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Hepactoprotective effect of *Piliostigma thonningii* leaves on male wistar albino rats

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ABSTRACT

Piliostigma thonningii is a medicinal plant with proven anti-malarial, anticough, antisnake, anti-inflammatory and antihelmintic potentials. This present study is aimed at ascertaining the effect of ethanol extract of P. thonningii leaf on liver function indices of albino rats. Twenty (20) male wistar albino rats ranging from180-200g were randomly assigned into group (A-D) of five animals each. Groups(B-D) were administered 1ml of ethanol extract via oral route corresponding to50, 100 and 200mg/Kg/body weight, while group A (control) received 1mlof distilled water orally. Rats in all groups were sacrificed 24hours after the experimental periods of 21days of oral administration of the extract. The extract significantly ($p<0.05$) increased serum Albumin, Globulin, Total protein and Total bilirubin concentration when compared with the control. The serum AST, ALT and GGT showed a significant decrease while serum ALP produced a significant ($p<0.05$) increase when compared with the control. Also the extract of p. thonningii produced a significant reduction ($p<0.05$) on liver AST, ALP and GGT when compared with the control. The alterations by the results are manifestation of its hepato protective effect.

Keywords: *Piliostigma thonningii*, hepatoprotective, anti-inflammatory, anticough, antisnakevenum

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INTRODUCTION

Piliostigma thonningii belongs to the family *Fabacea.* It is commonly called: camel's foot, foot tree, monkey bread, kamelspoor, wild bauhinia, picture-frame tree, *makolokote, molgoropo, nkolokotso*. Other names include: nyihar *(Tiv), abafe (Yoruba), kalgo (Hausa), okpoatu (Igbo) andejei-jei (Igala). P. thonningii* is a plant with a single stem. The tree is highly utilized by the local people. It flowers from December to February [1]. The preparation of different parts of this plant such as the bark, roots, seeds and leaf has been used traditionally in the treatment/management of different ailments [2].The bark of this plant has been used in the management of ear ache, toothache, diarrhoea, dysentery, intestinal problems, cough and other respiratory problems throughout tropical Africa. [3].The roots of *P. thonningii* are applied on wounds, ulcers as a haemostatic and to promote healing. [2].Unripe pods can be used as a substitute for soap making [4].Various leaf preparations are used as antiseptic and cicatrised to promote wound healing, against skin diseases, itching, snake bites, HIV virus, hepatitis B and C etc[5]. Therefore, the extensive use of this plant underscores the need to evaluate the effect of the leaf extract of *P. thonningii* on the liver. Thus, the primary aim of this study is to evaluate the effect of the ethanol extract of the leaf on liver functional indices in rats.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Piliostigma thonningii* was collected from Mkar Hills, Gboko, Benue State, 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.

ASSAY KITS

The assay kits for Albumin, Globulin, Bilirubin, Alkaline Phosphatase (ALP), Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were obtained from Randox Laboratories, Ltd, United Kingdom. Total protein concentration of the samples was assayed by the Biuret method [6]. All other reagents used were of analytical grade and were prepared in all glass distilled water.

Experimental Animals

Albino wistar rats were obtained from the animal holding unit of the Department of Chemical Sciences, University of Mkar, Mkar Nigeria. The animals were allowed to undergo an acclimatization period of seven (7) days. Each rat was housed in a wooden cage. The animal room was ventilated and kept at room temperature and relative humidity of29°c and 40-70% respectively with 12 hours natural light-dark cycle and were allowed free access to food and water *ad libitum*. Good hygiene was maintained by constant cleaning and removal of faeces and spilled from cages daily.

Preparation of ethanolic extracts of *Piliostigma thonningii* leaves

The leaves of *Piliostigma thonningii* were collected and air dried for 14days until constant weight was obtained. The dried leaves were then pulverized to coarse powder by blender machine and sieved. After which, 300g of the pulverized plant material (*P.thonningii*) was dissolved in 500mL of 70% ethanol for 72 hours. This was followed with vacuum filtration and extracts was concentrated using a rotary evaporator water bath at a 40°C. The concentrate was heated over a water bath to obtain a solvent free extract, which was stored in a refrigerator at 4°C.

Animal grouping and administration of extract

The animals were randomly assigned into four study groups (A-D) of five male rats in each cage. Rats in the control group were orally administered with 1ml of distilled water by gavage and standard feeds while the animals in the other groups (B-D) were administered orally with 1ml corresponding to 50,100 and 200 mg/kg of the ethanol leaf extract for 21days respectively. The animals in each group were sacrificed 24 hours after the completion of their respective doses by cardiac puncture procedure. The animals were handled humanly in accordance with the guidelines of European convention for the protection of vertebrate animals and other scientific purposes- ETS-123 [7].

Preparation of serum and tissue homogenates

The animals were anaesthetized in a jar containing cotton wool soaked in ether. When the animal became unconscious, they were brought out quickly of the jar, theabdominal region was opened along the linear Alba cut with scalpel blade to exposethe 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 300rpm x 10min 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 12hour of preparation as described by [8].Each ofthe organs (liver) was cut with a clean sterile blade and then homogenized in 0.25 M sucrose solution 1:5 (w/v) as described by [9]. The homogenates were later transferred into specimen bottles and kept frozen for 24 hours before being used for the biochemical analysis.

Statistical Analysis

Statistical analysis data used presentation as a means ± SD of five determinations. Statement analysis was carved out using one way analysis of variance (ANOVA). Differences were statistically significant at $P < 0.05$ [10].

RESULTS

The results below depict the effect of administration ofethanol leaf extract of*P. thonningii* on Liver indices of male wistar albino rats (table: 5).Fig 1-5 show a significant increase ($p<0.05$) in serum albumin, globulin, total protein and total bilirubin concentration when compared with the control. The serum AST, ALT and GGT showed a significant ($p>0.05$) decrease while ALP showed a significant increase when compared with the control. (Fig 6-

10).Also, the extract of *P. thonningii* produced a significant reduction ($p<0.05$) on Liver AST, ALP, ALT and GGT. When compared with the control (Fig 10-14).

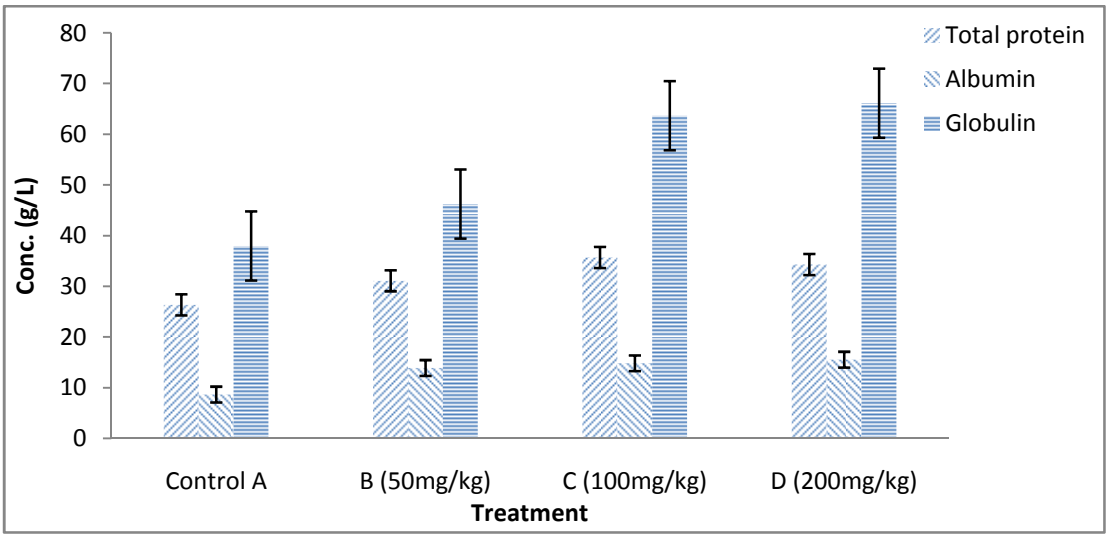

Figure 1.Effectsof ethanol extract of *P. thonningii* leaf on total protein, albumin and globulin of albino rats

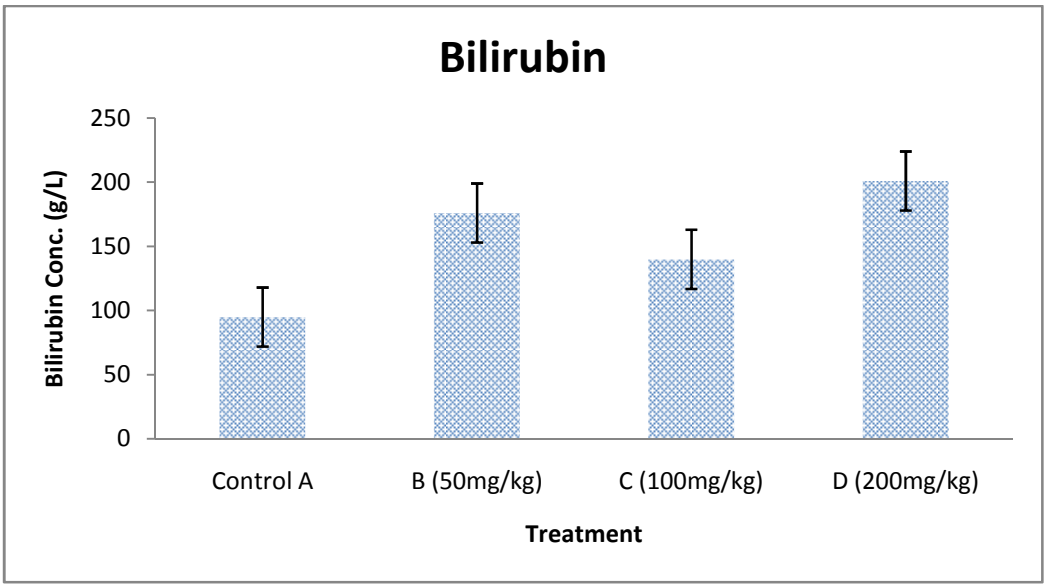

Figure 2. Effects of ethanol extract of *P. thonningii* leaf on total bilirubin of albino rats

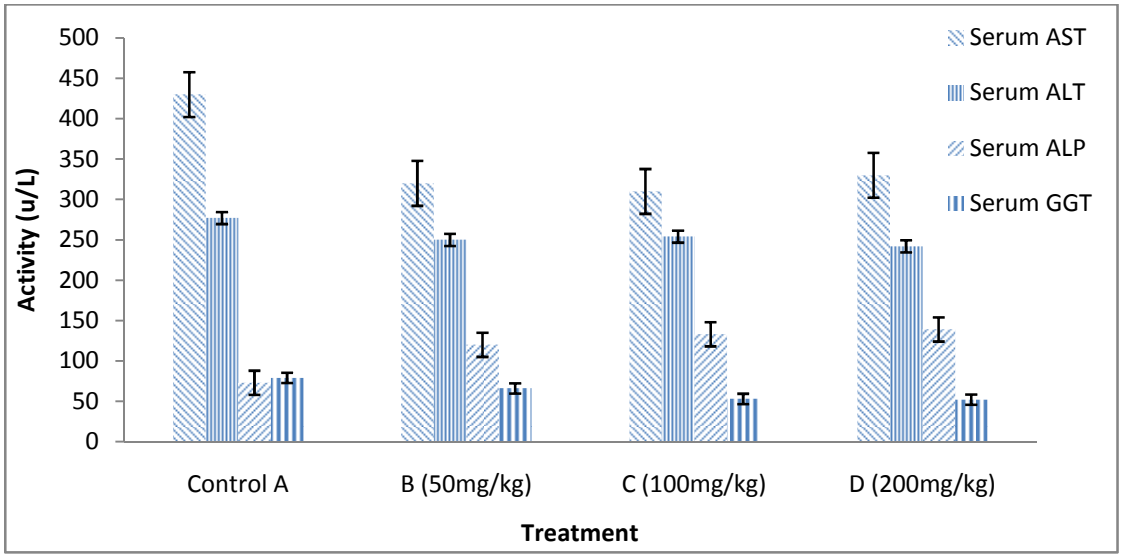

Figure 3.Effects of ethanol extract of *P. thonningii* leaf on serum ALT, AST, ALP and GGT of albino rats

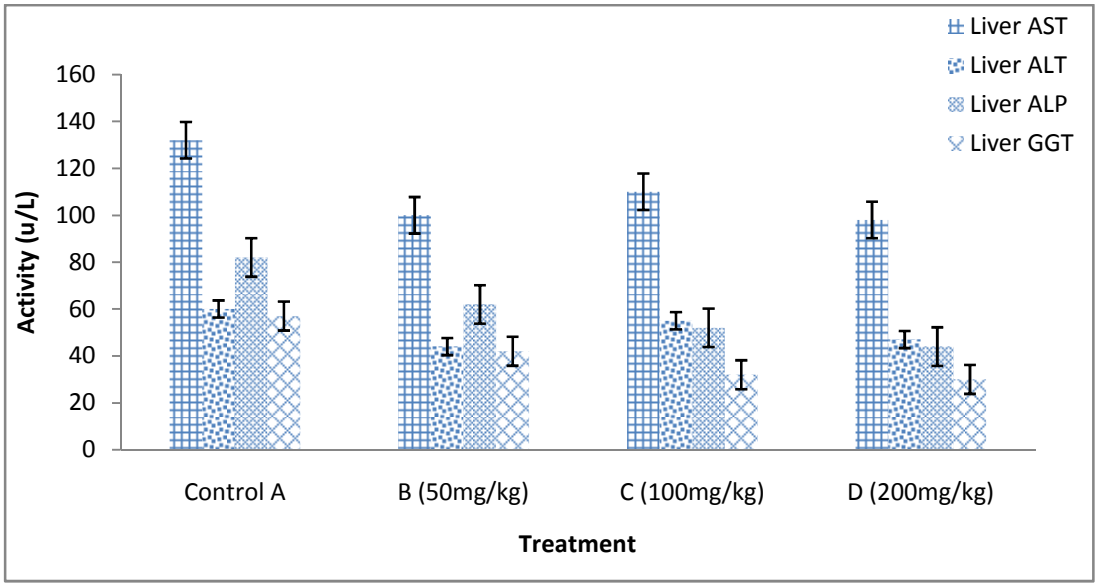

Figure 4.Effects of ethanol extract of *P. thonningii* leaf on liver ALT, AST, ALP and GGT of albino rats

DISCUSSION

The Liver has an incontrovertible influence on several functions of many organs in the body. It is prone to xenobiotic induced injury due to its central role in xenobiotic metabolism and its portal location within the system. [11].The liver plays important role in metabolism, detoxification and biotransformation. An alteration in the biomarkers of the liver function indices might be used to monitor the level of injury or damage by the plant extract before biopsy.The significant ($p<0.05$) increase caused by the Albumin, Globulin, Total protein and Total bilirubin concentration implied that the extract produced an increase in protein synthesis and (or) mobilization. The observed increase in Globulin level may indicate the efficiency of the plant extract to produce antibody [6,12]. Or due to the presence of bioactive constituent like flavonoids.

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 [14]. Albumin is a constituent of the total protein produced in the liver. Albumin levels are decreased in chronic liver disease such as cirrhosis or nephrotic syndrome. Therefore, the observed increase in serum albumin is an indication that the extract 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.

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 [15,14]. Hence, the observed decrease in serum ALT suggests that extract may be safe to the hepatic tissue at 50, 100 and 200mg/kg body weight. Serum Gamma GlutamylTranspeptidase (GGT) is a specific biomarker of the liver. It's more specific for cholestatic damage. Serum GGT may be elevated with even minor sub-clinical levels of liver dysfunctions. The observed significant ($p<0.05$) decrease in serum GGT may also support the hepato protective potential of the extract at the administered dosage.

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.Therefore, the significant ($p<0.05$) reduction in both serum and liver AST may suggest that no cytolysis or liver injury occurred.ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum [16] 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 [9,17] 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 [18].

An increase in serum ALP may suggest bile duct obstruction, intra hepatic cholestasis or infiltrative disease of the liver. Therefore, the observed decrease in both serum and tissue ALP may suggest that the extract produced no inimical injury that is capable of causing bile duct obstruction, intra hepatic cholestatasis or infiltrative disease of the liver.

CONCLUSION

The biochemical and liver functional indices studied suggest that the dosage of*p. thonningii* administered possess a hepato protective potential, since no injury was observed on the liver.

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