MODULATORY EFFECT OF LEAF EXTRACTS OF *Pterocarpus santalinoides* HOOK F. (RED SANDAL WOOD) ONACETAMINOPHEN-INDUCED HEPATOTOXICITY IN ALBINO RATS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

Michael Okey Enemali[1]*, Stanley Chidi Udedi[2], Kingsley Ikechukwu Ubaoji[2], Gambo Sunday Haruna[1] & Emmanuel Augustine[1]
[1]Department of Biochemistry & Molecular Biology, Nasarawa State University, PMB 1022, Keffi, Nigeria
[2]Department of Applied Biochemistry, Nnamdi Azikiwe University Awka, Anambra State, Nigeria
Corresponding author: mikenemali@nsuk.edu.ng

Received: April 12, 2019 **Accepted:** May 29, 2019

Abstract: There is increasing incidences of drug (paracetamol) toxicity resulting from its overdose and quests for the use of alternative medicinein the prevention and treatment of liver related ailments worldwide. Ethanol and aqueous leaf extracts of *Pterocarpus santalinoides* (Red Sandals wood) were screened for phytochemicals and *in vitro* antioxidant potentials. The extracts were administered to rats, to test for their protective effect on paracetamol-induced hepatotoxicity. Twenty five (25) rats were randomly selected and divided into five (5) groups of 5 animals each. Groups 1 and 2 animals were given feed and water only, group three were fed with the chow, water and pretreated with silymarin. Groups 4 and 5 were given feed, water with aqueous and ethanol leaf extract of the plant respectively. All the animals except those in group 1 were then administered with 2 g/kg of body weight single dose of acetaminophen. Using standard biochemical methods, the hepatoprotective effect of the extracts were assessed by determining the liver function and the activities of the liver antioxidant enzymes. Results show the presence of bioactive compounds in the extracts based on the solvent of extraction. The concentrations of proteins and the activities of the liver antioxidant enzymes were significantly increased, while the concentration of bilirubin and serum activities of ALT and ALP were markedly reduced by the extracts. In conclusion, both the aqueous and ethanol leaf extract of the plant showed the potency of protecting the liver from the toxicity induced by a single 2 g/kg body weight dose of acetaminophen.

Keywords: Antioxidant, hepatoprotective, hepatotoxicity, *Pterocarpus santalinoides*, silymarin

Introduction

Paracetamol is an analgesic and antipyretic drug used by people in all age brackets all over the world. It is safe at therapeutic dose, but cause severe liver injury when taken in overdose (Erica and Emily, 2014). The active principle in paracetamol is the acetaminophen. It is metabolically activated by cytochrome P_{450} enzymes to a reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI) that depletes glutathione (GSH) and covalently binds to proteins. There pletion of glutathione (GSH) has been found to prevent the toxicity (James *et al.*, 2009**).** NAPQI is formed by a direct two-electron oxidation of acetaminophen and can be detoxified by glutathione (GSH) to form anacetaminophen-GSH conjugate (Dahlin *et al*., 1984). After a toxic dose of acetaminophen, total hepatic GSH is depleted by as much as 90%, and as a result, the metabolite covalently binds to cysteine groups on protein, forming acetaminophen-protein adducts (Mitchell *et al*., 1973). Depletion of GSH which is an intrinsic antioxidant is capable of regeneration of reactive oxygen free radicals and hepatocellular fatty regeneration with central lobular necrosis of the liver. Overproduction of reactive oxygen species (ROS) can damage cellular biomolecules like nucleic acids, proteins, lipids, carbohydrates and enzymes resulting in several diseases (Halliwell and Guteridge, 1999). Living systems have specific pathways to overcome the adverse effects of these damages but sometimes these repair mechanisms fail to keep pace with such deleterious effects (Nilsson *et al*., 2004).

In chronic liver diseases caused by oxidative stress (alcoholic and non-alcoholic fatty liver diseases, drug- and chemical-induced hepatic toxicity), the antioxidant drugs such as silymarin can have beneficial effect, (Feher and Lengyey, 2017). Silymarin is the active ingredient in the branded drug, Sylibon 140 (a known hepatoprotective drug), manufactured by Micro Laboratory Ltd, India. Silymarin has cyto-protective activity mediated by its anti-oxidative and radical-scavenging properties (Křena and Walterovab, 2005). Silymarin focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants for treatment purposes or for the production of drugs (Dahanukar *et al*., 2001; Olamide and Mathew, 2013; Udochukwu *et al.*, 2015). Their use in ethnomedicine for the management of ailments stem from the presence of phytochemicals (Aja *et al.*, 2010). Various ailments had different methods of treatments ranging from the use of herbs, salts and animal parts and in some cases, plant parts or exudates which resemble a part in humans or secretions were used as medicines (Zaruwa *et al.*, 2016). Exploits with plants; *Cucurbita maxima* (Enemali,*et al* 2018), *Vernonia amygdalina* Delile/*Ocimum canum* Linn (Enemali and Udedi, 2018), *Allium cepa* Linn (Bamidele *et al.*, 2018), *Mormodica charantia* (Hague *et al*., 2011), *Glycyrrhiza glabra* (Duwiejua and Zeitlin, 2011), *Platycodon Radix* and *Bellis perennis* (Morikawa *et al*., 2008), *Bridellia ferruginea* (Iwu, 1983), *Leptedenia hastata* (Pers) Dec'ne. (Odugbemi, 2008), *Anisopus mannii* (Boye, 1983) were all products of the traditional health care giver's initiative, used as concoction, decoctions or inhaled. In Nigeria, vegetables have been proven to have medicinal values (Enemali *et al.*, 2018; Bamidele *et al.*, 2018; Enemali and Udedi, 2018). Pharmacological studies have demonstrated hepatoprotection, antioxidant and anti-inflammatory activities supporting the traditional uses of some vegetables (Perez, 2016; Ahmed, 2016). These plant extracts improve the functionality of the antioxidant system of the test rats (Rice-Evan *et al.*, 1996; Edeoga *et al.*, 2005; Sudha *et al.*, 2011; Imaga and Bamigbetan, 2013; Alamgir *et al.*, 2016).

Pterocarpus santalinoides is a tree specie in the legume family, fabaceae. It is usually an evergreen tree with a dense crown of more or less drooping branches, (Tropical plant) with compound leaves, flowers and bear fruits in the rainy season (Offor *et al*., 2015; Lemmens, 2008). The common names of *Pterocarpus santalinoides* are Red sandal wood (English), Ouokisse (French), Gunduru (Hausa), Nturukpa (Igbo), Gbengbe (Yoruba), Uturukpa (Igbede), (Lemmons, 2008; Offor *et al*., 2015).

The present study therefore, evaluated the modulation of paracetamol-induced hepatotoxicity by administration of leaf extracts of *P. santalinoides* Hook F (red sandal wood) to albino rats.

Materials and Methods

Materials

The plant material is *Pterocarpus santalinoides* (Red sandal wood) leaf obtained from Nzam, Anambra West Local Government Area of Anambra state, Nigeria.

The drug, acetaminophen was a research support from Emzor Pharmaceutical Ltd, Lagos.

Silymarin is a branded drug (Sylibon 140) fromMicro Laboratory Ltd, India, purchased from a local pharmaceutical shop in Keffi, Nasarawa State, Nigeria.

Twenty five (25) albino rats weighing between 160 – 180 g were used for the study. These rats were purchased from the animal house of the National Veterinary Research Institute (NVRI), Vom, in Plateau State, Nigeria.

Methods

Plant materials collection and preparations

The leaves of *Pterocarpus santaliniodes* collected from a fallowed farmland in Nzam, Anambra West Local Government Area of Anambra State, Nigeria. The leaves were authenticated atthe Department of Plant Science and Biotechnology of Nasarawa State University, Keffi, and assigned the voucher number NSUH-00286B and deposited at the University Herbarium. The leaves were rinsed in water to remove dust and sand particles, and then dried under room temperature for twenty one (21) days. The dried leaves were then pulverized using Waring laboratory blender. Absolute ethanol (99.9%) from Sigma Chemical Company, London and distilled water were separately used to extract the bioactive ingredients from the leaves.

Plant materials preparation

The leaves of the plant under study were rinsed in water to remove dust and sand particles, and then dried under room temperature for twenty one (21) days. The dried leaves were then ground into powder using electric blender and was used for preparation of aqueous and ethanol extracts.

Qualitative phytochemical screening of the leaf extracts

Portions of the concentrated extracts were used for phytochemical screening using standard procedures of the Association of Analytical Chemist to identify the constituents as described by Harborne (1973), Odebiyi and Sofowora (1978), Fadeyi (1983), and Sofowara (1993).

Handling of experimental animals

They were housed in clean, well ventilated metal cages in the animal house of the Department of Biochemistry and Molecular Biology in Nasarawa State University Keffi. The animals were kept under 24 h light/dark cycling. They were allowed access to unlimited food and water supply and allowed to acclimatize for two (2) weeks before the commencement of the study.

The animals were divided into five (5) groups of five animals each. These include three (3) control groups; Normal Control given water and feeds only, Negative Control fed with water, and feed plus administration with 2 g/kgb.w of Acetaminophen and Positive Control which had water, feed, 400 mg/kg body weight of Silymarin and 2 g/kg body weight of Acetaminophen. The other two groups were administered with 400 mg/kg b.w of the aqueous leaf extract or the ethanol leaf extract of the plant – *P. santalinoides* in addition to the feed, water and later intoxicated with 2 g/kg body weight of Acetaminophen

Collection of blood samples

At the end of the experimental period, (after 12 h of intoxication), the animals were made unconscious by exposure to chloroform in an enclosed container, according to the method described by Ekor *et al.* (2006). Incisions were quickly made into the animals' cervical region with the aid of sterile blades. Blood samples were collected by cervical decapitation into plain tubes. Serum was prepared by centrifuging the clotted blood in a HSC (1000-4000 rpm) bench centrifuge at 3000 rpm for 10 min.

Preparation of liver homogenate

After bleeding, the livers were carefully removed, trimmed of extraneous tissues and rinsed in ice-cold 1.15% KCl. The livers were then blotted dry, two grams (g) was weighed and homogenized in 8 milliliters (8 ml) of ice-cold phosphate buffer (100 mM, pH 7.4). The homogenate were then centrifuged first at 6,000 rpm for six minutes (6 min) to remove nuclear debris after which they obtained supernatant were centrifuged at 10,000 rpm for twenty minutes to obtain the post-mitochondrial supernatant (PMS),using a refrigerated centrifuge. This was used for the assay of the antioxidant enzymes (Super Oxide Dismutase, Catalase and Glutathione Peroxidase).

Determination of biochemical parameters

The prepared serum was analyzed for various biochemical parameters as stated below; Aspartate aminotransferase (AST)–King, (1965a), Alanine aminotransferase (ALT)–King, (1965a) and Alkaline phosphatase (ALP)–King, (1965b), lipid profile (*Triacyl glycerides*, Total Cholesterol, High and low density lipoproteins), total protein - Lowry *et al*., (1951), albumin and bilirubin - Malloy-Evelyn, (1937), using spectrophotometric procedures. Superoxide Dismutase activity was determined by the method described by Sun and Zigma (1978) and catalase activity was determined according to the method of Beers and Sizer as described by Usoh *et al*., (2005). While the method of Lawrence and Burk (1976) was employed to measure the activity of GSH-Px.

Statistical analysis

Data was expressed as means ± standard deviation (SD). The statistical tools used for the analysis was one way analysis of variance (ANOVA) and the post hoc Newmann Keul's multiple comparisons test. The computer software utilized were Microsoft excel 2016 edition and SPSS 16.0 for windows. Differences between means were considered significant at $p<0.05$.

Results and Discussion

The qualitative phytochemical analysis of the aqueous and ethanol leaf extracts of *P. santalinoides* showed the presence of alkaloids, tannins and sterols in both extracts. Saponins were detected only in aqueous extract while cardiac glycosides, triterpenoids, balsam and resins were found only in the ethanol extract as shown in Table 1.

A look at the (DPPH), scavenging activity of aqueous and ethanol extracts of the leaves used in this study using Ascorbic acid as standard (Fig. 1), showed that there was an increasing scavenging activity with increase in concentrations of the leaf extracts. The *in vitro* antioxidant property is comparable to that of Ascorbic acid at the concentration of 0.5 mg/L.

Table 1: The qualitative phytochemical compositions of aqueous and ethanol leaf extracts of *Pterocarpus santalinoides*

Parameters	Aqueous	Ethanol
Alkaloid	+	+
Tannin	+	+
Phenols	+	+
Cardiac glycosides	-	+
Triterpenoid	-	+
Sterol	+	+
Saponin	+	-
Balsam	-	+
Resin	-	+

+ … indicates presence; - … indicates absence

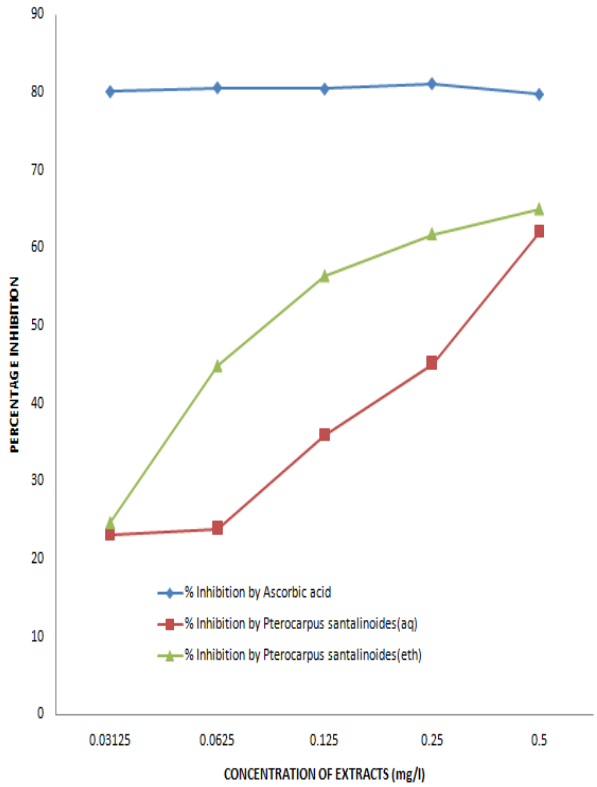

Fig. 1: The free radical, (DPPH), scavenging activity of aqueous and ethanol leaf extracts of *Pterocarpus santalinoides* using Ascorbic acid as a standard

The oral administration of a single 2 g/kg body weight dose of acetaminophen to the rats elicited a significant ($p<0.05$) decrease on the serum albumin and total protein concentration and increased the serum concentration of bilirubin (Table 3). The oral administration of 2 g/kg body weight single dose of acetaminophen to the rats (negative control), that were not pre-treated with either the aqueous or ethanol leaf extracts of the plants, induced some level of liver injury. This was evidenced by the significant ($p<0.05$) increase of the serum activities of the Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) of the rats in this group as shown in Table 2. Al-Mamary, (2002) reported that the aminotransferases are abundant in the liver and are released into the blood stream following hepatocellular damage, making them sensitive markers of liver damage. Therefore, a marked increase in the serum ALT and AST activities is indicative of liver damage. It has also been established that aminotransferases are cytoplasmicin location and are released into the circulating blood only after structural damage therefore, the serum activities are used as an indicator of damage to the liver structural integrity (Okediran *et al*, 2014). A marked rise in the serum activity of ALT, reduction in total serum protein and abnormal increase in serum bilirubin had been reported in hepatotoxicity (Martin and Friedman, 1992; Olorunnisola *et al.*, 2011; Olamide and Mattew, 2013). A decrease in total protein and albumin shows that the ability of the liver to synthesize/metabolize protein (example albumin), may have been impaired (Enemali *et al.*, 2018). This decrease in the albumin and total protein concentration could also be attributable to the binding of the proteins by the N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI is an oxidative product of acetaminophen metabolism that binds covalently to the sulphydryl groups of proteins resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver causing hepatotoxicity (Mitchel *et al.*, 1973). The 7 day pretreatment of the animals with 400 mg/kg body weight of either aqueous orethanol leaf extract of the plant, *Pterocarpus santalinoides*, elicited significant ($p<0.05$) decrease in the activity of ALT and ALP and a significant increase in serum activity of AST and the serum concentrations of the albumin of the animals administered with 2 g/kg body weight of acetaminophen (Table 2). The administration of 2g/kg body weight of acetaminophen reduced significantly ($p<0.05$) the activities of the SOD and GPx in the liver homogenate of the negative control group. The activity of the CAT was minimally affected by the intoxication with the stated dose of acetaminophen compared to the untreated animals. Results from the present study provides evidence of the induction of oxidative stress twelve hours (12 h), following acute acetaminophen (APAP), intoxication. However, the pre-treatment of the animals (rats), with 400 mg/kg body weight of either aqueous or ethanol leaf extracts of *P. santalinoides* gave protection against toxicity which may have occurred as a result of oxidative stress induced by the administration of a 2 g/kg body weight single dose of drug. This protection is attributable to the antioxidant properties of the plant, which stem from its bioactive components. Researchers have found that phytochemicals have the potential to reduce oxidative damage to cells (Huang *et al.*, 1992; Densie, 2013). However, it is not known whether the health benefits are the result of individual phytochemicals, the interaction of various phytochemicals, the fibre content of plant foods, or the interaction of phytochemicals and the vitamins and minerals found in the same foods. It was found in this study that *P. santalinoides* is a rich source of phytochemicals. Researches have suggested that there are no long-term stores of phytochemicals like the polyphenols in the body (Erdman *et al.*, 2007). Aside from inherent differences in the bioavailability of these compounds, absorption also is affected by the gut microflora and individuals' genetic makeup, both of which vary greatly (Da Costa *et al.*, 2012). In addition, processing, such as steaming, drying, freezing, and boiling, can reduce the levels of some phytochemicals found in the final food product (Mahn, 2012).

Table 2: Effect of pre-treatment with 400 gm/kg body weight aqueous and ethanol leaf extracts of *Pterocarpus santalinoides* on the serum activities of AST, ALT and ALP of rats intoxicated with 2 g/kg body weight single dose of acetaminophen

Treatment	AST (u/l)	ALT (u/l)	ALP (u/l)
Normal control	41.90±3.30	27.90±8.20	86.30±58.80
Standard control	42.80±10.80	17.90±3.10	167.30±107.70
Negative control	$67.40\pm26.40^{++}$	$117.30\pm57.50^{++}$	$209.80\pm67.00^{++}$
P. santalinoides (aq)	102.82±9.50*	51.75±9.60**	147.67±6.00**
P. santalinoides (eth)	182.75±44.90*	20.03±13.50**	101.00±42.80**

Values are mean ± SD of five (5) results, * and ** show values with significant increase and decrease respectively, compared to the Negative control while $^{++}$ and $^{-}$ indicate values with significant increase and decreases respectively compared to the Normal control.

Table 3: Effect of pre-treatment with 400 gm/kg body weight leaf extracts of *P. santalinoides* on the serum concentrations of total protein, albumin, and bilirubin of rats intoxicated with 2 g/kg body weight single dose of acetaminophen

Treatment	Total Protein (g/dl)	Albumin (g/dL)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Indirect. Bilirubin (mg/dl)
Normal control	75.10±5.70	18.67±5.00	12.50±2.70	5.30±1.40	5.90±0.70
Standard control	76.80±4.50	4.70±2.30	9.00±5.20	6.50±2.90	4.00±3.30
Negative control	62.80±1.20	$4.00\pm1.42^{--}$	$16.40\pm4.20^{++}$	$4.30\pm2.50^{--}$	$7.20\pm1.90^{++}$
P. santalinoides(aq)	76.15±4.80*	14.00±2.30*	3.97±1.80**	2.47±1.80**	1.50±1.10**
P. santalinoides(eth)	77.15±6.40*	16.00±5.70*	2.75±0.90**	1.9±1.10**	1.76±1.10**

Values are mean ± SD of five (5) results, * and ** show values with significant increase and decrease respectively, compared to the Negative control while $^{++}$ and $^{--}$ indicate values with significant increase and decreases respectively compared to the Normal control

The generation of superoxide radicals was reported to be inhibited by tannins and related compounds, (Chung, *et al.,* 1998). In this research, it was found that both the aqueous and ethanol leaf extracts contain tannins, phenols, and alkaloids, while only the aqueous extract contains the saponins. The non-sugar part of saponins has been reported to have a direct antioxidant activity (Alamgir *et al.,* 2016). The *in vitro* antioxidant assay of the aqueous leaf extracts of these plants showed a progressive increase in DPPH radical scavenging properties with increase in the concentration on the extracts. This suggests that the bioavailability of these phytochemicals that leads to the antioxidant properties is a factor in their *in vivo* activities. It can therefore, be adduced that within the concentration of extracts used in this study, the higher the concentration of the bioactive constituents in the extracts, the higher the *in vitro* antioxidant potential of the sample in question. One concentration of the extracts that were used throughout the work and the metabolic processes are the only factors that affected the resultant effect of the pre-treatment of these animals with the extracts. The effects of these leaf extracts on the biochemical parameters could be attributed to individual genetic differences of the organisms, the components of the plant and the metabolism of the materials (phytochemicals). This assertion is as a result of the fact that there was no defined pattern of effect based on the solvent of extraction in all the biochemical parameters assayed.

Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) are the primary intracellular defense mechanism to cope with increased oxidative stress. They eliminate superoxide anion and hydrogen peroxide that may oxidize cellular substrates and then prevent free radical chain reactions (Ekor *et al.,* 2006). Although only the activities of the SOD and GPx were significantly ($p<0.05$) reduced by the action of acetaminophen, in this study, all the antioxidant enzymes activities assayed in this study (CAT, SOD and GPx), were induced by the actions of the leaf extracts during the hepatic cell disruption induced by the administration of acetaminophen. The aqueous leaf extract of *P. santalinoides* significantly ($p<0.05$) increased the SOD, and GPx (even with higher values than Silymarin) when compared to the negative control. CAT activity was also significantly increased with lower than the effect elicited by Silymarin.

Table 4: Effect of pre-treatment with 400 mg/kg body weight of aqueous and ethanol leaf extracts of *P. santalinoides* on the activities of the liver antioxidant enzymes of rats intoxicated with a 2 g/kg body weight single dose of acetaminophen

Treatment	SOD (µ/mg)	CAT (µ/mg)	GPx (µ/mg)
Normal Control	0.05±0.01	3.00±2.30	347.00±13.20
Standard Control	0.20±0.40	10.21±3.90	174.60±16.30
Negative Control	0.02±0.01	3.00±2.60	$115.60\pm6.03^{--}$
P. santalinoides (aq)	0.07±0.02*	7.39±1.24*	313.79±37.58*
P. santalinoides (eth)	0.03±0.01*	8.1±3.00*	150.32±17.01*

Values are mean ± SD of five (5) results, * and ** show values with significant increase and decrease respectively, compared to the Negative control while $^{++}$ and $^{--}$ indicate values with significant increase and decreases respectively compared to the Normal control

Pre-treatment with ethanol leaf extract of all the plant elicited significant ($p<0.05$) increase in SOD and CAT activities of the rats although, the values were lower than what was obtained with pre-treatment with Silymarin. While pre-treatment with the ethanol extract markedly raised the activities of the GPx of the rats, even more than what was achieved by the standard drug.

Phenolic compounds including simple phenols and phenolic acids, hydroxyl cinnamic acid derivatives and flavonoids are bioactive substances occurring widely in food plants. Many phenolic compounds in plants are good sources of natural antioxidants. Phenol was found in the aqueous leaf extract and could be the cause of significant ($p<0.05$) increases in the activities of the antioxidant enzymes (SOD, CAT and GPx). Generally, the aqueous and ethanol leaf extracts of *P. Santalinoides* led to decrease of the serum activities of ALT and ALP of the animals pre-treated with them prior to the intoxication with acetaminophen, but markedly increased the serum activity of AST. However, the decreases in the activities of ALT and ALP in the serum of the rats pre-treated with these extracts were not as much as that caused by Silymarin. The extracts also markedly increased the serum albumin and total protein of the pre-treated rats suggesting the preservation of the hepatic lobular architecture. All these are indications of some degree of protection of the liver by the aqueous and ethanol leaf extracts of the plant under study. It can therefore, be suggested that these plants produced effects which may contribute to the preservation of cellular GSH levels in the acetaminophen-intoxicated rats, which further provides cellular defense both as a hydroxyl radical scavenger (Ekor *et al.,* 2006), and also a detoxifying agent against NAPQI, the toxic intermediate of acetaminophen in a GST-catalysed reaction.

From the foregoing, it could be suggested that *P. santalinoides* possess great deal of antioxidant potentials which can account for the preservation of the livers' lobular architecture resulting in the low activities of ALT and ALP in the serum of the pre-treated rats compared to the control group.

Conclusion

The present study provides evidence that *P. santalinoides* has antioxidant potentials and offered protection to the hepatic cells; thus encouraging and supporting, their use as edible vegetables and for therapy respectively. Therefore, they can be good sources of raw materials for drugs used for the prevention and treatment of liver diseases and other pathological conditions associated with oxidative stress. It is however, important to do an effective dose-response study to identify the dose range at which the leaf extracts are beneficial and to also clearly identify the specific compounds with the hepatoprotective properties in the leaf extracts of the plant.

Acknowledgement
Authors wish to acknowledge Emzor Pharmaceutical Ltd. Lagos, for providing the acetaminophen used in the work, as research assistance.

Conflict of Interest
The authors have declared that there is no conflict of interest on the work.

References

Ahmed A 2016. Nutrient, mineral and bioactive constituent evaluation of bamboo shoots grown in Masha area, South-West of Ethiopia. *Am. Scientific Res. J. Engr., Techn. and Sci.*, 15-25.

Aja PM, Okaka ANC, Onu PN, Ibiam U and Urako AJ 2010. hytochemical Composition of *Talinum triangulare* (Water Leaf) Leaves.*Pakistan Journal of Nutrition* 9 (6): 527-530.

Alamgir HM, Sheikh AM, Muniruddin A & Shahidulla KM 2016. Phytochemical and Pharmacological Investigation of *Lagenaria siceraria*, *Cucumis sativus* and *Cucurbita maxima. Eur. J. Medicinal Plants*, 12(3): 1-13.

Al-Mamary M, Al-Habori M, Al-aghbari AM & Basker MM 2002. Investigation into the toxicological effects of *Cathaedulis* leaves. A short term study in animals. *Phytotheraphy Research*, 16(2): 127-132.

Bamidele TO, Enemali MO & Ijeomah AU 2018. Hepatoprotective activity of ethanol extract of purple onion bulb (*Allium cepa* Linn) on CCl_4-induced hepatotoxicity in Wistar Rats. *FUW Trends in Sci. & Techn. J.*, 3(1): 149-152: www.ftstjournal.com.

Boye GL & Ampofo O 1983. In Proceedings of the first international symposium on Cryptolepine. University of Science and Tachnology, Kumasi, Ghana.

Chung KT, Wong TY, Wei CI, Haung YW & Lin Y 1998. US National Library of Medicine, National Institute of Health. *Critical Rev. in Food Sci. and Nutri.*, 38(6): 421-464.

Da Costa LA, Garcia-Bailo B, Badawi A & El-Sohemy A 2012. Genetic determinants of dietary antioxidant status. *Progress in Molecular Bio. and Translational Sci*., 108: 179-200.

Dahanukar A, Foster K, Van der Goes, Van Naters WM & Carlson JR 2001. A Gr eceptor is required for response to the sugar trehalose in taste neurons of *Drosophila*. *Nat. Neurosci.*, 4: 1182–1186.

Dahlin DC, Miwa GT, Lu AYH & Nelson SD 1984. N-Acetyl-p-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen. *Proceedings National Acad. Sci.*, USA, 81: 1327 – 1331.

Densie Webb 2013. Phytochemicals' role in good health. *Today's Dietitian*, 15(9): 70.

Edeoga HO, Okwu DE & Mbaebie BO 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotech.*, 4(7): 685 – 688.

Ekor M, Adekpoju GKA & Epoyun AA 2006. Protective effect of methanolic leaf extracts of *Persea americana* (Avocado) against paracetamol-induced acute hepatotoxicity in rats. *Int. J. Pharmacol.*, 2(4): 416-420.

Enemali MO & Udedi SC 2018. Comparative evaluation of the protective effect of leaf extracts of *Vernonia amygdalina* (bitter leaf) and *Ocimum canum* (curry) on acetaminophen induced acute liver toxicity. *Journal of Pharmacognosy and Phytotherapy*, 10(7): 116 – 125.

Enemali MO, Bamidele TO & Ubana MA 2018. Protective effect of ethanolic extract of *Cucurbita maxima* (Pumpkin) leaf on acetaminophen-induced acute liver toxicity. Journal of Pharmacognosy and Phytotherapy 10(8): 142-148.

Erdman JWJr, Balentin D & Arab L 2007. Flavonoids and heart health. *Journal of Nutrition*, 137(3 Suppl 1): 718S-737S.

Erica D & Emily MA 2014. Acetaminophen toxicity: What pharmacists need to know. *US Pharmacology*, 39(3): HS2-HS8.

Fadeyi M 1983. A handbook in traditional medicine practices inNigeria. Longman Nigeria, p. 35.

Halliwell B & Gutteridg JMC 1999. *Free Radicals in Biology and Medicine*. 3rd ed., Oxford University Press, New York, pp. 617–783.

Harborne JB 1973. Phytochemical methods. Chapman and Hall, London, p. 278.

Huang MT, Ho CT & Lee CY 1992. Phenolic Compounds in Food and their Effects on Health II. ACS Symposium Series; American Chemical Society: Washington, DC.

Imaga NOA & Bamigbetan DO 2013. *In vivo* biochemical assessment of aqueous extracts of *Vernonia amygdalina* (Bitter leaf). *Int. J. Nutr. and Metabolism*, 5(2): 22-27.

Iwu MM 1983. The hypoglycemic properties of *Bridellia ferruginea*. *Fitoterapia*, 54(6): 243-248.

James LP, Letzig L, Simpson PM, Capparelli E, Roberts DW, Hinson JA & Lee WM 2009. Pharmacokinetics of acetaminophen-protein adducts in adults with acetaminophen overdose and acute liver failure. *Drug Metabolism and Dispososal*, 37: 1779–1784.

Kanchana N & Mohamed SA 2011. Hepatoprotective effect of *Plumbago zeylanica*on paracetamol - induced liver toxicity in rats *Int. J. Pharmacy and Pharmace. Sci.*

King EJ 1965a. The Hydrolases-acid and Alkaline Phosphatases. In Van D (ed.): *Practical Clinical Enzymology*, Nostrand Co., London, pp. 199–208.

King EJ 1965b. The Transaminases: Alanine and Aspartate Transaminases. In Van D (ed.): *Practical Clinical Enzymology*, Nostrand Co., London, pp. 363–395.

Křena V & Walterovab D 2005. Silybin and silymarin – New effects and applications. *Biomedical Papers*, 149(1): 29–41.

Lemmens R 2008. .*Pterocarpus santalinoides*. In: Loupp D, Oteng A & Brink M (eds). PROTA4U (plant resource of tropical Africa/ Resources vegetables de l'Afrique tropicale) Wageningen, Netherlands. Retrieved from http://www.prota4u.org/search.aspon 5th March, 2016.

Lawrence R & Burk R 1976. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem. and Biophy. Res. Commun.*, 71: 952–958.

Lowry OH, Rosebrough NJ, Farr AL & Randal RJ 1951. Proteinmeasurement with Follin's phenol reagent. *Biological Chemistry*, 193: 265-275.

Mahn A & Reyes A 2012. An overview of health-promoting compounds of broccoli (*Brassica oleracea* var. Italic) and the effect of processing. *Food Sci. and Techn. Int.*, 18(6): 503-514.

Malloy HT and Evlyn EA 1937. The determination of bilirubin with photoelectric calorimeter. *J. Biol. Chem.*, 119: 481-485.

Martin P and Friedman LS 1992. Assessment of Liver Function on Diagnostic Studies in Friedman LS & Keefe EB (Eds). *Handbook of liver disease*. Churchill Livingstone, Philadelphia, pp. 1-14.

Mitchell JR, Jollow DJ, Potter WZ, Gillette JR and Brodie BB 1973. Acetaminophen-induced hepatic necrosis IV, Protective role of glutathione. *Journal of Pharmacological Experiment*, 187: 211-217.

Morikawa T, Li X, Nishida E, Ito Y, Matsuda H, Nakamura S, Muraoku O & Yoshikawa M 2008. Perennisoside I – VII, acytelated triterpene saponins with anti-hyperlipedemic activities from the flowers of *Bellis perennis. Journal of Natural Product* 71: 828 – 835.

Nilsson J, Stegmark R & Akesson B 2004. Total antioxidant capacity in different pea (*Pisum sativum*) varieties after blanching and freezing. *Food Chemistry*, 86, 501–507.

Odebiyi A, Sofowora AE 1978. Phytochemical screening of Nigeriamedicinal Plant Part III. *Lioydia* 4: 234-246.

Odubgemi T 2008. A Textbook of Medicinal Plants from Nigeria. University of Lagos Press, p. 107.

Offor CE, Nwankwegu NJ, Ugwu OPC & Aja PM 2015. The effects of ethanol leaf extract of *Pterocarpus santalinoides* on liver enzymes of albino rats. *American-Eurasian J. Agric. and Envtal. Sci.*, 15(5): 920-922.

Okediran BS, Olurotimi AE, Rahman SA, Michael OG & Olukunle JO 2014. Alterations in the lipid profile and liver enzymes of rats treated with monosodium glutamate. *Sokoto J. Veterinary Sci.*, 12(3): 42-46.

Olamide EA & Mathew OA 2013. Protective effects of *Enantia chlorantha* stem bark extracts on acetaminophen induced liver damage in rats. *Jordan J. Biol. Sci.* 6(4): 284-290.

Olorunnisola OS, Bradley G & Afolayan AJ 2011. Antioxidant properties and Cytotoxicity evaluation of methanolic extract of dried and fresh rhizomes of *Tulbaghia violacea*. *Afr. J. Pharmacy and Pharmaco.*, 5: 2390-2497.

Perez GRM 2016. Review of *Cucurbita pepo* (Pumpkin), its phytochemistry and pharmacology. *Perez Gutierrez, Medicinal Chemistry*. 6: 012-021.

Rice-Evans CA, Miller JM & Paganga G 1996. Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Bio. and Med.*, 20: 933-956.

Sofowora A 1993. Screening plants for bioactive agents. In: Medicinalplants and traditional medicine in Africa. 2nd Edition. Spectrum Books Limited, Ibadan, pp. 134-156.

Sudha M, Geetha RJS & Vaishnavi R 2011. Thephytochemical and antioxidant screening of *Justicia wynaadensis*. *Afr. J. Plant Sci.*, 5(9): 489-492.

Sun M & Zigma S 1978. An improved spectrophotometric assay of superoxide dismutase based on ephinephrine antioxidation. *Analytical Biochemistry*, 90: 81-89.

Udochukwu U, Omeje FI, Uloma IS & Oseiwe F D 2015. Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. *Am. J. Res. Commun.*, 3(5): 225-235.

Usoh FI, Akpan EJ, Etim EO & Farombi EO 2005. Antioxidant actions of dried flower of *Hibiscus sabidariffa* L. on sodium arsenite- induced oxidative stress. *Pak. J. Nutri.*, 4: 135-141.

Zaruwa ZM, Ibok NI, Ibok IU, Onyenonachi CE, Danchal C, Ahmed AG, Ahmed UM & Sudi YI 2016. Effects of *Sterculia setigera* Del. stem bark extract on haematological and biochemical parameters of wister rats. *Biochemistry Insight*, 9: 19–22.