EFFECTS OF AQUEOUS LEAF INFUSION OF *Pterocarpus santalinoides* DC ON THE SERUM LIPID PROFILE OF GUINEA PIGS (*Carvia porcellus*)

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Abstract

This study evaluated the effects of oral administration of aqueous leaf infusion of Pterocarpus santalinoides on serum lipid profile (SLP) of guinea pigs (GPs). Twenty GPs randomly assigned into four groups (A, B, C and D) of five each were used for the study. Group A GPs were given water placebo, while GPs in groups B, C and D were given infusion produced by dissolving 1.5, 3.0 and 4.5 g/kg bw of P. santalinoides leaf powder in 600ml of hot water, respectively for 28 days. Assay of the SLP was done on days 0 (before treatment), 14, and 28 of treatment following standard procedures. Results showed that there were no significant variations ($p > 0.05$) in the serum total cholesterol (TC) among the groups all through the study, but at day 28, 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, 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 (TAG) and very low density lipoprotein cholesterol (VLDLC) of groups B and C were significantly lower ($p < 0.05$) than that of group A at day 14 and 28 of treatment. It was concluded that oral administration of P. santalinoides leaf infusion as used in this study led to significant positive effects of enhancement of serum HDLC and decrease of serum LDLC, TAG and VLDLC of GPs.

Key words: *Pterocarpus santalinoides,* aqueous leaf infusion, serum lipid profile, guinea pigs.

Introduction

The complications of dyslipidaemia-induced atherosclerosis are the most common causes of death worldwide, and among the many genetic and environmental risk factors that have been identified by epidemiological studies, elevated levels of serum cholesterol and triglycerides are probably unique in being sufficient to drive the development of atherosclerosis in humans and experimental animals, even in the absence of other known risk factors (Brown & Goldstein, 1992; Oslon, 1998; Durrington, 2003). Although cholesterol is physiologically important in the body, high levels of it in the blood have been found to be associated with increased risk of atherosclerosis (Brown & Goldstein, 1992; Oslon, 1998). The major components of serum total cholesterol 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" (Libby *et al.*, 1998; Barter *et al.*, 2007). Several studies have shown the critical roles that LDLC, VLDLC, and HDLC play in the development, progression, diminution and /or management of atherosclerosis (Law, 1999; Drexel, 2006; Barter *et al.*, 2007). 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 total cholesterol (TC), LDLC and VLDLC, and enhance serum HDLC (Law, 1999; Drexel, 2006).

Pterocarpus santalinoides DC is an indigenous Nigerian plant in the family *Papilionaceae*. It is commonly referred to as "Red sandal wood" in English, 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. (Anowi *et al.*, 2012). Leaves of *P. santalinoides* are traditionally used as food (vegetable) and as medicine in the treatment of various ailments (Adesina, 1982; Shulz *et al.*, 2001; Okwu & Ekeke, 2003). Aged people traditionally use *P. santalinoides* leaves for soup and as medicine because it is believed to help them cope with some old age related cardiovascular and peripheral vascular diseases (myocardial infarction, cerebral infarction and oedema of the extremities) (Adesina, 1982; Akpanyung *et al.*, 1995). Scientific reports on the medicinal use of the

leaves of *P. santalinoides* for the treatment of cardiovascular diseases is however lacking in available literature. 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

Thirty two (32) adult female GPs (*C. porcellus*) of 12 weeks of age weighing between 300g - 400g, obtained from the Laboratory Animal Unit of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, were used for the study. Twelve of the GPs were used for the acute toxicity testing, while 20 were used for the *in vivo* study of the effects of the infusion on the serum lipid profile. The guinea pigs were acclimatized for 2 weeks, housed in standard clean cages in the animal house at room temperature, and fed commercial pelletized feed (Vital feed®, Grand Cereal Ltd, Jos, Nigeria), and were provided with clean water *ad libitum* all through the study. The 20 guinea pigs used for the *in vivo* testing of the effect of the infusion on serum lipid profile were randomly assigned into four groups (A, B, C and D) of five each. The handling, management and use of the guinea pigs in this experiment were in conformity with the Guide to Humane Laboratory Animal Use of the University of Nigeria, Nsukka.

Fresh leaves of *P. santalinoides* used for the study were collected in February 2015 from Nru Community in 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. The infusion was prepared by dissolving varied doses (1.5, 3.0 and 4.5 g/kg bw) of the dry powder of the leaves of *P. santalinoides* in 600 ml of hot water, for 10 minutes. The resulting infusion was filtered using a sieve and allowed to cool.

The oral acute toxicity test of the infusion was done following the new approach to practical acute toxicity testing as described by Lorke (1983), while the phytochemical analysis was done following the standard procedure described by Harborne (1998).

The four groups of GPs (A, B, C and D) used for the *in vivo* assessment of the effects of the infusion on SLP were treated as follows: group A was given 600 ml of water placebo and served as control. Group B, C and D were given infusions made from dissolving 1.5, 3.0 and 4.5 g/kg bw of *P. santalinoides* leaf powder in 600 ml of hot water respectively. Fresh infusions were prepared daily in the morning for the GPs, and were 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 serum lipid profile. Blood sample collection was by the orbital technique (Zimmerman *et al.*, 2010), while the assay of the serum lipid profile was done following standard procedures (Rifai *et al*, 2008), using Quimica Clinica Applicada (QCA) test kits (QCA, Spain).

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 infusion produced was golden brown in colour. It was well accepted by the guinea pigs as the treatment groups B, C and D drank as much in volume as the group A rats 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 body weight, and no signs of toxicity were observed either. The result of the acute toxicity study on the crude aqueous leaf infusion of *P. santalinoides* which showed that the guinea pigs exhibited no signs of toxicity or death even at the upper dose limit (5,000 mg/kg bw) implied that the infusion is not acutely toxic. An LD_{50} above 5,000 mg/kg bw lies within the World Health Organization's (WHO) category of substances "unlikely to present acute hazard in normal use" (WHO, 2001).

The phytochemical analysis showed that the infusion contained high levels (+++) of carbohydrates, glycosides, terpenes and sterols, moderate levels (++) of tannins, flavonoids and saponins, and low level (+) of alkaloids. The findings from the phytochemical analysis that the aqueous leaf infusion of *P. santalinoides* used in this study contained tannins, alkaloids, flavonoids, saponins, terpenes/sterols, glycosides and carbohydrates is in agreement with the reports of Anowi *et al.*, (2012) and Eze *et al.*, (2012) on the phytochemical constituents of the leaf extract of the plant. These phytochemicals, most especially flavonoids, had been reported to possess the ability to reduce free radical formation and scavenge free radicals *in vivo* (Robak & Gryglewski, 1988; Pietta, 2000), which are important for managing diseases associated with oxidative stress such as dyslipidaemia- induced atherosclerosis and cardiovascular diseases (Ferguson, 2001).

Table 1. Serum lipid profile of guinea pigs given oral doses of *Pterocarpus santaniloides* leaf

	Group A (Untreated control)	Group B (Treated with 1.5g/kg bw infusion)	Group C (Treated with 3.0g/kg bw infusion)	Group D (Treated with 4.5g/kg bw infusion)
	Total Cholesterol			
Day 0	78.73 ± 6.02	80.93 ± 7.96	78.30 ± 6.78	74.34 ± 3.27
Day 14	79.00 ± 4.13	76.45 ± 4.92	68.98 ± 4.88	78.09 ± 4.96
Day 28	79.58 ± 6.37	78.70 ± 6.63	73.30 ± 4.92	72.38 ± 4.87
	High density lipoprotein (HDL) cholesterol			
Day 0	14.44 ± 4.41	12.44 ± 1.64	14.72 ± 4.86	12.33 ± 1.96
Day 14	14.84 ± 5.96	20.39 ± 8.65	24.21 ± 4.82	14.34 ± 0.92
Day 28	14.80 ± 1.71^{a}	23.50 ± 2.08^{b}	21.32 ± 0.41^{b}	21.73 ± 1.33^{b}
	Low density lipoprotein (LDL) cholesterol			
Day 0	52.30 ± 11.44	56.39 ± 8.22	50.11 ± 7.15	50.31 ± 4.19
Day 14	52.01 ± 9.52	47.81 ± 8.86	36.99 ± 7.96	52.87 ± 9.46
Day 28	52.55 ± 8.86^{a}	47.43 ± 7.41^{ab}	46.54 ± 8.21^{ab}	37.81 ± 3.02^{b}
	Very low density lipoprotein (VLDL) cholesterol			
Day 0	12.00 ± 1.68	13.11 ± 0.65	13.28 ± 0.76	11.70 ± 1.81
Day 14	12.15 ± 1.75^{a}	8.24 ± 0.60^{b}	7.78 ± 0.16^{b}	10.88 ± 2.37^{ab}
Day 28	12.23 ± 1.44^{a}	7.73 ± 0.68^{b}	5.44 ± 0.82^{b}	12.84 ± 2.62^{a}
	Triglyceride			
Day 0	59.99 ± 8.39	65.54 ± 3.23	66.40 ± 3.79	58.49 ± 9.05
Day 14	60.75 ± 8.68^{a}	41.17 ± 2.91^{b}	35.88 ± 1.24^{b}	54.38 ± 11.85^{ab}
Day 28	61.10 ± 7.19^{a}	38.85 ± 3.42^{b}	27.20 ± 4.08^{b}	64.24 ± 13.12^{a}

^{a,b} Different superscripts in a row indicate significant difference between the means, $p < 0.05$

Results of the serum lipid profile assay are presented in Table 1. There were no significant ($p > 0.05$) variations between all the groups in their serum total cholesterol (TC) all through the study. 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. 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 guinea pigs was significantly ($p < 0.05$) lower than those of all other groups. The serum VLDLC and triglyceride of the different groups did not significantly ($p > 0.05$) vary at day 0, but at day 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. Though the total cholesterol levels of the treated groups was not affected by treatment with the infusion, 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 total cholesterol concentration (Drexel, 2006; Barter *et al*, 2007). The significantly lower LDLC in the group treated with 4.5 g/kg bw infusion, and significantly lower triglyceride and VLDLC recorded in the groups treated with 1.5 g/kg bw and 3.0 g/kg bw infusion may partly be attributed to the anti-oxidant activity of the phytochemicals in the infusion, as numerous studies had shown that anti-oxidant treatment protects against dyslipidaemia-induced atherogenesis and atherosclerosis (Steinberg & Witztum, 1990; Jha, 1995; Pietta, 2000). The significantly higher HDLC recorded for all the infusion treated groups is considered to be clinically relevant as low HDLC had been identified as an additional clinically important cardiovascular risk factor (Drexel, 2006; Barter *et al*, 2007). The beneficial effects on cardiovascular outcomes of agents that act mainly by raising HDLC had been reported (Drexel, 2006; Barter *et al*, 2007).

Based on the results of this study, it was concluded that oral administration of *P. santalinoides* leaf infusion as used in this study led to significantly higher serum HDLC and significantly lower LDLC, triglyceride and VLDLC in the treated GPs, depending on the dosage. These are clinically relevant positive effects that can help in the management and prevention of dyslipidaemia and atherosclerosis. These results validate the traditional use of the leaves of *P. santalinoides* in the management of cardiovascular diseases, and strongly suggest that the aqueous leaf infusion of *P. santalinoides* can be effectively used for the

management of dyslipidaemia and prevention of atherosclerosis.

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