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Liver Function Indices in Pregnant Wistar Rats Administered Leaf-Extract of *Pilliostigma thonningii*

Asuk A. A*[1], Dasofunjo K[1] and Agom, C. U[1].

[1]Department of Medical Biochemistry, Cross River University of Technology, Okuku Campus, P.M.B 1123 Calabar, Cross River State, Nigeria.
*Corresponding author's email: raphaelasuk@gmail.com

ABSTRACT

Pregnancy is a physiological condition associated with maternal changes. It may result in stress being impacted on the body as biochemical and physiological changes progress. This research evaluated the liver function indices of *Pilliostigma thonningii* leaf extract administration in pregnant Wistar rats. Twenty (20) pregnant female Wistar rats weighing 160-220g were assigned according to body weight to groups A, B, C and D of 5 rats each. Group A served as the control and received distilled water only. Animals in groups B, C and D were orally administered 200, 100 and 50mg/kg body weight respectively of *P. thonningii* extract. The extract administration was done for 14 days. Thereafter, the animals were sacrificed and blood collected via cardiac puncture for evaluation of liver function indices. On administration of *P. thonningii* extract, there was significant (P<0.05) increase and decrease in serum AST at 100 mg/kg bwt and 50 mg/kg bwt of extract respectively, whereas no change was observed at 200 mg/kg bwt when compared with the control. There were no changes in serum ALT at 200 mg/kg bwt and 50 mg/kg bwt of the extract but at 100 mg.kg bwt was significantly (P< 0.05) increased compared with the control. A significant (P<0.05) decrease and increase in serum ALP at 200 mg/kg bwt and 100 mg/kg bwt extract administration respectively was observed compared with the control. The extract administration also produced a significant (P<.0.05) increase in total serum protein, albumin, globulin and bilirubin at all dose levels when compared with the control. The outcome of the research showed that the extract did not exhibit dose-related effect. The extract also exhibited the potential to support growth of the foetus through increased protein. At selected dose levels, it may improve the integrity of the liverduring pregnancy.

Keywords: Pregnant rats. Aminotransferases, Alkaline phosphatase, Serum proteins, Plant extract.

INTRODUCTION

Pregnancy is a physiological condition associated with maternal changes. It may result in stress being impacted on the body as biochemical changes progress [1]**.** The pregnant women experience physiological changes to support foetal growth and development [2].The levels of oestrogens (estradiol) and progesterone increase progressively during pregnancy [3]. The liver is the most important organ after the heart, performing many important functions including metabolism, detoxification and formation of important compounds including blood clotting factors and albumin. These sex hormones have effects on hepatic metabolic synthesis and excretory functions of the liver. Serum albumin concentration has been reported to decrease during pregnancy and reaches a nadir towards the end of the pregnancy, prior to increase in plasma volume [4]. Alkaline phosphatase activity is increased during the third trimester both because of leakage of placental alkaline phosphatase into the maternal circulation and increase in maternal

bone turnover. Serum transaminases level gives normal values during pregnancy. Increase in transaminases levels have been reported to occur during labour and, most probably due to leakage from the contracting uterine muscle. Serum levels of total and free bilirubin are lowered during pregnancy [5]. These changes may also make the liver to be prone to diseases such as liver congestion/cholestasis, primary biliary cirrhosis and primary cholangitis, viral hepatitis, acute fatty liver disease which has been noted to occur in 3%-5% of pregnancies, with many potential causes, including coincidental liver disease (most common viral hepatitis or gallstones) and underlying chronic liver disease [6]. It is imperative to ensure that these physiological and biochemical changes be properly managed to prevent morbidity and mortality to either or both the mother and foetus.

In Africa, the use of medicinal plants has been found to be effective in handling several ailments. Researchers over the years have reported on the medicinal role of *Piliostigma thonningii* plant. The assemblage of different parts of this plant has been found to be traditionally useful. As a result, people have resorted to using parts of the plant in the management and treatment of different kinds of ailments such as toothache, diarrhoea, dysentery, intestinal problems, heart pain, ulcer among others including exercising hepato-protective effect [7]; [8]; [9], [2].It is therefore to evaluate the possible positive effect of *Piliostigma thonningii* extract on maternal liver function indices as well as other related parameters during gestation.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *P.thonningii* were collected from Okuku, Cross River University of Technology, Nigeria. The leaves were taken to the Federal College of Forestry (FCOFJ) Jos, Department of Herbarium for identification and authentication. The voucher number of #25 and has been deposited for future reference at the Department's (FCOFJ) herbarium.

Preparation of ethanolic leaf extract of *Pilliostigma thonningii*

The leaves of *P. thonningii* were collected and air dried for 14 days until constant weight was obtained. The dried leaves were then pulverized after which 300g was extracted in 1000mL of ethanol for 72 hours with constant shaking using the electric shaker. This was later filtered using Whatman No.1 filter paper. The filtrates were the concentrated in water bath at 45^{0}C. The resulting slurry was weighed and reconstituted in coil oil to administer the required dose.

Experimental animals

Twenty (20) virgin female Wistar rats were obtained from animal holding unit, Department of Medical Biochemistry, Okuku. The animal was acclimatized for a period of seven (7) days. Each rat was housed in a woodened cage. The animal room was well ventilated and kept at room temperature and relative humidity of 20 ± 2ºC and 70% respectively with 12-hour natural light – dark cycle. They were allowed free access to standard feed and water. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from cages daily. The animals were subcutaneously injected with 0.1mg/kg body weight of diethylstilboestrol in 0.5mL olive oil to ensure the female rats were in oestrous. The mature male rats were introduced in ratio 1:3 females until the females were confirmed pregnant.

Animal grouping and administration of extract

Twenty (20) pregnant female Wistar rats were picked and placed into wooden cages labelled A-D of 5 rats each. Group A-served as the control group while B, C and D were test groups. The animals in group B were administered orally high dose (200mg/kg body weight) of the ethanol leaf extract. Group C were administered medium dose (100mg/body weight) of the extract, Group D was administered low dose (50mg/body weight) while group A served as the control. All experimental groups used corn oil as vehicle. The oral administration was done for 14 days. The animal in each group was sacrificed

24 hours after the completion of their respective doses by cardiac puncture. The animals were handled humanly in accordance with the guideline of European convention for the protection of vertebrate animals and other scientific purpose – ETS-123 (2005).

Preparation of serum and tissue homogenates for biochemical assay The animals were anesthetized in a jar containing cotton wool soaked in ether. When the animal became unconscious they were brought out quickly of the jar, the abdominal region was opened along the linear Alba cut with scalpel blade to expose their organ 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 centrifuge at 300rpm × 10 min using UniscopeLaboratory Centrifuge.

The serum was thereafter aspirated into clean, dry sample bottles using pasture pipette and were kept or store in sample bottle and used within the next 12hour of preparation. Each of the organs (liver) was cut with clean sterile blade and then homogenized in 0.25M sucrose solution 1: 5 (W/V) as described by [10]. The homogenate was later transferred into specimen bottles and kept frozen for 24 hours before being used for biochemical analysis.

Biochemical assay

The assay for albumin, globulin, bilirubin, alkaline phosphatase (ALP), asparate amino transferase, and alanine amino transferase (ALT) was done using Redox Laboratory Kits, United Kingdom. Total protein concentration of the sample was assayed by the biuret method. All chemicals and reagents used in this research were of analytical grade.

Statistical analyses

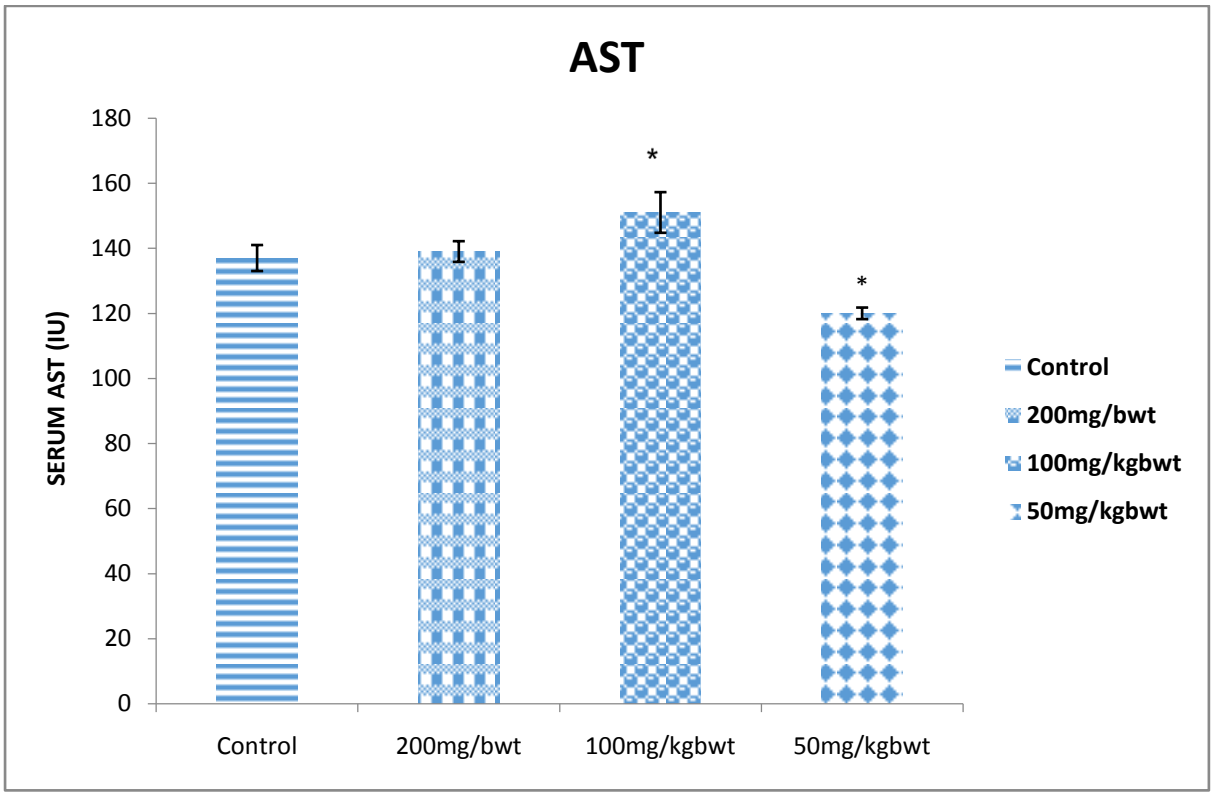
The data obtained from the experiment was used for the determination of the mean ± SEM using Microsoft Office Excel 2010. The values were then used to plot graphs using Microsoft Office 2010. Statistical significance was achieved using SPSS version 17.0 and the level of significance was accepted at $P<0.05$

RESULTS

The results below revealed the effect of ethanol leaf extract of *P. thonningii* on liver function indices of pregnant Wistar rats. The extract produced a significant ($P<0.05$) decrease in serum AST of group D with evidence of significant ($P<0.05$) increase of group C but none of group B when compared with the control (Figure 1). The results for serum ALT were similar to that of AST except that the extract did not produce any significant ($P \geq 0.05$) difference in group D when compared with the control (Figure 2). Similarly, the serum ALP result was comparable to serum ALT except serum ALP was significantly ($P<0.05$) decreased in group B (Figure 3).

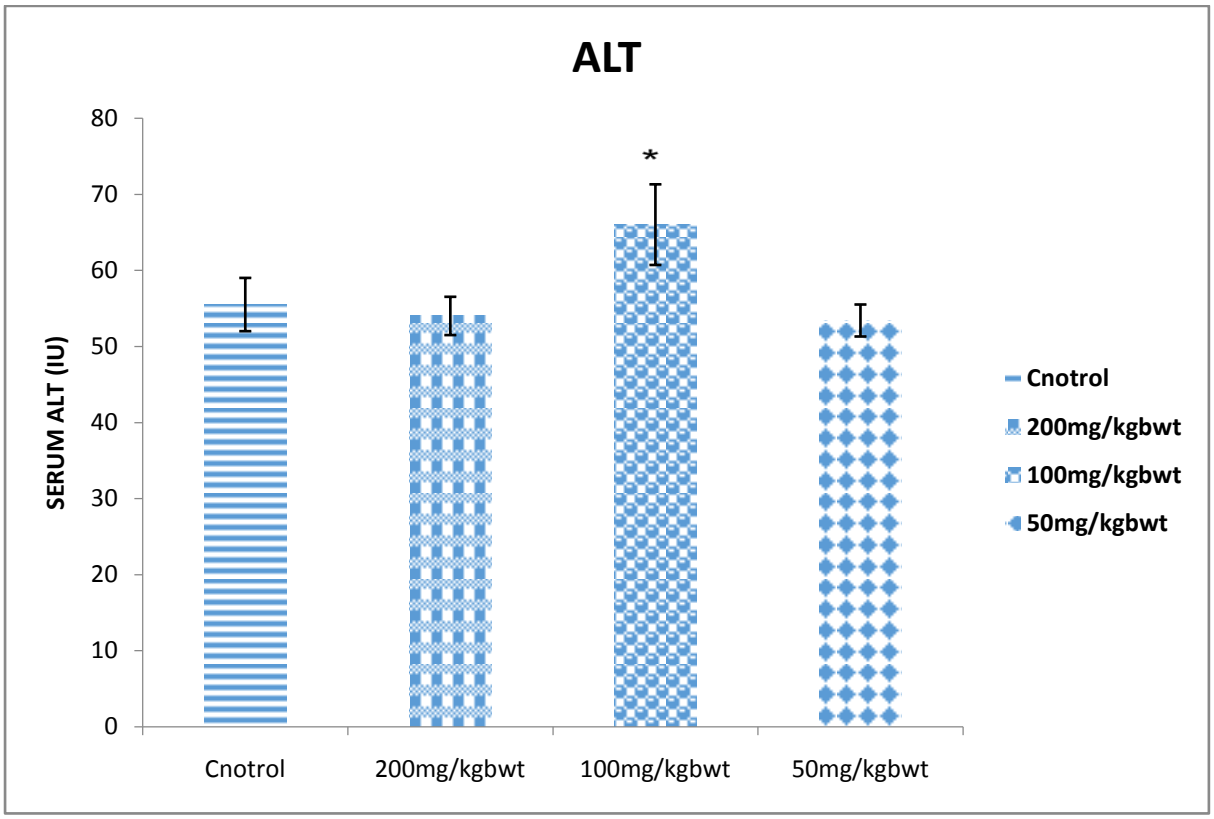
Consequently, following the administration the extract, a significant increase in total protein was produced when compared with the control (Figure 4)

More so, the extract caused a significant increase in serum bilirubin in groups B, C and D when compared with the control (Figure 5). Similar trend as that of serum total protein were also displayed by the extract on total albumin and globulin when compared with the control (Figure 6 and Figure 7).



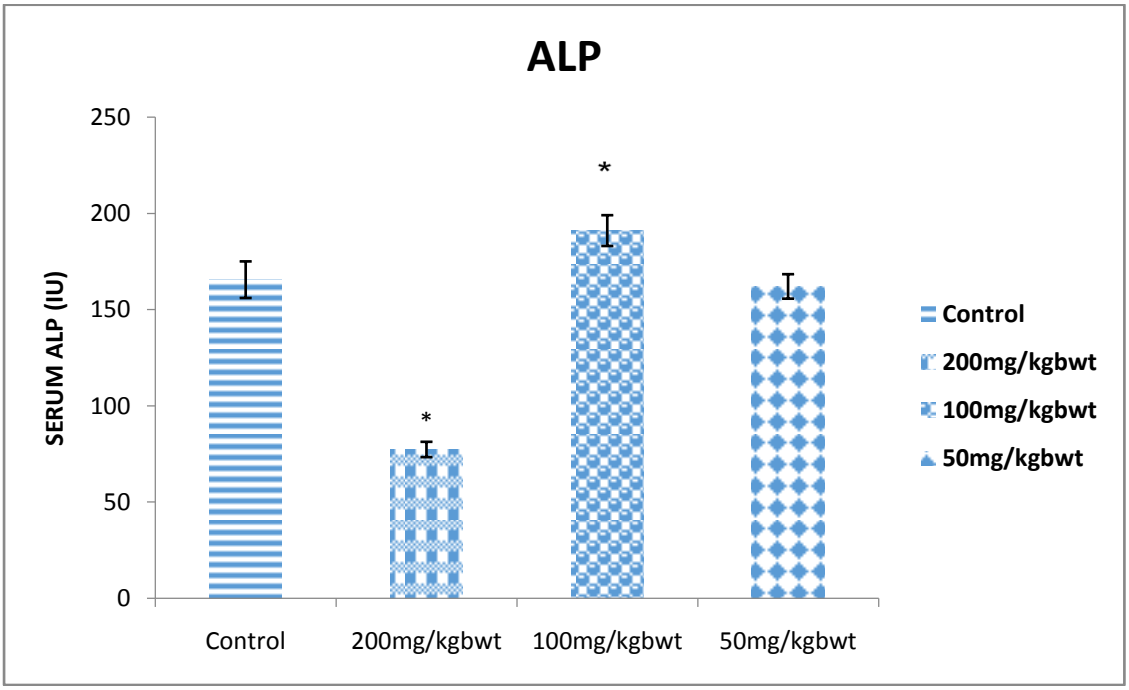
Values are mean ±SEM (n=5)
*Bars are significant at $P<0.05$ vs control

Fig 1.Serum AST levels on administration of *P.thonningii* leaf extract on pregnant Wistar rats.



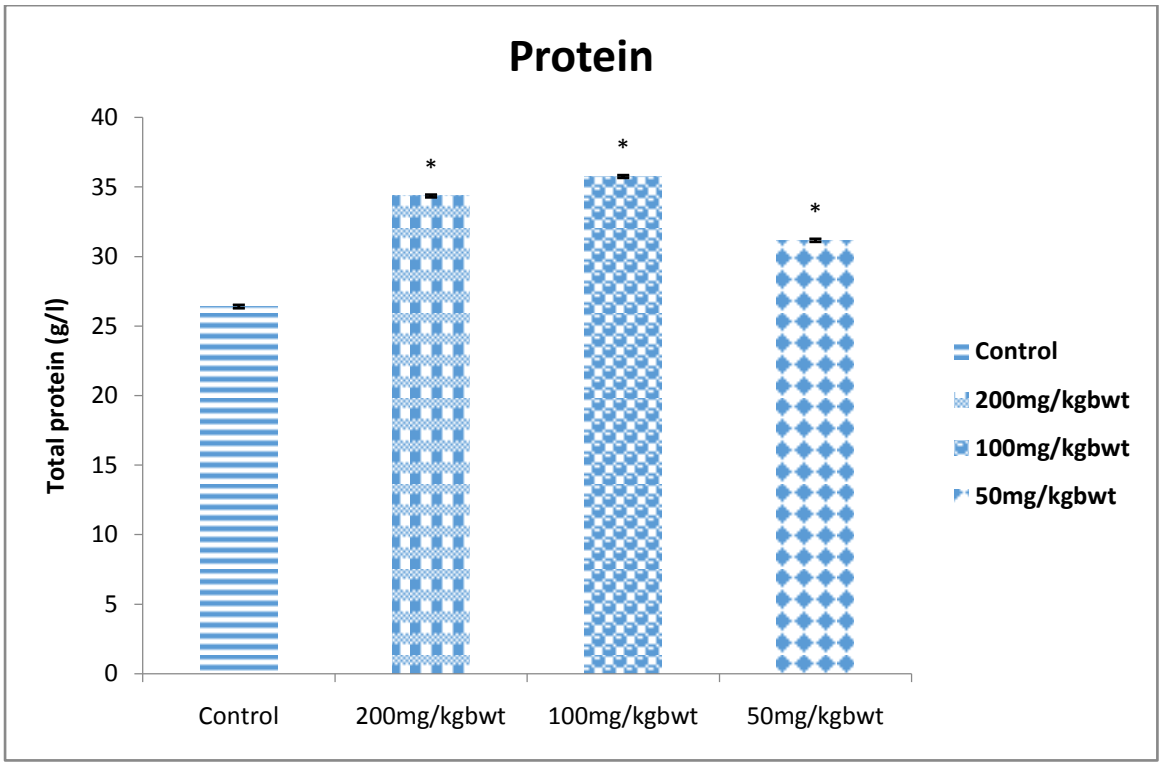
Values are mean ±SEM (n=5)
*Bars are significant at *P*<0.05 vs control

Fig 2.Serum ALT levels on administration of *P. thonningii* leaf extract on pregnant Wistar rats.



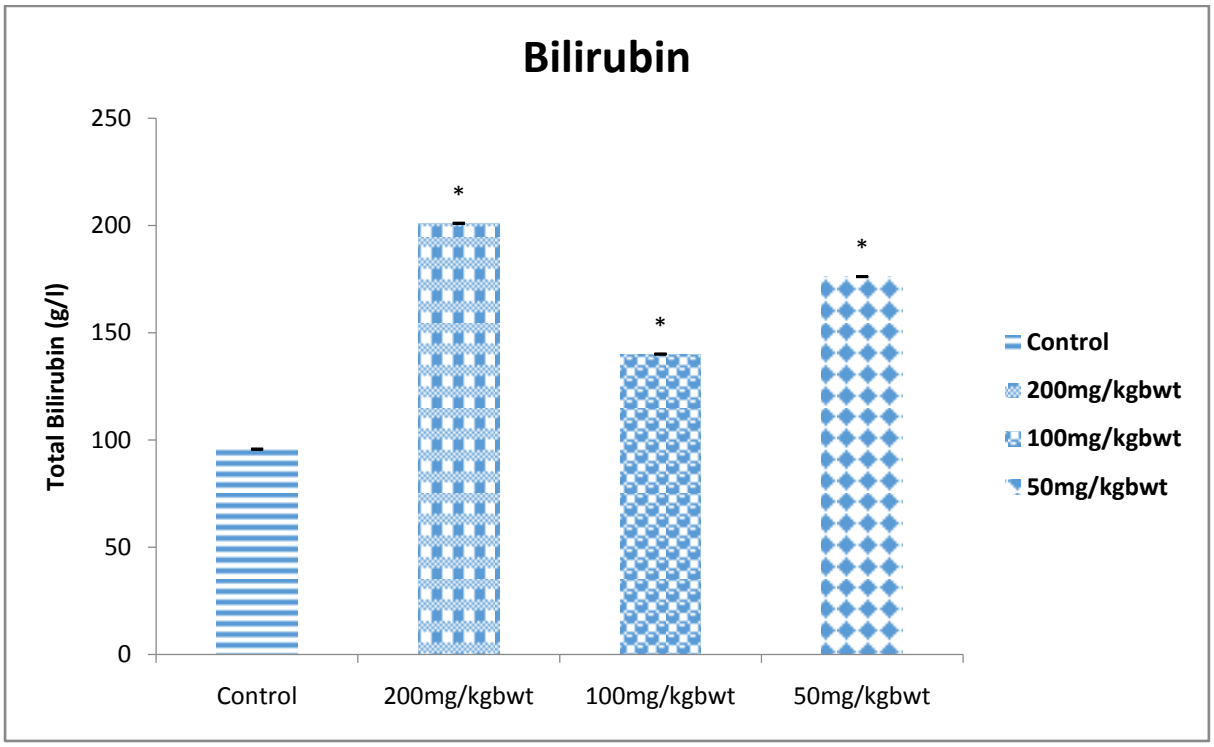
Values are mean ±SEM (n=5)
*Bars are significant at *P*<0.05 vs control

Fig 3. Serum ALP levels on administration of *P. thonningii* leaf extract on pregnant Wistar rats.



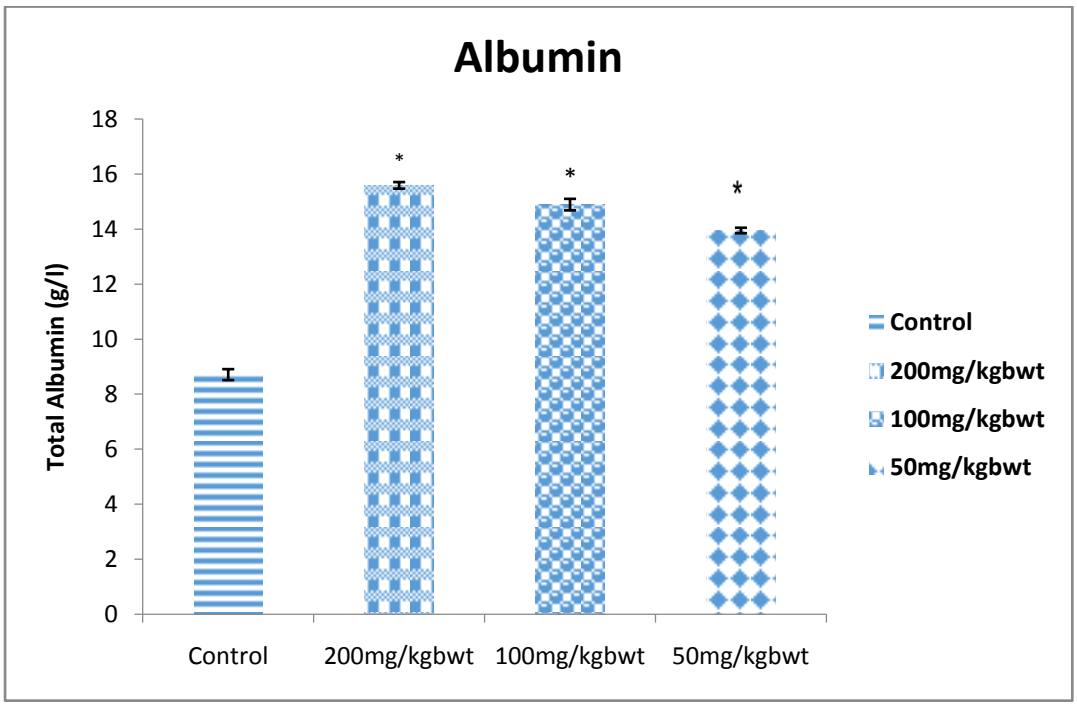
Values are mean ±SEM (n=5)
*Bars are significant at $P<0.05$ vs control

Fig 4.Serum protein concentration on administration of *P. thonningii* leaf extract on pregnant Wistar rats.



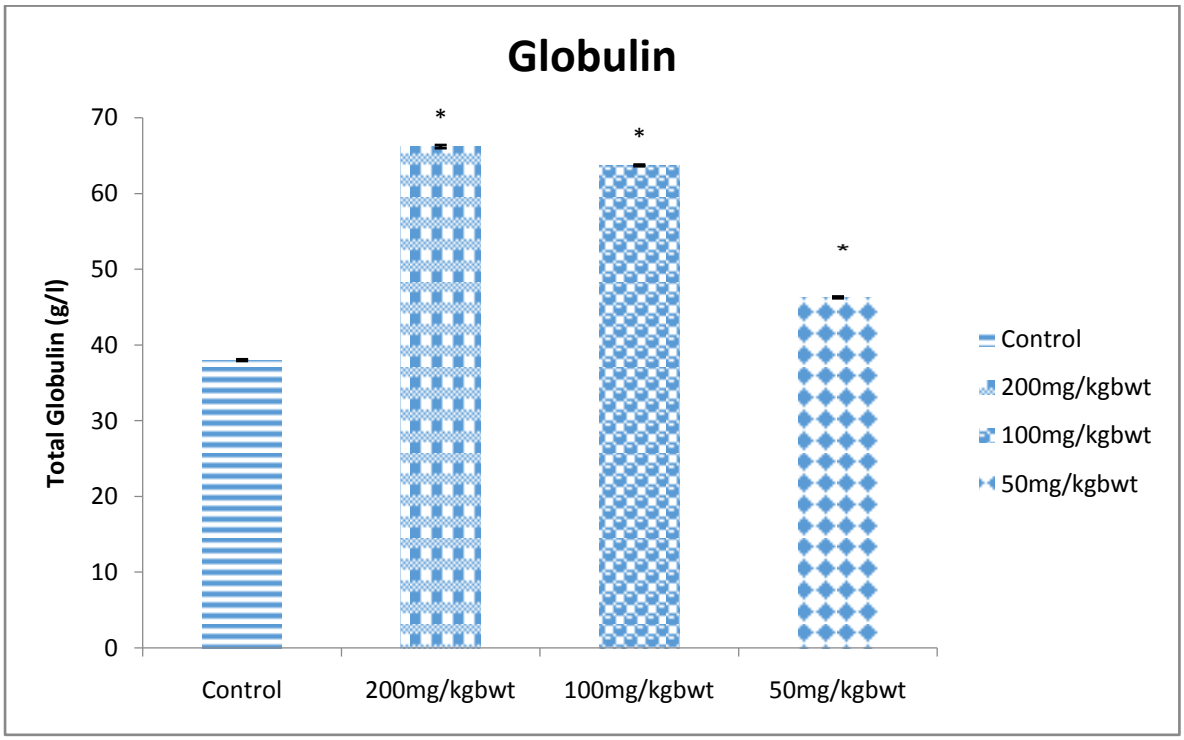
Values are mean ±SEM (n=5)
*Bars are significant at $P<0.05$ vs control

Fig 5.Serum Bilirubin concentration on administration of *P. thonningii* leaf extract on pregnant Wistar rats.



Values are mean ±SEM (n=5)
*Bars are significant at $P<0.05$ vs control

Fig 6. Serum Albumin concentration on administration of *P. thonningii* leaf extract on pregnant Wistar rats.



Values are mean ±SEM (n=5)
*Bars are significant at $P<0.05$ vs control

Fig 7.Serum globulin concentration on administration of *P. thonningii* leaf extract on pregnant Wistar rats.

DISCUSSION

The liver has an incontrovertible influence on several functions of many organs in the body and prone to xenobiotic induced injury due to its central role in xenobiotic metabolism within the system [11]. Alterations in certain biomolecules such as albumin, bilirubin and globulin as well as liver enzymes are used as indicators of impaired liver function [12]. These indices can be related to physiological changes in liver function during pregnancy. Disorders arising from pregnancy, such as acute fatty liver of pregnancy (AFLP), haemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome, pre-eclamptic liver dysfunction and cholestasis can have serious implications [13]. Proper interpretation of liver function tests (LFTs) can lead to proper diagnosis and timely management of diseases and may reduce complications in both mother and foetus.

Therefore, increase in serum albumin, globulin or total protein concentration caused by administered extract on the pregnant rat imply that protein synthesis and (or) mobilization for proper foetal growth during pregnancy may have occurred [14]. Furthermore, the observed increase in serum albumin is an indication that the extract may promote good functioning of the liver or possess a hepato-protective role as well as help calcium in the blood stream to regulate the movement of water through the blood stream into body tissue during pregnancy. More so, albumin is the protein with the highest concentration in the plasma produced by the liver. It transports many molecules in the blood and prevents fluid coalescence in the blood.

The AST and ALT are two of the most reliable markers of hepatocellular injury or necrosis whose levels are elevated in a variety of hepatic disorders or dysfunction. However, of these transaminases, ALT is more specific for hepatic injury because it is present mainly in the cytosol of the liver and

low concentrations elsewhere. On the other hand, aspartate amino transaminase (AST) is predominantly localized within the cells of the gills, kidney, muscle and liver parenchymal cells. An increase in serum AST might connote acute liver damage or liver cytolysis. Since the measurement of serum ALT and AST activities is the most useful test for the routine diagnosis of liver diseases, therefore in that context, serum ALT and AST activity were unaltered at high dose of the extract (200 mg/kg bwt) implying the extract was safe and did not induce hepatic injury during the gestation period as reported by [15]. Similarly, the non-alteration of serum AST level at 200 mg/kg bwt of extract admisnistartion may suggest that no cytolysis, injury or assault to the integrity of the liver during pregnancy occurred.

ALP is a ubiquitous enzyme localized within the plasma membrane and can be used to assess the integrity of the plasma membrane. It is a marker enzyme for the plasma membrane and endoplasmic reticulum cell lining of the biliary ducts of the liver, placental tissue and bone ALP is frequently used to access the plasma integrity of plasma membrane [16] such that any alteration in the activity of the enzymes in the tissue and serum would indicate likely damages to the external boundary of the plasma membrane of the cell [17].

Therefore, the increase in ALP at 100 mg/kg bwt can be attributed to the added placental secretion or due to increased production of the bone isoenzymes during pregnancy [18]; [16]. During pregnancy, there could be pregnancy-induced haemolysis leading to increased bilirubin levels [19]. The extract though produced increase in bilirubin but it is unclear since the liver parameters were quite in order. This might also imply that the extract had no inimical effect on the mother-foetal liver and might not predispose mother to complications associated with liver injury during pregnancy.

CONCLUSION

The outcome of the research showed that the extract exhibited no dose-related effect. Though the result of bilirubin was unclear, the extract has the potential to support growth of the foetus through increased protein. At selected dose levels, it may improve the integrity of the liverduring pregnancy.

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