Full Length Research Paper

Haematology, serum biochemistry and histopathological findings associated with sub-chronic administration of methanol leaf extract of *Pterocarpus santalinoides* DC in albino rats

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The effects of sub-chronic administration of *Pterocarpus santalinoides* methanol leaf extract (PSME) on haematology, serum biochemistry and histology of albino rats were evaluated. Twenty female albino rats, randomly assigned into four groups (A-D) of five rats each, were used for the study. Groups A-C were treated orally with 500, 250 and 50 mg/kg PSME, respectively, while Group D was given distilled water as placebo at the dose of 10 ml/kg (untreated control). Treatment was done daily for 3 months after which blood samples were collected for haematology and serum biochemistry. After blood sample collection, the rats were weighed and humanely euthanized. The liver, kidneys, heart and spleen were eviscerated and weighed, and relative organ weights calculated. Thin slices of the liver and kidneys were processed for histopathology. Results showed no significant (p > 0.05) differences between the groups in all the haematological parameters assayed. The PSME (50 mg/kg) led to significantly (p < 0.05) higher serum albumin level, and at 250 and 50 mg/kg, it led to significantly (p < 0.05) lower serum creatinine level and mild random vacoulation of the hepatocytes. The PSME at all doses used in the study led to significantly (p < 0.05) higher body weights and significantly (p < 0.05) lower relative spleen and heart weights. It also caused moderate hyperaemia of the renal cortex at 500 mg/kg. It was concluded that oral sub-chronic administration of graded doses of methanol leaf extract of *P. santalinoides* to albino rats is non-toxic.

Key words: *Pterocarpus santalinoides*, methanol extract, sub-chronic toxicity, haematology, serum biochemistry, histopathology.

INTRODUCTION

Medicinal plants are widely used for the prevention and treatment of various diseases and ailments worldwide.

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and more especially in Africa and other developing countries, where a reasonable percentage of the population rely on indigenous plants for the treatment of various ailments (Acaray and Anshu, 2008; Obidah et al., 2009). The rich floral biodiversity of Africa has provided herbal health practitioners with an impressive pool of ‘natural pharmacy’ from which plants are selected as remedies or as ingredients in the preparation of herbal medicines for the treatment, management and/or control of an array of disorders (Salawu et al., 2008). These herbs are generally accessible, affordable and acceptable to the consumers (Sofowora, 1985). However, intake of herbal medicines or drugs could cause alteration of the normal haematological and blood biochemical parameters of the body (Ayman, 2013). Blood acts as a pathological reflector of the status of exposed animals to toxicants and other conditions (Aderemi, 2004; Afolabi et al., 2010; Olafedehan et al., 2010).

Blood constituents change in relation to health conditions. These changes are of value in assessing the response of animals and humans to various physiological and pathological challenges and situations (Sancho et al., 2000). These alterations may also serve as indicators of adverse effects of these substances to the body system, thus, haematology and serum biochemistry parameters, and histopathology can be used as indicators of toxicity (Iwu, 1993; Ojiako and Nwanjo, 2006). The evaluation of toxic potentials of medicinal plants is thus crucial when considering such plants for public use.

Haematological, biochemical and histopathological changes are often used to evaluate the health status of the body and to determine the impact of stresses due to nutritional and/or pathological factors (Amaechi, 2009). In practice, evaluation of toxicity typically includes the assessment of acute, sub-chronic, chronic, carcinogenic and teratogenic effects (Subramanion et al., 2011; Asante-Duaah, 2002). The problem with claims about some medicinal plants has been that of insufficient scientific data on the efficacy and safety of the plants to back up the claims (Briejer et al., 2001). Therefore, there is need to validate/establish scientific bases for the safe ethno-medical uses of medicinal plants and to determine their toxicological profiles.

Pterocarpus santalinoides DC (Figure 1) is a plant indigenous in tropical Western and Southern Africa, and South America. It belongs in the Family Papilionaceae (Fabaceae) (Keay, 1989; Ogan, 2004; Adetunji, 2007). It is an evergreen small tree up to 15 metres tall (Keay, 1989). Pterocarpus santalinoides is commonly known in English language as “red sandal wood” (Adetunji, 2007; Anowi et al., 2012), and as nturukpa in Igbo language of Eastern Nigeria (Anowi et al., 2012). In Eastern Nigeria, the tender leaves are used as vegetable in soups, while the leaf extracts are used ethno-medically in the treatment of various ailments which include diarrhoea and vomiting, dysentery, elephantiasis, malaria, cold, cough, heart and liver diseases (Adesina, 1982; Okwu and Ekeke, 2003). Phytochemical analysis of the leaf extract of P. santalinoides DC showed the presence of flavonoids, tannins, terpenes, alkaloids, glycosides, saponins, carbohydrates, phenol and sterols, in varying concentrations (Anowi et al., 2012; Eze et al., 2012; Odeh and Tor-Anyim, 2014; Ihedioha et al., 2017; 2018; 2019; Enemali et al., 2019). The traditional use of the leaves of P. santalinoides both as a vegetable and medicament in humans, and as fodder or medicine for livestock has not been reported to be associated with any form of toxicity (Poppenga, 2007; Tiwari and Sinha, 2010; Anowi et al., 2012). Previous experimental studies have shown that methanol leaf extract of P. santalinoides is acutely non-toxic, has lipid and glucose lowering properties, and also possesses hepatoprotective and antioxidant activities (Anowi et al., 2012; Offor et al., 2015; Ihedioha et al., 2017; 2018; 2019; Obi et al., 2019). In view of the numerous traditional uses of P. santalinoides leaves in the treatment and management of various ailments (Adesina, 1982; Okwu and Ekeke, 2003) and the results of these previous studies (Anowi et al., 2012; Offor et al., 2015; Ihedioha et al., 2017; 2018; 2019; Obi et al., 2019), the purpose of this present study was to evaluate the effects of sub-chronic administration of graded doses of methanol leaf extract of P. santalinoides DC on haematology, serum biochemistry and histopathology of albino rats (Rattus norvegicus).

MATERIALS AND METHODS

Assay kits, chemicals and reagents

The serum biochemistry assay kits for the evaluation of the serum enzyme activity concentrations of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum levels of total cholesterol, total proteins and albumins, and kidney function test kits for serum creatinine determination were sourced from Quimica Clinica Applicada (QCA), Spain. The test kit for the evaluation of serum total bilirubin was sourced from Randox Laboratories Ltd, County Antrim, United Kingdom, while that for serum urea evaluation was procured from DiaLAB, Neudorf, Austria. The glucometer and blood glucose test strips used for the evaluation of fasting blood glucose levels were sourced from Roche Diagnostics GmbH, Mannheim, Germany. Sodium thiopentone was obtained from Chandra Bhagat Pharma Pvt., Ltd., Mumbai, India. All other routine chemicals and reagents used for the study were of analytical grade.

Collection and identification of plant material and preparation of plant extract

Fresh leaves of P. santalinoides DC were collected from Doidu Nru in Nsukka Local Government Area of Enugu State, Nigeria, in January 2018. The plant was identified by Mr. A.O. Oziko, a plant taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A voucher specimen (UNH/2018 No. 2) was deposited at the University of Nigeria herbarium. The leaves were spread under shade to dry, and then ground into powder. Five hundred grams (500 g) of the powdered leaves were extracted with 80% methanol using the cold maceration extraction technique, with intermittent shaking at 2-h interval for 48 h. The extract obtained
was filtered with Whatman size 1 filter paper, and the filtrate was evaporated to dryness in a rotary evaporator (Buchi, Switzerland). The dried extract was stored in a refrigerator at 4°C until time of use, and was referred to as *P. santalinoides* methanol extract (PSME).

**Experimental animals**

Twenty female albino rats (*R. norvegicus*) of twelve weeks of age, and body weight between 220 - 240 g, were used for the study. They were sourced from the Laboratory Animal Unit of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. The albino rats were housed in stainless steel cages in a fly proof animal house at room temperature between 24 – 28°C, and allowed 3 weeks to acclimatize before the commencement of the study. They were fed commercial rat pellets (Grand Cereals Nig. Ltd, Jos, Nigeria), which comprised 2600 Kcal/kg metabolizable energy, 8% fat, 13% crude protein, 0.35% phosphorus, 15% crude fiber and 0.9% calcium. Clean drinking water was made available to the rats *ad libitum*. The albino rats were humanely handled and well cared for all through the study period. Guidelines for the use of animals for laboratory experiments were strictly adhered to (Zimmermann, 1983; Ward and Elsea, 1997). The protocol for the laboratory animal experiment 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).

**Effects of PSME on haematology, serum biochemistry and histology of albino rats**

The twenty female albino rats used for the study were randomly assigned into four (4) groups (A – D) of 5 rats each. Groups A – C were treated daily with 500, 250, and 50 mg/kg PSME, respectively, while Group D was given distilled water as placebo at the dose of 10 ml/kg as untreated control. Treatment was done orally for 3 months. Blood samples were collected at the end of the study for haematology and serum biochemistry. Blood sample collection was...
Blood for serum biochemistry was collected into clean test tubes and allowed to stand at room temperature for 30 minutes to clot. It was then centrifuged at 3000 rpm for 10 min, after which the serum was aspirated with a syringe into clean labeled test tubes, and used immediately for the following tests based on the specified standard procedures. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by the Reitman-Frankel method (Reitman and Frankel, 1957; Colville, 2002), while the serum alkaline phosphatase (ALP) activity was determined by the phenolphthalein monophosphate method (Klein et al., 1960; Babson et al., 1966; Colville, 2002).

Serum total cholesterol was determined by the enzymatic colorimetric method (Rifai et al., 2008), while serum total bilirubin was determined by the Jendrassik-Grof method (Higgins et al., 2008b). Serum total protein was determined by the direct Biuret method, serum albumin was determined by the bromocresol green method and the serum globulin was calculated as the difference between the serum total protein and serum albumin (Johnson, 2008). The serum urea was determined by the modified Berthelot-Searcy method for the in-vitro determination of urea in serum (Fawcett and Scott, 1960; Searcy et al., 1967; Lamb and Price, 2008), while the serum creatinine level was determined by the modified Jaffe method (Blass et al., 1974; Lamb and Price, 2008).

Fasting blood glucose levels were determined on whole blood, using Accu-Chek Active\textsuperscript{®} glucometer and test strips (Roche Diagnostics, Mannheim, Germany), which was based on the glucose oxidase method (Sacks, 2008).

After blood sample collection, the rats were weighed and humanely sacrificed by intra-peritoneal injection of 250 mg/kg sodium thiopentone (AVMA, 2013). The liver, kidneys, heart and spleen samples were eviscerated and weighed, and relative organ weights were calculated. Thin slices of the liver and kidneys of rats from Groups A, B, C and D were fixed in 10\% buffered formal saline and processed for histopathology (Winsor, 1994; Nowacek, 2010). The slides were examined under a light microscope. Photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd.) attached to the microscope.

Data analysis

Data obtained from the study were analyzed using a one way analysis of variance (ANOVA). Variant means were separated post-hoc using the least significant difference (LSD) method. Significance was accepted at $p < 0.05$. Summary of the results were presented as tables of means with standard error and bar charts with standard error bars.

RESULTS

Results showed no significant ($p > 0.05$) differences between the groups in all the haematological parameters assayed (Table 1). The serum total protein levels of rats in Group C (50 mg/kg PSME) was significantly ($p < 0.05$) higher than that of rats in Group A (500 mg/kg PSME), while the serum albumin levels of rats in Group C was significantly ($p < 0.05$) higher than that of rats in Group B (250 mg/kg PSME) and Group D (untreated control) (Table 2). The serum creatinine levels of rats in Groups B and C were significantly ($p < 0.05$) lower than that of rats in Group D (Table 2). There were however no significant ($p > 0.05$) variations amongst the groups in their serum ALT, AST and ALP activities, and serum levels of globulins, bilirubins, cholesterol and urea, and blood glucose (Table 2).

The mean body weight of Groups B and C were significantly ($p < 0.05$) higher than that of Group D (Figure 2). No outward signs of abnormalities were recorded for each of the rat groups all through the study, and no mortality was recorded in any of the groups all through the study. There were no significant ($p > 0.05$) differences in relative liver and kidney weights amongst the groups (Figures 3 and 4). However, the relative spleen and heart weights of rats that received PSME at all the doses used in the study [50, 250 and 50 mg/kg (Groups B and C)] respectively, were significantly ($p < 0.05$) lower than those of rats in the untreated control (Group D) (Figures 5 and 6). Liver section from rats in Group A showed normal architecture of the liver with normal portal vein (PV), hepatic artery (HA) and bile duct (BD). Group B and Group C showed mild random vacuolation of the hepatocytes around the portal vein (PV), while Group D (untreated control) showed normal liver section, showing chords of hepatocytes around the central vein (CV) (Figure 7). Kidney section from Group A rats showed normal glomeruli and renal tubules with moderate hyperaemia of the renal cortex (arrow). Group B rats showed normal glomeruli and renal tubules with hyperaemic area at the center, while Group C and Group D rats showed normal architecture of the kidneys as indicated in normal glomeruli and renal tubules (Figure 8).

DISCUSSION

The results of haematology showed no variations amongst the rat groups in all the haematological parameters assayed. This implies that PSME at the doses used in the study had no significant effect on the hematopoietic system of the rats. The haematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status in both animals and humans (Adeneye et al., 2006; Kulkarni and Veeranjaneulu, 2012). This finding of no significant effect on haematology is in agreement with reports by Salawu et al. (2008) who also recorded no significant effect of ethanol stem bark extract of *Pterocarpus erinaceus* (a
related plant of the same genus) on all the haematological parameters studied. However, Offor and Ogbugo (2015) reported increases in the levels of haemoglobin and PCV in albino rats treated with ethanol leaf extract of *P. santalinoides* for two weeks. This elevation in the levels of haemoglobin and PCV after 2 weeks of administration, reported by Offor and Ogbugo (2015) was attributed to an immediate response to PSME administration because elevations in some haematological parameters often occur naturally in response to some medications (Kulkarni and Veeranjaneyulu, 2012).

The higher serum total protein level recorded in Group C (50 mg/kg PSME), and the higher serum albumin level recorded at the doses of 50 mg/kg and 500 mg/kg PSME implied that PSME enhanced total protein and albumin synthesis. This result implied that treatment with PSME especially at 50 mg/kg enhanced hepatic synthetic ability. It is thought that the earlier reported hepatoprotective activity of *P. santalinoides* methanol leaf extract (Ihedioha et al., 2017, 2019) may partly be due to this enhanced hepatic synthetic ability, as most liver toxicities and disorders are usually associated with poor hepatic synthetic ability (Thapa and Walia, 2007).

The significantly lower serum creatinine levels in the treated groups suggests that treatment with PSME as used in this study enhanced renal clearance of creatinine and thus kidney function. Creatinine, a product of

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### Table 1. The haematological profile of albino rats given graded daily oral doses of PSME for 3 months, presented as means with standard error of mean (SEM) in brackets.

| Haematological parameters                        | Group A (500 mg/kg PSME) | Group B (250 mg/kg PSME) | Group C (50 mg/kg PSME) | Group D (Untreated control) |
|--------------------------------------------------|--------------------------|--------------------------|-------------------------|-----------------------------|
| Packed cell volume (%)                           | 48.13(0.94)              | 47.50(0.74)              | 48.25(1.11)             | 47.83(0.81)                 |
| Haemoglobin concentration (g/dl)                 | 16.48(0.42)              | 16.44(0.19)              | 16.61(0.34)             | 15.29(0.63)                 |
| Red blood cell count (10³/µl)                    | 11.65(0.43)              | 11.09(0.92)              | 11.32(1.09)             | 11.08(0.28)                 |
| Total white blood cell count (10³/µl)            | 10.49(0.85)              | 10.13(0.80)              | 10.59(0.91)             | 10.93(1.04)                 |
| Lymphocyte counts (10³/µl)                       | 8.57(0.86)               | 7.08(0.74)               | 6.71(0.61)              | 6.56(0.71)                  |
| Neutrophil counts (10³/µl)                       | 1.44(0.15)               | 1.93(0.39)               | 2.20(0.20)              | 1.91(0.65)                  |
| Monocyte counts (10³/µl)                         | 0.44(0.19)               | 0.37(0.02)               | 1.03(0.32)              | 1.54(0.94)                  |
| Eosinophil counts (10³/µl)                       | 0.38(0.13)               | 0.70(0.18)               | 0.41(0.09)              | 0.89(0.44)                  |
| Basophil counts (10³/µl)                         | 0.00(0.00)               | 0.03(0.03)               | 0.03(0.03)              | 0.00(0.00)                  |

No significant differences between the means of the groups, p > 0.05.

### Table 2. The serum biochemistry profile of albino rats given graded daily oral doses of PSME for 3 months, presented as means with standard error of mean (SEM) in brackets.

| Clinical biochemistry parameters                  | Group A (500 mg/kg PSME) | Group B (250 mg/kg PSME) | Group C (50 mg/kg PSME) | Group D (Untreated control) |
|--------------------------------------------------|--------------------------|--------------------------|-------------------------|-----------------------------|
| Serum alanine aminotransferase (IU/L)             | 24.29(2.76)              | 27.11(2.36)              | 28.44(2.39)             | 23.84(3.35)                 |
| Serum aspartate aminotransferase (IU/L)          | 74.73(5.98)              | 67.56(3.89)              | 66.01(4.07)             | 63.56(3.01)                 |
| Serum alkaline phosphatase (IU/L)                | 124.06(15.50)            | 124.17(12.95)            | 148.29(9.30)            | 132.39(5.04)                |
| Serum total protein (g/dL)                       | 6.77(0.21)               | 7.16(ab)(0.14)           | 7.48(ab)(0.25)          | 7.19(ab)(0.16)              |
| Serum albumin (g/dL)                             | 4.05(ab)(0.09)           | 3.97(ab)(0.06)           | 4.26(ab)(0.11)          | 3.96(ab)(0.08)              |
| Serum globulin (g/dL)                            | 2.71(0.14)               | 3.25(0.19)               | 3.22(0.35)              | 3.23(0.16)                  |
| Serum total bilirubin (mg/dL)                    | 1.08(0.09)               | 1.06(0.04)               | 1.06(0.06)              | 1.15(0.02)                  |
| Serum direct bilirubin (mg/dL)                   | 0.21(0.01)               | 0.23(0.05)               | 0.19(0.01)              | 0.19(0.03)                  |
| Serum indirect bilirubin (mg/dL)                 | 0.88(0.10)               | 0.83(0.05)               | 0.87(0.05)              | 0.96(0.03)                  |
| Fasting blood glucose levels (mg/dL)             | 66.00(2.04)              | 65.75(3.88)              | 65.50(3.66)             | 68.33(5.46)                 |
| Serum total cholesterol (mg/dL)                  | 83.69(4.03)              | 75.61(4.63)              | 76.81(8.20)             | 87.12(2.71)                 |
| Serum creatinine (mg/dL)                         | 0.74(ab)(0.02)           | 0.67(ab)(0.02)           | 0.73(ab)(0.03)          | 0.79(ab)(0.04)              |
| Serum urea (mg/dL)                               | 46.40(0.87)              | 45.38(2.67)              | 47.92(1.03)             | 49.45(0.31)                 |

ab Different alphabetical superscripts in a row indicate significant differences between the means of the groups, p < 0.05.
Figure 2. The mean body weights of albino rats given graded daily oral doses of *P. santalinoides* methanol extract (PSME) for 3 months.

Figure 3. The relative liver weights of albino rats given graded daily oral doses of *P. santalinoides* methanol extract (PSME) for 3 months.
Mean kidney weight percentage of body weights, with standard error of mean (SEM) bars.

**Figure 4.** The relative kidney weights of albino rats given graded daily oral doses of *P. santalinoides* methanol extract (PSME) for 3 months.

Mean spleen weight percentage of body weight, with standard error of mean (SEM) bars.

**Figure 5.** The relative spleen weights of albino rats given graded daily oral doses of *P. santalinoides* methanol extract (PSME) for 3 months. *a,b* Different superscripts indicate significant difference (p < 0.05) between the means of the rat groups.
Figure 6. The relative heart weights of albino rats given graded daily oral doses of *P. santalinoides* methanol extract (PSME) for 3 months. Different superscripts indicate significant difference (p < 0.05) between the means of the rat groups.
Figure 7. Liver sections of albino rats after sub-chronic administration of graded oral doses of PSME; H & E X 100. A - 500 mg/kg PSME; B - 250 mg/kg PSME; C - 50 mg/kg PSME; D - Normal control (no treatment). PV - Portal Vein; HA - Hepatic Artery; BD - Bile Duct; CV - Central Vein.

Figure 8. Kidney sections of albino rats after sub-chronic administration of graded oral doses of PSME; H & E X 100. A - 500 mg/kg PSME; B - 250 mg/kg PSME; C - 50 mg/kg PSME; D - Untreated control; G - Glomeruli; Arrow indicates renal cortex.
pathologically significant, as it did not concur with the activities and levels of biochemical markers of liver function (ALT, AST and ALP), serum total cholesterol and bilirubin, which were not significantly altered at these doses. This result implies that the mild vacoulation was not significant as to cause a negative impact on the hepatocellular integrity and functional capacity of the liver. The mild to moderate hyperaemia of the glomeruli and renal tubules seen in rats given 500 mg/kg PSME (Group A) and 250 mg/kg PSME (Group B) respectively, however, did not result to higher relative kidney weight and higher serum creatinine and serum urea levels; rather these values were lowered. This suggests that long term (sub-chronic) therapy with PSME at high doses did not cause any significant damage to the kidneys, but may have acted as a nephroprotective agent.

In summary, sub-chronic oral administration of *P. santalinoides* methanol leaf extract to albino rats at the doses of 500, 250 and 50 mg/kg led to no significant effects on the haematopoietic system and the hepatocellular integrity of the albino rats, it enhanced serum total protein and albumin synthesis, and also enhanced kidney function of the albino rats. The general results of this present study concurs with earlier reports of acute toxicity studies on leaf extracts of *P. santalinoides* (Anowi et al., 2012; Ihedioha et al., 2017, 2018, 2019; Obi et al., 2019) which showed that leaf extracts of *P. santalinoides* were acutely safe up to the dose of 5000 mg/kg.

**Conclusion**

Based on the results of this study, it was concluded that sub-chronic oral administration of graded doses of methanol leaf extract of *P. santalinoides* DC to albino rats is nontoxic and may be safely used for the treatment and management of diseases for which it is effective.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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