HEPATOPROTECTIVE EFFECTS OF METHANOL LEAF EXTRACT OF *PTEROCARPUS SANTALINOIDES* DC ON ACETAMINOPHEN-INDUCED LIVER DAMAGE IN ALBINO RATS (RATTUS NORVEGICUS)

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ABSTRACT

This study investigated the effects of methanol leaf extract of *Pterocarpus santalinoides* on acetaminophen (APAP)-induced sub-acute liver damage in albino rats. Fresh leaves of *P. santalinoides* used for the study were collected, dried under shade, ground into powder, and extracted by cold maceration using absolute methanol. The extract obtained was filtered, dried, and subjected to phytochemical analysis. Forty two adult male albino rats were used for the study, 12 for the acute toxicity study and 30 for the *in vivo* evaluation of the hepatoprotective effects of the extract. The 30 rats used for the *in vivo* study were randomly assigned into 6 groups (A – F) of 5 rats each as follows: Group A – APAP + distilled water placebo (negative control), Groups B, C and D – APAP + 50, 250, and 500 mg/kg body weight (BW) *P. santalinoides* extract respectively, Group E – APAP + 100 mg/kg BW Silymarin (positive control), and Group F – distilled water placebo only (normal control). The APAP was given at 3000 mg/kg BW orally at the beginning of the study (day 0) and after every 72 hours for four more consecutive times (day 3, 6, 9, and 12), while treatment with the extract and Silymarin was done orally twice daily for 15 days. Blood for biochemical analysis was collected after the 15th day of treatment. All the rats were afterwards sacrificed humanely and the liver weight percentage of body weight determined. Biochemical assays, phytochemical analysis, and acute toxicity evaluation followed standard procedures. Results showed that the extract had high levels of tannins, moderate levels of flavonoids, saponins, carbohydrates and reducing sugars, and low levels of alkaloids. The LD_{50} was above 5000 mg/kg BW. Treatment with the extract at 500 mg/kg BW led to significantly lower ($P < 0.05$) serum ALT, AST, and ALP activities, cholesterol and bilirubin levels, and liver weight percentage of body weight when compared to the negative control, while the 250 mg/kg BW dose only led to significantly lower ($P < 0.05$) serum levels of cholesterol and bilirubin when compared to the negative control. It was concluded that treatment of acetaminophen-induced sub-acute liver damage in albino rats using 500 mg/kg BW methanol extract of *P. santalinoides* significantly protected the hepatocellular integrity and ameliorated impaired hepatic excretory function.

Key words – Hepatoprotection, *Pterocarpus santalinoides*, methanolic leaf extract.

INTRODUCTION

The liver is a vital organ responsible for the detoxification of various drugs and xenobiotics in the body. The central role the liver plays in the clearance and transformation of chemicals commonly exposes it to toxic injury (Singh *et al.*, 2011). Chemicals that cause liver injury (hepatotoxicants and/or hepatotoxins) include a wide variety of pharmaceutical agents, natural products, industrial chemicals, microbial toxins, environmental pollutants, herbal remedies and dietary supplements (Willett *et al.*, 2004; Papay *et al.*, 2009). Hepatotoxicity-induced disorders are reversible at early stages upon cessation of exposure to the hepatotoxicants. However, severe intoxication with these hepatotoxicants can lead to liver necrosis and death of the organism if left untreated. Despite various hepatoprotective drugs and herbal treatments available today, therapy for liver disease remains unsatisfactory and inadequate. Moreover, increasing incidences of xenobiotic-induced hepatotoxicity has become a driving force behind the search for more promising and possibly better hepatoprotective therapy.

Pterocarpus santalinoides DC is an indigenous Nigerian plant in the family *Papilionaceae*. It is commonly referred to as "Red Sandal wood" in English (Anowai *et al.*, 2012), and locally, it is known as *nturukpa* in Igbo, *gunduru*, *gyadar*, or *kurmi* in Hausa, *gbengbe* in Yoruba, *akumeze* in Edo, *nja* in Efik, *kereke* in Tiv, and *maganchi* in Nupe. Leaves of *P. santalinoides* are traditionally used as food (vegetable) and as medicine in the treatment of various ailments which includes diarrhoea and vomiting, dysentery, elephantiasis, malaria, cold, cough, and liver diseases (Adesina, 1982). Scientific reports on the medicinal use of the leaves of *P. santalinoides* for the treatment of liver diseases is however lacking in available scientific literature. The objective of this study therefore was to evaluate the effects of methanol leaf extract of *P. santalinoides* DC on acetaminophen-induced liver damage in albino rats.

MATERIALS AND METHODS

Fresh leaves of *P. santalinoides* used for the study were collected in February 2016 from Nsukka, Enugu state, Nigeria. The plant was identified by a plant taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The leaves were dried under shade, pulverized, and ground into powder. Five hundred (500 g) of the powdered leaves was extracted with absolute methanol using the cold maceration method. The resulting extract was filtered using Whatmann size 1 filter paper and concentrated to dryness with a rotary evaporator. Phytochemical analysis was carried out on the extract following the standard procedure described by Harborne (1998).

Forty two (42) adult male albino rats (*Rattus norvegicus*) of 12 weeks of age weighing between 250 g – 300 g, were obtained from the Laboratory Animal Unit of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, and used for the study. The albino rats were housed in stainless steel cages in a fly proof animal house at room temperature, and allowed 2 weeks to acclimatize before the commencement of the study. They were fed commercial pelletized feed (Grand Cereals Ltd, Jos, Nigeria), and were provided with clean water *ad libitum* all through the study. Twelve of the albino rats were used for acute toxicity test, and median lethal dose (LD_{50}) of the extract was determined following the new approach to practical acute toxicity testing as described by Lorke (1983). Thirty (30) male albino rats were used for the *in vivo* study of the effects of the extract on acetaminophen-induced experimental liver damage. The rats were randomly assigned into six (6) groups (A, B, C, D, E and F) of five rats each. Groups A, B, C, D, and E were given 3000 mg/kg body weight (BW) acetaminophen orally at the beginning of the experiment (day 0), and after every 72 hours for 12 days (days 0, 3, 6, 9 and 12). Treatment with the extract was done *per os* twice daily for 15 days as follows: group A served as negative control (given acetaminophen and treated with 10 ml/kg BW distilled water placebo), groups B, C and D were given acetaminophen and treated with 50, 250, and 500 mg/kg BW extract respectively, group E was given acetaminophen and treated with silymarin (a standard hepatoprotective drug) at the dose of 100 mg/kg BW *per os* twice daily for the 15 days as positive control, while group F was not given acetaminophen and was treated with 10 ml/kg BW of distilled water placebo and served as normal control. Blood samples were collected from the albino rats on day 15 (at the end of the experiment) for serum biochemistry assay. Blood sample collection was by the orbital technique, while the serum biochemistry assay was done immediately upon separation of the serum from blood clot, following standard procedures (Colville, 2002) using Quimica Clinica Applicada (QCA) test kits (QCA, Spain). The serum enzymes and bio-chemicals evaluated were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol, total bilirubin, total proteins, albumin and globulin. The rats were humanely sacrificed afterwards on the 15th day. Gross changes in the liver were observed, and relative liver weight of body weight of the individual rats was determined. The handling, management and use of the albino rats in this experiment were in conformity with the Guide to Humane Laboratory Animal Use.

Data obtained from the study were subjected to one way analysis of variance (ANOVA), and variant means were separated post hoc using the least significant difference (LSD) method. Significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

The methanolic leaf extract of *P. santalinoides* was dark green in color and soluble in water. The percentage yield of the extract was 18% weight/weight. The phytochemical analysis of the extract showed the presence of high levels (+++) of tannins, moderate levels (++) of flavonoids, saponins, carbohydrates and reducing sugars and low levels (+) of alkaloids. These phytochemicals are essential compounds commonly found in various herbs used for medicinal purposes. Phytochemicals similar and comparable to the ones we recorded in this study had also been reported by Anowai *et al.* (2012), Eze *et al.* (2012) and Odeh *et al.* (2014) in the ethanol extracts of the leaf and stem bark of *P. santalinoides*.

The acute toxicity test showed that there was no mortality or any obvious sign of toxicity in all the rats treated with the extract, and that the LD_{50} is above 5000 mg/kg bw. This result places *P. santalinoides* methanol extract within the World Health Organization's (WHO) category of substances "unlikely to present acute hazard in normal use" (WHO, 2001). This implies that the methanolic leaf extract of *P. santalinoides* is safe for acute use as treatment of ailments and diseases for which it is effective. This finding is in agreement with the reports of Anowai *et al.* (2012) and Eze *et al.* (2012) on *P. santalinoides* leaf and stem bark extracts, respectively.

The serum ALT activity of rats in groups A, B and C were significantly higher ($P < 0.05$) than and ranged from two to three times that of groups D, E and F, while the serum AST activity of groups A and B were significantly higher ($P < 0.05$) and almost two times those of groups D, E and F (Table 1). The serum ALP activity of rats in groups A, B, C, D and E were significantly higher ($P < 0.05$) than, and ranged from two to three times that of group F rats, though that of groups D and E were significantly lower ($P < 0.05$) than those of groups A, B and C (Table 1). The serum total cholesterol levels of group A rats were significantly lower ($P < 0.05$) than those of group C, D, E and F rats, while the serum bilirubin levels of rats in groups A and B were about two times those of rats in groups D, E and F, and was found to be significantly higher ($P < 0.05$) than those of groups C, D, E and F (Table 1).The significantly higher serum enzyme activity of ALT, AST and ALP, and higher serum levels of cholesterol and bilirubin in the group A rats and some others that were given acetaminophen and treated are indicators of the ability of acetaminophen to induce liver damage by disrupting hepatocellular integrity and liver function (Sun *et al.*, 2009). The finding in this study that rats in group D which were given acetaminophen and treated with 500 mg/kg bw extract had serum ALT, AST and ALP activities significantly lower than that of group A and comparable to that recorded for groups E and F implied that the administration of the extract at the dose of 500 mg/kg bw was protective of the hepatocellular integrity of the liver and compared effectively with silymarin (a standard hepatoprotective drug). This finding is in agreement with the reports of Offor *et al.* (2015) who studied the effects of ethanol leaf extract of the plant on liver enzymes. The significantly higher serum total cholesterol (TC) of rats in group A, and total bilirubin of rats in groups A and B when compared to other groups is an indication that the excretory function of the liver of rats that were given acetaminophen were impaired, and that treatment with the extract ameliorated the impairment.

There were no significant variations ($P > 0.05$) in the levels of serum proteins and albumins between the rat groups, but serum globulin levels of rats in group A and B were significantly lower ($P < 0.05$) than that of the group F rats (Table 2). The liver weight percentage of rats in groups A and B were however significantly higher ($P < 0.05$) than those of rats in groups D, E and F (Table 2). The lack of significant variation between the rat groups in their serum levels of total proteins and albumins indicates that sub-acute administration of acetaminophen as used in the study did not significantly affect the protein synthetic function of the liver. The significantly lower serum globulin levels of the group A rats when compared to group F rats may be attributed to the negative effects of acetaminophen on other body organs (including the lymphoid organs) apart from the liver (Sun *et al.*, 2009). The higher liver weight percentage of body weight of rats in groups A and B is an indication of inflammation and/or degeneration which accompanies hepatotoxicity (Saukkonen *et al.*, 2006; Sun *et al.*, 2009). Treatment with the extract at 500 mg/kg BW and silymarin at 100 mg/kg BW significantly reduced this

inflammation/degeneration induced enlargement caused by acetaminophen toxicity.

CONCLUSION

Based on the results of the study, it was concluded that treatment of acetaminophen-induced hepatic injury in albino rats with the methanolic leaf extract of *P. santalinoides* at the dose of 500 mg/kg BW led to significantly lower ($P < 0.05$) serum ALT, AST, and ALP activity, cholesterol and bilirubin levels, and liver weight percentage of body weight of the treated rats when compared to the negative control. Also treatment with the extract at 250 mg/kg BW and 500 mg/kg BW led to significantly lower ($P < 0.05$) serum levels of total cholesterol and bilirubin of the treated rats when compared to the negative control. These results substantiate the validity of the traditional use of the leaves of *P. santalinoides* in the treatment of liver diseases, and also strongly suggests that the methanolic leaf extract of *P. santalinoides* can be safely used for the treatment of sub-acute toxic liver injury.

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Table 1. Effects of oral administration of *Pterocarpus santaniloides* extracts (PSE) on serum enzymes activity, cholesterol and bilirubin levels of rats given sub-acute toxic doses of acetaminophen (APAP).
[ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatase; TChol. = Total Cholesterol; TBil. = Total bilirubin]

Groups	Mean ± standard error				
	ALT (U/L)	AST (U/L)	ALP (U/L)	TChol. (mg/dl)	TBil. (mg/dl)
Group A	96.38±4.79^{a}	108.43±6.83^{a}	345.16±43.27^{a}	78.23±6.87^{a}	0.64±0.04^{a}
Group B	89.74±6.86^{a}	106.94±7.02^{a}	363.21±30.67^{a}	68.74±8.28^{ab}	0.58±0.06^{a}
Group C	72.43±5.37^{a}	83.24±6.44^{ab}	339.83±29.42^{a}	63.44±3.76^{b}	0.43±0.05^{b}
Group D	46.32±4.28^{b}	61.11±5.23^{b}	286.33±30.26^{b}	59.25±4.21^{b}	0.32±0.04^{c}
Group E	43.65±5.02^{b}	62.43±4.79^{b}	256.39±18.21^{b}	64.32±5.43^{b}	0.30±0.04^{c}
Group F	33.66±4.73^{b}	57.29±5.03^{b}	105.28±24.23^{c}	61.77±3.48^{b}	0.31±0.05^{c}

Alphabetical superscripts in a column indicate significant differences between the groups, $P < 0.05$
* Group treatments: Group A - APAP + distilled water placebo; Group B - APAP + 50mg/kg bw of PSE; Group C - APAP + 250mg/kg bw of PSE; Group D - APAP + 500mg/kg bw of PSE; Group E = APAP + 100mg/kg bw Silymarin; Group F - Distilled water placebo only

Table 2. Effects of oral administration of *Pterocarpus santaniloides* extracts (PSE) on serum proteins and liver weight percentage of body weight of rats given sub-acute toxic doses of acetaminophen (APAP).

Groups	Mean ± standard error			
	Total proteins (g/dl)	Albumins (g/dl)	Globulins (g/dl)	Liver weight % of body weight (%)
Group A	7.03±0.32	3.20±0.18	3.82±0.12^{a}	4.53±0.18^{a}
Group B	7.01±0.27	3.27±0.20	3.75±0.16^{a}	4.37±0.14^{a}
Group C	7.25±0.18	3.32±0.14	3.93±0.15^{ab}	4.29±0.12^{ab}
Group D	7.36±0.20	3.34±0.18	4.01±0.17^{ab}	4.11±0.14^{b}
Group E	7.41±0.19	3.38±0.16	4.02±0.16^{ab}	4.02±0.19^{b}
Group F	7.43±0.21	3.36±0.12	4.07±0.15^{b}	3.93±0.24^{b}

Alphabetical superscripts in a column indicate significant differences between the groups, $P < 0.05$
* Group treatments: Group A - APAP + distilled water placebo; Group B - APAP + 50mg/kg bw of PSE; Group C - APAP + 250mg/kg bw of PSE; Group D - APAP + 500mg/kg bw of PSE; Group E = APAP + 100mg/kg bw Silymarin; Group F - Distilled water placebo only