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Hepatoprotective and antioxidant activities of *Pterocarpus santalinoides* methanol leaf extract

Thelma Ebele Ihedioha^{1*}, Isaac Uzoma Asuzu¹, Aruh Ottah Anaga¹ and John Ikechukwu Ihedioha²

¹Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

²Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

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This study evaluated the hepatoprotective and antioxidant activities (AA) of *Pterocarpus santalinoides* methanol leaf extract (PSMLE) on carbon tetrachloride (CCl₄)-induced hepatotoxicity in albino rats. Thirty male albino rats randomly assigned into 6 groups (A – F) of 5 rats each were used for the *in vivo* study. Hepatotoxicity was induced in groups A – E using CCl₄. Group A served as negative control. Groups B, C and D were treated with 50, 250 and 500 mg/kg PSMLE, respectively. Group E was treated with 100 mg/kg Silymarin, while Group F served as normal control. Treatment was given orally twice daily for 15 days, after which markers of hepatotoxicity and oxidative stress were evaluated. The *in vitro* AA of PSMLE was also evaluated using 1, 1-diphenyl 2-picryl hydrazyl. Results showed that treatment with PSMLE at 250 and 500 mg/kg led to significantly ($p < 0.05$) lower serum alanine aminotransferase and malondialdehyde, significantly ($p < 0.05$) higher superoxide dismutase and glutathione peroxidase levels, while 250 mg/kg dose further led to significantly ($p < 0.05$) lower serum aspartate aminotransferase and serum total bilirubin levels, and significantly ($p < 0.05$) higher serum total protein and serum globulin levels. 500 mg/kg dose treatment additionally led to significantly ($p < 0.05$) lower serum total cholesterol. Treatment at all doses led to significantly ($p < 0.05$) lower liver weight and relative liver weights and significantly ($p < 0.05$) higher catalase and total glutathione levels. The PSMLE exhibited significantly ($p < 0.05$) higher AA at concentrations ≥ 50 $\mu\text{g/ml}$ *in vitro*. It was concluded that PSMLE was hepatoprotective and possesses significant antioxidant activity *in vivo* and *in vitro*.

Key words: Hepatotoxicity, oxidative stress, antioxidants, *Pterocarpus santalinoides* leaf extract, carbon tetrachloride.

INTRODUCTION

The liver is a vital organ for metabolism, excretion, clearance and transformation of chemicals in the body (Singh et al., 2011). It is responsible for the detoxification of drugs and xenobiotics; thus, it is constantly and

*Corresponding author. E-mail: thelma.ihedioha@unn.edu.ng. Tel: +2348036868258.

variedly exposed to xenobiotics which may induce liver damage (Saukkonen et al., 2006). Most absorbed toxins and toxicants will first pass through liver, and the possible response elicited may range from inflammation to degeneration and/or neoplasia of the hepatocytes (Schiff and Schiff, 1987). Hepatotoxicity is a major health problem, and the manifestations vary from asymptomatic elevation of liver enzymes to fulminant liver failure (Saukkonen et al., 2006). Toxic liver damage is commonly oxidative stress mediated, and constitutes a large proportion of liver disorders/diseases; its occurrence has been steadily increasing over the years (Suk and Kim, 2012; Rehm et al., 2013; Nwokediuko et al., 2013).

Carbon tetrachloride (CCl_4) is a commonly used model chemical for the experimental induction of hepatotoxicity (Kim et al., 2010). It is metabolized to trichloromethyl (CCl_3) free radical which induces hepatotoxicity by causing peroxidative degradation in the adipose tissue, resulting in fatty infiltration of the hepatocytes (Boll et al., 2001). Following administration, CCl_4 is activated by cytochrome $\text{CYP}_{2\text{E}1}$ and $\text{CYP}_{2\text{B}1}$ to form CCl_3 radical which binds to cellular molecules such as nucleic acids, proteins and lipids, thereby impairing crucial cellular processes like lipid metabolism, with the potential outcome of fatty degeneration (Boll et al., 2001). The CCl_3 radical reacts with oxygen to form highly reactive species, the trichloromethylperoxy (CCl_3OO) radical, which initiates the chain reaction of lipid peroxidation culminating in destruction of polyunsaturated fatty acids (Boll et al., 2001). This causes alteration in permeability of the mitochondria, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular calcium, disruption of calcium homeostasis and damage/death of hepatocytes (Weber et al., 2003).

Oxidative stress is a state in which oxidation and oxidants exceed the antioxidant systems in the body leading to imbalance between the generation of reactive oxygen species (ROS) and the level of antioxidants in the biological system (Yoshikawa and Naito, 2002). It occurs when free radicals which are not neutralized by antioxidants go on to create more volatile free radicals and damage cell membranes, vessels, proteins, fats and DNA. Biological free radicals are highly unstable reactive molecules that have electrons available to react with various organ substrates such as DNA, proteins and lipids. Oxidative stress is known to be involved in the pathogenesis of a variety of diseases including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, liver diseases and malignancies (Yoshikawa and Naito, 2002), or may exacerbate their symptoms (Halliwell and Gutteridge, 1989; Valko et al., 2007).

Antioxidants are compounds that inhibit the oxidation of other compounds and prevent chemical damage caused by free radicals (Sies, 1997). Oxidation reactions in living organisms produce free radicals which can initiate chain reactions that may cause damage or death to cells.

Antioxidants terminate these chain reactions by removing free radical intermediates and inhibiting other oxidation reactions (Sies, 1997; Valko et al., 2007). Insufficient levels of anti-oxidants or inhibition of the antioxidant enzymes in living organisms cause oxidative stress which may lead to injury and/or death of cells (Davies, 1995; Valko et al., 2007). Many of the natural antioxidants such as tannins, flavonoids and glycosides are very important in the prevention of diseases associated with oxidative stress (Yi-Fang et al., 2002; Aruoma, 2003).

Some plants such as *Cussonia barteri* (leaves), *Lannea vilutina* (leaves), *Sacoglottis gabonensis* (stem bark), *Trichilia roka* (roots), *Tinospora cordifolia* (whole plant), *Piptadeniastrum africanum* (stem bark) and *Gongronema latifolium* (leaf) amongst others, have been reported to be rich sources of natural antioxidants that can protect against oxidative stress and thus play important role in the chemoprevention of diseases that have their etiology and pathophysiology in ROS (Ames et al., 1993; Atawodi, 2005; Karamalakova et al., 2018; Dlamini et al., 2019). There has been an increase in interest in the therapeutic potential of plants as antioxidants that may reduce free radical-induced tissue injury (Schuler, 1990; Karamalakova et al., 2018). A number of plants such as *Ipomoea batatas* (leaves), *Allium cepa* (leaves), *Cnestus ferruginea* (leaves stem and roots), *Splenacentrum jollyanum* (leaves and roots) and *Voacanga africana* (leaves) had been investigated in the search for novel antioxidants (Chu, 2000; Mantle et al., 2000; Koleva et al., 2002; Oke and Hamburger, 2002), while a lot more are still under investigation.

Pterocarpus santalinoides DC is an indigenous Nigerian plant in the family *Papilionaceae* (Keay, 1989). It is commonly known as "red sandal wood" in English language and "nturukpa" in Igbo language (Adetunji, 2007; Anowi et al., 2012). Leaves of *P. santalinoides* are used traditionally as vegetable for making soup, and also in folk medicine for the treatment of various ailments including heart and liver diseases (Adesina, 1982; Okwu and Ekeke, 2003). Leaves of *P. santalinoides* contain considerable amounts of tannins, flavonoids and glycosides which are known natural antioxidants (Anowi et al., 2012; Eze et al., 2012; Ihedioha et al., 2017; 2018). Previous studies by Ihedioha et al. (2017) suggested that the hepatoprotective properties of methanol leaf extract of *P. santalinoides* in acetaminophen-induced hepatotoxicity may be attributed to its antioxidant phytochemical composition. Also, reports from earlier studies on the lipid lowering effects of aqueous leaf infusion of *P. santalinoides* in guinea pigs showed that it may be related to its antioxidant properties (Ihedioha et al., 2018). Based on the various traditional medicinal uses of *P. santalinoides* especially in the treatment of diseases in which oxidative stress is known to play critical role and the results of these earlier cited studies, the purpose of the present study was to evaluate the

hepatoprotective and antioxidant activities of methanol leaf extract of *Pterocarpus santalinoides* on CCl₄-induced sub-acute hepatotoxicity *in vivo* in albino rats, and *in vitro* using the DPPH assay method.

MATERIALS AND METHODS

Plant collection, identification and extract preparation

Fresh leaves of *Pterocarpus santalinoides* (Figure 1) used for the study were collected from Nsukka Local Government Area of Enugu State in November 2017. The plant was identified and authenticated by a plant taxonomist (Mr. A.O. Ozioko) at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, a voucher specimen [UNH (University of Nigeria Herbarium) No. 02] was deposited at the University of Nigeria, Nsukka, herbarium. The leaves were dried under shade and pulverized. Five hundred grammes of the pulverized leaves were extracted with 80% methanol using the cold maceration extraction technique. The resulting extract was filtered with Whatman size 1 filter paper, concentrated to dryness with a Rotary Evaporator (Buchi, Switzerland), and referred to as *Pterocarpus santalinoides* methanol leaf extract (PSMLE).

Experimental animals

Thirty adult male albino rats (*Rattus norvegicus*) of 12 weeks of age, weighing between 200 and 250 g, were obtained from the Laboratory Animal House of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, and used for the *in vivo* hepatotoxicity study. Fifteen adult female rats of 12 weeks of age weighing between 172- 190 g were also obtained from the same source and used for the acute toxicity study. The albino rats were housed in stainless steel cages in a fly proof Animal House at room temperature between 23 -29°C, and allowed 2 weeks to acclimatize before the commencement of the study. They were fed commercial rat pellets (Grand Cereals Nig. Ltd, Jos, Nigeria), composed of 13% crude protein, 8% fat, 15% crude fibre, 0.9% calcium, 0.35% phosphorus and 2600 Kcal/kg metabolizable energy, and clean drinking water *ad libitum*. They were cared for and handled humanely all through the study. Stipulated guidelines governing the use of animals for laboratory experiments were strictly adhered to (Zimmermann, 1983; Ward and Elsea, 1997). The protocol for the laboratory animal study was approved by the Faculty of Veterinary Medicine Institutional Animal Care and Use Committee, University of Nigeria, Nsukka, (Approval No: FVM-UNN-IACUC/2018/0814).

Acute toxicity study

The acute toxicity and median lethal dose (LD₅₀) of PSMLE were determined in albino rats following the OECD Acute Toxic Class method (OECD, 2001). Fifteen adult female albino rats, randomly assigned into 5 groups of 3 rats each (Groups 1, 2, 3, 4 and 5) were used for testing at 0, 50, 300, 2000 and 5000 mg/kg, respectively. The albino rats were fasted for 12 hours before the test commenced, but water was made available to them *ad libitum*. The extracts were each dissolved in 1 ml of distilled water and administered orally with a gastric tube. The rats were observed for 14 days for any sign of toxicity or mortality. Their body weights were

measured at intervals, and at the end of the 14 days of observation, they were humanely sacrificed and the weights of their vital organs (liver, kidney, spleen and heart) were measured (OECD, 2001).

Phytochemical analysis of PSMLE

Phytochemical analysis was done to determine the phytochemical constituents of PSMLE, following standard procedures as described by Trease and Evans (1996), Harborne (1998). One gramme (1 g) of PSMLE was dissolved in 100 ml of distilled water in a beaker. The solution was filtered with Whatmann no. 1 filter paper to obtain a clear filtrate which was used to test for the presence of tannins, flavonoids, alkaloids, saponins, phenols, carbohydrates, glycosides, starch, polyuronides, sterols and terpenes. High level presence of each phytochemical was scored +++, moderate levels were scored ++, low levels were scored + (Trease and Evans, 1996; Harborne, 1998).

Evaluation of the effects of PSMLE on blood levels of markers of hepatotoxicity, oxidative stress and antioxidant marker levels in albino rats given sub-acute toxic doses of CCl₄

The thirty male albino rats used for the hepatotoxicity and oxidative stress study were randomly assigned to 6 groups A – F of five rats each. Hepatotoxicity and oxidative stress were induced in rats in groups A – E using the CCl₄ model (Robin et al., 2012; Singh et al., 2012). A mixture of 1 ml/kg CCl₄ in equal volume of olive oil (50% v/v) was injected intraperitoneally to groups A – E at 3 day intervals (days 0, 3, 6, 9 and 12) for 12 days. Group A was treated with 10 ml/kg distilled water as placebo and served as negative (untreated) control, Groups B, C and D were treated with 50, 250 and 500 mg/kg PSMLE, respectively. Group E was treated with 100 mg/kg Silymarin (a known hepatoprotective and antioxidant drug) as positive control, while Group F was also given 10 ml/kg distilled water as placebo and served as normal control (not given CCl₄). Treatment started 24 h post-initial CCl₄ administration and was done twice daily for 15 days. On day 15 post-initial CCl₄ administration, two milliliters of blood sample was collected from each rat and used immediately for evaluation of blood levels of enzyme markers of liver damage, oxidative stress and antioxidant markers, following standard procedures. Blood sample collection was done using the orbital technique (Bolliger and Everds, 2010). A portion of the blood (1.5 ml) was dispensed into a glass test tube and allowed to stand at room temperature for 45 min to clot; then centrifuged at 3000 revolutions per minute for ten minutes using a table centrifuge (Jenalab Medical, England), after which the serum was harvested and assayed immediately. The remaining 0.5 ml was dispensed into a heparinized sample bottle for use in the determination of glutathione peroxidase activity.

Evaluation of effects of PSMLE on blood levels of markers of hepatotoxicity

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were evaluated following the Reitman and Frankel method (Colville, 2002), using the Quimica Clinica Applicada (QCA) serum ALT and AST test kits. The ALT in the serum sample and standards catalyzed the reaction of L-alanine and alpha-ketoglutaric acid to form pyruvic acid and L-glutamic acid, while the AST catalyzed the reaction of L-aspartic acid with alpha- ketoglutaric acid to form oxaloacetic acid and L-glutamic acid. These ketonic acids reacted with 2,4, dinitrophenyl hydrazine to form corresponding colored hydrazones, the optical



Figure 1. Leaves of *Pterocarpus santalinoides*.

density of which was measured and ALT/AST quantified at 505 nm wavelengths, using the Diatek[®] semi-automated blood biochemistry analyzer (Diatek Instruments, Wuxi, China).

The serum alkaline phosphatase (ALP) activity was quantified using the QCA alkaline phosphatase test kit, based on the phenolphthalein monophosphate method (Colville, 2002). The alkaline phosphatase in the serum and a standard (containing 30 IU/L alkaline phosphatase) hydrolyzed a colorless substrate of phenolphthalein monophosphate and gave rise to phosphoric acid and phenolphthalein which at alkaline pH turned into pink color. The optical density was measured and the alkaline phosphatase activity was quantified at 546 nm wavelength, using the semi-automated biochemistry analyzer (Diatek Instruments, Wuxi, China).

Serum total protein levels were determined using the QCA total protein test kit which was based on the direct Biuret method

(Lubran, 1978; Johnson, 2008). A reaction of the proteins in the serum samples and a standard (containing 5 g/dl of proteins), with copper ions in the Biuret reagent in an alkaline medium, resulted in the formation of a stable colored complex. The optical density of the colored complex was measured at 546 nm wavelength and quantified using the Diatek[®] semi-automated blood biochemistry analyzer (Diatek Instruments, Wuxi, China).

Assay of serum albumin was done using the QCA albumin test kit based on the bromocresol green method (Doumas and Peters, 1997; Johnson, 2008). This involved the reaction of the albumin in the serum samples and standard (containing 5 g/dl of albumin) with bromocresol reagent at acid pH to form a colored complex. The optical density of the colored complex was measured at 630 nm wavelength and the serum albumin was quantified using the Diatek[®] semi-automated blood biochemistry analyzer (Diatek

Instruments, Wuxi, China). The globulin levels were calculated by subtracting the serum albumin levels from the total protein levels (Johnson, 2008).

The serum total cholesterol levels were determined using the QCA total cholesterol test kit, which is based on the enzymatic colorimetric method (Allain et al., 1974; Rifai et al., 2008). Total cholesterol in the serum samples and standard (containing 200 mg/dl of cholesterol) was enzymatically hydrolyzed by cholesterol esterase and further oxidized by cholesterol oxidase contained in the QCA total cholesterol working reagent. The reactions resulted to formation of a colored quinonic derivative. The optical density of the colored quinonic solution was measured at 505 nm wavelength and the total cholesterol was quantified using the Diatek[®] semi-automated blood biochemistry analyzer (Diatek Instruments, Wuxi, China).

The total bilirubin levels in the serum samples was assayed using the Randox[®] bilirubin test kit (Randox Laboratories Ltd, County Antrim, United Kingdom), which is based on the Jendrassik and Grof method (Doumas et al., 1973; Higgins et al., 2008). The serum samples were reacted with diazotized sulfanilic acid in the presence of caffeine to produce an azopigment. Their optical densities were measured at 578 nm and quantified a Chem5V3[®] Semi-automated Clinical Chemistry Analyzer, (Erba Diagnostics, Mannheim GmbH, Mannheim, Germany).

Evaluation of effects of PSMLE on oxidative stress and antioxidant markers

The serum malondialdehyde (MDA) levels of the rats were determined following the modified thiobarbituric acid method (Draper and Hadley, 1990). The serum was first mixed with trichloroacetic acid (TCA) and centrifuged to obtain the protein-free supernatant, to which was added 1% thiobarbituric acid and incubated for one hour at 95°C. The free MDA present in the supernatant reacted with thiobarbituric acid (TBA) to generate an MDA-TBA adduct, which was quantified colorimetrically at 532 nm wavelength using a SpectrumLab[®] spectrophotometer (HME Global Medical, England).

The serum catalase (CAT) activity was determined by the visible light method (Weydert and Cullen, 2010), using ElabScience catalase assay kit (ElabScience Biotechnology Co., Ltd., South Africa). In this determination, the decomposition of H₂O₂ by catalase in the plasma sample was quickly stopped by ammonium molybdate, and the rest of H₂O₂ reacted with the ammonium molybdate to generate a yellowish complex. The absorbance of the yellowish complex was measured at the wavelength of 405 nm using a Chem5V3[®] Semi-automated Clinical Chemistry Analyzer, (Erba Diagnostics, Mannheim GmbH, Mannheim, Germany) and the catalase activity was calculated from the absorbance.

The superoxide dismutase (SOD) activity was determined on serum, using the ElabScience SOD Assay kit (ElabScience Biotechnology Company Ltd., South Africa), which was based on the hydroxylamine method that adopted xanthine oxidase to measure SOD activity (Weydert and Cullen, 2010) with a Chem5V3[®] Semi-automated Clinical Chemistry Analyzer, (Erba Diagnostics, Mannheim GmbH, Mannheim, Germany), set at 550 nm wavelength.

The glutathione peroxidase (GPx) activity was determined on heparinized whole blood using a Fortress Diagnostics GPx test kit (Fortress Diagnostics Ltd, Antrim, UK.), based on the method described by Weydert and Cullen (2010). In this determination, the GPx in blood catalysed the oxidation of glutathione (GSH) by cumene hydroperoxide. The oxidized glutathione was converted to the reduced form in the presence of glutathione reductase and NADPH, and the NADPH was oxidized to NADP⁺ simultaneously.

The absorbance of the solution at 340 nm wavelength was measured using a Chem5V3[®] Semi-automated Clinical Chemistry Analyzer, (Erba Diagnostics, Mannheim GmbH, Mannheim Germany) and the GPx activity concentration was calculated from the absorbance.

The serum total glutathione (GSH) was determined using RayBio[®] Glutathione Colorimetric Detection Kit (RayBiotech Inc., Georgia, USA), which was based on the modified enzyme recycling system by 5, 5-dithiobis-(2-nitrobenzoic acid) (DNTB) and glutathione reductase (Tipple and Rogers, 2012). The DNTB and glutathione reacted to generate 2-nitro-5-thiobenzoic acid which has a yellow color. The glutathione concentration was determined by measuring the absorbance of the generated yellow solution at 412 nm using Diatek[®] DR-3508G Microplate (ELISA) Reader, (Wuxi Hiwell Diatek Instruments Co. Ltd., China). The total glutathione concentration in the plasma was obtained from the standard total glutathione calibration curve.

Measurement of the liver weight and the calculation of the relative liver weight (liver weight percentage of body weight)

After blood sample collection, the rats were euthanized by intraperitoneal injection of 250 mg/kg Thiopentone sodium and confirmatory exsanguination (AVMA, 2013). The liver of each rat was carefully eviscerated and weighed, and the relative liver weight (liver weight percentage of the body weight) was calculated.

Evaluation of the *in vitro* antioxidant activity (AA) of PSMLE using (DPPH) assay method

The *in vitro* anti-oxidant activity of PSMLE was analyzed following the 1, 1-diphenyl 2-picryl hydrazyl (DPPH) assay method (Mensor et al., 2001). Two milliliters of PSMLE in distilled water at concentrations of 10, 50, 100, 200 and 400 µg/ml were each mixed with 1 ml of 0.5 mM DPPH (in methanol) in a cuvette. The DPPH reagent formed yellow coloured complexes with the free hydroxyl group present in the crude extract. The absorbance of the coloured complex was read at 517 nm wavelength after 30 minutes of incubation in the dark at room temperature, with the aid of a spectrophotometer (HME Global Medical, England). The tests were done in triplicates. A mixture of 1 ml of methanol and 2 ml of PSMLE served as blank, while 1 ml of 0.5 mM DPPH solution and 2 ml of methanol served as negative control. Ascorbic acid was used as the reference standard. The percentage AA was calculated as follows:

$$\% \text{ AA} = 100 - \left[\frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of control}} \times 100 \right]$$

(Mensor et al., 2001; Oke and Hamburger, 2002).

Data analysis

Data obtained from the *in vivo* experiment with rats, and the results of PSMLE and ascorbic acid across the different concentrations, were subjected to one way analysis of variance (ANOVA). Variant means were separated post-hoc using the least significant difference method. Significance was accepted at $p < 0.05$. The *in vitro* anti-oxidant activities of PSMLE at the varied concentrations were compared with that of ascorbic acid using the student's t-test. A summary of the results are presented as bar charts with standard deviation bars and as table of means with standard error.

Table 1. Mean \pm standard error of the body weights (g) and percentage change in body weights of albino rat groups* given graded acute oral doses of *Pterocarpus santalinoides* methanol leaf extract (PSMLE).

Rat groups	Body weights (g) of the rats				% change in body weights
	Day 0	Day 3	Day 7	Day 14	
Group 1	183.17 \pm 8.02	186.37 \pm 8.09	187.57 \pm 8.51	189.47 \pm 8.45	3.42 \pm 1.31
Group 2	181.23 \pm 10.96	182.57 \pm 10.63	184.20 \pm 10.34	187.73 \pm 8.92	3.83 \pm 1.30
Group 3	179.73 \pm 6.06	182.10 \pm 6.30	182.33 \pm 4.91	186.40 \pm 4.73	3.71 \pm 0.83
Group 4	182.36 \pm 7.84	184.03 \pm 6.93	184.88 \pm 8.47	189.41 \pm 7.52	3.85 \pm 1.29
Group 5	180.30 \pm 8.19	181.43 \pm 7.74	183.78 \pm 8.01	187.58 \pm 7.57	4.04 \pm 1.40

No significant differences ($p > 0.05$) in the body weight and percentage change in body weight of the rats groups. * Groups: Group 1 - 0 mg/kg PSMLE (untreated); Group 2 - 50 mg/kg PSMLE; Group 3 - 300 mg/kg PSMLE; Group 4 - 2000 mg/kg PSMLE; and Group 5 - 5000 mg/kg PSMLE.

Table 2. Mean \pm standard error of the organ weights (g) of albino rat groups* given graded acute oral doses of *Pterocarpus santalinoides* methanol leaf extract (PSMLE).

Rat groups	Organ weights (g)			
	Liver weight	Average kidney weight	Spleen weight	Heart weight
Group 1	6.85 \pm 0.34	0.61 \pm 0.02	0.72 \pm 0.05	0.63 \pm 0.05
Group 2	6.76 \pm 0.22	0.60 \pm 0.05	0.70 \pm 0.03	0.62 \pm 0.04
Group 3	6.69 \pm 0.23	0.58 \pm 0.01	0.69 \pm 0.05	0.65 \pm 0.03
Group 4	6.78 \pm 0.24	0.60 \pm 0.06	0.71 \pm 0.04	0.63 \pm 0.04
Group 5	6.74 \pm 0.21	0.59 \pm 0.02	0.67 \pm 0.05	0.62 \pm 0.05

No significant differences ($p > 0.05$) in the organ weights of the rat groups. * Groups: Group 1 - 0 mg/kg PSMLE (untreated); Group 2 - 50 mg/kg PSMLE; Group 3 - 300 mg/kg PSMLE; Group 4 - 2000 mg/kg PSMLE; and Group 5 - 5000 mg/kg PSMLE.

RESULTS

Acute toxicity

There was no sign of toxicity or abnormality and no mortality in all the rat groups given the varied acute doses (0, 50, 300, 200 and 5000 mg/kg) of PSMLE all through the 14 day observation/monitoring period. All the rats which were given the varied acute doses were normal, with no sign of changes in the behavioral pattern, mucous membrane, skin and eyes. There were no significant differences ($p > 0.05$) in the body weights and the percentage change in body weights of the rat groups treated with the varied acute doses of PSMLE when compared to the untreated control (Table 1). There were also no significant differences ($p > 0.05$) between the groups given the acute doses of PSMLE and the untreated in their organ weights and relative organ weights (organ weight percentages of body weights) (Tables 2 and 3).

Phytochemical analysis

Phytochemical analysis revealed the presence of high

(+++ levels of tannins, glycosides, carbohydrates, saponins, phenols, sterols and terpenes; moderate (++) levels of flavonoids and low (+) level of alkaloid (Table 4).

Effects of PSMLE on blood levels of markers of hepatotoxicity

Treatment with 250 and 500 mg/kg PSMLE (Groups C and D) led to significantly ($p < 0.05$) lower serum ALT activity (Table 5). The 250 mg/kg PSMLE dose also led to significantly ($p < 0.05$) lower serum AST activity when compared to the negative control (Group A), and compared favorably with that of Group E (100 mg/kg silymarin) and Group F (normal control) rats (Table 5). There was no significant ($p > 0.05$) differences in the ALP activity between all the PSMLE-treated groups (Groups B, C and D) and the negative control (Group A), though the recorded lowering of the ALP activity in the treated groups occurred in a dose dependent manner (Table 5). The ALP activity of the normal control (Group F) was significantly ($p < 0.05$) lower than that of the negative control and all the PSMLE-treated groups (Table 5). Treatment with PSMLE at 250 mg/kg (Group C) led to

Table 3. Mean \pm standard error of the organ weight percentages (relative organ weights) of albino rats groups* given graded acute oral doses of *P. santalinoides* methanol leaf extract (PSMLE).

Rat groups	Relative organ weights (%)			
	Liver weight %	Kidney weight %	Spleen weight %	Heart weight %
Group 1	3.54 \pm 0.03	0.32 \pm 0.01	0.37 \pm 0.02	0.33 \pm 0.01
Group 2	3.61 \pm 0.05	0.32 \pm 0.01	0.38 \pm 0.02	0.32 \pm 0.01
Group 3	3.59 \pm 0.05	0.31 \pm 0.02	0.37 \pm 0.02	0.35 \pm 0.02
Group 4	3.60 \pm 0.04	0.32 \pm 0.01	0.38 \pm 0.02	0.33 \pm 0.02
Group 5	3.60 \pm 0.04	0.32 \pm 0.01	0.36 \pm 0.02	0.33 \pm 0.02

No significant differences ($p > 0.05$) in the relative organ weights of the rats groups. * Groups: Group 1 - 0 mg/kg PSMLE (untreated); Group 2 - 50 mg/kg PSMLE; Group 3 - 300 mg/kg PSMLE; Group 4 - 2000 mg/kg PSMLE; and Group 5 - 5000 mg/kg PSMLE.

Table 4. Phytochemical constituents of *P. santalinoides* methanol leaf extract (PSMLE).

Phytochemical constituents	Semi-quantitative composition
Tannins	+++
Terpenes and sterols	+++
Alkaloids	+
Flavonoids	++
Saponins	+++
Carbohydrates	+++
Glycosides	+++
Phenol	+++

+++ = High levels present; ++ = Moderate levels present; + = Low levels present.

Table 5. Effects of oral administration of graded doses of PSMLE on serum enzyme activities of albino rats given sub-acute toxic doses of CCl_4

Group	Mean \pm standard error (U/L)		
	ALT	AST	ALP
Group A	102.4 \pm 4.79 ^a	121.03 \pm 8.03 ^a	384.46 \pm 60.81 ^a
Group B	98.78 \pm 6.83 ^a	120.82 \pm 7.41 ^a	391.01 \pm 46.11 ^a
Group C	48.36 \pm 7.02 ^b	77.28 \pm 7.96 ^b	358.42 \pm 10.08 ^a
Group D	79.11 \pm 6.08 ^c	106.92 \pm 5.16 ^a	338.41 \pm 18.93 ^a
Group E	46.96 \pm 7.48 ^b	75.03 \pm 6.74 ^b	279.97 \pm 22.86 ^{ab}
Group F	30.83 \pm 4.61 ^b	63.01 \pm 6.42 ^b	104.11 \pm 21.04 ^b

^{a, b, c} Different alphabetical superscripts in a column indicate significant ($p < 0.05$) differences between the groups.

*Groups: Group A – CCl_4 + 10 ml/kg distilled water (negative control); Group B – CCl_4 + 50 mg/kg PSMLE; Group C – CCl_4 + 250 mg/kg PSMLE; Group D – CCl_4 + 500 mg/kg PSMLE; Group E – CCl_4 + 100 mg/kg silymarin; Group F – No CCl_4 and no treatment.

significantly ($p < 0.05$) higher serum total protein levels when compared to the negative control (Group A) (Figure 2). Treatment with PSMLE at all the doses used in the study had no significant ($p > 0.05$) effect on the serum albumin levels (Figure 2). There was significantly ($p <$

0.05) higher serum globulin level in albino rats treated with 250 mg/kg PSMLE (Group C) when compared to the negative control (Group A). There was no significant ($p > 0.05$) differences in serum globulin levels between 250 mg/kg PSMLE-treated rats (Group C), 100 mg/kg

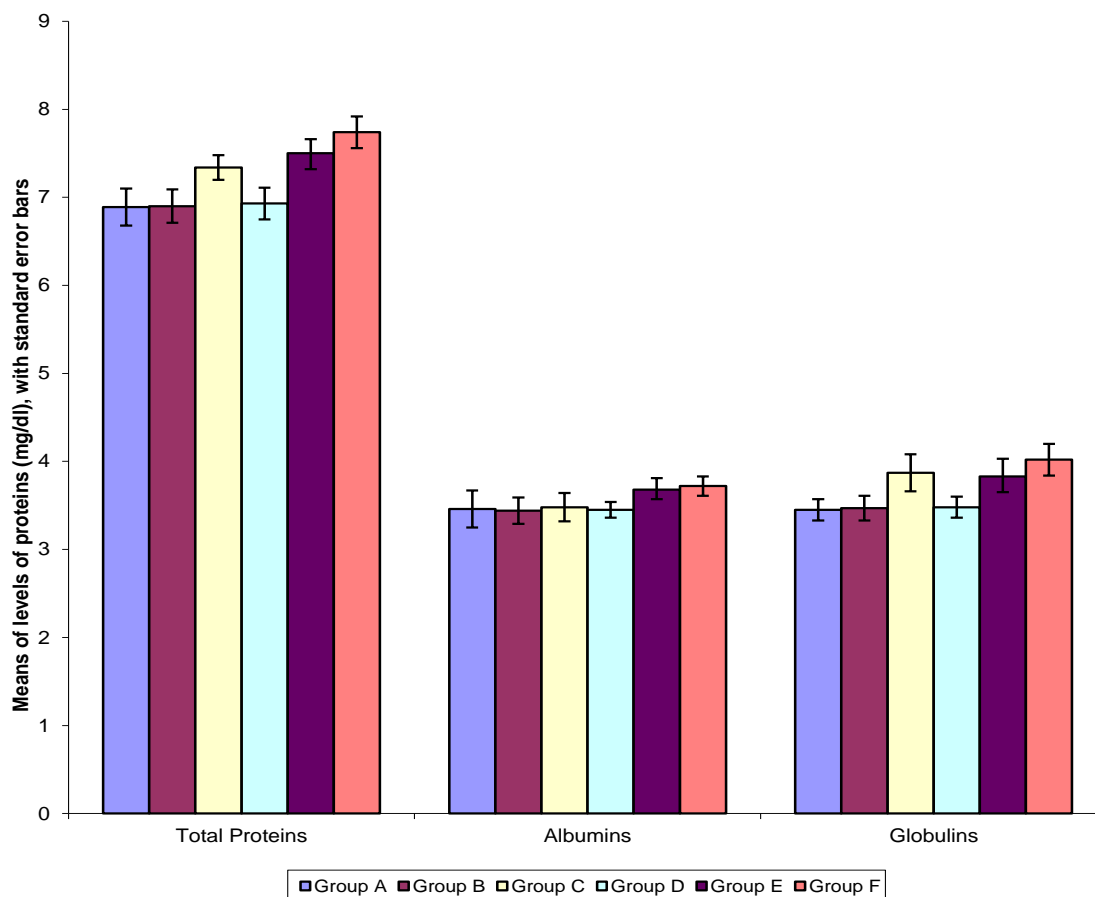


Figure 2. Effects of oral administration of graded doses of PSMLE on serum proteins of albino rats given sub-acute toxic doses of CCl_4 ; [Group A – CCl_4 + 10 ml/kg distilled water (negative control); Group B - CCl_4 + 50 mg/kg PSMLE; Group C - CCl_4 + 250 mg/kg PSMLE; Group D - CCl_4 + 500 mg/kg PSMLE; Group E - CCl_4 + 100 mg/kg Silymarin; Group F – No CCl_4 , No treatment (normal control)].

silymarin-treated (Group E) and the normal control (Group F) (Figure 2).

The serum total cholesterol level of Group D rats (500 mg/kg PSMLE) was significantly ($p < 0.05$) lower than that of the negative control (Group A), and there were no significant ($p > 0.05$) differences in serum total cholesterol levels between Group D and Groups B, C, E and F (Table 6). Treatment with 250 mg/kg PSMLE (Group C) and 100 mg/kg silymarin (Group E) significantly ($p < 0.05$) lowered serum total bilirubin levels when compared to the negative control (Group A) and other PSMLE-treated groups (Table 6). There was no significant ($p > 0.05$) difference in serum total bilirubin level between the rat groups treated with 250 mg/kg PSMLE, 100 mg/kg silymarin and the normal control (Table 6). The liver weight of the rat groups treated with 50, 250 and 500 mg/kg PSMLE and 100 mg/kg silymarin (Groups B, C, D and E) were significantly ($p < 0.05$) lower than that of the negative control (Group A), with Groups C, D and E being significantly ($p < 0.05$) lower than

Group B, and comparing favorably with the normal control (Group F) (Figure 3). Treatment with PSMLE at all doses used in the study (Groups B, C and D) and 100 mg/kg silymarin (Group E) led to significantly ($p < 0.05$) lower relative liver weights of the albino rats when compared to the negative control (Group A) (Figure 3).

Effects of PSMLE on oxidative stress and antioxidant markers

Treatment with PSMLE caused a dose-dependent reduction in MDA levels across the groups, with the negative control (Group A) being significantly ($p < 0.05$) higher than 250 and 500 mg/kg PSMLE, 100 mg/kg silymarin and normal control (Groups C, D, E and F), but not significantly ($p > 0.05$) different from 50 mg/kg PSMLE (Group B) (Table 7). The CAT activity of Groups B, C, D and E was significantly ($p < 0.05$) higher than that of Group A rats, and there were no significant ($p > 0.05$)

Table 6. Effects of oral administration of graded doses of PSMLE on serum total cholesterol and serum total bilirubin levels of albino rats given sub-acute toxic doses of CCl₄.

Group	Mean \pm standard error (mg/dl)	
	Total cholesterol	Total bilirubin
Group A	81.23 \pm 8.04 ^a	0.70 \pm 0.06 ^a
Group B	63.48 \pm 10.84 ^{ab}	0.63 \pm 0.14 ^a
Group C	61.64 \pm 3.02 ^{ab}	0.36 \pm 0.07 ^b
Group D	56.48 \pm 2.42 ^b	0.66 \pm 0.03 ^a
Group E	64.19 \pm 5.76 ^{ab}	0.37 \pm 0.04 ^b
Group F	62.49 \pm 3.54 ^{ab}	0.38 \pm 0.03 ^b

^{a, b, c} Different alphabetical superscripts in a column indicate significant ($p < 0.05$) differences between the groups. *Groups: Group A – CCl₄ + 10 ml/kg distilled water (negative control); Group B – CCl₄ + 50 mg/kg PSMLE; Group C – CCl₄ + 250 mg/kg PSMLE; Group D – CCl₄ + 500 mg/kg PSMLE; Group E – CCl₄ + 100 mg/kg silymarin; Group F – No CCl₄ and no treatment.

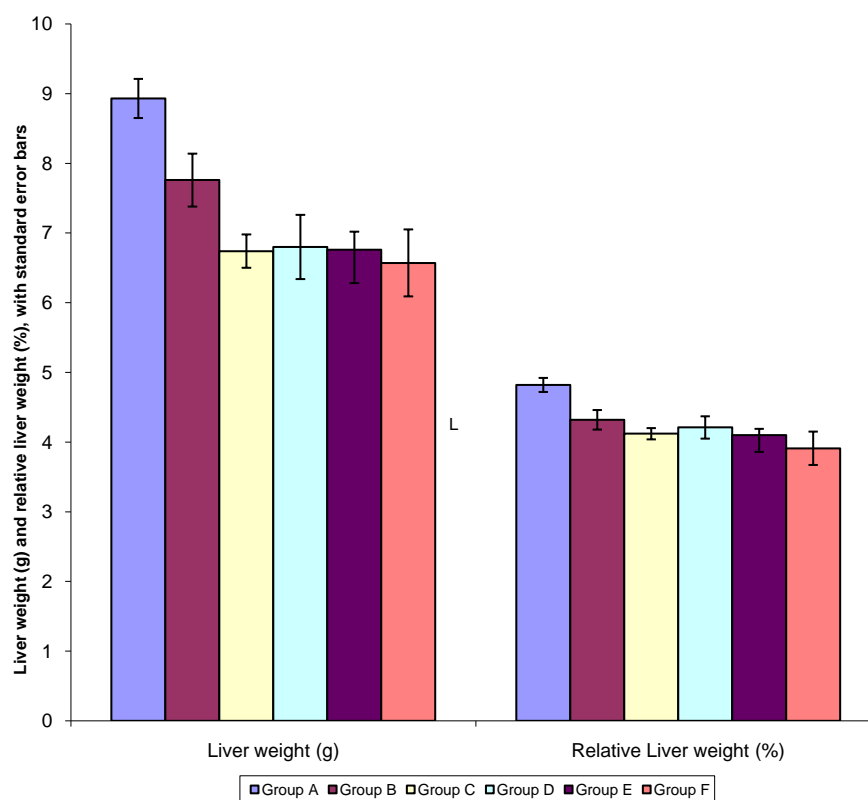


Figure 3. Effects of oral administration of graded doses of PSMLE on liver weight and relative liver weight of albino rats given sub-acute toxic doses of CCl₄. [Group A – CCl₄ + 10 ml/kg distilled water (negative control), B - CCl₄ + 50 mg/kg PSMLE; Group C - CCl₄ + 250 mg/kg PSMLE; Group D - CCl₄ + 500 mg/kg PSMLE; Group E - CCl₄ + 100 mg/kg Silymarin; Group F – No CCl₄, No treatment (normal control)]

differences between Groups B, C, D and E in their CAT activity (Table 7). The SOD activity was also significantly ($p < 0.05$) lower in Groups A and B compared to other

groups (Table 7). Glutathione peroxidase (GPx) activity followed the same pattern as SOD with Groups A and B being significantly ($p < 0.05$) lower than Groups C, D, E

Table 7. Effects of oral administration of graded doses of PSMLE on *in vivo* oxidative stress and blood anti-oxidant marker levels of albino rats given sub-acute toxic doses of CCl₄.

Oxidative stress and antioxidant parameter	Means, with standard deviation in brackets					
	Group A	Group B	Group C	Group D	Group E	Group F
MDA (µmol/L)	17.61 (3.32) ^a	14.19(4.29) ^{ab}	9.65 (2.87) ^{bc}	7.52 (3.30) ^{cd}	7.65(2.88) ^{cd}	4.05 (1.90) ^d
CAT (IU/ml)	12.18(2.36) ^a	21.55(2.38) ^b	20.92 (3.35) ^b	20.35(3.49) ^b	23.14(4.40) ^{bc}	29.76(7.99) ^c
SOD (IU/ml)	0.221 (0.020) ^a	0.257(0.049) ^{ab}	0.300(0.024) ^c	0.278(0.020) ^{bc}	0.294 (0.027) ^{bc}	0.299 (0.019) ^c
GPx (IU/L)	82.50 (10.74) ^a	88.41(12.89) ^a	119.35 (20.17) ^b	124.29(23.90) ^b	127.16 (17.27) ^b	190.25(18.58) ^c
GSH (µg/µl)	0.016 (0.006) ^a	0.029(0.004) ^b	0.039(0.005) ^{bc}	0.041(0.005) ^{bc}	0.046 (0.006) ^c	0.068(0.015) ^d

^{a,b,c} Different alphabetical superscripts in a row indicate significant differences between the groups ($p < 0.05$). *Groups: Group A – CCl₄ + 10 ml/kg distilled water (negative control); Group B – CCl₄ + 50 mg/kg PSMLE; Group C – CCl₄ + 250 mg/kg PSMLE; Group D – CCl₄ + 500 mg/kg PSMLE; Group E – CCl₄ + 100 mg/kg silymarin; Group F – No CCl₄ and no treatment.

and F (Table 7). Treatment with PSMLE at all doses (50, 250, 500 mg/kg) and silymarin (100 mg/kg) (Groups B, C, D and E) led to significantly ($p < 0.05$) higher GSH levels when compared to the negative control (Group A) (Table 7). There was significant ($p < 0.05$) dose-dependent higher levels of GSH when all the PSMLE-treated groups were compared. However, there were significantly ($p < 0.05$) lower GSH levels in all the PSMLE-treated groups when compared with the normal control (Group F) (Table 7).

***In vitro* antioxidant activity of PSMLE**

The antioxidant activity of PSMLE at 10 µg/ml concentration was significantly ($p < 0.05$) lower than that of ascorbic acid (Figure 4). However, 50, 100, 200 and 400 µg/ml concentration of PSMLE produced significantly ($p < 0.05$) higher antioxidant activity when compared to the same concentrations of ascorbic acid (Figure 4). The PSMLE showed maximum anti-oxidant activity at 400 µg/ml and minimum activity at 10 µg/ml. There were no significant ($p > 0.05$) differences between the anti-oxidant activity values obtained at 50 µg/ml, 100 µg/ml, and 200 µg/ml concentrations (Figure 4). Also, there were no significant ($p > 0.05$) differences between the anti-oxidant activity values of the ascorbic acid standard at varied concentrations (Figure 4).

DISCUSSION

In the acute toxicity study, the rats tolerated the extract up to 5000 mg/kg, without any significant/adverse changes in their body and organ weights. This shows that the LD₅₀ is above 5000 mg/kg (OECD, 2001). An LD₅₀ above 5000 mg/kg is within the World Health Organization's category of substances "unlikely to present acute hazard in normal use" (WHO, 2001). This implies that PSMLE is safe for acute use in the treatment

of ailments and diseases for which it is effective (OECD, 2001). This concurs with earlier reports by Ihedioha et al. (2017; 2018) that methanol leaf extracts of *P. santalinoides* is not acutely toxic.

The phytochemicals observed in PSMLE are essential bioactive compounds commonly found in various herbs used for medicinal purposes. Similar compounds as recorded in this study have been identified in the methanol and ethanol extracts of the leaf and stem bark of *P. santalinoides* (Anowi et al., 2012; Eze et al., 2012; Odeh and Tor-Anyim, 2014; Enemali et al., 2019).

The higher serum ALT, AST and ALP activities in all the groups that were given CCl₄ showed that CCl₄ administration damaged the liver cells and altered the integrity of the hepatocytes (Boll et al., 2001; Kim et al., 2010). Elevation in serum transaminases (AST and ALT) is a biomarker of hepatocellular necrosis and hepatotoxicity (Friedman et al., 1996). The administration of PSMLE at the doses of 250 and 500 mg/kg led to significantly lower serum ALT activity, and at 250 mg/kg it led to significantly lower serum AST activity, and these were comparable to that of silymarin treatment (a known hepatoprotective drug). These findings are in agreement with the reports of Offor et al. (2015) who also recorded significantly lower ALT and AST activities in CCl₄-induced hepatotoxic albino rats treated with ethanol leaf extract of *P. santalinoides*. Reports by Ihedioha et al. (2017) also showed that methanol leaf extract of *P. santalinoides* restored hepatocellular integrity in acetaminophen-induced hepatotoxicity in albino rats. In another study on a related species of *Pterocarpus*, Ihedioha et al. (2019) also reported significantly lower serum ALT and AST activities in CCl₄-induced hepatotoxic albino rats treated with methanol leaf extract of *Pterocarpus mildbraedii* (a plant species in the same Genus *Pterocarpus*). The ability of the PSMLE to protect hepatocellular integrity from CCl₄-induced damage is believed to be due to its phytochemical constituents such as tannins, flavonoids and glycosides (Muriel et al., 2001; Ihedioha et al., 2017), which have been reported to be hepatoprotective. Other

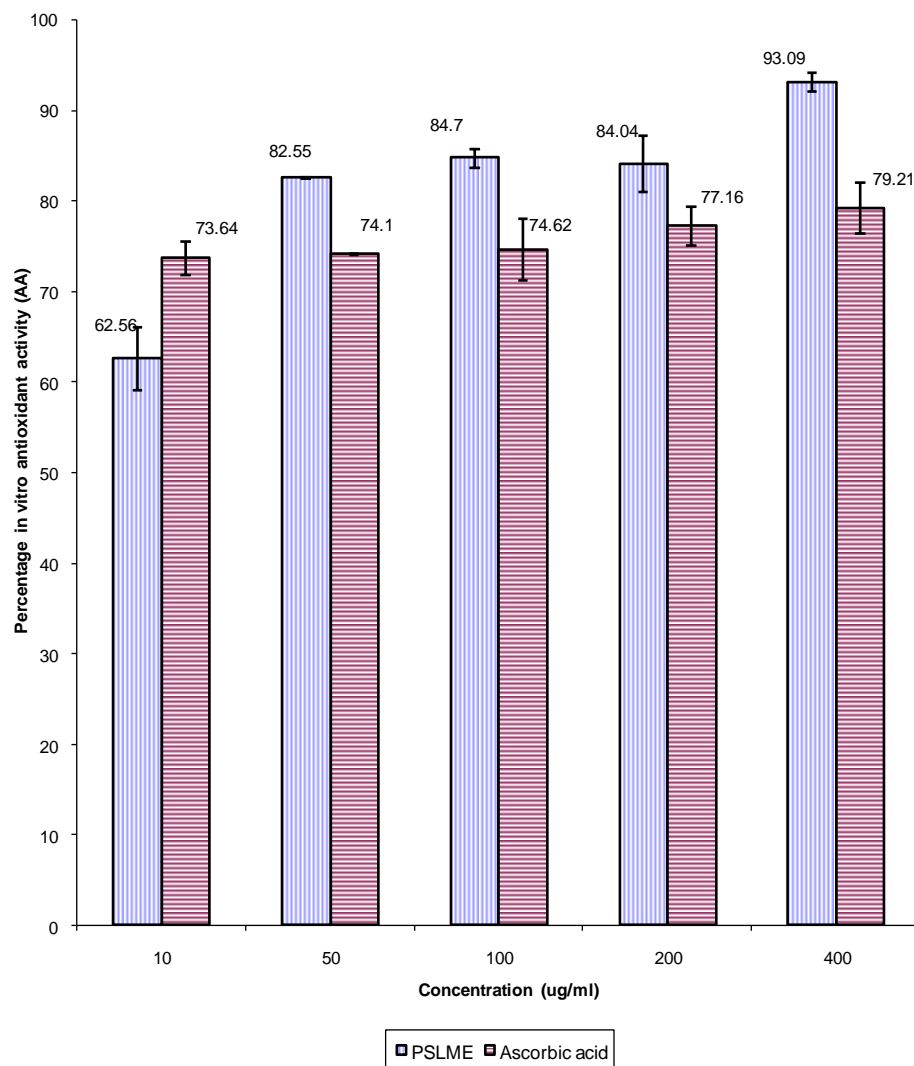


Figure 4. The *in vitro* antioxidant activity values of varied concentrations of PSLME and ascorbic acid using 1, 1-diphenyl 2 picryl hydrazyl (DPPH) assay.

natural products that possess similar phytochemical constituents have also been reported to protect against CCl₄-induced liver damage (Hsiao et al., 2003).

The significantly lower serum total proteins and serum globulin in the negative control group and other groups that were given CCl₄ are indications of the ability of CCl₄ to damage hepatocytes and impair protein synthesis (Navarro and Senior, 2006). Proteins form the major portion of dissolved substances in the plasma (Singh et al., 2011), and act as transport agents for a wide variety of substances such as hormones, lipids and vitamins. Proteins can be used as a supplementary test for hepatic biosynthetic functions (Friedman et al., 1996; Thapa and Walia, 2007; Singh et al., 2011). Decreased levels of proteins are found in liver disorders (Kipple, 2003). The

PSMLE (250 mg/kg) ameliorated the impaired protein synthetic function by leading to significantly higher serum total protein and serum globulins of the treated rats.

Elevation of serum globulin in CCl₄-induced hepatotoxic albino rats treated with *P. mildbraedii* (a plant of the same genus as *P. santalinoides*) methanol extract has also been reported (Ihedioha et al., 2019). Stimulation of protein synthesis is known to be a hepatoprotective mechanism. Protein synthesis accelerates the hepatocyte regeneration process and helps in the production of replacement liver cells (Rip et al., 1985; Tadeusz et al, 2001).

The significantly higher serum total cholesterol in the negative control group and other groups that were given CCl₄ are indications of alterations in serum lipid profile

caused by CCl₄ administration. The liver is the major site for the synthesis and clearance of lipoproteins; therefore, hepatotoxicity or damage to the hepatocytes can affect plasma lipids leading to alterations in lipid profile, and this has been found to be instrumental to the development of atherosclerosis (Ihedioha et al., 2013; Ihedioha et al., 2018). Hypercholesterolaemia occurs in hepatotoxicity as dyslipidaemia consequent upon dysfunction and alterations in hepatic lipid synthesis and clearance (Mandal et al., 2013). Treatment with PSMLE (500 mg/kg) and silymarin (100 mg/kg) ameliorated these effects by significantly lowering the serum total cholesterol of the treated albino rats at these doses. Ihedioha et al. (2017) also reported significantly lower serum total cholesterol levels in acetaminophen-induced hepatotoxic albino rats treated with methanol leaf extract of *P. santalinoides*.

Administration of CCl₄ also adversely affected bilirubin excretion as observed in all the groups that were given CCl₄. Bilirubin is an endogenous anion derived from the regular degradation of haemoglobin from the red blood cells. It is a chemical normally present in the blood in small amounts and excreted from the liver in form of bile. When the liver cells are damaged, they may not be able to excrete bilirubin in the normal way, thus causing a build-up of bilirubin in the blood and extracellular fluid (Singh et al., 2011). Increased levels of bilirubin may also result from decreased hepatic clearance and lead to jaundice and other hepatotoxicity symptoms (Saukkonen et al., 2006). Treatment with PSMLE (500 mg/kg) and silymarin (100 mg/kg) led to significantly lower serum total bilirubin in albino rats given toxic doses of CCl₄. They ameliorated the impaired excretory function induced by the CCl₄ administration, and enhanced hepatic clearance of bilirubin. Ihedioha et al. (2017) reported that *P. santalinoides* methanol extract significantly lowered total bilirubin level in acetaminophen-induced hepatotoxicity in albino rats. The ethanol extract of a related species (*Pterocarpus milbraedii*) had been reported by other researchers to significantly lower serum total bilirubin level in propanil and CCl₄-induced hepatotoxicity in albino rats (Otuechere and Farombi, 2015; Hamza et al., 2017; Ihedioha et al., 2019).

The relatively higher liver weights in albino rats given CCl₄ are indications of inflammation and/or degeneration which accompany CCl₄ toxicity (Tahashi et al., 2002; Bukhsh et al., 2014). Inflammation is a complex catalogue of vascular and tissue changes that develop as a response of tissue to injury (Ihedioha, 2003). Degeneration on the other hand, is a retrogressive change in tissues characterized by abnormal changes and decreases in function. Organs undergoing acute degenerative changes tend to be larger and heavier than normal (Ihedioha, 2003). Enlargement of the liver (hepatomegaly) is a common evidence of hepatic injury (Ihedioha and Chineme, 2005). Treatment with PSMLE at

all the doses used in the study and silymarin (100 mg/kg) significantly lowered this inflammation/degeneration-induced enlargement caused by CCl₄ toxicity and this suggests their amelioration of this inflammatory enlargement of the liver. Relatively lower liver weight has also been reported in hepatotoxic albino rats treated with *P. mildbraedii* methanol leaf extract (Ihedioha et al., 2019).

In the *in vivo* oxidative stress and antioxidants evaluation results, the higher doses of PSMLE (250 mg/kg and 500 mg/kg) were able to mop up more free radicals more than the lower dose (50 mg/kg). This implies that the PSMLE possesses anti-oxidant activity which is dose-related. Malondialdehyde (MDA) is one of the final products of the peroxidation of polyunsaturated fatty acids in cells and is a known marker of oxidative stress status in several diseases (Draper and Hadley, 1990; Gawel et al., 2004; DelRio et al., 2005). The MDA result in this present study concurs with the reports of Maruthupandian and Mohan (2011) on the lowering of plasma lipid peroxides in diabetic rats given ethanol extract of *Pterocarpus marsupium* wood and bark (a related plant belonging to the same genus). Administration of PSMLE enhanced catalase (CAT) activity. This is evident by the significantly higher catalase activity in all the groups treated with PSMLE. Catalase is a very important enzyme in protecting the cell from oxidative damage (Chelikani et al., 2004). It is the main enzyme that removes hydrogen peroxide (a reactive oxygen species), and blocks oxidative stress (Gaetani et al., 1996). The findings in this present study are similar to the reports by Maruthupandian and Mohan (2011) on the effects of a related plant of the same genus (*Pterocarpus marsupium*) on catalase activity of alloxan-induced diabetic rats. The superoxide dismutase (SOD) assay result in this present study is also an indication that the negative control (Groups A) and 50 mg/kg PSMLE-treated (Group B) rats had no significant protection from oxidative damage as compared to other groups. Treatment with PSMLE at the doses of 250 and 500 mg/kg (Groups C and D) effectively restored the SOD activity of these rats to a level comparable to that of normal rats not given CCl₄ (Group F). Superoxide dismutase (SOD) is an important anti-oxidant defense in living cells exposed to oxygen radicals. Earlier studies had shown that treatment with SOD decreased the generation of reactive oxygen species and oxidative stress (Fridovich, 1997; Gongora et al., 2006). Treatment with PSMLE at the doses of 250 and 500 mg/kg also restored the glutathione peroxidase (GPx) activity of the albino rats. The main biological role of GPx is to protect the organism from oxidative damage. Its biochemical function is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Blood levels of GPx have been reported to be low in some important diseases, and had

significantly improved when antioxidants were administered (Lubos et al., 2011). The findings in this present study of significantly higher GPx activity in PSMLE-treated groups is comparable to the reports by Maruthupandian and Mohan (2011) on the effects of *P. marsupium* wood and bark extracts on GPx of alloxan-induced diabetic rats. Plasma total glutathione (GSH) is one of the major endogenous anti-oxidants produced by cells, which participates directly in the neutralization of free radicals and reactive oxygen species, as well as maintaining exogenous anti-oxidants such as vitamins C and E in their reduced (active) forms (Dringen, 2000). The higher total glutathione level recorded in this study for Group B (50 mg/kg PSMLE), Group C (250 mg/kg PSMLE), and Group D (500 mg/kg PSMLE) implies a dose-dependent anti-oxidant activity in these groups. Maruthupandian and Mohan (2011) also reported the enhancing effects by *P. marsupium* extracts on the plasma total glutathione of alloxan-induced diabetic rats, but their results did not show dose-dependence.

In vitro, the PSMLE evoked significant concentration-dependent antioxidant activity, from 50 to 400 µg/ml concentrations, suggesting that it could be of benefit in ameliorating tissue damaging effect of increased concentration of reactive oxygen species (ROS) seen in toxic liver diseases. This result concurs with the reports of Bothon et al. (2014), Kabine et al. (2015) and Akaniro-Ejim et al. (2018) who also reported that the aqueous and hydro-ethanol leaf and fruit extracts, and aqueous-ethanol leaf extract of *P. santalinoides* respectively, possess anti-oxidant activity *in vitro*.

The antioxidant properties of PSMLE may be attributed to its flavonoids, tannins and/or glycoside contents (Pietta, 2000; Hodek et al., 2002; Yi-Fang et al., 2002; Aruoma, 2003). The results obtained from the present study also concurred with the reports by Ihedioha et al. (2017), who attributed the ability of PSMLE in lowering serum ALT and AST activities to its antioxidant activity. Ihedioha et al. (2018) also reported that the lipid lowering capability of aqueous leaf infusion of *P. santalinoides* may be related to its antioxidant properties. This present result is also in agreement with reports by other researchers on the *in vitro* antioxidant activity of other species in the same genus *Pterocarpus*, such as *Pterocarpus marsupium* (Mohammadi et al., 2009; Tippani et al., 2010; Maruthupandian and Mohan, 2011), *Pterocarpus mildbraedii* (Nwozo et al., 2015), *Pterocarpus angolensis* (Traore et al., 2016) and *Pterocarpus erinaceus* (Patrick et al., 2016). These findings are significant because foods, spices and herbal formulations containing antioxidants are used pharmacologically to prevent, manage and /or treat diseases in which oxidative stress play critical roles such as liver diseases, heart diseases and diabetes mellitus (Sies, 1997; Halliwell and Gutteridge, 1989; Valko et al., 2007). The present study was limited to the evaluation of

the hepatoprotective and antioxidant activity of the crude methanol extract of the leaves of *P. santalinoides*. Further studies to elucidate the active fractions and pure compound(s) responsible for the hepatoprotective and antioxidant activity are ongoing, and shall be reported in future.

Conclusion

The findings in this present study confirm that extracts of the leaves of *P. santalinoides* significantly ameliorated hepatocellular injury and relieved oxidative stress, and may thus be beneficial in the treatment and management of toxic liver damage and diseases mediated by and/or associated with oxidative stress.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Adesina SK (1982). Studies on Nigeria herbal medicinal plants. International Journal of Crude Drug Research 20:93-100.
- Adetunji JA (2007). Reviewing *Pterocarpus* species and their distribution. African Journal of Traditional Complementary and Alternative Medicine 4(2):23-36.
- Akaniro-Ejim EN, Ibe MI, Engwa GA (2018). Polyphenol content and *in vitro* anti-oxidant activity of aqueous-ethanol extract of *Pterocarpus soyauxii* and *Pterocarpus santalinoides*. Global Journal of Medical Research 18(2):1-8.
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC (1974). Enzymatic determination of total cholesterol. Clinical Chemistry 20(4):470-475.
- Ames BM, Shigena MK, Hagen TM (1993). Oxidants, antioxidants and degenerative disease of aging. Proceedings of the National Academy of Science 90:7915-7922.
- Anowi CF, Okonkwo C, Agbata CA, Ezeokafor E (2012). Preliminary phytochemical screening, evaluation of acute toxicity and antipyretic activity of methanolic extract of *Pterocarpus santalinoides* (Fabaceae). International Journal of Pharmaceutical and Phytopharmacological Research 1(6):343-346.
- Aruoma OI (2003). Methodological considerations for characterizing potential antioxidant actions of bioactive compounds in food plants. Mutation Research 523:9-20.
- Atawodi SE (2005). Antioxidant potential of African medicinal plants. African Journal of Biotechnology 4:28-133.
- American Veterinary Medical Association (AVMA) (2013). AVMA

- guidelines for the euthanasia of animals: 2013 edition. Illinois, USA pp. 48-50.
- Boll M, Weber LWD, Becker LE, Stampfl A (2001). Mechanism of carbon tetrachloride induced hepatotoxicity. Hepatocellular damage by reactive CCl₄ metabolites. *Zeitschrift für Naturforschung C* 56:649-659.
- Bolliger AP, Everds NE (2010). Hematology of laboratory rodents: mouse (*Mus musculus*) and rat (*Rattus norvegicus*). In: Weiss DJ and Wardrop KJ (eds), *Schalm's Veterinary Hematology*, 6th edn. Wiley-Blackwell, Iowa pp. 852-862.
- Bothon FTD, Moustapha M, Bogninou GS, Dossa CPA, Yehouenou B, Medoatinsa SE, Noudogbessi JP, Avlessi F, Sohounhloue DCK (2014). Chemical characterization and biological activities of *Newbouldia laevis* and *Pterocarpus santalinoides* leaves. *Bulletin of Environment Pharmacology and Life Sciences* 3(11):9-15.
- Bukhsh E, Malik SA, Ahmad SS, Erum S (2014). Hepatoprotective and hepatocurative properties of alcoholic extract of *Carthamus oxyacantha* seeds. *African Journal of Plant Sciences* 8(1):31-41
- Chelikani P, Fita I, Loewen PC (2004). Diversity of structures and properties among catalases. *Cellular and Molecular Life Sciences* 61:192-208.
- Chu Y (2000). Flavonoids content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture* 80:561-566.
- Colville J (2002). Blood chemistry. In: Hendrix CM (ed), *Laboratory procedures for Veterinary Technicians*. 4th edn. Mosby, St Louis pp. 75-103.
- Davies KJ (1995). Oxidative stress: the paradox of aerobic life. *Biochemical Society Symposia* 61:1-31.
- DelRio D, Stewart AJ, Pellegrina N (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrition, Metabolism and Cardiovascular Diseases* 15:316-328.
- Dlamini LM, Tata CM, Djuidje MCF, Ikhile MI, Nikolova GD, Karamalakova YD, Gadjeva VG, Zheleva AM, Njoh PB, Ndinteh DT (2019). Antioxidant and prooxidant effects of *Piptadeniastrum africanum* as the possible rationale behind its broad scale application in African ethnomedicine. *Journal of Ethnopharmacology* 231:429-437.
- Doumas BT, Perry BW, Sasse EA, Straumfjord Jr. JV (1973). Standardization in bilirubin assays: Evaluation of selected methods and stability of bilirubin solutions. *Clinical Chemistry* 19:984-993.
- Doumas BT, Peters T Jr. (1997). Serum and urine albumin: A progress report on their measurement and clinical significance. *Clinica Chimica Acta* 258(1):3-20.
- Draper HH, Hadley M (1990). Malondialdehyde determination as an index of lipid peroxidation. *Methods and Enzymology* 186:421-431.
- Dringen R (2000). Metabolism and functions of glutathione in brain. *Progress in Neurobiology* 62(6):649-671.
- Enemali MO, Udedi SC, Ubaoji KI, Haruna GS, Augustine E (2019). Modulatory effect of leaf extracts of *Pterocarpus santalinoides* Hook F. (red sandal wood) on acetaminophen-induced hepatotoxicity in albino rats. *FUW Trends in Science and Technology* 4(2):366-371.
- Eze SO, Cornelius C, Okereke HC (2012). Phytochemical and antimicrobial analyses of the stem bark of *Pterocarpus santalinoides* (Nturu ukpa). *Asian Journal of Natural and Applied Sciences* 1(3):26-32.
- Fridovich I (1997). Superoxide anion radical (O₂⁻), superoxide dismutases and related matters. *Journal of Biological Chemistry* 272:18515-18517.
- Friedman LS, Martin P, Munoz SJ (1996). Liver function tests and the objective evaluation of the patient with liver diseases. In: Zakin D, Boyer TD (eds). *Hepatology: A Textbook of Liver Diseases*. 3rd edn. WB Saunders, Philadelphia pp. 79-833.
- Gaetani G, Ferraris AM, Rolfo M, Mangerini R, Arena S, Kirkman HN (1996). Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. *Blood* 87(4):1595-1599.
- Gawel S, Wardas M, Neidworok E, Wardas P (2004). Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiadomosci Lekarskie* 57(9):453-455.
- Gongora MC, Qin Z, Laude K, Kim HW, McCann L, Folz JR, Dikalov S, Harrison DG (2006). Role of extracellular superoxide dismutase in hypertension. *Hypertension* 48:473-481.
- Halliwell B, Gutteridge JMC (1989). *Free radicals in biology and medicine*, 2nd edn. Clarendon Press, Oxford.
- Hamza RU, Jigam AA, Makun HA, Egwim EC, Ibikunle GK (2017). Effects of EF, EG and EI subfractions of *Pterocarpus mildbraedii* Harms leaf extract against CCL₄-induced hepatic damage in albino rats. *Medicine* 65:1-6.
- Harborne JB. (1998). *Phytochemical methods – A guide to modern techniques of plant analysis*. London: Chapman and Hall pp. 129-134.
- Higgins T, Beutler E, Doumas BT (2008). Analytical methodology – Serum bilirubin. In: Burtis CA, Ashwood ER, Bruns DE (Eds). *Tietz Fundamentals of Clinical Chemistry*. 6th edn. Saunders Elsevier, Missouri pp. 524-525.
- Hodek P, Trefil P, Stiborova M (2002). Flavonoids – potent and versatile biologically active compounds interacting with cytochrome P₄₅₀. *Chemico-Biological International* 139(1):1-21.
- Hsiao G, Shen MY, Lin KH, Lan MH, Wu LY, Chou DS, Lin CH, Su CH, Sheu JR (2003). Antioxidative and hepatoprotective effects of *Androdia camphorate* extract. *Journal of Agriculture and Food Chemistry* 51:3302-3308.
- Ihedioha JI (2003). *Basic Principles of General Pathology*, (1st edn.). AP Express Publishers Ltd., Nsukka, Nigeria pp. 25-59.
- Ihedioha JI, Chineme CN (2005). *Fundamentals of Systemic Veterinary Pathology*, Vol.2. AP Express Publishers Ltd., Nsukka, Nigeria pp. 231.
- Ihedioha JI, Noel-Uneke OA, Ihedioha TE (2013). Reference values for the serum lipid profile of albino rats (*Rattus norvegicus*) of varied ages and sexes. *Comparative Clinical Pathology* 22 (1):93-99.
- Ihedioha TE, Odo RI, Onoja US, Nwagu CA, Ihedioha JI (2019). Hepatoprotective properties of methanol leaf extract of *Pterocarpus mildbraedii* Harms on carbon tetrachloride-induced hepatotoxicity in albino rats (*Rattus norvegicus*). *Journal of Complementary Medicine Research* 10(4):162-169.
- Ihedioha TE, Okechukwu VN, Ihedioha JI (2018). Effects of aqueous leaf infusion of *Pterocarpus santalinoides* DC on serum lipid profile of guinea pigs (*Carvia porcellus*). *Journal of Complementary Medicine Research* 7(2):154-160.
- Ihedioha TE, Onwuegbuka LU, Ihedioha JI (2017). Hepatoprotective effects of methanol leaf extract of *Pterocarpus santalinoides* DC on acetaminophen-induced liver damage in albino rats (*Rattus norvegicus*) *Nigerian Journal of Animal Production* 44(5):34-39.
- Johnson AM (2008). Amino acids and proteins. In: Burtis CA, Ashwood ER, Bruns DE (Eds). *Tietz Fundamentals of Clinical Chemistry*. 6th edn. Saunders Elsevier, Missouri pp. 206-316.
- Kabine O, Samba BM, Fatoumata B, Namagan K, Luopou HN, Mamadou BA (2015). Anti-oxidative activity of fruit extract of some medicinal plants used against chronic diseases (diabetes, hypertension) in Kankan, Guinea. *Journal of Plant Sciences* 3(2):1-5.
- Karamalakova YD, Nikolova GD, Adhikari M, Stoev SD, Agarwal P, Gadjeva VG, Zhelev Z (2018). Oxidative-protective effects of *Tinospora cordifolia* extract on plasma and spleen cells after experimental ochratoxycosis. *Comparative Clinical Pathology* 27(6):1487-1495.
- Keay RWJ (1989). *Trees of Nigeria. A revised version of Nigeria trees (1960 – 1964)*. Keay RWJ, Onochie CFA, Stanfield DP (Eds). Clarendon Press, Oxford, United Kingdom P 476.
- Kim H, Joon-Ki K, Jun-Ho C, Joo-Yeon J, Woo-Yong O, Dong CK, Hee SL, Yeong-Shik K, Sam SK, Seung-Ho L, Sun-Mee L (2010). Hepatoprotective effect of pinosresinol on carbon tetrachloride-induced hepatic damage in mice. *Journal of Pharmacological Sciences* 112:105-112.
- Kipple KF (2003). *The Cambridge historical dictionary of disease*, (1st Ed.). Cambridge University Press, New York P 428.
- Koleva II, Van-Beek TA, Linssen JPH, Degroot A, Evstatieva LN (2002). Screening of plant extracts for antioxidant activity, a comparative study on three testing methods. *Phytochemical Analysis* 13:8-17.
- Lubos E, Loscalzo J, Handy DE (2011). Glutathione peroxidase-1 in

- health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxidants and Redox Signal* 15(7):1957-1997.
- Lubran MM (1978). The measurement of total serum proteins by the Biuret method. *Annals of Clinical and Laboratory Sciences* 8(2):106-110.
- Mandal SK, Sil K, Chatterjee S, Ganguly J, Chatterjee K, Sarkar P, Hazra S, Sardar D (2013). A study on lipid profile in chronic liver diseases. *National Journal of Medical Research* 7(1): 70-72.
- Mantle D, Eddeb F, Pickering AT (2000). Comparison of relative antioxidant activities of British medicinal plant species – *in vitro*. *Journal of Ethnopharmacology* 72:47-55.
- Maruthupandian A, Mohan VR (2011). Antidiabetic, antihyperlipidaemic and antioxidant activities of *Pterocarpus marsupium* Roxb. in alloxan induced diabetic rats. *International Journal of Pharmtech Research* 3(3):1681-1687.
- Mensor LL, Fabio S, Gilder G, Aleixandre S, Teresa C (2001). Screening of Brazilian plant extract for antioxidant activity by DPPH free radical method. *Phytotherapy Research* 15:127-130.
- Mohammadi M, Khole S, Devasagayam TPA, Ghaskadbi SS (2009). *Pterocarpus marsupium* extract reveals strong *in vitro* antioxidant activity. *Drug Discovery and Therapeutics* 3(4):151-161.
- Muriel P, Alba N, Perez-Alvarez VM, Shibayama M, Tsutsumi VK (2001). Kupffer cell inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 130:219-266.
- Navarro VJ, Senior JR (2006). Drug-related hepatotoxicity. *New England Journal of Medicine* 354:731-739.
- Nwokediuko SC, Osuala PC, Uduma UV, Onwuka CC, Mesigo C (2013). Patterns of liver disease admission in a Nigerian tertiary hospital. *Nigerian Journal of Clinical Practice* 16(3):339-342.
- Nwozo SO, Oso BJ, Oyinloye BE (2015). Effect of heat on antioxidant activity of some tropical leafy vegetables. *Nigerian Journal of Basic and Applied Sciences* 23(2):93-101.
- Odeh IC, Tor-Anyiin A (2014). Phytochemical and antimicrobial evaluation of leaf-extracts of *Pterocarpus santalinoides*. *European Journal of Medicinal Plants* 4(1):105-110.
- OECD (2001). Guideline for Testing of Chemicals; Acute Oral Toxicity – Acute Toxic Class Method. OECD Test Guideline 423. Organisation for Economic Cooperation and Development (OECD), Paris, France.
- Offor CE, Nwankwegu NJ, Ugwu-Okechukwu PC, Aja PM (2015). The effects of ethanol Leaf extract of *Pterocarpus santalinoides* on liver enzymes of albino rats. *American-Eurasian Journal of Agriculture and Environmental Sciences* 15(5):920-922.
- Oke JM, Hamburger MO (2002). Screening of some Nigerian medicinal plants for antioxidant activity, using 2, 2-Diphenyl-1-picryl hydrazyl radical. *African Journal of Biomedical Research* 5:77-79.
- Okwu DE, Ekeke T (2003). Phytochemical screening and medicinal composition of chewing sticks in South Eastern Nigeria. *Global Journal of Pure and Applied Sciences* 9:235-238.
- Otuechere CA, Farombi EO (2015). Evaluation of the protective effect of *Pterocarpus mildbraedii* extract on propanil-induced hepatotoxicity. *International Journal Medical and Health Sciences* 2(2):12-20.
- Patrick AT, Samson FP, Thagriki D, Umaru HA, Madusolumuo MA (2016). *In vitro* antioxidant activity and phytochemical evaluation of aqueous and methanolic stem bark extracts of *Pterocarpus erinaceus*. *World Journal of Pharmaceutical Research* 5(4):134-151.
- Pietta PG (2000). Flavonoids as antioxidants. *Journal of Natural Products* 63:1035-1042.
- Rehm J, Somokhvalov AV, Shield KD (2013). Global burden of alcoholic liver diseases. *Journal of Hepatology* 59:160-168.
- Rifai N, Warnick GR, Remaley AT (2008). Analysis of lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, Bruns DE (Eds). *Tietz Fundamentals of Clinical Chemistry*, 6th edn. Saunders Elsevier, Missouri pp. 422-427.
- Rip JW, Rupar CA, Ravi K, Carroll KK (1985). Distribution, metabolism and function of dolichol and polyprenols. *Progress in Lipid Research* 24:269-309.
- Robin S, Sunil K, Rana AC, Nidhi S (2012). Different models of hepatotoxicity and related liver diseases: a review. *International Research Journal of Pharmacy* 3(7):86-95.
- Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA, Nolan CM, Peloquin CA, Gordin FM, Nunes D, Strader DB, Bernardo J, Venkataramanan R, Sterling TR (2006). An Official ATS Statement: Hepatotoxicity of antituberculosis therapy. *American Journal of Respiratory and Critical Care Medicine* 174:935-952.
- Schiff L, Schiff ER (1987). *Diseases of the liver*, 6th edn. J.P. Lippincott, Philadelphia.
- Schuler P (1990). Natural antioxidants exploited commercially. In: *Food antioxidants*. Hudson BJF (Ed.). Elsevier, London, p. 99-170.
- Sies H (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology* 82:291-295.
- Singh A, Bhat TK, Sharma OP (2011). Clinical Biochemistry of Hepatotoxicity. *Journal of Clinical Toxicology* S4:1-19.
- Singh GS, Goyal R, Sharma PL (2012). Pharmacological potential of silymarin in combination with hepatoprotective plants against experimental hepatotoxicity in rats. *Asian Journal of Pharmaceutical and Clinical Research* 5(1):128-133.
- Suk KT, Kim DJ (2012). Drug-induced liver injury: present and future. *Clinical and Molecular Hepatology* 18:249-257.
- Tadeusz J, Teresa J, Krzysztof N (2001). The role of polyprenol in modulation of physical properties of model membranes. *Current Topics in Biophysics* 25:33-38.
- Tahashi Y, Matsuzaki K, Date M, Yoshida K, Furukawa F, Sugano Y, Matsushita M, Himeno Y, Inagak Y, Inoue K (2002). Differential regulation of TGF- β signal in hepatic stellate cells between acute and chronic liver injury. *Hepatology* 35:49-61.
- Thapa BR, Walia A (2007). Liver function tests and their interpretation. *Indian Journal of Pediatrics* 74:663-671.
- Tippani R, Porika M, Allenki V, Anreddy RNR, Reddy YN, Devaraconda K, Thammdala C, Abbagani S (2010). Antioxidant and analgesic activities of *Pterocarpus marsupium* Roxb. *Journal of Herbs Spices and Medicinal Plants* 16(1):63-68.
- Tipple TE, Rogers LK (2012). Methods for the determination of plasma and tissue glutathione levels. *Methods in Molecular Biology* 889:315-324.
- Traore AN, Anokwuru CP, Sigidi MT, Zininga T, Tshisikaw MP, Shonhai A, Ramaite IDI, Potgieter N (2016). Phenolic contents, antioxidant activity and spectroscopic characteristics of *Pterocarpus angolensis* DC stem bark fractions. *Journal of Traditional Medicine and Clinical Naturopathy* 5(2):61.
- Trease CE, Evans WC (1996). *Textbook of Pharmacology*, 14th ed. USA, W.B. Saunders Coy pp. 365-650.
- Valko M, Leibfricit D, Moncol J, Cronin MTD, Mazur M, Telser J (2007). Free radicals and antioxidants in normal physiological functions and human diseases. *International Journal of Biochemistry and Cell Biology* 39:44-84.
- Ward JW, Elsea JR (1997). *Animal care and use in drug fate and metabolism. Methods and techniques* Vol. 1. Edward RG, Jean LH (Eds.). Marcel Dekker, New York pp. 372-390.
- Weber LWD, Meinrad B, Andreas S (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Critical Reviews in Toxicology* 33(2):105-136.
- Weydert CJ, Cullen JJ (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissues. *Nature Protocols* 5(1):51-66.
- World Health Organisation (WHO) (2001). The World Health Organisation (WHO) recommended classification of pesticides by hazards and guidelines to classification 2000-2001, WHO, Geneva.
- Yi-Fang C, Jie S, Xian-Hong WU, Rui-Hai L (2002). Antioxidant and anti-proliferative activities of common vegetables. *Journal of Agriculture and Food Chemistry* 50:6910-6916.
- Yoshikawa T, Naito Y (2002). What is oxidative stress? *Japan Medical Association Journal* 45(7):271-276.
- Zimmermann M (1983). Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16(2):109-110.