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Maternal Alterations in Lipid Profile and Free Radical Scavenging Activity Following Exposure to Ethanol Extract of *Piliostigma thonningii* in Pregnant Wistar Rats

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Abstract: *During pregnancy, there is a change in the pro-oxidant and antioxidant balance and some metabolic processes due to circulation modifications that are inherent to the body. This present research determined the effect of extract ethanol leaf extract of Piliostigma thonningii on the lipid profile and oxidative stress of pregnant Wistar rats. The leaves of P. thonningii were collected and air dried for 14 days until constant weight was obtained. Twenty (20) pregnant female albino rats (160-220g) were randomly assigned to groups labelled A-D and treated thus. Animals in group A, B, C respectively were administered orally 200, 100, 50mg/kg body weight of the extract of Piliostigma thonningii while group D which served as the control received distilled water. The extract administration was done for 14 days consecutively. Thereafter, the animals were sacrificed and blood collected via cardiac puncture and tissues inclusive for assessment of the lipid profile and oxidative stress.The result revealed that the extract produced a significant (P<0.05) increase in liver and kidney SOD at 50, 100 and 200mg/kg bodyweight when compared with the control. The ethanol extract of P. thonningii leaves caused a significant (P<0.05) decrease in serum, liver and kidney lipid peroxidation, catalase and serum total protein for all the treated groups when compared with the control .**A**lso, the extract caused a significant (P<0.05) decrease in serum triacylglycerol, LDL and cholesterol concentration but a significant increase in HDL in a dosage dependent manner when compared with the control .The results suggest that the extract of P.thonningii leaves has an anti-preeclampsia effect and might act as good adjuvant therapy in controlling the oxidative stress generated during normal pregnancy.*

Keywords*: Adjuvant therapy, free radical scavenging activity, pregnancy, preeclampsia.*

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I. Introduction

Pregnancy is characterized by dynamic changes in multiple body systems resulting in increased basal oxygen consumption and in changes in energy substrate used by different organs including the foeto-placental unit. From early pregnancy the human placenta influences maternal homeostasis; it is rich in mitochondria and when fully developed consumes about 1% of the basal metabolic rate of the pregnant woman [1]. It is also highly vascular and is exposed to high maternal oxygen partial pressure. These characteristics explain, in part, the generation of superoxide, because about 5% of all electrons in the mitochondrial respiratory chain leak out of the mitochondria [2]

Oxidative stress peaks by the second trimester of pregnancy, ending what appears to be a vulnerable period for foetal health and gestational progress. Conditions restricted to pregnancy, such as gestational hypertension, insulin resistance and diabetes, exhibit exaggerated indications of free radical damage [3]. Antioxidants as well as avoidance of iron excess ameliorate maternal and early foetal damage of the mitochondria [3].

Pregnancy greatly increases demand for metabolic fuels that are needed for growth and development of the foetus and its support structures. It is known that high concentrations of many of the steroids occur as normal pregnancy advances. Since cholesterol is the source of most of the steroids found in increased amounts

in the circulation of normal pregnant patients, the part played by lipid metabolism in pregnancy, becomes all the more intriguing as cholesterol is a major factor for the development of atherosclerosis [4]. With the development of more modern techniques various studies observed an increase in various lipid fractions, though the increase was neither consistent in time of appearance nor proportion of changes in various fractions. Most authors believe that the increase in blood lipids is related to the requirements of the foetus and to development of the mammary apparatus [5].

However, herbs and plant such as *Piliostigma thonningii* has been used in treatment and management of several disease conditions. These plants have demonstrated its contributions to the treatment of diseases such as, malaria, diabetes, sickle cell anaemia, mental disorders and some microbial infections [6,7]

Piliostigma thonningii is a plant with numerous ethno medicinal importance which ranges from hematopoietic, a ntilididaemic, hepatoprotective, aphrodisiac, antimalarial, anti-venom [8, 9,10]. Metabolic, physiological and biochemical changes associated with pregnancy has been implicated to be accompanied with complications leading to mother –foetal mortality. Therefore, this research work determines the effect of ethanol leaf extract on lipid profile and oxidative stress in pregnant albino Wistar rats.

II. Materials and methods

2.1 Plant Material

Fresh leaves of *Piliostigma 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 Herbarium for identification and authentication with the Voucher number #25 has been deposited for future reference at the department's (FCOF J) Herbarium.

2.2 Preparation of Plant Material

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

2.3 Ethanol Extraction

Three hundred grams (300g) of powdered *P. thonningii*, leaves were dissolved in 1200mls of distilled water for 24 hours in a refrigerator. Thereafter, it was filtered with muslin cloth and filtered using Whatman filter No1.The filtrate was evaporated to dryness to obtain the slurry and calculate the percentage yield. The resulting slurry was used to evaluate the effects plant extract on lipid profile and oxidative stressand oxidative stress.

2.4Chemical Materials

All the reagents used in this study were of analytical grade and were sought from various companies. The solvents were obtained from E. Merck Germany, and were HPLC grade. The ascorbic acid and β-carotene was purchased from Sigma Aldrich, USA. Thiamine HCL Lot No 36020 was obtained from Serra Heidelberg, Germany. The α- tocopherol standard was obtained from Fluka, Switzerland, and niacinamide Lot 37F-0018, was obtained from Sigma Aldrich, USA.

2.5 Experimental animal

Twenty (20) Wistar rats ranging from (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 (7) days before the commencement of the research. Rats were grouped and housed in a plastic cage. The animal room was ventilated and kept at room temperature and relative humidity 29^0 C and 70% 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.To achieve pregnancy, female Wistar rats were mated with proven male breeder (ratio 2: 1) during their proestrous and the presence of sperm in their vaginal smear was taking as the first day of gestation.

2.6 Extract administration and serum collection

The animals were randomly assigned into four groups (A-D) of five female rats each in the cage. Rats in the control group (D) were orally administered with corn oil and standard feeds while the animals in group (C) was administered 50mg/kg body weight of *P. thonningii* leaf extract, group (B) was administered 100mg/kg body weight of *P. thonningii* leaf extract and group (A) was administered 200mg/kg body weight of P. thonningii leaf extract respectively.Administration of the extract was between gestation days 1-14. Seven (7) days (gestation day 21) after *P. thonningii* administration all rats were sacrificed after been anaesthetized with chloroform. Blood was collected by cardiac puncture in a plane bottle for the determination of their superoxide dismutase (SOD), catalase, and lipid peroxidation (MDA) activity.

2.7Preparation of tissue homogenate

The kidneys and livers of the rats were removed under the same condition (i.e. under chloroform as anesthesia), and the surrounding fatty tissues were removed from the organs, as they could make the homogenization process more difficult.The process was carried out by blending each organ of each rat separately in 2mls of 1% glucose solution until a relatively smooth homogenate was formed. The homogenate of each organ was centrifuge for 15mins followed by extraction of the liquid homogenate into a sterile plane tube.

2.8 Biochemical analysis

The levels of total SOD activity in the tissues were determined by the method of [11], Catalase activity was determined according to the method of [12],A breakdown product of lipid peroxidation thiobarbituric acid reactive substances (TBARS) was measured by the method of [13],serum cholesterol, serum triglycerides,HDL and LDL was determined using Randox cholesterol Kit based on the cholesterol oxidase method as described by [14].

2.9 **Statistical analysis**

Data were analysed using SPSS (v 16; IBM, Armonk, NY, USA). The descriptive data are presented here in as means ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for analytic assessment and the differences were considered statistically significant when the *P* value obtained was <0.05.

III. Results

The result below indicates the effect of ethanol extract of *Piliostigma thonningii* leaves on serum lipid profile and oxidative stress of pregnant Wistar albino rats.The extract produced a significant (P<0.05) increase on serum liver and kidney SOD at 50, 100 and 200mg/kg bodyweight when compared with the control (fig. 1). The ethanol extract of P. thonningii caused a significant (P<0.05) decrease in serum malonyldialdehyde (MDA) for all the treated groups for both the liver and kidney except for the serum kidney MDA which produced a significant increase (P<0.05) when compared with the control (fig. 2).More so, following the administration of graded doses of *P. thonningii*, the extract produced a significant (P<0.05) increase on kidney catalase activity when compared with the control (fig. 3).

Similar trend was also showed for liver catalase activity following the administration of the extract when compared with the control (fig .4).Likewise, the extract produced a significant increase (P<0.05) in serum total protein at all the graded doses for the liver, except for kidney which produced no significant decrease at 200mg/kg bodyweight for kidney tissue protein when compared to the control (fig .5).Also, the extract caused a significant (p<0.05) decrease on serum triacylglycerol and cholesterol concentration in all the experimental doses when compared with the control (fig. 6).

The extract also produced a significant (P<0.05) decrease on serum low density lipoproteins (LDL) at 50, 100 and 200mg/kg bodyweight. Alternatively, the extract caused a significant (P<0.05) increase in serum high density lipoprotein (HDL) in all graded doses when compared with the control (fig.7)

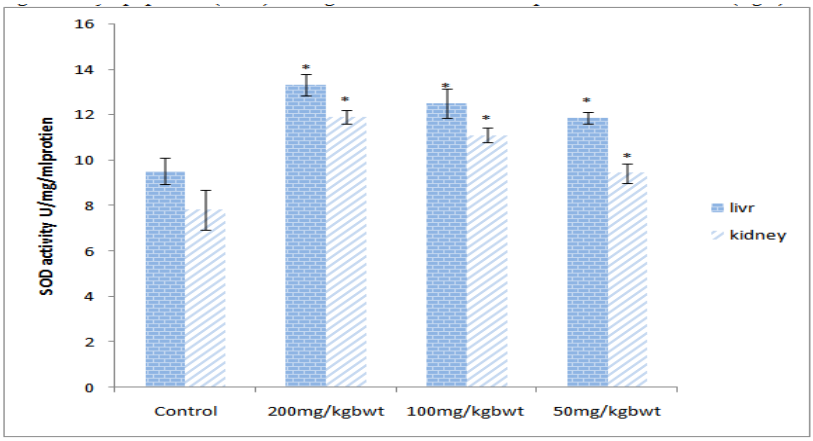

Fig. 1. The effect of Piliostigma thonningii leaves extract on serum and tissues superoxide dismutase (SOD) of pregnant Wistar rats. *p<0.05 (significant different from control)

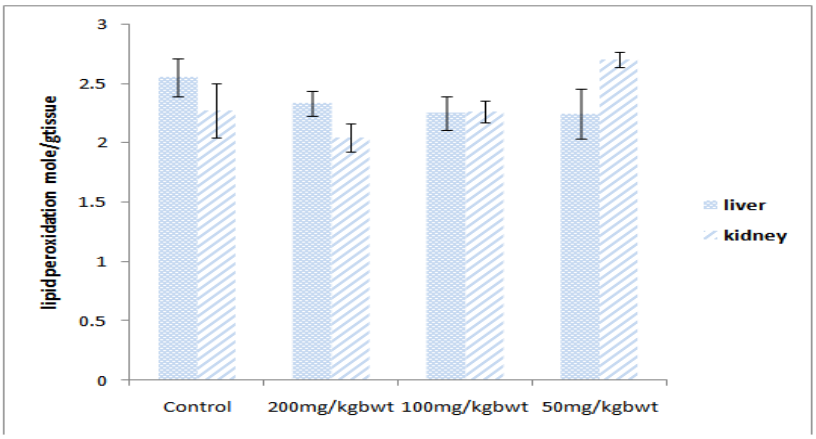

Fig.2. Effect of Piliostigma thonningii ethanol leaves extract on serum and tissues MDA of pregnant Wistar rats.

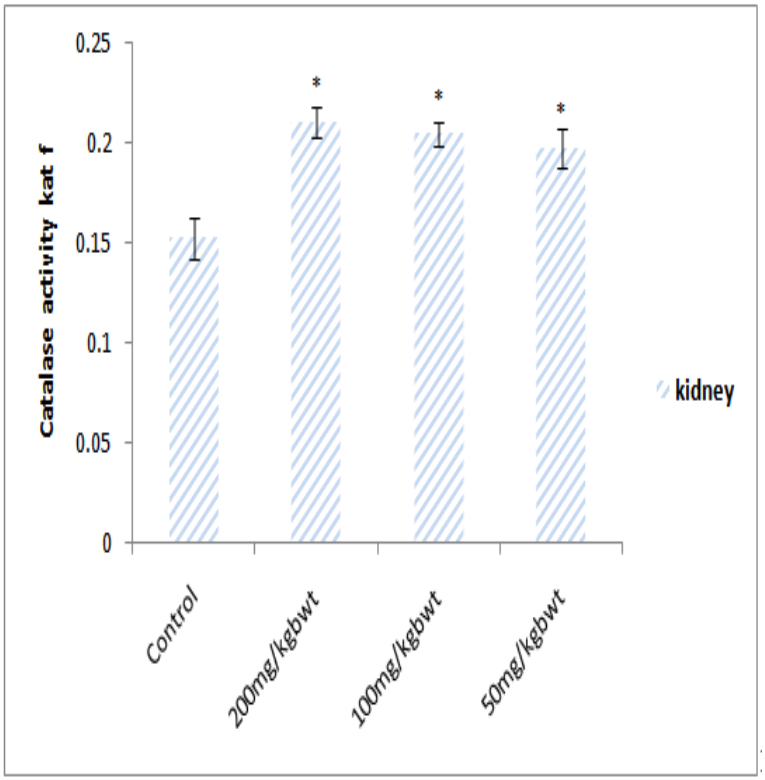

Fig. 3. Effect of ethanol extract of *Piliostigma thonningii* leaves on serum catalase activity on the kidney of pregnant Wistar albino rats.*p<0.05 (significant different from control)

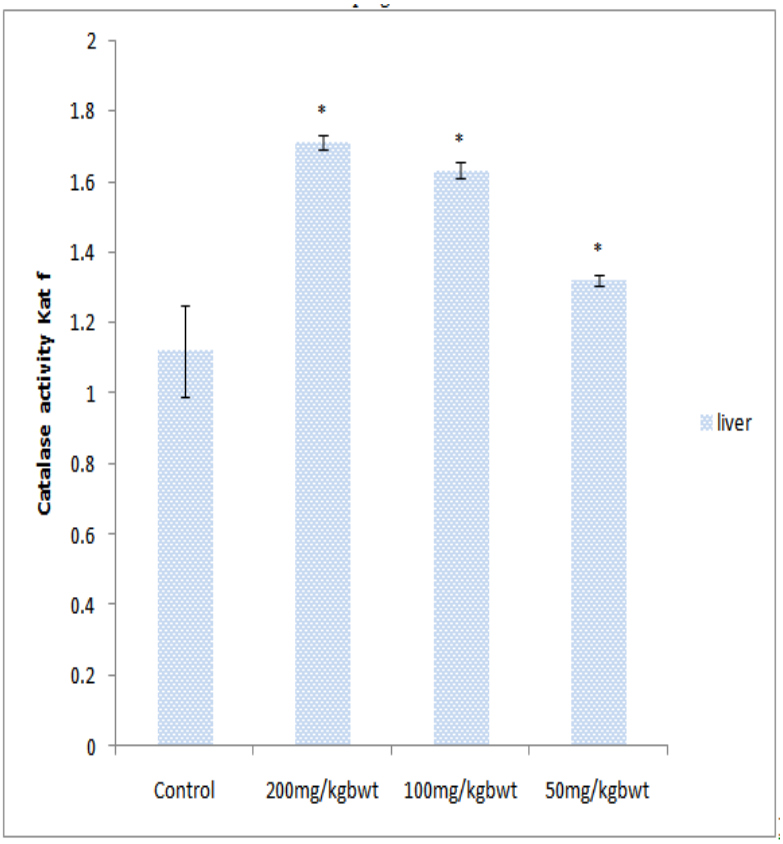

Fig. 4. Effect of ethanol extract of *Piliostigma thonningii* leaves on serum catalase activity of the liver of pregnant Wistar rats. *p<0.05 (significant different from control)

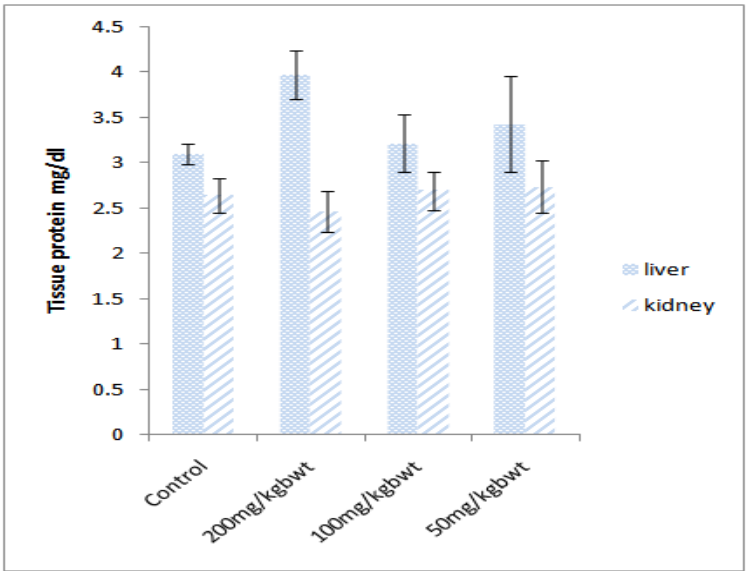

Fig.5. The effect of ethanol extract of *Piliostigma thonningii* leaves on tissue protein of pregnant Wistar rats. *p<0.05 (significant different from control)

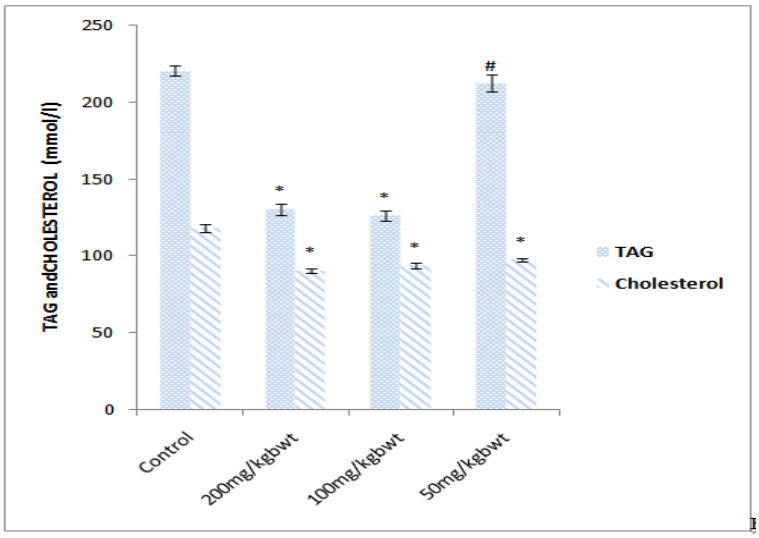

Fig. 6. Effect of Piliostigma thonningii ethanol leaves extract on serum triacylglcerol (TAG) and cholesterol of pregnant Wistar albino rats. *p<0.05 (significant different from control)

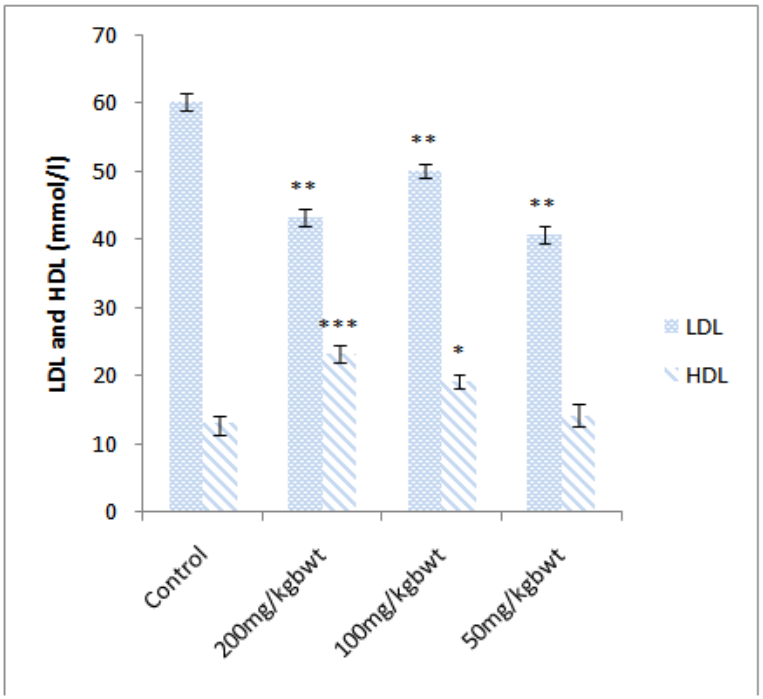

Fig. 7. Effect of ethanol extract of *Piliostigma thonningii* leaves on serum high and low density lipoprotein (HDL and LDL) of pregnant Wistar rats.*p<0.05, **p<0.01, ***p<0.001 (significant different from control)

IV. Discussion

The establishment of pregnancy requires a receptive uterus able to respond to a variety of biochemical and molecular signals produced by the developing conceptus, as well as specific interactions between the uterine endometrium and the extra-embryonic membranes. Therefore, placental development and function are prerequisites for an adequate supply of nutrients and oxygen to the foetus and successful establishment of pregnancy. Pregnancy-induced hypertension (PIH) or pre-eclampsia is a toxic condition that is accompanied by increased oxidative stress [15]. It is also one of the major risk factors in present day health care practice because it does not only causes maternal mortality but also impairs foetal development during pregnancy [16,17].Hypertension is directly associated with increased levels of total cholesterol (TC), triglycerols (TGs), low density lipoproteins (LDL) and very low density lipoproteins (VLDL). PIH may cause several critical problems in pregnancy such as premature delivery, intrauterine growth restriction, foetal death, maternal mortality and morbidity [18]. Several factors responsible for PIH have been identified [18] but among them the

most important are lipid profiles [16;19;20]. It has been reported that metabolism of lipoproteins is directly associated with PIH [20; 21; 22].

From this present study, Under pregnancy condition, the decrease in Cholesterol, TAG, and LDL following the administration of the extract to the pregnant albino rats might be due to response to the maternal switch from carbohydrate to fat metabolism which is an alternative pathway for energy generation due to high energy demand or accelerated breakdown of fat depots, which plays an important role in foetal development and it also suggest that the extract might not predispose the animal to preeclampsia or pregnancy induced hypertension or might possess a bioactive ingredient against pregnancy induced hypertension.Moreso, 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 [23]. Also, HDL-C is considered to have anti atherogenic properties [24]. It has also been shown that an increase in HDL-Cholesterol correlates inversely to coronary heart disease [25]. It can therefore be inferred that the significant increase 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 or suggests that the extract might exert a protective effect against atherosclerosis or increases estradiol which might promote apolipoprotein A1 (Apo A1) production, and reduce hepatic lipase activity, resulting in increased production of high-density lipoproteins (HDL) during pregnancy their by inhibiting pregnancy induced hypertension.

Thus Pregnancy is a physiological state accompanied by high-energy demand of many functions and increased oxygen requirement due to increased intake and utilization of oxygen which results in increased level of oxidative stress. Pregnancy induced hypertension also known preeclampsia is a toxic condition that is accompanied by increased oxidative stress [26]. Preeclampsia is a hypertensive disorder of pregnancy in which antioxidant defences system fail and tissues are injured. Oxidative stress corresponds to event resulting from an imbalance between the production of reactive species (oxygen, ROS, and nitrogen, RNS) and antioxidant capacity. Regarding its high ability to oxidize important cellular components (lipids, proteins, and deoxyribonucleic acid - DNA), oxidative stress is now recognized as one of the most common mechanisms involved in the development of a variety of diseases [27] as well as natural events, such as gestation [28].
Therefore it can be inferred from this present study that the markers of oxidative stress, SOD and Catalase increases significantly and also a significant decrease in MDA activities on both the liver and kidney following the administration of the extract suggest that the extract possess a free radical scavenging potential either by decreasing in lipid peroxidation or decreasing the generation of free radical oxygen species (ROS) or by reducing oxidative damage and might be a good adjuvant therapy in controlling the oxidative stress generated during pregnancy possibly due to its anti-oxidant activity.

V. Conclusion

From the results above it appears that the extract of *P. thonningii* leaves has an anti-preeclampsia effect and might act as good adjuvant therapy in controlling the oxidative stress generated during pregnancy.

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