

Full Length Research Paper

Effects of ethanolic and aqueous leaf extracts of *Landolphia owariensis* on the serum lipid profile of rats

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The lipid profile of normal adult male rats administered both ethanolic and aqueous leaf extracts of *Landolphia owariensis* (P. Beauv) were determined. The animals were distributed into two sets of four groups with five animals in each group. Each set had one group, which served as control while the other three groups in the two sets were administered different concentrations of the ethanolic and aqueous leaf extracts. The control groups were administered normal saline and the other groups' 100, 200 and 300 mgkg⁻¹ of the ethanolic and aqueous extracts respectively, twice daily for two weeks. The Total cholesterol (TC), Triacylglyceride (TAG), High Density Lipoprotein-Cholesterol (HDL-C), Low Density Lipoprotein-Cholesterol (LDL-C), and Very Low Density Lipoprotein (VLDL) levels were determined in both sets by colorimetric methods. The ethanolic extract showed a marked reduction of 87.45% in LDL-C level with the 100 mgkg⁻¹ dose, though effect of all the three concentrations were significant but depreciated with increase in concentration. Animals administered both the extracts at all three concentrations increased in their HDL-C levels, but effect was pronounced in 100 and 200 mgkg⁻¹ with 15 and 150% increases respectively, in the aqueous extract group. There were dose-dependent reductions of TC levels, with the 100, 200 and 300 mgkg⁻¹ with reduction of 40.78, 37.59 and 34.56% respectively, in ethanolic extracts. There were 50.55 and 55.33% reduction in 100 and 200 mgkg⁻¹ of the aqueous extracts on TAG level. The results are indicative of the hypocholesterolaemic potentials of *L. owariensis* leaf extracts.

Key words: Aqueous extract, ethanolic extract, Hypocholesterolaemia, *Landolphia owariensis*.

INTRODUCTION

Most primitive tribes possess expert knowledge of medicinal plants, which number at times in hundreds (Singer, 1951). Although, modern medicine may be available in developing countries, but the use of herbs for treatment and management of diseases has often maintained popularity for historical and cultural reason. The practice has gained more grounds, as traditional medicine has become a topic of global importance (Zhang, 1999). This age long practice (Sofowora, 1982) encouraged research into pharmacologic activities of plant secondary metabolites and has improved modern

pharmacotherapeutics around the world (Nwaogu et al., 2007).

Landolphia owariensis commonly called vine rubber is widely used for treatment of many ailments (Owoyele et al., 2001). It is widely used in the sub-Sahara of Africa. The decoction of its leaves is used as a purgative and treatment of malaria (Gill, 1992). In some tribes, extracts of roots soaked in local gin is used to cure gonorrhoea (Gill, 1992). The leaf extracts has been proven to have anti-inflammatory and analgesic activities (Owoyele et al., 2001).

Also, Lewis and Lewis (1977) demonstrated the use of the stem bark as vermifuge. People of the French Equatorial Africa use the latex as enema for intestinal worms (Irvine, 1961). The latex is also used as natural preserva-

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Table 1. Effect of ethanolic extracts of *L. owariensis* on the serum TC, TAG, HDL-C, LDL-C and VLDL levels (mg/dl).

	Control	100 mg/kg	200 mg/kg	300 mg/kg
TC	92.09 ± 13.2	54.54 ± 4.8*	57.47 ± 9.2*	60.26 ± 10.3*
TAG	93.70 ± 7.1	131.13 ± 12.5	68.13 ± 11.0	88.15 ± 12.7
HDL-C	11.03 ± 8.2	18.85 ± 10.4*	28.47 ± 18.3*	19.07 ± 12.1*
LDL-C	75.31 ± 3.1	09.45 ± 4.7*	15.33 ± 8.2*	23.06 ± 10.4*
VLDL	17.70 ± 2.9	26.23 ± 7.2*	13.63 ± 23.7	17.63 ± 3.6

Values * given as mean ± standard deviation had significant differences, when compared with the control ($p \leq 0.05$) as determined by t-test.

preservative (Anthony, 1995). The leaf extracts has been shown to have antimicrobial activity (Ebi and Ofoefule, 1997). This was corroborated by the works of Nwaogu et al. (2007).

This study was aimed at the investigation of the effect of different concentrations of aqueous and ethanolic leaf extracts of *L. owariensis* on the serum lipid profile of rats. This is with the view of exercising restraint in the use and exploitation of other possible potentials of the plant that is beginning to gain wider acceptance in Nigeria.

MATERIALS AND METHODS

Experimental animal

The experimental animals (*Rattus norvegicus*), all male, which weighed 120 - 160 g used for the research work was obtained from the animal house of the College of Health Sciences, Igbinedion University, Okada. They were acclimatized and housed in plastic cages. The rats were fed and given water *ad libitum*. The temperature of the room was maintained at $25 \pm 2^\circ\text{C}$ throughout the whole experimental period.

Experimental design

The animals were randomly selected and grouped. There were a total of eight groups with five animals per group. The animals were distributed into two sets of four groups. Each set had one group which served as control while the other three groups in the two sets were administered different concentrations of the ethanolic and aqueous leaf extracts. The control groups were administered normal saline while the other groups were administered 100, 200 and 300 mgkg⁻¹ of the ethanolic and aqueous extracts respectively twice daily for two weeks. The ethanolic and aqueous extracts were administered orally in normal saline. The animals were sacrificed after two weeks by cervical dislocation and blood samples were collected by cardiac puncture.

Lipid profile assay

The determination of the Total cholesterol (TC) in the serum was by the method of Searcy and Berquist (1960), which utilized the cholesterol oxidase. The LDL-cholesterol and HDL-cholesterol were determined according to the methods described by Friedwald et al. (1972). Serum triacylglyceride (TAG) was determined using the method of Tiez (1990) while VLDL-cholesterol was calculated using the formula of Friedwald et al. (1972).

Statistical analysis

The results obtained in the research work were expressed as mean ± standard deviation. The difference between mean values was assessed for significance by student T-test (SPC-XL, 2000) at $P \leq 0.05$ level of significance.

RESULTS

The results of the effect of ethanolic extracts of *L. owariensis* on lipid profile of rats as shown in Table 1 shows that the ethanolic extract of *L. owariensis* reduced the serum Total cholesterol (TC) of rats at all three concentration. This result suggests that, the reducing ability of the extract increased as the concentration of extract reduced making the list concentration administered (100 mgkg⁻¹) the most potent. The same pattern of result was seen with Low Density Lipoprotein- Cholesterol (LDL-C). Though the ethanolic extract of *L. owariensis* increased the serum High Density Lipoprotein-Cholesterol (HDL-C), the result did not show any dependence on concentration. These results show fluctuations in the serum Triacylglyceride (TAG), which was not significant at any concentration when compared with the control. The Very Low Density Lipoprotein (VLDL) serum level increased significantly only at the 100 mgkg⁻¹ and fluctuated non-significantly with other concentrations.

The results of the effect of aqueous extracts of *L. owariensis* on lipid profile of rats as shown in table 2 shows that the serum Total cholesterol (TC) reduced significantly at 100 and 300 mgkg⁻¹ concentrations. The reduction was concentration dependent, though there was no reduction with 200 mgkg⁻¹ concentration. The Low Density Lipoprotein-Cholesterol (LDL-C) serum levels reduced significantly at all three concentrations administered.

The 100 mgkg⁻¹ extract reduction was drastic as shown in Table 2. The administration of aqueous extracts of *L. owariensis* showed an increase in the serum High Density Lipoprotein-Cholesterol (HDL-C). The increase was concentration dependent. The Triacylglyceride (TAG) and Very Low Density Lipoprotein (VLDL) serum levels as shown in Table 2 reduced significantly with the 100 and 200 mgkg⁻¹. The reduction seems to be concentration dependent with the two concentrations.

Table 2. Effect of aqueous extracts of *L. owariensis* on the serum TC, TAG, HDL-C, LDL-C and VLDL levels (mg/dl).

	Control	100 mg/kg	200 mg/kg	300 mg/kg
TC	93.29 ± 11.4	61.17 ± 4.7*	92.85 ± 19.9	48.42 ± 10.3*
TAG	95.50 ± 14.3	48.18 ± 13.2*	43.66 ± 12.6*	92.68 ± 12.7
HDL-C	13.02 ± 9.2	29.7 ± 19.8*	29.06 ± 18.3*	25.85 ± 10.1*
LDL-C	77.21 ± 3.1	24.9 ± 8.6*	58.11 ± 11.1*	52.07 ± 10.4*
VLDL	19.81 ± 2.9	11.38 ± 13.2*	10.48 ± 2.6*	20.28 ± 7.5

Values * given as mean ± standard deviation had significant differences, when compared with the control ($p \leq 0.05$) as determined by t-test.

DISCUSSION

The results obtained from this work suggests that though the two extracts affected the TC, LDL-C, HDL-C, TAG and VLDL serum levels in the rats, the degree varied from one extract to the other. The aqueous extracts seem to increase the HDL-C and reduce TAG and VLDL more effectively when compared with the ethanolic extracts. The ethanolic extracts were found to reduce the TC and LDL-C better than the aqueous extracts. To that effect, it will not be out of place to suggest that this degree of variation is dependent on the solvent used for extraction. It is well known that, Cardiovascular Diseases (CVD) is the leading cause of death of men and women in developed countries (Kummerow, 1982). Hypercholesterolemia has been identified as a primary risk factor in the development of CVD. Therefore, preventing or reducing the increase in serum cholesterol is associated with reducing the risk of CVD (Onyeneke et al., 2008). In this work the ethanolic and aqueous extracts showed reduction TC and LDL-C, while there was an increase in the HDL-C serum levels. This may be attributed to changes in serum lipids, which are controlled by the enzymes responsible for lipid metabolism (Onyeneke et al., 2008).

There was increase in HDL-C serum levels of rats administered both ethanolic and aqueous extracts at all three concentration, which was not dose dependent. The aqueous extracts seem to be more effective in increasing the HDL-C levels from the result. This may be as result of the influence of the extracts on the activities of Lecithin Cholesterol acyl transferase (LCAT), a serum enzyme that esterifies free cholesterol, primarily at the surface of the HDL particle after which the cholesteryl ester molecules migrate to the inner core of this lipoprotein. Through this action, LCAT plays a key role in the maturation of HDL particles (Glomset, 1968).

This may also be attributed activities secondary plant metabolites, such as saponins, alkaloids, phenols, flavonoids and tannins detected in *L. owariensis* leaf extract, which exhibit varied biochemical and pharmacological actions in animals and even micro organisms when ingested (Trease and Evans, 1983). The ethanolic extracts of *L. owariensis* at all doses were able to reduce

the serum TC more effectively than the aqueous extract. The activity may have resulted from lowering effect that can be attributed to the gut intra-luminal interactive effect of saponins (Igwe et al., 2008).

LDL-C serum levels of rats administered both ethanolic and aqueous extracts showed decrease at all three concentration, which was dose dependent, increasing with dose. Unlike in the HDL-C, the aqueous extracts were more effective in the reduction of serum LDL-C. This agrees with Igwe et al. (2008) who surmise the extract even at the lowest dose used significantly reduced LDL-C concentration. This may affect the Low-density lipoprotein transport of cholesterol to the arteries where they can be retained by arterial proteoglycans, starting the formation of plaques. LDL-C poses a risk for cardiovascular disease when it invades the endothelium and become oxidized, since the oxidized form is more easily retained by the proteoglycans (Cromwell and Otvos, 2004).

Hypertriglyceridemia, a major risk factor for CVD which has been demonstrated to be associated with increased LPL and TGL activity (Richards et al., 1985; Kanter et al., 1985). The effect may be alleviated by effect of aqueous extracts of *L. owariensis*, which reduce TAG levels in rat serum in the experiment. The ethanolic extracts had no significant effect on the TAG levels indicating the polarity of the active ingredients.

The different extracts, showed varied effects on the lipid profile indicating variation in polarity of active ingredients. Therefore, improved extraction methods will improve efficacy of extracts. In consideration of the availability of plants and the popularity gained by phytomedicine, it is necessary to trade the path of caution in the use of these extracts whose full potentials has not been harnessed.

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REFERENCES

- Anthony CD (1995). Natural preservatives from *Landolphia owariensis*. Afr. Dev. J. 2(1): 21-22.

- Cromwell WC, Otvos,JD (2004). Low density lipoprotein particles number and risk for cardiovascular disease. *Curr. Atheroscler. Rep.* 6: 381-387.
- Doerge RF, Wilson CO, Grivol O (1971). *Textbook of Organic Medicine and Pharmaceutical Chemistry*. 6th edition, Lippincott Company, Philadelphia. pp. 34-37.
- Ebi GC, Ofoefule, SI (1997). Investigation into the Folkloric Antimicrobial Activities of *Landolphia owariensis*. *Phytother. Res.* 11(2): 149-151.
- Friedwald WT, Levy RT, Fredrickson DS (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. *Clin. Chem.* 18:499-502.
- Gill LS (1992). *Ethnomedical uses of Plants in Nigeria*. Uniben Press. pp. 145-146.
- Glomset JA (1968). The Plasma Lecithin: Cholesterol acyl transferase reaction. *J. Lipid. Res.* 9:155-167.
- Igwe CU, Ojiako OA, Nwaogu LA, Onyeze GOC (2008). Lipid Lowering Effect Of Aqueous Leaf Extract Of *Spondias Mombin* Linn . *Internet J. Pharmacol.*(6) 1.
- Irvine FR (1961). *The Woody Plants of Ghana*. Oxford University Press, London. pp. 26-28.
- Kanter MA, Bianchini A, Bernier D, Sady SP, Thompson PD (1985). Androgen reduce HDL2-cholesterol and increase hepatic triglyceride lipase activity. *Med. – Sci. Sports Exerc.* 17:462-465.
- Kummerow FA (1982). The possible involvement of dietary fats in atherosclerosis. *Prog. Lipid. Res.* 21: 743-746.
- Lewis WH, Lewis MPE (1977). *Medical Botany*. John Wiley and Sons Publishers, New York. pp. 21-22.
- Nwaogu LA, Alisi CS, Ibegbulem CO, Igwe CU (2007). Phytochemical and antimicrobial activity of ethanolic extract of *Landolphia owariensis* leaf. *African J. Biotechnol.* 6(7): 890-893.
- Onyeneke EC, Oluba OM, Adeyemi O, Aboluwoye C, Eriyamremu CE, Ojeaburu SI, Adebisi K E, Adeyemi O (2008). Effect of Soy Protein On Serum Lipid Profile And Some Lipid-Metabolizing Enzymes In Cholesterol Fed Rats. *Internet J. Alternative Med.*(5) 2.
- Owoyele BV, Olaleye SB, Oke JM, Elegbe RA (2001). Anti-inflammatory and Analgesic Activities of Leaf Extracts of *Landolphia owariensis*. *African. J. Biomed. Res.* 4: 131-133.
- Richards EG, Grundy SM, Cooper K (1989). Influence of plasma triglycerides on lipoprotein patterns in normal subjects and in patients with coronary artery disease. *Am. J. Cardiol*; 63: 1214-1220.
- Searcy RL, Berquist LM (1960). A new colour reaction for the quantification of serum cholesterol. *Clin. Chim. Acta*, 5, 192-199.
- Singerist HE (1951). *A history of medicine*. Oxford University Press, New York. (1) 180
- Sofowara, EA (1982). *Medicinal Plants and Traditional Medicines in Africa*. John Wiley and Sons Ltd, Nigeria. pp 64-79.
- Tiez NW (1990). *Clinical guide to laboratory tests*. 2nd edn. W.B. Saunders company, Philadelphia, U.S.A. 554.
- Trease GE, Evans WC (1983). *Textbook of Pharmacognosy*. 12th ed. Balliese Tindall and Company Publishers, London. pp. 343-383.
- Zhang T(1999). *WHO monograph on selected medicinal plants*. 1: 4-1.