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Research Article

MATERNAL CHANGES ON HISTOLOGICAL AND LIVER FUNCTION INDICES EXPOSED TO *PILIOSTIGMA THONNINGII* EXTRACT FOLLOWING ACETAMINOPHEN TOXICITY IN PREGNANT RATS

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ABSTRACT
Acetaminophen is the most commonly used over-the-counter pain reliever and antipyretic agent among pregnant women. It has been considered to be one of the safest analgesics, even for pregnant women. It is known that paracetamol crosses the placenta and that its metabolites enter the foetal blood flow. This present research work determines the effect of maternal alteration of extract of *P.thonningii* on Liver function indices following acetaminophen induced toxicity in pregnant rats. Twenty five (25) pregnant female Wistar rats ranging from 180-200g were assigned on the basis of their body weight and were grouped into five of five rats each. A served as the control, B was administered with 200mg/kg body weight of acetaminophen, C was administered with 200mg/kgbwt of *P.thonningii*, D -100 mg/kgbwt *P.thonningii* + 200 mg/kgbwt acetaminophen, E-200 mg/kgbwt *P.thonningii* + 200 mg/kgbwt acetaminophen. The administration was done for 21 days. Thereafter, the animals were sacrificed and blood collected via cardiac puncture into a plane tube for the assessment of the liver function indices. The liver tissues were also removed for histological examination. The result shows significant ($P<0.005$) increase in serum albumin, globulin and total protein following the administration of the extract while groups treated with acetaminophen alone shows significant ($P<0.005$) decrease when compared with the control. Also the extract produced a significant ($P<0.005$) decrease on serum ALT, AST and ALP when compared with the control. The acetaminophen treated groups were found to be significantly ($P<0.005$) higher on serum ALP, AST and ALT. Likewise, groups co-administered with both acetaminophen and *P.thonningii* were found to be significantly ($P<0.005$) lowered on serum ALT,AST and ALP. The biochemical changes on maternal liver functional indices studied suggested that ethanol leaf extract of *P. thonningii* administered had a hepatoprotective effect, but with evidence of hepatic injury/assault in groups treated with acetaminophen during pregnancy.

***Keywords**: Acetaminophen, hepatic injury, hepatoprotective, histopathology*

INTRODUCTION
Acetaminophen is an organic compound that inhibits synthesis of prostaglandins in the central nervous system, thus raising the body's pain threshold, and further impacts the temperature-regulating centre of the brain, thus reducing fever. Its exact mechanism is still poorly understood (Karai *et al.*, 2004). Unlike other common analgesics, such as aspirin and ibuprofen, acetaminophen has no anti-inflammatory properties, and so it is *not* a member of the class of drugs known as *Non-Steroidal Anti-Inflammatory Drugs* (NSAIDs) (Page and Henry,2000). Acetaminophen in normal doses is less likely than NSAIDs to irritate the lining of the stomach and cause peptic ulcers, and does not affect blood coagulation, the kidneys, or the foetal ductus arteriosus (as NSAIDS can) (Doan, 1994). Thus, it is a possible alternative for people allergic to NSAIDs or who are using anticoagulants (Peters *et al.*, 1999). Like NSAIDs acetaminophen does not cause euphoria or alter mood. Acetaminophen has long been suspected of having

a similar mechanism of action to aspirin because of the similarity in structure. That is, it has been assumed that acetaminophen acts by reducing production of prostaglandins, which are involved in the pain and fever processes, by inhibiting the cyclooxygenase (COX) enzyme (Aderogba *et al*., 2006).Report also suggests that acetaminophen selectively blocks a variant of the COX enzyme that is different from the then-known variants $COX\text{-}_1$ and $COX\text{-}_2$. This enzyme is now referred to as COX-3.

As with almost every drug, administration of acetaminophen is not without side effects or undesired effects especially in cases of over-dose hyper-toxicity (Ouellet and Percival, 2001). Previous studies on hepatotoxicity associated with acetaminophen usage reveal that acetaminophen has a narrow therapeutic index. This means that the common dose is close to the overdose, making it a relatively dangerous substance (Swierkosz *et al.,* 2002). In general, medical experts also warn that patients should not take acetaminophen after engaging in alcohol consumption, because the liver, when engaged with alcohol breakdown cannot properly dispose of acetaminophen, thus increasing the risk of hepatotoxicity (Zimmerman and Maddrey,1995). The use of a pain-killing drug like acetaminophen is just one of a multitude of approaches to ameliorating pain, which has varied causes and influences. Some commonly consumed herbs have been reported to have analgesics and protect the functional integrity of the liver is *Piliostigma thonningii* (Dasofunjo *et al*., 2012).

Piliostigma thonningii is an under-explored leguminous plant that belongs to the family, *Leguminosae-caesalpiniodae*. Report shows that in many African countries various parts of *Piliostigma thonningii* (leaf root, bark, seed, and fruit) are used for various medicinal purposes such as anti-inflammatory and analgesic activity, anti-inflammatory and antibacterial activities among others (Omole 2012;Dasofunjo *et al.,*2013).

However, there is yet a single therapy option to manage the undesirable effects of acetaminophen toxicity and given the fact that there is still an unquenchable desire for consumption of herbal medicine due to its vague and undefined dosage, there is need to proffer more efficient solutions that are widely tolerable across age brackets as well as special cases as in pregnant and breast feeding women (Neuwinger, 2000). Therefore, this present research is target towards determining the maternal changes in histological and liver function indices on pregnant Wistar rats following the administration of ethanol extract of *P. thonningii* and acetaminophen.

MATERIALS AND METHODS

Materials

Plant material

Fresh *P.thonningii* leaves were obtained from Igoli/Okuku road, Cross River State, Nigeria in February, 2017.Identification and authentication was done at the Federal College of Forestry Jos, Plateau state, Nigeria, with the voucher number #25.

Experimental animals

Twenty five (25) 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 were well ventilated and kept at room temperature and relative humidity of 27^oC and 70% respectively with 12 hours natural light – dark cycle and 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 diethylstilbestrol in 0.5ml olive oil to ensure the female rats were in oestrous. The mature male rats were introduced in ratio 1:3 until they have been confirmed pregnant.

Preparation of ethanol extract of Piliostigma thonningii leaf

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^{o}c. The resulting slurry was weighed and reconstituted in coil oil to administer the required dose.

Animal grouping and administration of extract

Twenty five (25) pregnant female albino rats were picked at random and placed into wooden cages labelled A-E. Group A served as control while groups B -E were test groups. The animals in group A were administered orally with distilled water. Group B were administered 200mg/body weight of acetaminophen, Group C was administered 200 mg/body weight of the extract while group D was administered with 100 mg/kg body weight of acetaminophen and 200 *P. thonningii* respectively while group E was administrated 200 mg/kg body weight of acetaminophen 200 *p. thonningii* respectively. All experimental groups used corn oil as vehicle. The oral administration was done for 14 days .The animals in each group were sacrificed after 24 hours by cardiac puncture procedure. The animals were handled humanely in accordance with the guidelines of European convention for the protection of vertebrate animals and other scientific purposes.

Blood sample collection

Blood was collected from all the test rats and control by cardiac puncture using disposable syringe and needle draw blood dispensed into plane tubes. The specimens were labelled with the identification alphabets/ number. The samples were refrigerated for 24 hours before analysis.

Preparation of liver homogenate

The liver of the rats were removed under the same condition 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 1g of liver of each animal 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 separation of the liquid homogenate into a sterile plane test tube.

The enzymes assayed in the course of this study included the aspartate amino transferase, alanine transaminase and alkaline phosphatase. These enzymes were assayed in the homogenates of liver and the serum.

Statistical analysis

Data collected from the laboratory analysis of the liver and serum specimen was analysed using graph pad prism 5.0 (USA).

RESULTS

The result below shows the mean concentration of the liver enzymes across the different experimental groups. It was observed that the mean concentration of ALT in the acetaminophen only group and P.T. only group was significantly different from control at $p<0.001$ and $p<0.05$ respectively. ALT level of A. only was significantly different from P.T only (0.001), from A+P.T LD (0.01) and from A+P.T HD (0.01). It was also observed that the ALT level of A+P.T LD was significantly greater than that of A+P.T HD at $p<0.01$ when compared with the control (fig 1-3).

It was observed that the mean concentration of AST in acetaminophen only group and P.T only group was significantly different from control at $p<0.001$ and $p<0.01$ respectively. AST level of Acetaminophen. only was significantly different from P.T only ($p<0.01$), from A+P.T LD ($p<0.001$ & $p<0.01$), and from Aceta+P.T HD ($p<0.001$ & $p<0.01$). It was also observed that the AST level of A+P.T HD was significantly greater than that of A+P.T LD at $p<0.001$ (fig 1-3).

It was also observed that the mean concentration of ALP in acetaminophen only group and P.T only group was different from control at $p<0.001$ and $p<0.001$ respectively. ALP level of A.only was significantly different from P.T only ($p<0.001$), from A+P.T LD ($p<0.01$, $p<0.001$ and $p<0.001$), and from A+P.T HD ($p<0.001$, $p<0.001$ and $p<0.05$). It was also observed that ALP level of A+P.T LD was greater than that of A+P.T HD at $p<0.01$.

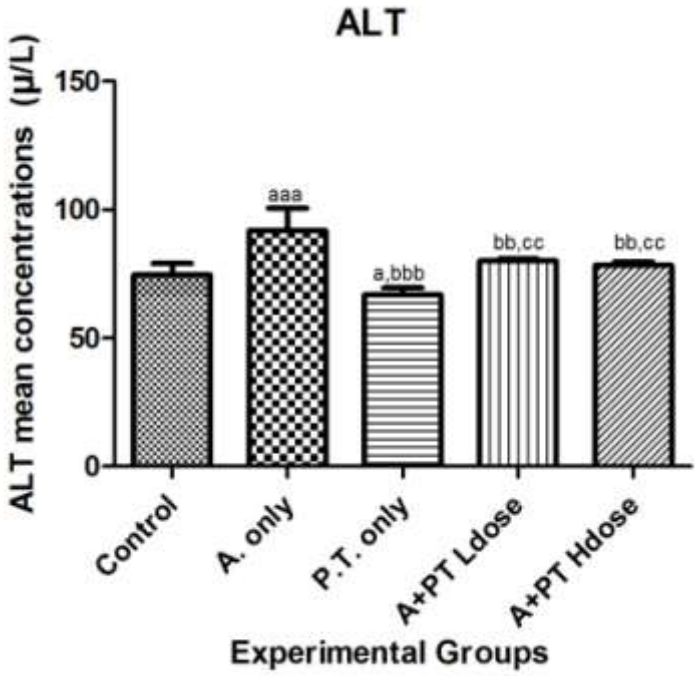

Figure 1: Effect of administration of ethanol extract of *P.thonningii* on serum ALT following acetaminophen induced toxicity on Wistar rats

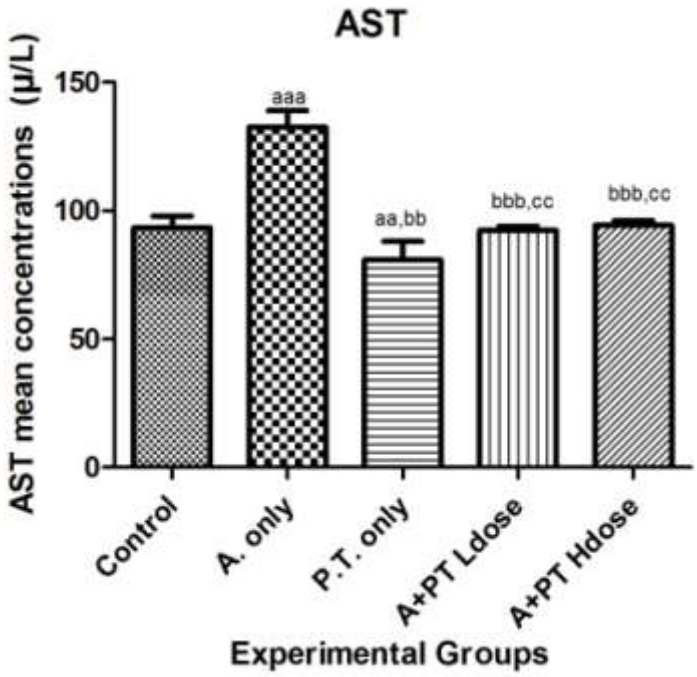

Figure 2: Effect of administration of ethanol extract of *P.thonningii* extract on serum AST following acetaminophen induced toxicity on Wistar rats

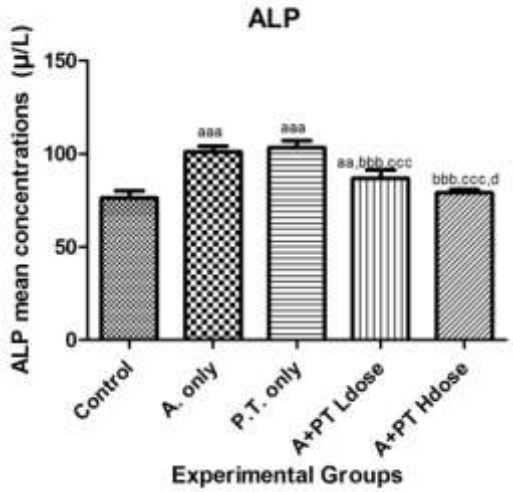

Figure 3: Effect of administration of ethanol extract of *P.thonningii* extract of *P.* on serum ALP following acetaminophen induced toxicity on Wistar rats

It was observed that the mean concentration of Albumin in the acetaminophen only group and P.T only group was significantly different from control at $p<0.001$ and $p<0.001$ respectively, albumin level of Acet.only was significantly different from P.T only ($p<0.001$), from A+P.T LD ($p<0.001$), and from Acet+P.T HD ($p<0.001$). It was also observed that the Albumin level of A+P.T LD was significantly greater than that of Acet+P.T HD at $p<0.001$.

It was observed that the mean concentration of globulin in the acetaminophen only group and P.T only group was significantly different from control at $p<0.001$ and $p<0.01$ respectively, globulin level of acetaminophen only was significantly different from P.T only ($p<0.01$ and $p<0.001$), from Acet+P.T LD ($p<0.01$), and from Acet+P.T HD ($p<0.01$), it was also observed that the globulin level of Acet.+P.T LD was significantly greater than that of Acet+PT HD at $p<0.01$ (fig 4-6).

It was observed that the mean concentration of total protein in the acetaminophen only group and P.T only group was significantly different from control at $p<0.001$ and $p<0.001$ respectively, Total Protein level of Acet.only was significantly different from P.T only ($p<0.001$), from Acet.+P.T LD ($p<0.001$) and from Acet.+P.T HD ($p<0.001$), It was also observed that the total protein level of A+P.T HD was significantly greater than that of Acet+P.T LD at $p<0.001$ (fig 4-6).

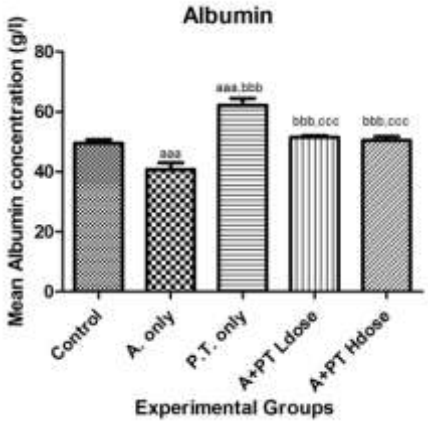

Figure 4: Effect of administration of ethanol extract of *P.thonningii* extract on serum albumin following acetaminophen induced toxicity on Wistar rats

In figure 4-
a represent significantly different from control
b represent significantly different from acetaminophen only
c represent significantly different from P.T only
d represent significantly different from A+P.T Ldose
one superscript represent $p<0.05$
two superscripts represent $p<0.01$
three superscripts represent $p<0.001$

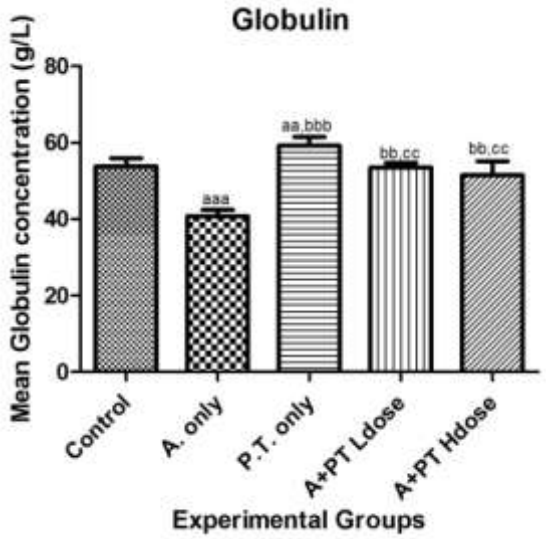

Figure 5: Effect of administration of ethanol extract of *P.thonningii* extract on serum globulin following acetaminophen induced toxicity on Wistar rats

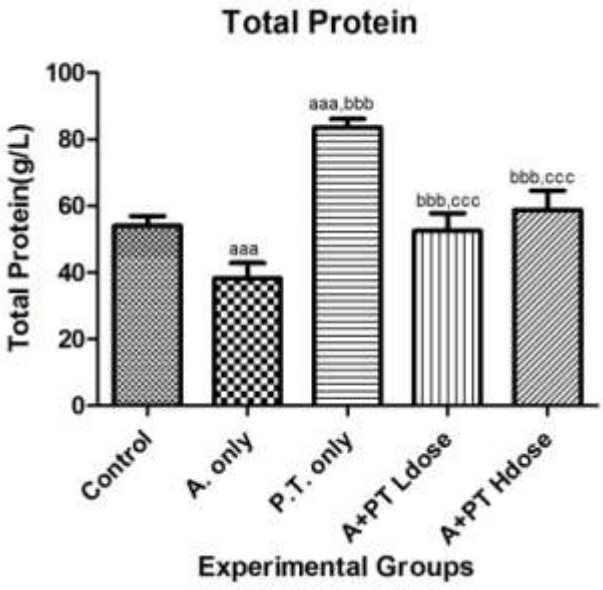

Figure 6: Effect of administration of ethanol extract of *P.thonningii* extract on serum total protein following acetaminophen induced toxicity on Wistar rats

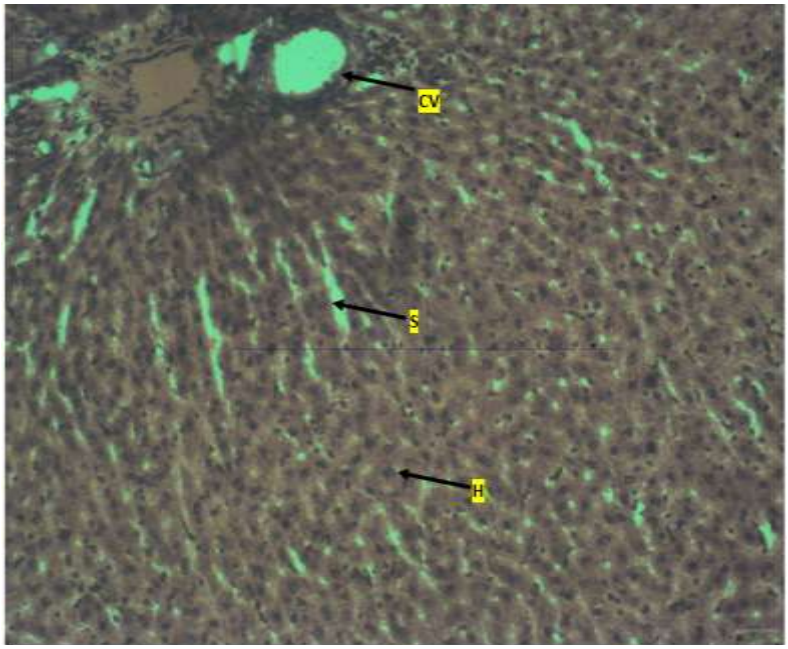

Figure 7: Photomicrograph of liver of Control group showing a central vein (CV) with a normal parenchyma. Hepatic cells (H) and Sinusoids (S) appear normal. No pathology seen
H & E. X100

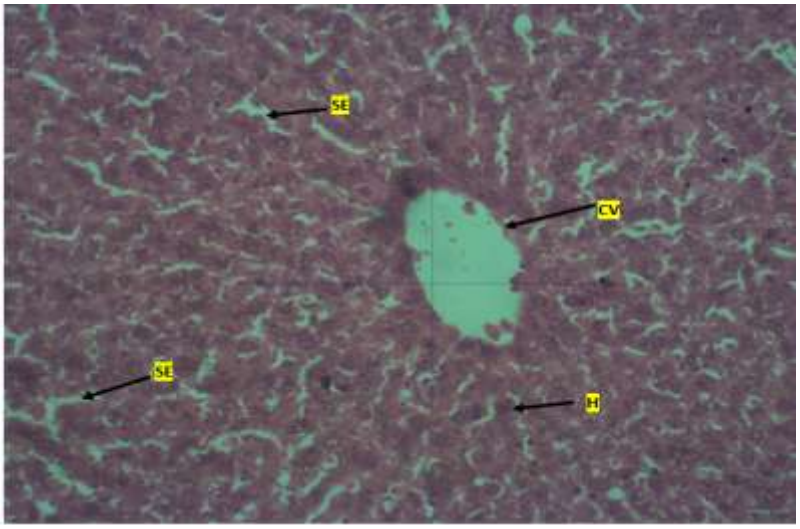

Figure 8: Photomicrograph of the liver of group administered with acetaminophen showing a central vein (CV) with a slight enlargement of the sinusoidal arrangement. Hepatic cells (H) appear normal. This enlargement of the sinusoidal space is a prognosis of pseudopeliotic steatosis (H & E. X100).

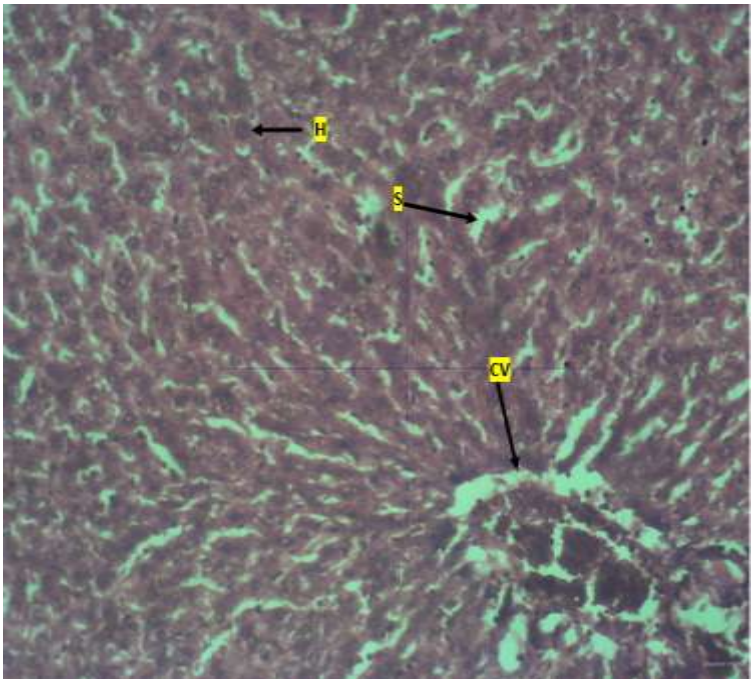

Figure 9: Photomicrograph of the liver of group administered with P.thonningii showing a central vein (CV) with an onset of venous peliosis hepatis (blood filled cysts in the liver). Sinusoidal spaces (S) appear enlarged (H & E. X100)

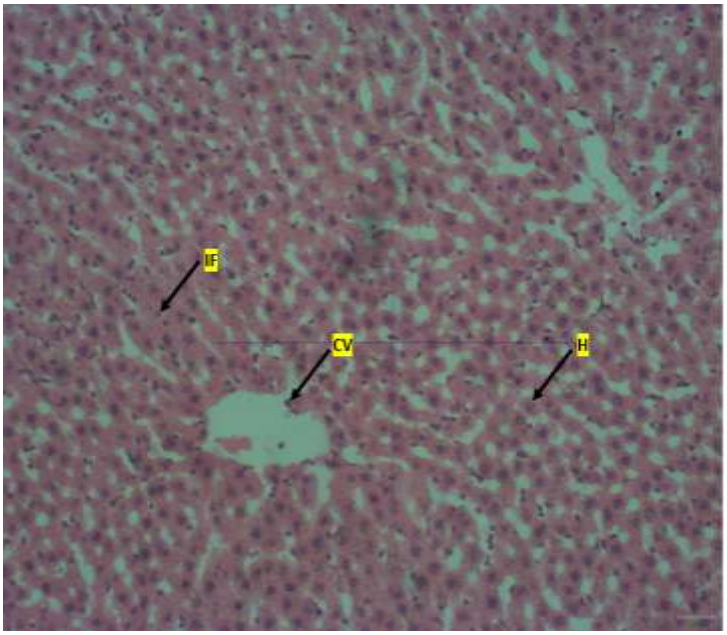

Figure 10: Photomicrograph of the liver of group administered with *P.thonningii* and low dose acetaminophen showing a central vein (CV) with inflammatory cells (IF) adjoining the hepatic cells (H). Presence of inflammatory cells suggests a prognosis of metabolic imbalance.

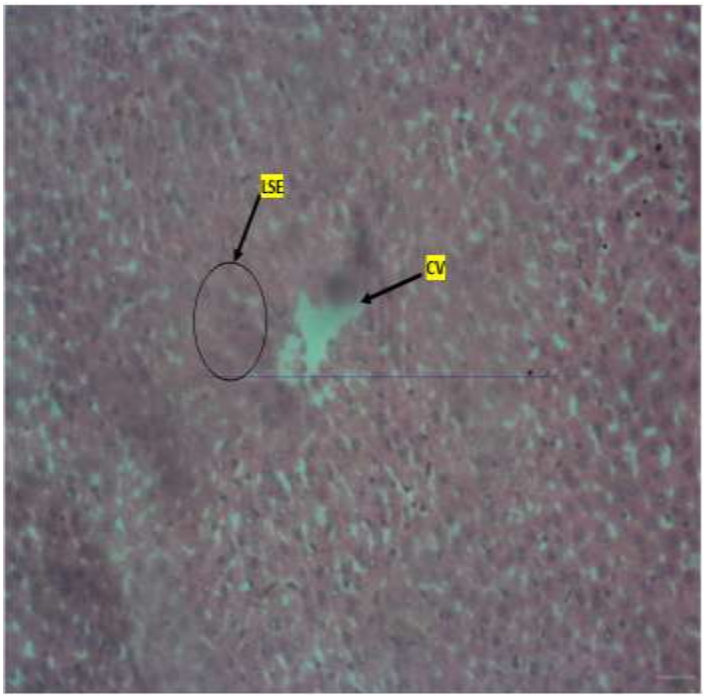

Figure 11: Photomicrograph of the liver of group administered with *P.thonningii* and high dose acetaminophen showing a diffused cental vein (CV) and a granulomatous infection of the parenchyma of the liver. There is loss of sinusoidal space (LSE). This also suggests a primary bilary cholangitis (H & E. X100).

DISCUSION

The biochemical indices studied in this research are sensitive and useful parameters to indicate the alterations caused by the drugs on the hepatic capacity or integrity of the rats. More so, alteration in the biomarkers of the liver function indices might be useful tool to monitor the level of injury or damage by the plant extract before biopsy (Dasofunjo *et al*., 2013).

Albumin and globulin are among the indices that can be used to evaluate the normal functioning of the liver of animals (Asuk *et al*., 2018). Albumin is the protein with the highest concentration in the plasma. It transports many molecules in the blood. It prevents the fluid in the blood from leaking out the tissue (Duncan *et al* 2006). Albumin is a constituent of the total protein produced in the liver. Albumin levels are decreased in chronic liver disease such as cirrhosis .The reduction in the levels of serum albumin and globulin by acetaminophen treated group may be an indication of diminished synthetic function of the liver, resulting probably from hepatocellular damage. This is similar to the findings of (Pendota *et al*., 2010) who reported similar decrease in albumin content of the serum of male rats following the administration of 200 mg/kg body weight of aqueous extract of *Hippobromus pauciflorus* leaves to male rats. The significant ($P<0.05$) increase caused by the Albumin and Globulin level following the administration of the extract implied that the extract produced an increase in protein synthesis and (or) mobilization or antibody during pregnancy (Adewuyi and Afolayan, 2009). Therefore, the observed maternal and prenatal increase in serum albumin and globulin in all the treated groups is an indication that the extract/drug may promote good functioning of the liver or possess a hepatoprotective role and may help calcium in the blood stream to regulate the movement of water blood stream into body tissue.

Aspartate amino transaminase (AST) is predominantly localized within the cells of the gills, kidney, muscle and liver parenchamal cells. An increase in serum AST might connote acute liver damage or liver

cytolysis. Serum Alanine Amino Transferase (ALT) is known to increase when there is liver disease and it has been used as a tool for measuring hepatic necrosis (Duncan *et al.,* 1994) while ALP 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 (Dasofunjo *et al.,* 2013) 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 (Yakubu, 2006).

However, of this marker enzymes, ALT is the most reliable. AST is known to be abundance in the cardiac muscles, skeletal muscles, kidneys and testes. Thus, any disease state affecting any of these extra hepatic tissues significantly elevates the serum level of enzymes. Therefore, the observed significant increase maternal and prenatal alteration in serum ALT, AST and ALP following the administration of acetaminophen when compared with the control suggest that the drug might induce hepatic injury or assault or damage or hepatotoxicity during pregnancy. These findings are similar to the findings of other researchers (Puri *et al.,* 2008). Alternatively, the decrease in serum and liver AST, ALT support the report of (Dasofunjo *et al.,* 2013) that extract of *P. thonningii* leaf exhibits a hepatoprotective effect. Likewise, this present work showed that the co -administration of the ethanol leaf extract of *P. thonningii* and acetaminophen reduced significantly Serum Liver ALP, ALT and AST. Though the mechanism of action was not studied but it appears that co administration of ethanol leaf extract of *P. thonningii* and acetaminophen possess a synergistic effect which was able to buffer or ameliorate the hepatotoxic effect of the drug. Thus, suggesting its hepatoprotective effect during pregnancy.

CONCLUSION

The biochemical changes on maternal liver functional indices studied suggest that ethanol leaf extract of *P. thonningii* administered possess a hepatoprotective effect, since no injury was observed on the liver but with the evidence of hepatic injury/assault in groups treated with acetaminophen which was ameliorated in groups co- administered with both the acetaminophen and the extract

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