Original Paper

Effect of ethanol extract of *Piliostigma thonningii* leaf on serum lipid profile following indomethacin induced mucosa onslaught in male wistar albino rats.

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Running Title: Effect of *Piliostigma thonningii* leaf extract on lipid profile of ulcerated rats

**Abstract**

In determining the effect of *P. thonningii* leaf extract on serum lipid profile in male Wistar albino rats following indomethacin induced mucosa onslaught. Thirty six (36) male rats were divided into six (6) groups of 6 rats each. Group one (1) served as control and was given 0.5ml of normal saline (vehicle). Group three (3), five (5) and six (6) were given 100, 100 and 200mg/kg body weight of the extract while group two (2) was treated with 100mg/kg body weight of the standard drug (Cimetidine). The vehicle and extract were administered orally while the drug was administered intra-muscularly for 12 days. After 12 days of administration all rats were fasted for 24 hours, gastric ulceration was then induced by the administration of 40mg/kg indomethacin orally to group 2, 4, 5 and 6 respectively. Twelve (12) hours after indomethacin administration all rats were sacrificed after been anaesthetized with chloroform, blood was collected by cardiac puncture and serum collected for the determination of lipid profile using standard methods. The result depicted a significant (P<0.05) increases in serum total cholesterol when compared with the control. The treatment also produced a significant (P<0.05) increase throughout the experimental group for serum triglyceride when compared with the control. The biochemical and physiological alterations in this study following indomethacin mediated mucosa onslaught suggest that the extract treated groups might be shielded against cardiovascular related disorder but it appears that cimetidine and indomethacin treated groups might be predisposed to cardiovascular derangements such as atherosclerosis, myocardial infarction and coronary heart diseases but the mechanism is not yet fully understood.

**Keywords**: Lipid profile, Atherosclerosis, Coronary heart diseases, Myocardial infarction and *Piliostigma thonningii*

**Introduction**

Ulcer and Cardiovascular diseases and related disorders are a major cause of mortality both in men and women all over the world [1]. They are commonly characterized by high levels of total cholesterol, triglycerides, and low-density lipoprotein cholesterol in the serum. Increased total cholesterol and more significantly LDL cholesterol in the serum have been implicated in the etiology of cardiovascular diseases and are seen as primary risk factors. Also, high level of lipids in the blood has been associated with hypertension and lipid peroxidation. According to World Health Organization, about 80% of folks in developing countries depend mainly on traditional medicine for their primary health care, and about 85% of such traditional medicine involves the use of plant extracts and some commonly consumed herbs have been reported to promote reduction in blood lipids [2, 3].

Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic
origin and functional groups into primary and secondary metabolites. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be valuable in the management of most degenerative diseases. One of these plants is *Piliostigma thonningii*.

*P. thonningii* is an under-explored leguminous plant that belongs to the family, *Leguminosae*-Caesalpiniodae. The tree is perennial in nature, and the petals vary from white to pink in color and are produced between November and April [4]. *P. thonningii* is used for various medicinal purposes such as to treat wounds, gastric/heart pain, gingivitis, antipyretic, a cough remedy and anti-inflammatory/analgesic and antibacterial activities [5-7]. Preliminary phytochemical studies on *P. thonningii* reveals high levels of flavonoids, tannins, and alkaloids [8]. The plant is also reported to contain nutritionally important vitamins (such as C, E, and beta-carotene) and minerals (such as calcium, magnesium, zinc, and potassium) all of which contribute to its high-antioxidant properties). Against high incidence of cardiovascular diseases, there is paucity of information on scientifically verified plants with anti lipidaemic and anticholesterolaemic properties [7, 9].

In light of the chemical constituents of *P. thonningii*, this study was designed to evaluate the effect of its ethanol leaf extract on the serum lipid profile following indomethacin induced ulcer in rats.

### Materials and Methods

#### Plant material

Fresh leaves of *P. thonningii* were collected from Igoli Road, Cross River University of Technology, Cross River State, Nigeria. The leaves were taken to Federal College of Forestry (FCOFJ) Jos in Plateau State, Department of Herbarium for identification and authentication with the Voucher number #25 has been deposited for future reference at the department’s (FCOF J) Herbarium.

#### Preparation of plant material

Fresh leaves of *P. thonningii* were air-dried at room temperature for twenty (20) days, macerated and pulverized into powdery form using the blender and then sieved.

### Ethanol extraction

Three hundred (300) g of powdered *P. thonningii*, leaves were dissolved with 1200 mls of 75% ethanol for 24 hours in refrigerator. Thereafter, it was filtered with muslin cloth and filtered using Whatman filter No 1. The filtrate was evaporated to dryness and the percentage yield was reconstituted into dosage and administered into rats.

#### Experimental animal

Thirty-Six (36) male Wistar albino rats (120-200) g were obtained from the Animal Holding Unit of the Department of Medical Biochemistry, Cross River University of Technology. Cross River State Nigeria. The animals were allowed to undergo acclimatization period for seven days before the commencement of the research. The rats were housed in wooden cage. The animal room was ventilated and kept at room temperature and relative humidity 29°C and 70% relative humidity with 12 hours natural light dark cycle and were allowed free access to standard feed and water *ad libitum*. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from cages daily. The study was approved by the Animal Welfare and Ethics Committee of CRUTECH, Cross River State, Nigeria. All conditions of animal use were also as approved by United States National Institute of Health (NIH) guide for Care and Use of Laboratory Animals.

### Anti-ulcer activity

The experiment was carried out on 36 male rats that were divided into six (6) groups of 6 rats each. Group one (1) served as control and was given 0.5ml of normal saline (vehicle). Group three (3), five (5) and six (6) were given 100, 100 and 200mg/kg body weight of the extract while group two (2) was treated with 100mg/kg body weight. The vehicle and extract were administered orally while the drug was administered intra-muscularly for 12days. After 12days of administration all rats were fasted for 24 hours, gastric ulceration was then induced by the administration of 40mg/kg indomethacin orally to group 2, 4, 5 and 6. 12 hours after indomethacin administration all rats were sacrificed after been anaesthetized with chloroform, blood was collected by cardiac puncture.
Preparation of serum

The animals were anaesthetized in a desiccator containing cotton wool soaked in ether and chloroform in ratio 1:1. When the animal became unconscious, they were brought out quickly of the desiccator, the abdominal region was opened along the linear Alba and diaphragm cut with scalpel blade to expose the organs and blood was collected into a sterile sample container by cardiac puncture. Blood was collected into a clean, dry centrifuge tube and allowed to clot for 30 min before centrifuging at 300 rpm x 10 min using Uniscope Laboratory Centrifuge. The serum was thereafter aspirated into clean, dry, sample bottles using Pasteur pipette and was kept or stored in sample bottles and used within 12 hours of preparation. Later it was transferred into specimen bottles before being used for analysis of the lipid profile.

Statistical analysis

Statistically analysed data used was presented as mean ± SD of five (5) determinations. Statement analysis was done using one way analysis of variance (ANOVA). Differences were statistically significant at P<0.05.

Result

The result below depict the effect of *P. thonningii* leaf extract on lipid profile of male Wistar albino rat following indomethacin mediated gastric mucosa onslaught. The treated dose showed a significant (P<0.05) increases in serum total cholesterol when compared with the control (fig 1). Likewise the standard drug (cimetidine), the extract and other treated groups showed a significant (P<0.05) increase in serum high density lipoprotein (HDL) when compared with the control while the group induced with ulcer without treatment showed a significant (P<0.05) decrease in serum HDL when compared with the control (fig 2). The extract also produced a significant (P<0.05) decrease of serum very low density lipoprotein (VLDL) while the group treated with cimetidine and indomethacin produced a significant (P<0.05) increase when compared with the control (fig 3). Similar pattern was shown with serum low density lipoprotein (LDL) (fig 4). The treatment also produced a significant (P<0.05) increase throughout the experimental group for serum triglyceride when compared with the control (fig 5).

Fig 1: Effect of extract of *Piliostigma thonningii* leaf on serum total cholesterol following indomethacin mediated mucosa onslaught.

Values are expressed as mean ± SEM, n=5.
* = p<0.05 vs control
a = p<0.05 vs ulcer control

Fig 2: Effect of extract of *P. thonningii* leaf on serum HDL following indomethacin mediated mucosa onslaught.

Values are expressed as mean ± SEM, n=5
*= P<0.05 vs control
Discussion

Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are among the most commonly prescribed medications worldwide. Over 300 million patients use non-steroidal anti-inflammatory drugs (NSAIDs) in the world to treat pain, arthritis, fever and other diseases. Nearly 30% of the users suffer from gastric lesions and bleeding. Mechanisms for such actions of NSAIDs seem to be complex and multifactorial, including the inhibition of prostaglandin (PG) synthesis, induction of apoptosis and necrosis of gastric mucosal cells [10], neutrophil penetration, dysfunction of micro vessels, reduced secretion of bicarbonate and mucus, and increased gastric motility [11]. However, NSAIDs have adverse effects on the gastric mucosa, resulting in various clinical presentations, ranging from nonspecific dyspepsia to ulceration, upper gastrointestinal bleeding, and death, summarized by the term “NSAID gastropathy” [10]. NSAIDs-induced gastric damage is the major side effect of this kind of drug. The main action of NSAIDs is to inhibit prostaglandin synthesis. There is substantial evidence supporting the view that the ulcerogenic effect of this medication correlates with its ability to suppress
prostaglandin synthesis [11]. Endogenous prostaglandins normally regulate mucosal blood flow, epithelial cell proliferation, epithelial restitution, mucosal immunocyte function, mucus and bicarbonate secretion, and basal acid secretion. Therefore, decreases in prostaglandins, protective factors for ulcer formation, lead to gastric mucosal injury [12].

In this study, the sera obtained were used for the biochemical analysis of total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol, total cholesterol and triglycerides following indomethacin mediated gastric mucosa onslaught. The assessment of serum lipid profile reveals the clinical basis for understanding the metabolism of lipids and their role in predisposing humans to atherosclerosis, coronary heart diseases and other cardiovascular related disorders [7, 9].

Lipids are generally characterized by insolubility in aqueous or polar solvents but highly soluble nonpolar or organic solvents. Biochemical reactions and transportations of molecules generally occur in aqueous medium. Hence, lipids are normally combined with specific proteins to form structures called lipoproteins which possess substantial degree of hydrophilicity. Low density lipoproteins, high- density lipoproteins, and chylomicrons which are basically composed of triglycerides are integral parts of the serum lipoproteins. Except for the HDL cholesterol, high level of all lipids in the blood is arguably a high risk factor in the onset of cardiovascular disorders. High serum concentrations of triglycerides and LDLS have been reported to cause atherosclerosis and coronary heart diseases [13]. Cholesterol is the principal sterol in animal tissues and occurs mainly in the cell membrane due to its amphipathic nature. It is also found in the adrenal gland, liver, brain, and nervous system [13]. The molecule is synthesized basically from acetyl CoA in the liver from where it is distributed through the blood to extra hepatic tissues where it is utilized for the synthesis of bile acids and steroid hormones as well as regulation of membrane fluidity. However, high level of cholesterol in the blood has adverse effects on human health. It is reportedly a major cause of cardiovascular derangements such as atherosclerosis, myocardial infarction and coronary heart diseases.

The significant increase in HDL and corresponding decrease in LDL in the extract treated groups supports the antilipemic or cholesterol lowering or hypocholesterolemic effect of P. thonningii leaf as reported by [9]. Therefore despite the indomethacin mucosa ulcerations, the observed significant increase in HDL and decrease in LDL in the extract treated groups suggests that P. thonningii possess some bioactive compounds, possibly flavonoids or alkaloids that aids such pharmacological activities. There is also the possibility that the extract possess the ability to facilitate the transport of cholesterol and triglycerides from the blood into tissues. This may have probably occurred through the induction or suppression of certain enzymes critical to the metabolism of these lipids. This observation is similar to the report of [3, 14, 15]. Hence, might not be predisposed to atherosclerosis and other cardiovascular diseases. On the other hand, the observed significant increase in LDL and decrease in HDL in indomethacin and cimetidine treated group suggest that the drugs might alter the transportation of lipids in the blood which might predisposes the animals to cardiovascular related disorders. More so, the significant increase in serum cholesterol in all the treated male albino rats may be attributed to an increased concentration of acetyl-CoA is a key substrate in the biosynthesis of cholesterol or increase in absorption from the intestine by binding with bile acid within the intestine and increasing bile acid secretion due to the drug.

The observed increased in triglyceride in all the indomethacin treated groups suggest that it can induce hypertriglyceridemia which may be due to increase in fatty acid synthesis, enhanced catabolism of LDL, activation of Lipid catabolism and tissue lipase and or increased of acetyl-CoA carboxylase and production of triglycerides precursors such as acetyl-CoA and glycerol phosphate.

**Conclusion**

The biochemical and physiological alterations in this study following indomethacin mediated mucosa onslaught suggest that the extract treated groups are shielded against cardiovascular related disorder but it appears that cimetidine and indomethacin treated groups are predisposed to cardiovascular derangements such as atherosclerosis, myocardial infarction and coronary heart diseases.

**Conflict of interest**

The authors declare that there is no conflict of interest to reveal.
References


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