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Piliostigma thonningii leaf extract potentiates remedy to pregnancy-induced hypertension

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Abstract

This research investigated the effect of *Piliostigma thonningii* leaf extract on maternal and offspring lipid profiles following acetaminophen-induced toxicity in Wistar rats. Twenty-five pregnant rats (180-200 g) were assigned based on their body weight to groups I-V and treated thus: animals in groups II-V were orally administered 200 mg/kg b.w acetaminophen, 200 mg/kg b.w. *P. thonningii*, 100 mg/kg b.w. *P. thonningii* +200 mg/kg b.w. acetaminophen, and 200 mg/kg *P.thonningii* + 200 mg/kg b.w. acetaminophen, respectively, while group I served as the control. Lipid profile assay was performed after 28 days of animal experimentation. We observed that the maternal triglyceride (TG) and HDL concentrations of group II were significantly (P<0.001) higher and lower respectively than the control and test groups, while the TG and HDL of group II offspring were significantly higher (P<0.05) than the control and group V and lower (P<0.01) than control and test groups, respectively. The maternal total cholesterol (TC) concentration of group II was significantly reduced to normal in groups IV (P<0.001) and V (P<0.01) and for LDL of groups IV (P<0.001) and V (P<0.05). The TC of group II offspring was significantly reduced in groups IV (P<0.05) and V (P<0.001) of the offspring but LDL was not altered. This report revealed that *P. thonningii* leaf extract reverses acetaminophen-induced toxicity in pregnancy, which is reflected in dyslipidemia vis-à-vis pregnancy-induced hypertension (PIH). Hence, *P. thonningii* leaf extract possesses the potential to reverse PIH and related effects on offspring with a possible reduction in the risk of preeclampsia.

Keywords: Acetaminophen; Dams; Maternal; Prenatal; Preeclampsia; lipid profile.

Introduction

The use of medicinal plants is among the oldest and most varied of all therapeutic systems as an essential component of the African health care system (Mahomoodally, 2013; Ahmad, *et al.*, 2018). Traditional healers prescribing medicinal plants are the most inexpensive and readily accessible health resources available to the world's people in many parts of rural Africa and often the only surviving therapy (Ukwuani *et al.*, 2012; Benzineb, *et al.*, 2018).

Cardiovascular diseases and related disorders are a major cause of mortality in both men and women worldwide (Omole and Ighodaro, 2012; Gao *et al.*, 2019); commonly characterized by high levels of total cholesterol, triglycerides, and low-density lipoprotein cholesterol in the serum. Elevated total cholesterol and more importantly LDL cholesterol within the serum are implicated within the etiology of cardiovascular diseases and are seen as primary risk factors (Omole and Ighodaro, 2012). Also, high levels of lipids in the blood have been associated with hypertension and lipid peroxidation (Moriel *et al.*, 2000). Though orthodox medicine is acceptable and preferred, it is highly trusted in traditional medicine (Gurib-Fakim and Mahomoodally, 2013). Traditional medicine is common in developing countries where the cost of orthodox medicine is astronomical and unaffordable to a large number of people. According to the World Health Organization, approximately 80% of folks in developing countries depend mainly on traditional medicine for their primary health care (Ekor, 2014; Almaiman, and Al Wutayd, 2019).

Some commonly consumed herbs lower blood lipids (Ighodaro and Omole, 2012; Rouhi-Boroujeni *et al.*, 2015; Farhan, 2018). Preliminary phytochemical research on *Piliostigma thonningii* revealed high levels of flavonoids, tannins, and alkaloids, and cholesterol-lowering effect (Dasofunjo *et al.*, 2013). This plant has also been reported to exhibit antioxidative, hematopoietic, and hepatoprotective roles among others (Dasofunjo *et al.*, 2016). Therefore, this research aimed at assessing the effect of *Piliostigma thonningii* on the serum lipid profile of maternal and offspring rats following acetaminophen-induced toxicity.

Materials and Methods

Plant material

Fresh *P. thonningii* leaves were obtained from Igoli/Okuku road, Cross River State, Nigeria. Identification and authentication were performed at the Federal College of Forestry Jos, Plateau State, Nigeria, with the voucher number #25.

Experimental animals

Twenty-five (25) virgin female Wister rats were obtained from the animal holding unit, Department of Medical Biochemistry, Okuku Campus, acclimated for 7 days and housed in wooden cages. The animal room was well ventilated and kept at relative humidity and

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room temperature of 70% and 27 ± 2°C, respectively, with a 12-h natural light-dark cycle. They were allowed free access to standard feed and water with the maintenance of good hygiene through constant cleaning and removal of feces as well as spilled feeds from cages daily. The animals were subcutaneously injected with 0.1mg/kg bodyweight of diethylstilbestrol in 0.5 mL olive oil to ensure the female rats were in estrous. Mature male rats were introduced in the ratio of 1:3 until they were confirmed pregnant.

Preparation of ethanol extract of Piliostigma thonningii leaves

The *P. thonningii* leaves were collected and dried in air for 14 days to obtain the constant weight. The dried leaves were then pulverized, after which 300 g was extracted in 1000-mL ethanol for 72 h with constant shaking using an electric shaker. It was later filtered using Whatman No.1 filter paper. The filtrates were then concentrated in a water bath at 45°C. The resulting slurry was weighed and reconstituted in maize oil to administer the required dose.

Animal Grouping and Extract Administration

Twenty-five pregnant female albino rats were chosen according to body weight and placed in wooden cages labeled A-E. Group A served as control and groups B to E were test groups. The animals in group A were orally administered with distilled water. Groups B to E was administered with 200mg/kg bodyweight of acetaminophen, 200 mg/kg body weight of *P. thonningii extract,* 200 mg/kg body weight of acetaminophen + 100mg/kg b.w. *P. thonningii* extract*,* 200 mg/kg body weight of acetaminophen + 200mg/kg b.w. *P. thonningii* extract, respectively. All experimental groups used maize oil as the vehicle. The oral administration was performed for 28 days till parturition (i.e till they gave birth). The offspring were carefully separated and cared until they were weaned on day 21. After 24 h, a cardiac puncture procedure was used to sacrifice the animals in each group. The animals were handled humanely under the guidelines of the European Convention for protecting vertebrate animals and other scientific purposes (2005). Ethical approval for the study was obtained from the Faculty of Basic Medical Sciences Animal Research Ethical Committee of the Cross River University of Technology, Calabar, Nigeria (approval number FBMS/CRUTECH/12/021).

Blood Sample Collection

Cardiac puncture procedure was used to collect blood from the test rats and control using disposable syringes and needle to draw blood into plane tubes for the determination of lipid profile.

Statistical analysis

Data obtained from the experiment were statistically analyzed using graph pad prism 5.0 software and presented in tables. A statistically significant difference was taken at $p<0.05$, $p<0.01$, and $p<0.001$.

Results

The results of the lipid profile of maternal and offspring rats on prenatal administration of *P. thonningii* following acetaminophen-induced toxicity are presented here. The maternal lipid profile is given in Table 1 while that of offspring in Table 2.

We observed that the maternal triglyceride (TG) concentration of acetaminophen only (Ac) group was significantly ($P<0.001$) higher than the control, *P. thonningii* only (PT), acetaminophen plus *P. thonningii* low dose (Ac + PT LD), and acetaminophen plus *P. thonningii* high dose (Ac + PT HD). Conversely, the TG of the test groups was significantly ($P<0.05$) decreased compared with the control. Maternal total cholesterol (TC) concentration of acetaminophen only group was significantly greater than control ($P<0.01$), PT ($P<0.01$), Ac + PT LD ($P<0.001$), and Ac + PT HD ($P<0.01$)

We observed that the maternal high-density lipoprotein (HDL) concentration of acetaminophen only (Ac) group was significantly ($P< 0.001$) lower than control, Ac + PT LD, Ac + PT HD but for PT ($P<0.01$). Maternal low-density lipoprotein (LDL) of Ac was significantly greater than control ($P<0.01$), PT ($P<0.01$), Ac + PT LD ($P<0.001$), and Ac + PT HD ($P<0.05$).

Furthermore, it was observed that offspring triglycerides (TG) of Ac were significantly ($P<0.05$) higher than the control, PT, and Ac + PT HD. The total cholesterol (TC) of the offspring was significantly greater than the control ($P<0.01$), PT ($P<0.001$), A+PT LD ($P<0.05$), and Ac + PT HD ($P<0.001$). The offspring HDL concentration of Ac was significantly different from the control ($P<0.01$), PT ($P<0.001$), Ac + PT LD ($P<0.01$), and Ac + PT HD ($P<0.01$). The offspring LDL of all groups showed no significant ($P>0.05$) difference between each other.

Table 1. Effect of *P. thonningii* extract on serum lipid profile of pregnant rats following acetaminophen-induced toxicity

Group Parameters	I (Control)	II (Ac)	III (PT)	IV (Ac + PT LD)	V (Ac + PT HD)
TG (mg/dl)	85.00 ± 6.13	143.3 ± 8.65^{aaa}	67.00 ± 3. 44^{aabbb}	72.75± 4. 57^{abbb}	72.00 ± 4.04^{abbb}
TC (mg/dl)	125.30 ± 4.11	139.3 ± 2.99^{aa}	125.00 ± 2.16^{bb}	117.8 ± 3.38^{bbb}	127.80 ± 1.71^{bb}
HDL (mg/dl)	57.25 ± 2.21	35.75 ± 3.30^{aaa}	51.75 ± 3.86^{bb}	63.75 ± 4.35^{bbb}	58.75 ± 3.46^{bbb}
LDL (mg/dl)	56.25 ± 4.50	68.75 ± 3.50^{aa}	55.75 ± 1.71^{bb}	50.50 ± 3.11^{bbb}	59.25 ± 3.63^{b}

Values are presented as mean ± SD (n = 5);
Superscript (a) represents significantly different from control
Superscript (b) represents significantly different from Acetaminophen only

One superscript represents a significant difference at p<0.05
Two similar superscripts represent significant difference at p<0.01
Three similar superscripts represent significant difference at p<0.001
Legend: Ac, Acetaminophen; PT, *Piliostigma thonningii*; LD, Low dose; HD, High dose

Table 2. Impact of *P. thonningii* extract on serum lipid profile of offspring rats following acetaminophen-induced toxicity in mother rats.

Group Parameters	I (Control)	II (Ac)	III (PT)	IV (Ac + PT LD)	V (Ac + PT HD)
TG (mg/dl)	50.50 ± 3.70	59.25 ± 2.69^{a}	50.50 ± 2.38^{b}	55.25 ± 2.75	52.75 ± 2.50^{b}
TC (mg/dl)	136.0 ± 3.16	149.5 ± 2.65^{aa}	133.8 ± 2.99^{bbb}	144.0 ± 2.68^{a}	132.0 ± 3.65^{bbb}
HDL (mg/dl)	52.50 ± 4.05	43.50 ± 2.65^{aa}	60.00 ± 3.56^{bbb}	54.25 ± 3.30^{bb}	54.75 ± 2.50^{bb}
LDL (mg/dl)	46.00 ± 1.83	44.25 ± 3.35	44.25 ± 3.35	43.50 ± 3.416	45.50 ± 2.08

Values are presented as mean ± SD (n = 5);
Superscript (a) represents significantly different from control
Superscript (b) represents significantly different from Acetaminophen only
One superscript represents a significant difference at p<0.05
Two similar superscripts represent significant difference at p<0.01
Three similar superscripts represent significant difference at p<0.001
Legend: Ac, Acetaminophen; PT, *Piliostigma thonningii*; LD, Low dose; HD, High dose

Discussion

The establishment of pregnancy requires a receptive uterus able to respond to various molecular and biochemical signals produced by the developing conceptus, and also specific interactions between the extra-embryonic membranes and the uterine endometrium (Dasofunjo *et al.*, 2018). It has been reported that pregnancy not only requires the use of more metabolic fuels but also causes hormonal imbalance, which affects the lipid profile (Mankuta *et al.*, 2010; Pusukuru *et al.*, 2016).

Changes in the lipid profile of lipids such as TC, HDL–C, LDL–C, and TG hold relevant information on cardiovascular health and related diseases (Gao *et al.*, 2019).

Hypertension is high blood pressure. The ratio of systolic BP (the pressure the blood exerts on the arterial wall when the heart contracts) and diastolic BP (the pressure when the heart relaxes) is often expressed as blood pressure (BP). A systolic pressure of 70 - 90 mmHg and diastolic of 120-140mmHg are still considered normal depending on one's activity (Oparil *et al.*, 2018). The alteration, therefore, of either systolic or diastolic pressure or both above this range is considered hypertension. Hypertension is the most common cardiovascular disease (CVD) risk factor that can be avoided (including coronary heart disease, heart failure, stroke, myocardial infarction, atrial fibrillation, and peripheral artery disease), chronic kidney disease (CKD), and cognitive decline, and is the world's leading contributor to all-cause death and disability (Oparil *et al.*, 2018).

Hypertension is a prominent preventable cause of premature mortality and morbidity worldwide (Saiz *et al.*, 2017). It is a key independent risk factor for high morbidity and mortality of cardiovascular diseases (Gao *et al.*, 2016). Hence, there is a need to pay urgent attention to the causes of hypertension and address them. Hypertension occurs during pregnancy as pregnancy-induced hypertension (PIH), which is a new hypertension that appears at ≥20 weeks of gestational age with or without proteinuria (Berhe *et al.*, 2020). PIH is a significant Global public threat in both developing and developed countries contributing to high maternal and perinatal morbidity and mortality (Berhe *et al.*, 2020).

A relationship exists between hypertension and dyslipidemia, which is associated with increased serum levels of TC, TG, LDL, and decreased levels of HDL (Adamu *et al.*, 2013). The risk of cardiovascular diseases associated with hypertension coexisting with dyslipidemia is more multiplicative than the sum of the individual risk factors (Adamu *et al.*, 2013). It has been reported that maternal lipid profile in the second trimester is an excellent non-invasive test that can be utilized to predict PIH before its clinical onset (Yadav *et al.*, 2014). Murmu and Dwivedi (2020), further stressed and reported that the serum lipid profile and beta-hCG are useful indicators to identify women who are to develop PIH, preeclampsia, or eclampsia in the 2nd trimester. The TG, TC, LDL, and VLDL of women who developed PIH were found to be significantly higher than normotensive women (Yadav *et al.*, 2014). Our present results recorded a similar outcome corroborated this report.

Acetaminophen or paracetamol is the most commonly used analgesic and antipyretic drug worldwide, with a long record of use in chronic and acute pain (McCrae *et al.*, 2018). McCrae *et al.* (2018), reported that prolonged use of acetaminophen causes hypertension and gastrointestinal (GI) bleeding. Acetaminophen, a prostaglandin G2 synthase inhibitor presents an increased risk of preeclampsia in the third trimester, which is further increased among women with early preeclampsia (Rebordosa *et al.*, 2010). Acetaminophen was used in this work to induce hypertension in pregnancy (PIH). Acetaminophen and NSAIDs can cross the placenta into the fetal circulation and consequently affect fetal development (Hurtado-Gonzalez *et al.*, 2018). The relationship between acetaminophen and lipid profile is similar to that of hypertension or PIH and lipid profile. A report by Madi Almajwal

and Farouk Elsadek (2015) revealed that TC, TG, LDL, and VLDL significantly increase with a concomitant decrease in HDL on the administration of 750 mg/kg BW of acetaminophen (paracetamol). A similar result was obtained from our research even though 200 mg/kg body weight of acetaminophen was used.

The administration of 100 mg/kg body weight of *P. thonningii* (considered as a low dose) and 200 mg/kg body weight (considered as high dose) to pregnant rats administered acetaminophen reversed the effect of dyslipidemia of acetaminophen in maternal rats as the TC, TG, and LDL were significantly decreased with a concomitant increase in HDL. Acetaminophen did not affect the LDL of the offspring rats but caused a significant upward change in TC and TG and downward change in HDL, while the reverse was the case on the addition of *P. thonningii* extract at both high and low doses. It was inferred that since the extracts reversed dyslipidemia from acetaminophen, and acetaminophen induces hypertension in pregnancy, which on the other is predicted by dyslipidemia, therefore, *P. thonninigii* exhibits the potential to normalize blood pressure during pregnancy, thereby treat pregnancy-induced hypertension and prevent associated effects such as maternal and perinatal mortality and morbidity.

Conclusion

This research showed that the extract of *P. thonningii* leaf reverses acetaminophen-induced toxicity in pregnancy, which is reflected in dyslipidemia vis-à-vis pregnancy-induced hypertension (PIH). Hence, *P. thonningii* leaf extract possesses the potential to reverse PIH and related effects in the offspring.

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Conflict of interest

The authors declare there is no conflict of interest.

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