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J. Nat. Prod. Plant Resour., 2013, 3 (2):5-9
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Phytochemical screening and effect of ethanolic leaf extract of *Piliostigma thonningii* on serum lipid profile of male albino rats

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ABSTRACT

The quest for a safe herbal remedy for the management of cardiovascular diseases prompted this research. It is aimed at determining the effect of orally administered ethanol extract of Piliostigma thonningiileaves on male albino rats. Extract of the leaf were first screened for the presence of bioactive phytoconstituents using standard methods and result reveals the presence of flavonids, tannins, steroids, phlobatannin, terpenoid, cardioglycosides and saponin at varying concentrations. Twenty (20) male albino Wistar rats were randomly assigned on the basis of average body weight into four (4) groups of five (5) rats each. While group A served as the control and received distilled water, group B, C and D were administered with 50,100 and 200mg/kg body weight of the extract respectively via oral gavages and treatment lasted for twenty-one (21) days. Results showed a dose dependent ($P<0.05$) decrease in serum LDL –cholesterol and Total-Cholesterol. However, a significance ($P<0.05$) increased was observed for HDL-cholesterol at 100mg and 200mg/kg body weight but no significance ($P<0.05$) changes was recorded for TG levels in all treatment groups. The Atherogenic risk predictor indices HDL-cholesterol/total cholesterol (HDL-C/T.C) increases while LDL-cholesterol/HDL-cholesterol (LDL–C/ HDL–C) decrease with increasing dosage respectively. There are manifestations of hypo-cholesterelemia, hypo-triglyceridemia and antilipidemic activity in the serum of the treated animals which might be due to the bioactive constituents in the extract. It is speculated that the extract might serve as therapeutic tool for the management of the risk factor of atherosclerosis, coronary heart disease, and other cardiovascular related disorders.

Keywords: *Ethanoilc, Leaf extract, Piliostigma thonningii, phytoconstituents, Lipid Profile.*

INTRODUCTION

Total lipid profile or lipid panel of an individual is a contributory factor resulting from blood cholesterol along with its associated varieties of lipoproteins i.e. high-density lipoproteins (HDL or α-lipoproteins), very low density lipoproteins (VLDL or pre-β-lipoproteins) and triglycerides (Maruf *et al*., 2006). Elevation of serum total cholesterol and more importantly low density lipoprotein (LDL) cholesterol have been implicated as primary risk factor for cardiovascular diseases (Edijala *et al*., 2005). Disposition of blood pressure and coronary heart disease has been found to be in strong correlation with lipid profile particularly with blood cholesterol level (Cotaran, 1999).

Medical plants have been used in Africa for many centuries and today almost every part of the world uses herbal plants for the treatment of different diseases (Adewunmi and Ojewole, 2004). In fact, natural products, medicines from plant sources of wide diversity have been used effectively in the treatment of blood pressure and higher lipid level (Anderson *et al*., 2000; Castano*et al*., 2001; Quiles *et al*., 2002; De Laney *et al*., 2003; Rose *et al*., 2004). A number of studies have shown that reduction of LDL-cholesterol with medicinal plants has reduced the incidence of cardiovascular diseases and overall death rate (Adebayo *et al*., 2006).

Piliostigma thonningii also known as schumcamel's foot / monkey bread (English), abafe (Yoruba), kalgo (Hausa), Okpoatu (Igbo), nyihar (Tiv).ejei –jei (Igala) and omepa(Igede)**(**Dasofunjo,Ipav and Ezegwu,2012) is one of the plants with diverse ethno medical and economic applications. *P.thonningii* has been reported in literatures to have age-long folkloric use in traditional medicine, especially in the management of malaria, fever, wound, ulcers, dysentery, diarrhoea, infections, snake bites and cough (Fakae*et al*.,2000;Ajali ,2002;Jimoh and Oladiji,2005;Igoli *et al*., 2005;Madara*et al*., 2010). Therefore, since studies on the effect of ethanolic leaf extract of *Piliostigma thonningii* on the serum lipid profile is lacking and the search for suitable medicinal plants against atherosclerosis and other cardiovascular related disorders are highly imperative. Thus, this study assessed the effect of graded dosage of *P.thonningii* ethanolic leaf extract on some lipid profile in rat.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh *P. thonningii* leaves were obtained from Mkar hills, Gboko, Benue State, North Central Nigeria. Identification and authentication was done at the Federal College of Forestry Jos, Plateau State, Nigeria, with the voucher number #25. The leaves were collected and air dried for 14 days until constant weight was obtained. The dried leaves were then pulverised after which 300g was soaked in 1000mls of Ethanol and agitated, then allowed to stay in refrigerator for 48 hours at 4oC. The mixtures were first filtered with cheese cloth, then with WhatMan No 1 filter paper (24cm). The filtrates were separately concentrated using water bath (Model RE52A, China) to 10% of its original volume at 40^{o} C.

Laboratory Animals

Twenty (20) male albino rats of wistar strain were obtained from the animal holding unit, Department of Chemical Sciences, University of Mkar, Mkar, Nigeria and were allowed acclimatization period of seven (7) days in well ventilated room with a temperature and relative humidity of 29±2ºc and 70% respectively. They were maintained with rat chow (Vital Feeds LMT) and water *ad libitum*. The animals were housed in a cage and were exposed to 12 hour light-dark cycle and handled according to standard protocol. At the end of the acclimatization period, they were divided into four groups of five (5) each. Group A serve as the control, while B, C and D were the test groups. The control group was treated with distil water using orogastric tube. Test groups B, C and D were treated with 50,100 and 200 mg/kg body weight of the extract respectively. The administration of the extract lasted for 21 days period after which the animals sacrificed 24hrs after the last administration in accordance with the guidelines of the European Convention for the Protection of Vertebrate animals and other scientific purposes –ETS-123 (European Treaty Series, 2005).

PHYTOCHEMICAL ANALYSIS

Qualitative analysis was carried out on each of the test samples using diverse methods viz:

The phytochemicals; flavonoids, tannins, steroids, phlobatannins, saponins, terpenoids, cardiac glycosides and alkaloids were tested for, using the method of Trease and Evans (1989); modified by Harbone (1996) and Sofowora (1993).

Determination of Serum Lipid Profile

At the end of the treatment period, the animals were anaesthetized in chloroform vapour and the blood collected via cardiac puncture into a plane tube. The blood was allowed a clotting period of two hours and then centrifuged at 3000rpm for ten minutes, using a model 0412-1 centrifuge (Cole medical instrument co.LTD, England). The serum of the centrifuged blood was collected into a clean plane tube using a syringe, and used for lipid profile determination. The lipid profile analysis was done using kits and an AJ-1222 semi-auto Biochemistry Analyzer (Easy way medical equipments LTD, made in England)

STATISTICAL ANALYSIS

Data were presented as a mean ± SD of five determinations. Statistical analysis was carried out using one way analysis of variance (ANOVA). Differences were statistically significant at $p < 0.05$. (Mahaja, 1997).

RESULTS

The results of assessment of the effect of ethanolic leaf extract of *P. thonningii* on the lipid profile of albino rats and phytochemical screening are presented in table 1 and 2 below respectively. The extract produced a significant (P<0.05) reduction on the TG levels for all treatment groups; B (128.25±0.22), C (130.60±0.21) and D (95.80±0.11) body weight compared with the control (165.00±2.11). More so, similar trend was observed for LDL-cholesterol levels where all treatment groups B (7.50±0.12), C (5.90±2.11), and D (2.48±0.23) showed significant (P<0.05) decreased compared with the control (19.71±1.11). However, HDL-cholesterol levels for group C (62.69±0.11) and D (62.42±0.31) recorded significant (P<0.05) increased compared with the control (51.140.0±3). Statistical evaluation also shows that no marked changes was observed for total cholesterol in all treatment groups compared with the control all at (P<0.05).

The phytochemical screening result reveals that the ethanolic leaf extract of *Piliostigma thonningii contains* some bioactive constituents such as flavonids, tannins, steroids, phlobatannin, terpenoid, cardioglycosides and saponin at varying concentrations and hence may responsible for the striking results observed above.

Table 1 Effect of ethanol leaf extract of *Piliostigma thonningii* on serum lipid profile.

Groups	T.C (mg/l)	HDL-C (mg/l)	LDL-C (mg/l)	T.G (mg/l)
A(Control)	87.60±0.02	51.140.0±3	19.71±1.11	165.00±2.11
B (treated with 50 mg/kg bwt)	79.80±0.27	47.64±0.21	7.50±0.12*	128.25±0.22*
C (treated with 100 mg/kg bwt)	77.80±0.22	62.69±0.11*	5.90±2.11*	130.60±0.21*
D (treated with 200 mg/kg bwt)	95.80±0.21	62.42±0.31*	2.48±0.23*	95.80±0.11*

*Results are expressed in mean ±SEM (n=5). *signifi cant at P<0.05 compared with the control.*

T.C = *Total cholesterol*
HDL-C = *HDL cholesterol*
LDL-C = *LDL cholesterol*
T.G = *Triacylglyceride*

Table 2 Phytoconstituents of the ethanolic leaf extract of *Piliostigma thonningii.*

Parameters	Ethanol extract
Flavonoid	+
Tannins	+++
Steroid	++
Phlobatannin	++
Saponin	+
Terpenoid	++++
Cardioglycoside	+++

\+ = *Sparingly present*
\++ = *Present*
\+++ = *Highly present*
\++++ = *Very present*

DISCUSSION

The recent shift from orthodox medicine to ethnobotanicals and pharmacognosy or plants with medical potentials is a major contributive factor to a decline in the prevalence of atherosclerosis and other cardiovascular related diseases or management of the disposable risk factor in most developing nations especially in Africa and Asia. Over the years, herbal extracts have been reported to improve the lipid profile of humans (Chaltapadhyay and Bandy Opdyay, 2005). Dyslipodemia, which can range from hypercholesterolemia to hypolipiproteinemia is one of the many modifiable risk factors for coronary artery disease (CAD), stroke and peripheral vascular disease (Chong and Bacheheimer, 2000; Maysone*t al*., 2011). Assessment of serum lipid panel will reveal a clinical basis to understanding of the metabolism of lipids and it role in predisposing humans to atherosclerosis, coronary heart diseases and other cardiovascular related disorders.

Therefore, the increase in serum cholesterol at 200mg/kg body weight of the external may be due to increase in concentration of acetyl-CoA arising probably from enhanced α-oxidation stem of fatty acids, since acetyl CoA is a key substrate in the biosynthesis of cholesterol (Ray, Dale and Relter, 1995; Yakubu and Afolayan, 2009). While the observed decrease ($P<0.05$) in serum cholesterol at 50 and 100mg/kg body weight might be due to a decrease in absorption from the intestine by binding with bile acid within the intestine and increasing bile acid secretion.

HDL-Cholesterol is known to have a protective effect against cardiovascular disease, since it removes excess cholesterol from circulation and carries it back to the liver where it is degraded or converted into bile acid (Ahmed *et al*., 1992). Also, HDL-C is considered to have anti atherogenic properties. It has also been shown that an increase in HDL-Cholesterol correlates inversely to coronary heart disease (Ghaji, Nwobodo and Ofili, 2002). It can therefore be inferred that the significant increase ($P<0.05$) in serum level HDL-C suggests that the ethanol leaf extract of *P.thonningii* may be used to reduce the risk factor of atherosclerosis and other cardiovascular related disorders. It also suggests that the extract might exert a protective, shielding or effect against atherosclerosis.The observed hypotriglyceridemic effect of the extract of *P.thonningii* may be due to a decrease of fatty acid synthesis (Bopanna *et al*., 1997) enhanced catabolism of LDL, activation of lipid catabolism and tissue lipase (Khanna *et al*., 2002) and/or inhabitation of acetyl-CoA carboxylase (Mccarly, 2001) and production of triglycerides precursors such as acetyl-CoA and glycerol-phosphate (Kabir, 2010). This hypotriglyceridemic potential of the extract might be responsible for its continual usage in folk medicine.

More so, the observed reduction in low density lipoprotein cholesterol (LDL-Cholesterol) following the administration of the extract at all dosage suggest that extract possess cholesterol lowering or clearing ability or an hypocholesterolemic agent which might be of great significant in the management of atherosclerosis and other cardiovascular related disorders.

Atherogenic risk predictors indices HDL-C/T.C which increased with increasing dosage of the ethanol leaf extract of *P.thonningii* and LDL-C/HDL-C which decreased at all the treated groups with increasing dosage suggests its beneficial role to reduce coronary heart disease, atherosclerosis and other related disease which appear to be dosage dependent.

The presence of bioactive phytoconstituents such as saponins, tannins, flavoniods, terpenoid, cardioglycocides and steroids are known to perform several general and specific function in plants and many exhibits different biochemical and pharmacological actions in different species of animals when injected. The action ranges from cell toxicity to cell protective effect (Trease and Evan, 1996). Saponin is known to lower triglyceride level due to it lytic role and as it is well established that VLDL cholesterol are the main transporter of triglycerides in serum (Hertog*et al*., 1993). Phytosterol are reported to displace intestinal cholesterol and reduce cholesterol absorption from intestine (Ikeda and Sugano, 1998; Mayson, 2011). Saponins are capable of precipitating anterohepatic circulation of bile acid, making it unavailable for intestinal absorption (Fuhrma*et al*., 2002). Therefore, the decline in both trigylceride and LDL-Cholesterol in treated groups may indicate that the extract of *P.thonningii* produce possible effect due to the presence of phytosterol, Saponin and other phytoconstituents leading to decreased absorption of dietary cholesterol. Thus, the observed reduction in serum cholesterol and triglyceride may be attributed to the bioactive phytoconstituents of the extracts like phytosterol, flavonoids and Saponin as also reported by Johnson, et al., (2012). It will be logical to conclude that the extract possessan hypocholesterelemic, hypotriglyceridemic and antilipidemic activity which suggest that extracts of *P.thonningii* might be a therapeutic tool for the management of atherosclerosis and other cardiovascular related disorders.

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