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Effects of aqueous leaf infusion of *Pterocarpus santalinoides* DC. on the serum lipid profile of guinea pigs (*Carvia porcellus*)

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ABSTRACT

Aim: This study investigated the effects of aqueous leaf infusion of *Pterocarpus santalinoides* on serum lipid profile (SLP) of guinea pigs (GPs).

Methods: Fresh leaves of *Pterocarpus santalinoides* were collected in February 2015. Aqueous leaf infusion was prepared daily by soaking dried ground leaves of *P. santalinoides* in hot water for 10 minutes. Twenty female GPs were randomly assigned to four groups of five GPs each, treated as follows: Group A—water as placebo (control), Groups B, C, and D—1.5, 3.0, and 4.5 g/kg body weight of ground *P. santalinoides* leaf soaked in 600 ml of hot water, respectively. Treatment was given orally daily for 28 days. Assay of SLP was done on days 0 (before treatment), 14, and 28 of treatment, following standard procedures.

Results: The mean serum high density lipoprotein cholesterol (HDLC) of Groups B, C, and D rose to almost double its baseline values and was significantly ($p < 0.05$) higher than that of Group A on day 28, while the mean serum low density lipoprotein cholesterol (LDLC) of Group D was significantly lower ($p < 0.05$) than those of other groups. The mean serum triglyceride and very low density lipoprotein cholesterol (VLDLC) of Groups B and C were significantly lower ($p < 0.05$) than that of Group A at days 14 and 28 of treatment.

Conclusion: Administration of *P. santalinoides* aqueous leaf infusion as used in this study led to significant positive effects of enhancement of serum HDLC and decrease of serum LDLC, triglyceride, and VLDLC.

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Introduction

Cholesterol and triglycerides are the major clinically significant lipids commonly assayed for in the blood of humans and animals, because alterations in certain components of them have been found to be instrumental to the development of atherosclerosis and its clinical complications of cardiovascular diseases such as myocardial infarction (heart attack), cerebral infarction (stroke), and gangrene of the extremities [1–4]. The major components of serum total cholesterol (TC) associated with increased risk of atherosclerosis are low density lipoprotein cholesterol (LDLC) and very low density lipoprotein cholesterol (VLDLC), which play the physiologic role of vehicles for the delivery of cholesterol to peripheral tissues; in contrast, high density lipoprotein cholesterol (HDLC) mobilizes cholesterol from developing and existing atheromas and transports them to the liver for excretion in bile in a process known as "reverse cholesterol transport" [5,6]. Several studies have shown the critical roles that LDLC, VLDLC, and HDLC play in the development, progression, diminution, and/or management of atherosclerosis [6,7]. Thus, efforts/strategies aimed at prevention and management of atherosclerosis have been centered on the development of drugs, supplements, diets, and lifestyle adjustments that will reduce serum TC, LDLC, and VLDLC, and enhance serum HDLC [6,7].

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Pterocarpus santalinoides DC (Fig. 1) is an indigenous Nigerian plant in the family *Papilionaceae*. It is commonly referred to as "Red sandal wood" in English [8,9]. Leaves of *P. santalinoides* are traditionally used as food (vegetable) and as medicine in the treatment of various ailments, including inflammatory and cardiovascular diseases (heart attack and stroke) [10–12]. Aged people traditionally use *P. santalinoides* leaves for soup and as medicine because it is believed to help them cope with old age-related cardiovascular diseases such as weak/failing heart and stroke [10,12,13]. Scientific reports on the medicinal use of the leaves of *P. santalinoides* for the treatment or management of cardiovascular diseases is, however, lacking in the available literature. Based on the various medicinal uses of *P. santalinoides* especially in the treatment of cardiovascular diseases and the role that serum lipids play in the evolution, development, and progression of cardiovascular diseases, the objective of this study was to evaluate the effects of aqueous leaf infusion of *P. santalinoides* on the serum lipid profile (SLP) of guinea pigs (GPs).

Materials and Methods

Chemicals, reagents and assay kits

The clinical biochemistry assay kit for the evaluation of the SLP was procured from Quimica Clinica Applicada (QCA), Spain. All other routine reagents and chemicals were of analytical grade.

Figure 1. Picture showing leaves, flowers, and stem of *Pterocarpus santalinoides*.

Plant collection, identification, and preparation

This study was conducted in 2015. Fresh leaves of *P. santalinoides* used for the study were collected in February 2015 from Nsukka, Enugu State, Nigeria. The plant was identified by a plant taxonomist (Mr. A. O. Ozioko) at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka (Voucher Specimen Number—University of Nigeria Herbarium no. 2). The leaves were dried under shade, and ground into coarse powder. The infusion was prepared by dissolving varied quantities [1.5, 3.0, and 4.5 g/kg body weight (BW)] of the ground leaves of *P. santalinoides* each in 600 ml of hot water (70°C–90°C) for 10 minutes. The resulting infusion was filtered using a domestic tea sieve (0.63 mm pore size) and allowed to cool.

Experimental animals

Thirty-two adult female GPs (*Carvia porcellus*) of 12 weeks of age, weighing between 300– and 400 g, obtained from the Laboratory Animal Unit of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, were used for the study. The GPs were housed in a fly-proof animal house at room temperature (23°C–29°C), and allowed for 2 weeks to acclimatize before the commencement of the study. All through the study, the GPs were fed commercial pelletized feed (Grand Cereals Ltd, Jos, Nigeria), composed of 13% crude protein, 8% fat, 15% crude fiber, 0.9% calcium, 0.35% phosphorus, and 2,600 Kcal/kg metabolizable energy, and were provided with clean water *ad libitum*. Twelve of the GPs were used for the acute toxicity study, while 20 were used for the study of the effects of the infusion on the SLP.

GPs were chosen as the experimental animal model for this study because of the numerous documented metabolic similarities to humans especially in lipid metabolism and response to hypocholesterolaemic agents [14–16]. Like humans, GPs carry the majority of plasma cholesterol in low density lipoprotein (LDL) and have been shown to vary cholesterol and lipoprotein metabolism in response to dietary interventions [17–19]. GPs also have a tissue distribution of whole body cholesterol synthesis similar to that of humans and express plasma cholesteryl ester transfer protein activity [20,21].

The animal experimental protocol was approved by the Experimental Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka and in compliance with the Federation of European Laboratory Animal Science

Association and the European Community Council Directive of November 24, 1986 (86/609/EEC).

Experimental design

Acute toxicity study

The acute toxicity and median lethal dose (LD_{50}) of the infusion was determined following Lorke's two-step method of acute toxicity testing [22]. Nine of the 12 GPs used for the acute toxicity testing were used for the first step of the acute toxicity testing. These nine GPs were randomly assigned into three groups (A, B, and C) of three GPs each, and were given the 10, 100, and 1,000 mg/kg BW of the *P. santalinoides* leaf powder infusion *per os*. These were observed for 3 days, and in the absence of any signs of abnormalites or mortality, the remaining three GPs were used for the second step which involved these three being given 1,600, 2,500, and 5,000 mg/kg BW of the *P. santalinoides* leaf powder infusion according to Lorke [22]. The GPs used for the second step were also observed for signs of abnormality and mortality for up to day 14 post-administration [22].

Phytochemical analysis

Semi-quantitative phytochemical analysis was carried out on the infusion to test for the presence of tannins, flavonoids, alkaloids, saponins, glycosides, terpenes, and sterols following the standard procedures [23,24]. One gram of the *P. santalinoides* leaf powder was dissolved in 100 ml of distilled water in a beaker. The solution was filtered with Whatman Filter Paper No. 1 to obtain a clear filtrate, which was used to test for the presence and semi-quantity of the phytochemicals–high levels of specific phytochemicals were scored +++, moderate levels were scored ++, low level were scored +, while phytochemicals that were absent were not scored [23,24].

Evaluation of the effects of the aqueous leaf infusion on SLP of GPs

The 20 GPs used for the *in vivo* testing of the effect of the aqueous leaf infusion on SLP were randomly assigned into four groups (A, B, C, and D) of five each. The four groups of GPs were treated as follows: Group A was given 600 ml of water as placebo and served as control. Groups B, C, and D were given infusions made from soaking 1.5, 3.0, and 4.5 g/kg BW of ground *P. santalinoides* leaf in 600 ml of hot water, respectively. Fresh infusions were prepared daily in the morning for the GPs, and were made freely available to them all through the 28 days of the study. Blood samples were collected from the GPs after a 12-hour overnight fast before the commencement of treatments (day 0), and on days 14 and 28 of the treatment for the assay of the SLP. Blood sample collection was by the orbital technique [25], while the assay of the SLP was done using commercially available QCA test kits (QCA, Spain), following standard colorimetric methods [26].

Serum TC was determined based on the enzymatic colorimetric method [27], which involved the enzymatic hydrolysis and oxidation of cholesterol in the serum samples by cholesterol esterase and oxidase, respectively, contained in the QCA cholesterol working reagent, leading to formation of a colored quinonic derivative the optical density of which was measured at 505 nm wavelength and compared with that of a standard containing 200 mg/dl of cholesterol [28] using a Spectrum lab 23A spectrophotometer (HME Global Medical, England). The serum HDLC was evaluated based on the dextran sulphate-magnesium (II) precipitation method [29], which involved the precipitation of LDLC and VLDLC in the serum sample using dextran sulphate in the presence of magnesium acetate, leaving only the HDLC in the supernatant after centrifugation. The supernatant containing only HDLC was then subjected to cholesterol determination procedure as described above [30]. The serum triglyceride was determined based on glycerol phosphate oxidase enzymatic method [31]. In the triglyceride determination procedure, lipases, glycerol kinase, glycerol 3-phosphate oxidase, and peroxidase in the QCA triglyceride working reagent catalyzed the conversion of triglyceride in the serum sample to a colored indicator compound (quinoneimine), the optical density of which is measured at 505 nm wavelength and compared with standards containing 200 mg/dl of triglyceride [32] using a Spectrum lab 23A spectrophotometer (HME Global Medical, England). Diacal Auto (Dialab, Wiener Neudorf, Austria) lyophilized calibration serum was used as control for the quantitative *in vitro* clinical chemistry determinations. The serum VLDLC was calculated as 1/5 of the serum triglyceride, while the serum LDLC was calculated using the Friedewald formula [26].

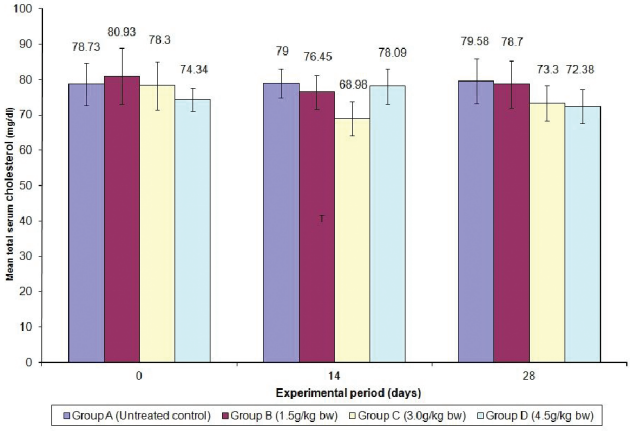

Figure 2. Serum TC of GPs given graded oral doses of *Pterocarpus santalinoides* leaf infusion.

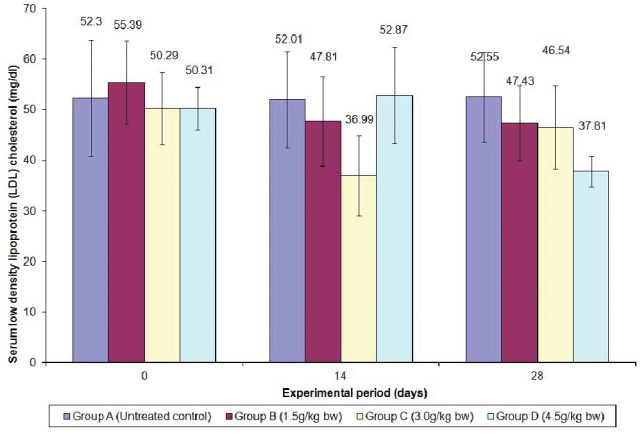

Figure 4. Serum LDL-cholesterol levels of GPs given graded oral doses of *Pterocarpus santalinoides* leaf infusion.

Statistical analysis

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

Results and Discussion

The infusion produced was golden brown in colour. It was well accepted by the GPs as there was no significant difference ($p > 0.05$) between the volume of the infusion consumed by GPs in the treatment Groups B (78.60 ± 3.45 ml/day), C (83.00 ± 2.37 ml/day), and D (79.30 ± 4.01 ml/day) and the volume of water consumed by the control group A (78.82 ± 4.16 ml/day) that were given plain drinking water.

In the acute toxicity study, no mortality was recorded even at the highest dose of 5,000 mg/kg BW. No signs of abnormality/toxicity were observed either, and there were no significant variations between the GPs when given the varied doses. This result of the acute toxicity study implied that the infusion is not acutely toxic. An LD_{50} above 5,000 mg/kg is within the World Health Organization's category of substances "unlikely to present acute hazard in normal use" [33]. Thus, the aqueous leaf infusion of *P. santalinoides* is considered safe. This is in agreement with the works carried out by Anowi et al. [9], and Eze et al. [34], who respectively reported that *P. santalinoides* leaf and stem bark extracts are acutely non-toxic.

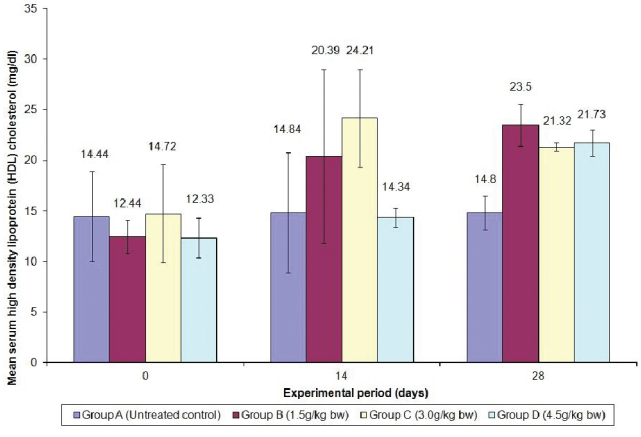

Figure 3. Serum HDL-cholesterol levels of GPs given graded oral doses of *Pterocarpus santalinoides* leaf infusion.

The phytochemical analysis showed that the infusion contained high levels (+++) of glycosides, terpenes and sterols, moderate levels (++) of tannins, flavonoids and saponins, and low level (+) of alkaloids. The results of the phytochemical analysis in this study is in agreement with the reports of Anowi et al. [9] and Eze et al. [34] who reported on the phytochemical constituents of the ethanol leaf extract, and aqueous stem bark extract of *P. santalinoides*, respectively. Also, alkaloids, saponins, and flavonoids were present in varying quantities in the leaves of *P. santalinoides* as reported by Odeh et al. [35]. Heterogeneous phytoconstituents of crude extracts have been reported to have synergistic effect [36]. These phytochemicals, most especially flavonoids, have been reported to possess the ability to reduce free radical formation and scavenge free radicals *in vivo* [37,38], and this is important in the management of diseases associated with oxidative stress such as atherosclerosis and other cardiovascular diseases [39].

There were no significant ($p > 0.05$) variations between all the groups in their serum TC all through the study (Fig. 2). There were also no significant ($p > 0.05$) variations in the serum HDLC on days 0 and 14, but by day 28, the serum HDLC of Groups B,

C, and D had risen to nearly double their baseline values and were significantly ($p < 0.05$) higher than that of Group A (Fig. 3). The serum LDLC of all the groups did not significantly ($p > 0.05$) vary at day 0 and 14, but by day 28, the serum LDLC of the Group D GPs was significantly ($p < 0.05$) lower than those of all other groups (Fig. 4). The serum VLDLC and triglyceride of the different groups did not significantly ($p > 0.05$) vary at day 0, but at days 14 and 28, the serum VLDLC and triglyceride of Groups B and C were significantly ($p < 0.05$) lower than that of all other groups (Figs. 5 and 6).

Though the serum TC levels of the treated groups were not affected by treatment with the aqueous leaf infusion of *P. santalinoides*, the treated groups had significantly higher levels of HDLC and lower levels of LDLC, under which condition atheroma growth rate had been reported to be low, or even negative for any given TC concentration [6,7]. It is thought that the administered infusion may have modulated the lipoprotein synthetic capability of the liver in such a way that relatively more HDLC was synthesized by the liver, while more LDLC and VLDLC were catabolized by the liver. The significantly lower LDLC in the group treated with 4.5 g/kg BW aqueous leaf infusion of *P. santalinoides*, and significantly lower triglyceride and VLDLC recorded in the groups treated with 1.5 and 3.0 g/kg BW aqueous leaf infusion of *P. santalinoides* may partly be attributed to the antioxidant activity of the phytochemicals in the aqueous leaf infusion of *P. santalinoides*, as numerous studies had shown that antioxidant treatment protects against dyslipidaemia-induced atherogenesis and atherosclerosis [38,40,41]. The significantly higher HDLC recorded for all the aqueous leaf infusion of *P. santalinoides* treated groups is considered to be

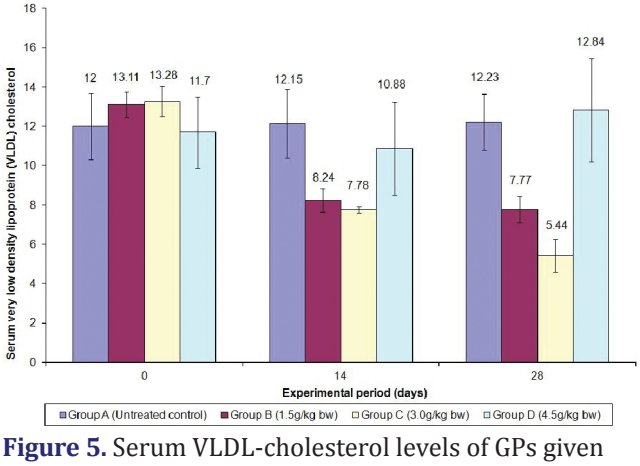

Figure 5. Serum VLDL-cholesterol levels of GPs given graded oral doses of *Pterocarpus santalinoides* leaf infusion.

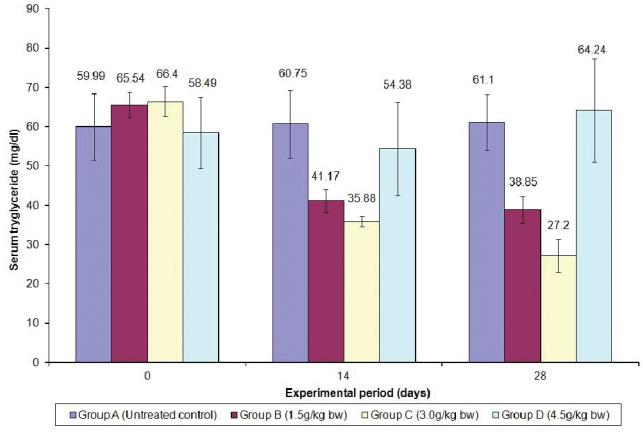

Figure 6. Serum triglyceride levels of GPs given graded oral doses of *Pterocarpus santalinoides* leaf infusion.

clinically relevant as low HDLC had been identified as an additional clinically important cardiovascular risk factor [6,7]. The beneficial effects on cardiovascular outcome of agents that act mainly by raising HDLC have been reported [6,7].

Conclusion

Based on the results of this study, it was concluded that oral administration of *P. santalinoides* aqueous leaf infusion as used in this study led to significant positive effects of enhancement of serum HDLC and decrease of serum LDLC, triglyceride, and VLDLC in the treated GPs. These are clinically relevant positive effects that can help in the prevention and management of dyslipidaemia-induced atherosclerosis and other cardiovascular diseases.

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