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J. Nat. Prod. Plant Resour., 2012, 2 (6):665-669

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ISSN : 2231 – 3184

CODEN (USA): JNPPB7

### The effect of ethanolic leaf extract of *Piliostigma thonningii* on serum lipid profile of male wistar albino rats

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#### ABSTRACT

The phytochemical screening and the effect of orally administered ethanolic leaf extract of *Piliostigma thonningii* on Serum lipid profile of male albino wistar rats were determined. Extracts of the leaf were first screened for presence of phytochemicals using chemical methods. Fifteen (15) albino wistar rats were randomly assigned on the basis of average body weights into three groups of five (5) rats each, following acclimatization to laboratory and handling conditions. Animals in group I (control) were administered placebo (1mls) of distilled water. Group II was administered with 200mg/kg body weight of the extract and group III was administered 400 mg/kg body weight. Extract administration lasted for twenty one (21) days. Water and feeds were allowed ad libitum. At the end of dose administration, animals were sacrificed and blood obtained for lipid profile analysis of Total-cholesterol, Triglyceride, HDL-cholesterol, and LDL-cholesterol and Atherogenic risk predictor using standard methods. Results showed a dose dependent increase in total cholesterol, HDL – cholesterol, and Triacylglyceride and decrease in LDL – cholesterol. Phytochemical results revealed the presence of Flavonoids, Saponins, Tannins, Steroids, Phlobatannins, Terpenoids, Cardioglycosides, with steroids and flavonoids in excess. The Atherogenic risk predictor indices HDL – C/TC, increase with increasing dosage but LDL – C/ HDL – C decrease only at the treated with 200mg/kg body. There was hypercholesterolemia and hypertriglyceridemia, although at 200mg/kg body weight there appear to be a favourable impact on good cholesterol, (HDL). These was not so at 400mg/kg body weight.

**Keywords:** *Piliostigma thonningii*, Photochemical, Total Cholesterol, HDL, LDL, Triacylglyceride and Atherogenic risk predictor indices.

#### INTRODUCTION

Traditional medicinal plants are therapeutic resource used by the population of the African continent specifically for health care, which serve as starting materials for drugs [1]. Iwu, Duncan and Okunji reported that infectious diseases account for one-half of all deaths in the tropical countries [2]. As a result, people have imbibed the use of indigenous plants dating back to prehistory for health purposes [3]. These medicinal plants are herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal product [4].

However, traditional medicines have been the focus for a wide coverage of primary health care delivery in Africa and the rest of the world. These plants have demonstrated its contributions to the treatment of diseases such as HIV/AIDS, malaria, diabetes, sickle cell anemia, mental disorders and some microbial infections [5][6]. The primary benefit of using plant derived medicines is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment.

A variety of plants have been used for therapeutic purposes over the years and one of such plants is *Piliostigma thonningii*. *Piliostigma thonningii* is a leguminous plant belonging to the family *caesalpiniacea*, a family that comprises of trees, shrubs or very rarely scramblers. [7]. The plant is locally called abafe in Yoruba, Kalgo in Hausa, Okpoatu in Igbo and Monkey bread or Camel's foot in English [8] and nyihar in Tiv and omepa in Igede.

*Piliostigma thonningii* has been used traditionally in the management of fever, cough, wounds and various ulceration [9]. Also, different parts of *P. thonningii* such as the bark, root, trunk and leaves have been described as useful in the treatment of many pathologies of viral origin such as Herpes, Influenza, Broncho-pulmonary diseases and HIV virus [10]. The bark of this plant has been used in the treatment of diarrhea, dysentery, intestinal upset, sore throat, toothache, stomach-ache, ear ache and as analgesics [3][11].

Although there are many reported folkloric claims on the medicinal usefulness of *P.thonningii* plant and some research reports on selected parts such as root, stem, bark and leaf, there is no report yet on the effect of the ethanolic extract of *P.thonningii* leaves on the lipid profile of albino rats.

## MATERIALS AND METHODS

### PLANT MATERIALS

*Piliostigma thonningii* leaves were obtained from Mkar area in Benue State, Nigeria. The plant was identified and authenticated at the forestry department of the University of Jos, Jos Plateau State Nigeria with the voucher number #25.

### ASSAY KITS

Lipid profile cholesterol, HDL-cholesterol and Triglyceride was assayed by a colorimetric method by Hiller [12] – enzymatic and point method (CHOD – PAP) by Braun [13] and precipitation colorimetric method by Stein and Meyers, [14] respectively. While LDL – cholesterol was assayed using direct immune – Inhibition method by Burtis and Ashwood [15].

### EXPERIMENTAL ANIMALS

Albino rats were obtained from the animal holding unit of the college of Health Sciences, department of Biochemistry, Benue State University Makurdi Nigeria. The animals were allowed to undergo acclimatization period for seven days. The rats were housed in a wooden cage with well ventilation. They were kept at room temperature and relative humidity of  $29\pm 2^{\circ}\text{C}$  and 70% respectively with 12 hours natural light and dark cycle. They were also allowed free access to standard feed from vital feed Gboko, Benue State, and water. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from cages daily.

### PREPARATION OF PLANT MATERIALS

The leaves of *Piliostigma thonningii* was collected and air dried for 14 days until constant weight was obtained. It was pulverized using a blender machine and sieved to a powdery form. 300g was obtained after pulverization and dissolved in 1,000mls of ethanol solvent for 72 hours so that maximum extraction was achieved. The solution was filtered using whatman No.1 filter paper and the filtrate concentrated in a water bath at  $40^{\circ}\text{C}$ . The resulting slurry was later weighed and reconstituted in distilled water to the required dosage.

### PHYTOCHEMICAL ANALYSIS

The phytochemicals (flavonoids, tannins, steroids, phlobatannins, saponins, terpenoids, cardiac glycosides and alkaloids) were tested for, using the method of Trease and Evans [16]; modified by Harbone and Sofowora [1][17].

### ORAL ADMINISTRATION

Fifteen (15) male albino rats with an average weight of 199g were randomly selected and divided into three groups of five (5) rats each (A, B, C). A, served as the control, while B and C were used as test. The control group was

administered only with water and standard feed, while B and C received 200 and 400mg/kg body weight of the ethanolic extract of *P.thonningii* leaves, standard feed and water respectively for the period of 21 days. The rats were subsequently sacrificed and the serum collected in an EDTA bottle via cardiac puncture for lipid profile assessment.

### STATISTICAL ANALYSIS

Data were presented as a mean  $\pm$  SD of five determinations. Statistical analysis was carried out using one way analysis of variance (ANOVA). Differences were statistically significant at  $p < 0.05$  [18].

### RESULTS

The effect of the graded doses of the ethanolic leaves extract *P.thonningii* on the lipid profile of albino rats are shown in (table 1). While there was only a significant decrease ( $p < 0.05$ ) for the group administered with 200mg/kg body weight for serum LDL – cholesterol, the total cholesterol, HDL – cholesterol, LDL – cholesterol and Triacylglyceride shows a significant increase ( $p < 0.05$ ) for both the groups administered with 200mg/kg body weight and 400mg/kg body weight when compared with the control respectively. (Table 2) also show the atherogenic risk predictor indices for the ethanolic leaf extract of *Piliostigma thonningii*; the HDL – C/T.C increased with increasing dosage of *P.thonningii*, while LDL/HDL – C reveals only a significant reduction in the groups administered with 200mg/kg body weight. Phytochemical screening of the ethanolic leaf extract of *Piliostigma thonningii* is as shown in table 3.

**Table 1: Effect of ethanolic leaves extract of *Piliostigma thonningii*.**

Groups	T.C (mg/l)	HDL – C (mg/l)	LDL – C (mg/l)	T.G (mg/l)
A Control)	1.75 $\pm$ 0.10	1.10 $\pm$ 0.01	0.15 $\pm$ 0.11	1.15 $\pm$ 0.11
B (treated 200mg/wt)	1.98 $\pm$ 0.21*	1.53 $\pm$ 0.11*	0.08 $\pm$ 0.21*	1.38 $\pm$ 0.11*
C (treated 400mg/wt)	2.00 $\pm$ 0.02*	1.50 $\pm$ 0.1*	0.21 $\pm$ 0.01*	1.20 $\pm$ 0.01

Results were expressed in mean  $\pm$  (n=5). \*Significant 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: Atherogenic risk predictor of ethanolic leaves extract of *Piliostigma thonningii***

Groups	HDL-C/T.C(mg/l)	LDL-C/HDL-C(mg/l)
A (control)	0.63 $\pm$ 0.02	0.14 $\pm$ 0.01
B (treated-200mg/wt)	0.77 $\pm$ 0.01*	0.05 $\pm$ 0.02*
C (treated-400mg/wt)	0.75 $\pm$ 0.02*	0.14 $\pm$ 0.01

Results were expressed in mean  $\pm$  (n=5). \*Significant at  $P < 0.05$  Compared with the control.

T.C	=	Total cholesterol
HDL – C	=	HDL cholesterol
LDL – C	=	LDL cholesterol
T.G	=	Triacylglyceride

**Table 3: Phytochemical screening of the ethanolic leaf extract of *Piliostigma thonningii*.**

Parameters	Results
Tannins	++
Phlobatannin	+
Saponin	++
Steroid	++++
Flavonoid	+++
Terpenoid	++
Cardioglycoside	++

  

+	=	sparingly present
++	=	present
+++	=	highly present
++++	=	very highly present

## DISCUSSION

An assessment of the alterations on the lipid profile of major lipids like total cholesterol (T.C), high density lipoprotein cholesterol (HDL – C), low density lipoprotein cholesterol (LDL – C) and triglycerides could give relevant information on cardiovascular, atherosclerosis and other atherosclerosis related diseases.

Therefore, the increase in serum cholesterol with increase ethanolic extract may be due to increase in the concentration of acetyl – CoA arising probably from enhanced  $\beta$  – oxidation stem of fatty acids, since acetyl – CoA is a key substrate in the biosynthesis of cholesterol [19][20].

Also, HDL – C is considered to have anti-therogenic properties. It has also been shown that an increase in HDL – C correlates inversely with coronary heart disease [21]. It can therefore be inferred that the significant increase in serum level HDL – C suggests that the plant leaves ethanolic extract may be used to reduce the risk factors of cardiovascular related diseases. Hence, it may exert a protective effect against atherosclerosis.

Though there was a significant increase in LDL – C of the groups administered with 400 mg/kg body weight, lowering of serum lipid concentration particularly LDL and VLDL fractions was considered as one of the strategies that can delay the on-set of chronic disorders associated with hyperlipidemia in human [22].

Therefore, significant decrease in the group administered with 200mg/kg body weight at the end of the experimental period suggests that the extracts is dosage dependent and might have hypolipidaemic and hypocholesterolaemic potential at a dosage lower than 400mg/kg body weight. An atherogenic risk predictor indices HDL – C/T.C which increased with increasing dosage of the ethanolic leave extract of *Piliostigma thonningii* and LDL – C/HDL – C which decreased only at the group treated with 200mg/kg body weight suggest that its beneficial role to reduce coronary heart diseases, atherosclerosis and other related diseases might be dosage dependent.

The presence of Phytochemicals such as Saponins, Tannins, Flavonoid, Terpenoid, Cardioglycosides and Steroid are known to perform several general and specific functions in plants and may exhibit different biochemical and pharmacological actions in different species of animal when ingested. These action ranges from cell toxicity to cell protective effect [23]. flavonoids have antioxidant property [24]. Hypocholesterolemic and antilipidaemic activity of the ethanolic leaf extract of *P.thonningii* might be due to the presence of Tannin and Saponin [25].

The ethanolic leaf extract of *Piliostigma thonningii* can be utilized for the prevention of atherosclerosis in hypercholesterolemic patients and related disorders. Although, at 200mg/kg body weight dose of the extract, there was a reduction in LDL- cholesterol and increase in LDL –cholesterol relative to control but this is not enough to conclude its usage in the management or prevention of hypercholesteramia as triglyceride levels were raised.

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