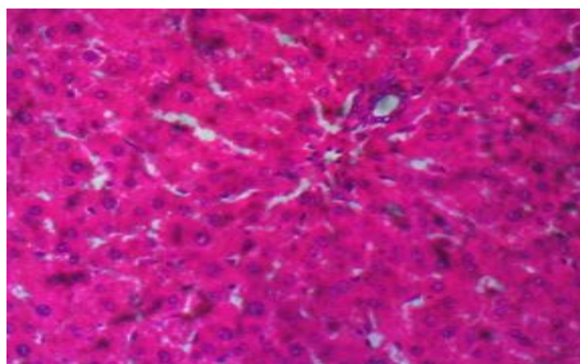

Figure 14: The photomicrograph of a liver section of group fed with 20mg of cyclophosphamide and methylcholanthrene stained by Hematoxylin and Eosin. The photomicrograph shows that there is no observable lesion

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DISCUSSION

This study evaluated the hepatoprotective effect of *Alchornea laxilora* compared to cyclophosphamide on 3-methylcholanthrene-induced cancer in male wistar rat. The liver is an essential organ in the biotransformation and detoxification of drugs (Correia, 2018). Cyclophosphamide is administered either as intermittent pulses (intravenous) or a continuous oral low-dose regimen, depending on the indication for it use. Two well recognized dose-related adverse effects of cyclophosphamide are bone marrow suppression and hepatic injury (Turnbull and Harper, 2009; Subramaniam, *et al.,* 2013). The majority of the world's population gets its life-saving medications from medicinal plants, which are its primary source. The use of plants and plant extracts is the foundation of the ancient medical practice known as herbalism (Acharaya *et al*., 2008). The primary components of medications in conventional medical systems are plants. For both their safety and effectiveness, these medications have undergone extensive standardization (Fabricant and Fainsworth, 2009). Any plant that has been traditionally used for therapeutic purposes must be safe, especially in terms of mutagenicity, nephrotoxicity, carcinogenicity, and hepatotoxicity (Ashafa *et al*., 2009). *Alchornea laxiflora* is reported to treat hepatitis and other liver-related disorders as toxic hepatitis is often associated with the oxidative destruction of lipids and proteins.

The study of phytochemicals, free radicals and antioxidants is important in medicine due to their implications in different disease conditions. Antioxidants are substances that inhibit oxidation and are capable of counteracting the damaging effects of oxidation in body tissues (Adwas *et al.,* 2019). They create a barrier from free radical damage that results in delaying process of oxidation. The literatures show that FRAPS method is sensitive in the measurement of total antioxidant power of the fresh biological fluids, such as plant homogenates and pharmacological plant products (Gohari *et al.,* 2011). The FRAP assay utilizes antioxidants to reduce Ferric (Fe^{3+}) ion to ferrous (Fe^{2+}) ion. In this study, the n-hexane extract of *Alchornea laxiflora* had a significantly higher ($p<0.05$) FRAP value than methanol extract of *Alchornea laxiflora* and ascorbic acid standard, while ascorbic acid standard is significantly higher ($p<0.05$) than that of methanol extract (Figure 1). This showed that *Alchornea laxiflora* has a strong antioxidant capacity due to its ability to prevent the reduction of Fe^{3+} to Fe^{2+} which generate ROS that are sources of various disease like cancer.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical. The principle of DPPH antioxidant assay lies on the ability of a potential antioxidant to scavenge the stable radical of DPPH (Kedare and Singh, 2011). In the presence of antioxidants, DPPH can accept an electron or a hydrogen atom from the antioxidant scavenger molecule, to be converted to a more stable DPPH molecule. It is usually used to estimate the *in-vitro* antioxidant activity of natural compounds or plant extracts (Philips *et al*., 2010). The n-hexane extract of *Alchornea laxiflora* was a better scavenger of DPPH radical than methanol extract of *Alchornea laxiflora* and it also compete favourably with the ascorbic acid standard showing appreciable activity in this regard (Figure 2).

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule formed in living cells (Ogunboye *et al.,* 2022). Hydroxyl radicals are also known to initiate peroxidation of lipid membranes (Onur and Ayhanci, 2021). The radical has the capacity to form adducts with nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity (Thirunavukkarasu *et al.,* 2011). The present study reveals that n-hexane extract of *Alchornea laxiflora* is a potent scavenger of deleterious free radicals, such as OH, at very low concentrations as it showed significant inhibition of hydroxyl radicals (Figure 3). The results obtained from *in vitro* antioxidant research showed that n-hexane extract of *Alchornea laxiflora* root contained higher amount of phytochemicals, consequently, it was a more powerful antioxidant than methanol extract of *Alchornea laxiflora* root.

According to Omage *et al*., (2011), diagnostic enzymes are those that are utilized to diagnose or distinguish between certain or specific disorders. Enzymes are typically found in little amounts in the blood, but when an organ is damaged, the enzymes that are already there (inside the cells) seep out into the blood. Increased serum enzyme activities signify damages to the liver tissues (Kalas *et al.,* 2021). Aspartate aminotransferase (AST) is present in all tissues except bone, with highest levels in liver and skeletal muscle (Adeyemi *et al.,* 2015). In this study, serum activity of Aspartate aminotransferase (AST) was significantly elevated ($p<0.05$) in the rats administered with 3-methylcholanthrene only which was suggestive of liver injury. Rats administered n-hexane extract of *Alchornea laxiflora* however, showed significant decrease ($p<0.05$) in the serum AST activity when compared with the group administered cyclohosphamide (Figure 4). Serum activity of Alanine aminotransferase (ALT) was significantly elevated ($p<0.05$) in the rats administered with 3-methylcholanthrene only which was suggestive of hepatic injury. Rats pre-treated with feed containing n-hexane extract of *Alchornea laxiflora* however, showed significant decrease ($p<0.05$) in the serum ALT activity when compared with the group administered cyclohosphamide (Figure 5). Serum activity of Alkaline phosphatase (ALP) was significantly elevated ($p<0.05$) in the rats administered with 3-methylcholanthrene only which was suggestive of liver injury. Rats pre-treated with feed containing n-hexane extract of *Alchornea laxiflora* however, showed significant decrease ($p<0.05$)

in the serum AST activity when compared with the group administered cyclohosphamide (Figure 6). The reducing effects of the plant extract on the activities of the enzymes indicate a protective function of the plant. This may suggest that intake of this plant extract offer a better protection against liver damage than treatment with cyclophosphamide which is a standard drug.

Antioxidants have been shown to function in vivo by decreasing the $CytP_{450}$ bioactivation of chemicals and medicines to reactive metabolites (Uadia *et al*., 2012). This inhibits the production of reactive oxygen species. Additionally, antioxidants work by up-regulating the expression of the genes encoding for superoxide dismutase, catalase activity, glutathione peroxidase, and glutathione reductase in order to directly scavenge free radicals, a process known as mopping up (Aruoma, 1999; Ojeaburu, and Oriakhi, 2021). In this work, 3-methylcholanthrene induced cancer in rats result in a significant decrease in the antioxidant enzymes. Reduced glutathione (GSH), a naturally occurring chemical, is present in large amounts in many different types of living things. It is an intracellular reductant and is important for transport, metabolism, and catalysis. According to Ulusu and Tandoan (2007), the cellular antioxidant glutathione (GSH) is essential for preserving the redox state of cells. From the result obtained in this study, there was significant reduction in the GSH levels (Figure 7) in untreated group compared to the control group. It shows that the free radicals generated due to the 3-methylcholanthrene induced cancer were at higher concentrations compared to the enzyme antioxidant. The glutathione content in the group pretreated with extract of *Alchornea laxiflora* increased compared to the group administered cyclophosphamide shows the antioxidant effect of this *Alchornea laxiflora* root extract as it was capable of enhancing the activity of the enzyme in scavenging free radicals which cause hepatocellular damage.

Lipid peroxidation is a chain phenomenon resulting in the formation of various active compounds that result in cellular damage (Wadhwa *et al.,* 2012). Lipid peroxidation can be initiated by any chemical species that can extract a hydrogen atom from side chain of a polyunsaturated fatty acid (PUFA) which is generally present in the cell membranes (Prisacaru, 2016). Lipid peroxides, which are derived from polyunsaturated fatty acids, are unstable. They readily decompose to form a complex series of compounds, which include malondialdehyde (MDA). MDA is the major metabolite of arachidonic acid and serves as a reliable biomarker for oxidative stress (Singh *et al.,* 2014). According to this study, there was a significant increase ($p<0.05$) in the MDA level of rats administered 3-methylcholanthrene only relative to the control group which was suggestive of increased lipid peroxidation causing cellular damage. However, rats pre-treated with n-hexane extract of *Alchornea laxiflora* root showed a significant decrease ($p<0.05$) in the MDA level when compared to the group administered cyclophosphamide which suggests the antioxidant potential of *Alchornea laxiflora* root (Figure 8).

The observed ameliorative effects of *Alchornea laxiflora* from biochemical evaluations were further corroborated by the histopathology of the liver sections of the experimental animals. Photomicrograph of a liver section of control group (fed water and rat pellet only) stained by Haematoxylin and Eosin showed normal morphology and architecture with no observable lesion (Figure 9) whereas the photomicrograph of a liver section of group administered methylcholanthrene only (negative control) showed plates of hepatocellular atrophy and Kupffer cell hyperplasia (blue arrows) (Figure 10) indicating severe damage caused by it. Pretreatment with different dosages of n-hexane extract of *Alchornea laxiflora* (50mg, 100mg, 200mg), cyclophosphamide (20mg) and methylcholanthrene (50mg) showed foci of vasculitis and portal inflammation (blue arrow) (Figure 11) and no observable lesions (Figures 12, 13 and 14). This fact validates the results obtained from biochemical analyses, and could imply that the extract was able to prevent the damages caused by methylcholanthrene on the organs at the doses tested except at 50mg.

CONCLUSION

Our research showed that the oral administration of hexane root extract of Alchornea laxiflora at varying doses (50, 100 and 200mg/kg body weight) caused reduction in the serum (AST, ALT, ALP), liver homogenate (GSH, MDA) enzyme activities and Histopathological studies of the experimental animals when compared to the group administered cyclophosphamide (20mg/kg body weight), intoxicated with 3-methylcholanthre. This suggests that the ability of the root extract to reverse the adverse effect of cancer on the liver is better than when administered cyclophosphamide.

Abbreviations

AST – Aspartate aminotransferase
ALT – Alanine aminotransferase
ALP – Alkaline phosphatase
MDA – Malondialdehyde
GSH – Reduced glutathione
WHO – World health organization
PAHs – Polycyclic aromatic hydrocarbons
3-MC – 3-Methylcholanthrene
UAREC – university animal regulatory committee
DPPH –1,1-Diphenyl-2-picrylhydraxyl
FRAP – Ferric reducing antipoxidant power
TBARS – Thiobarbituric acid reactive substances
ANOVA – Analysis of variance

Ethics Approval and Consent to Participate

An ethical approval from UAREC University of Medical Sciences, Ondo was received for this work.

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