Effect of Ethanol Leaf Extract of *Piliostigma thonningii* on Liver Function indices following Pefloxacin induced Toxicity in Wistar Albino Rats.

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ABSTRACT.

Purpose: Pefloxacin is a broad spectrum antibiotic that is active against Gram-negative bacteria by inhibiting DNA gyrase. This present research determines the effect of ethanol leaf extract of *Piliostigma thonningii* following Pefloxacin induced toxicity in wistar albino rats. **Methodology**: Twenty (20) male wistar albino rats ranging from 180-200g were acclimatized to laboratory conditions for 7days, following which they were randomly assigned into 4 groups A, B, C and D of five animals each. Group B was administered 0.5 ml of ethanol extract via oral route corresponding to 200mg/Kg/body weight, group C was administered with Pefloxacin (400mg/5ml) only, group D was co administered with ethanol leaf extract of *P. thonningii* and Pefloxacin (1:1) while group A. (control) received 0.5ml of distilled water orally. The rats was housed in wooden cages. The animal room was ventilated and kept at room temperature and relative humidity of 29°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. Rats in all groups were sacrificed 24 hours after the experimental periods of 21days of oral administration. **Results**: The result reveals a significant ($P<0.05$) increase in serum albumin in all the experimental groups when compared with the control. Likewise, serum globulin depicts a significant increase ($P<0.05$) while groups treated with Pefloxacin and mixture of Pefloxacin and ethanol leaf extract of *Piliostigma thonningii* showed significant ($P<0.05$) decrease when compared with the control. Except for group B which reveals a significant ($P< 0.05$) increase in serum bilirubin, Pefloxacin and mixture of Pefloxacin and ethanol leaf extract of *Piliostigma thonningii* showed a significant decrease when compared with the control. The serum AST concentration showed a significant ($P<0.05$) decrease when compared with the control. Liver AST and ALP reveals a significant decrease in groups administered with ethanol leaf extract of *Piliostigma thonningii* and mixture of Pefloxacin and ethanol leaf extract of *Piliostigma thonningii* while groups administered with the drug showed a significant ($P<0.05$) increase when compared with the control. Likewise, the Liver ALP showed a significant ($P<0.05$) increase in groups treated with the extract and drug alone while groups treated with both the extract and Pefloxacin showed a significant decrease ($P<0.05$) when compared with the control. **Conclusion**: The alterations on the liver functional indices studied suggest that ethanol leaf extract of *p. thonningii* possess a hepato protective effect, but with the evidence of hepatic injury/assault in groups treated with Pefloxacin but was significantly ameliorated in groups co- administered with the drug and the extract.

KEY WORDS: DNA gyrase, Hepatic injury, Hepatoprotective, Pefloxacin, *Piliostigma thonningii*,

1. INTRODUCTION

The use of chemotherapeutic agents to manage severe and life threatening bacterial infections has grown increasingly over the years because of its effectiveness, mode of administration, packaging, and ease of carriage made it a more usable therapy [1].Due to its curative nature, more people ascribes to the usage of this therapy knowing a little about the side effect it may exert on the body.

One of this chemotherapeutic agent which is popularly used in the treatment of typhoid fever both in the developed and underdeveloped countries of the world like the tropical Africa is known as Pefloxacin or the fluoroquinolone which belongs to the fluoroquinolone class of antibacterial [2].

Pefloxacin (1-ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid) with the formula $C_{17}H_2OFN_3O_3$ is to be considered a drug of last resort when all antibiotics have failed. This drug is well

absorbed by the oral route. The bioavailability is 100%, protein binding 20-30%, it undergoes hepatic metabolism with a half-life of 8.6 hours and is excreted mostly through renal and biliary clearance [3]. Fluoroquinolones such as Pefloxacin possess excellent activity against gram-negative aerobic bacteria such as *E.coli* and *Neisseria gonorrhoea* as well as gram-positive bacteria including *S.pneumoniae* and *Staphylococcus aureus* .They also possess effective activity against *Shigella, Salmonella, Campylobacter, Gonococcal* organisms, and multi drug resistant *Pseudomonas* and *Enterobacter* [4].Its half-life is 8.6 hours unchanged Pefloxacin and its metabolites may be identified in the urine 84 hours after the intake of this product [4]. Pefloxacin has been reported to have some side effect including the displacement of Y-amino butyric acid from its receptor, destruction of proteoglycan and collagen. Some of the serious adverse effects which occur more commonly with fluoroquinolones than with other antibiotic drug classes, includes central nervous system and tendon toxicity psychosis and cholera(involuntary muscle movements) [5].Though, the currently marketed quinolones have safety profile similar to that of other antimicrobial classes. Photo toxicity, neurological symptoms, impaired colour vision, peripheral neuropathy, exanthema, abdominal pain, malaise, drug fever, dysesthesia and eosinophilia all have been observed as adverse effects of Pefloxacin [6].Recently, research have been going on to see how some plants which have an antioxidant antimicrobial properties which can help reduced these harmful effects by using the active ingredients of this plants to reduce the side effects that have been observed over the years, one of such plant is known as *Piliostigma thonningii.*

The assemblage of different parts of this plant has been found traditionally useful. As a result, people have resorted to using parts of this plant in the management or treatment of different kinds of ailments. The stem of this plant have been used in the management of earache, toothache, diarrhoea, dysentery, intestinal problems in tropical Africa [8].The back of *P.thonningii* could be used to manage cough; this is done by chewing the bark or through infusion. The infusion or maceration of the bark also includes the treatment of malaria and leprosy [9]. The analgesic properties of this plant is also ascribed to the bark [10]. The leaf also serves as a laxative, sometimes giving to neonates as tonic and to massage mother's abdomen [11]. The barks are used in phytochemistry as elements like alkaloids, antibiotics, bacteriostatic, antifungal, tannins and astringents. The pods root and bark produces dyes, stains, inks, as bi-products. *P. thonningii* is also used medicinally in many topical African countries to treat wounds, ulcers, chest pain among others [9, 10]. With these broad spectrum antibiotic properties that Pefloxacin is possessed with, you cannot deny the fact that it has a very harmful adverse effect that is generally referred to as the fluoro quinolone toxicity which is our major interest in this work .Therefore paucity demands the assessment of hepatoprotective role ethanol extract of *P. thonningii* leaf following Pefloxacin induced toxicity.

2.0 MATERIALS AND METHODS

2.1 MATERIALS

2.2 PLANT MATERIALS

Fresh leaves of *P. thonningii* was 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.

2.3 ASSAY KITS

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

2.4 EXPERIMENTAL ANIMALS

Wistar albino rats were obtained from the animal holding unit of the Department of Medical Biochemistry, Cross River University of Technology, Okuku, Cross River State- Nigeria after the faculty ethical committee has granted permission. The animals were allowed to undergo an acclimatization period of seven (7) days. Each rat was housed in a plastic cage. The animal room was ventilated and kept at room temperature and relative humidity of 29°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.

2.5 METHOD

2.5.1 PREPARATION OF ETHANOLIC EXTRACTS OF *P. thonningii* LEAVES

The leaves of *P. 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 using a white clean plastic container.

2.5.2. ANIMAL GROUPING AND ADMINISTRATION OF EXTRACT

The animals were randomly assigned into four groups (A-D) of five male rats each in the cage house. Rats in the control group (A) were orally administered with 0.5 ml distilled water and standard feeds while the animals in group B was administered orally with same of 0.5ml volume corresponding 200 mg/kg body weight of the ethanol leaf extract group C was administered with Pefloxacin (400mg/5ml) alone while group D was co-administered with Pefloxacin and the extract (1:1) for 21days respectively. The animals in each group were sacrificed 24 hours after the completion of their respective doses and blood collected by cardiac puncture procedure. The study was approved by the Animal Welfare and Ethics Committee of CRUTECH, Cross River State, Nigeria. All conditions of animal use were also as approved by United States National Institute of Health (NIH) guide for Care and Use of Laboratory Animals and in accordance with the recommendation of IASP [12].

2.5.3 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, the abdominal region was opened along the linear alba cut with scalpel blade to expose the organs and blood was collected into a sterile sample container by a cardiac puncture 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 were thereafter aspirated into clean, dry, sample bottles using Pasteur pipette and were kept or store in sample bottle and used within 24 hours of preparation. Each of the organs (liver) was cut with a clean sterile blade and then homogenized in 0.25 M sucrose solution 1:5 (w/v). The homogenates were later transferred into specimen bottles and kept frozen for 24 hours before being used for the biochemical analysis.

2.5.6. ENZYME ASSAY

The enzymes assayed in the course of this project work included, Alkaline Phosphatase, Aspartate Amino Transaminase and Alanine Amino Transferase. These enzymes were assayed in the homogenates of liver and the serum.

3.0 RESULTS.

The result of the effect of ethanol leaf extract of *P. thonningii* on liver function indices following Pefloxacin induced toxicity reveals a significant ($P<0.05$) increase in serum albumin in all the experimental groups when compared with the control (Fig 1) .Likewise, serum globulin depicts a significant increase ($P<0.05$) while groups treated with Pefloxacin and mixture of Pefloxacin and ethanol leaf extract of *P. thonningii showed significant (P<0.05)* decrease when compared with the control (Fig 2). Except for group B which reveals a significant increase in serum bilirubin when compared with the control, group C and D showed a significant decrease when compared with the control (Fig 3). Effect of ethanol leaf extract of *P. thonningii* and Pefloxacin on serum AST, ALT and, ALP concentration showed a significant ($P<0.05$) decrease when compared with the control *(Fig 4- 6)*. Following the administration of the extract and drug respectively ,Liver AST and ALT reveals a significant decrease in groups administered with ethanol leaf extract of *P. thonningii and* mixture of Pefloxacin and ethanol leaf extract of *P. thonningii* while groups administered with the drug showed a significant ($P<0.05$) increase when compared with the control (Fig 7 and 8).Following the administration of the drug and the extract, the Liver ALP showed a significant ($P<0.05$) increase in groups treated with the extract and drug alone while groups treated with both the extract and Pefloxacin showed a significant decrease ($P<0.05$) when compared with the control(Fig 9).

4.0 DISCUSSION

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 [11]. The significant ($P<0.05$) increase caused by the Albumin and Globulin level following the administration of the drug and extract implied that the extract/drug produced an increase in protein synthesis and (or) mobilization or antibody [13,14). 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 [15]. Albumin is a constituent of the total protein produced in the liver. Albumin levels are decreased in chronic liver disease such as cirrhosis or nephrotics syndrome. Therefore, the observed 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.

The hepatocyte membrane distortion is associated with membrane leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of serum/plasma enzymes of acute hepatocellular damage namely

ALT, AST and ALP as a marker hepatocellular damage [16].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 in serum ALT, AST and ALP when compared with the control for the groups treated with the drug suggest that the drug might induce hepatic injury or assault or damage or hepatotoxicity. These findings are similar to the findings of other researchers [11, 17, 18, 19]. Alternatively, the decrease in serum and liver AST, ALT support the report of [11] that extract of *P. thonningii l*eaf exhibits a hepatoprotective effect. Likewise, this present work showed that the co -administration of the ethanol leaf extract of *P. thonningii* and Pefloxacin 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 Pefloxacin possess a synergistic effect which was able to buffer or ameliorate the hepatotoxic effect of the drug. Thus, suggesting its hepatoprotective effect. Likewise, the significant decrease in the serum level of total bilirubin in groups treated with both drug and the extract, is an indication that the drug might not induce injury to the hepatic tissue or caused conjugated hepatobiliary injury on the wistar albino rats.

5.0 CONCLUSION.

The biochemical alterations on the 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 Pefloxacin which was ameliorated in groups co-administered with both the drug and the extract

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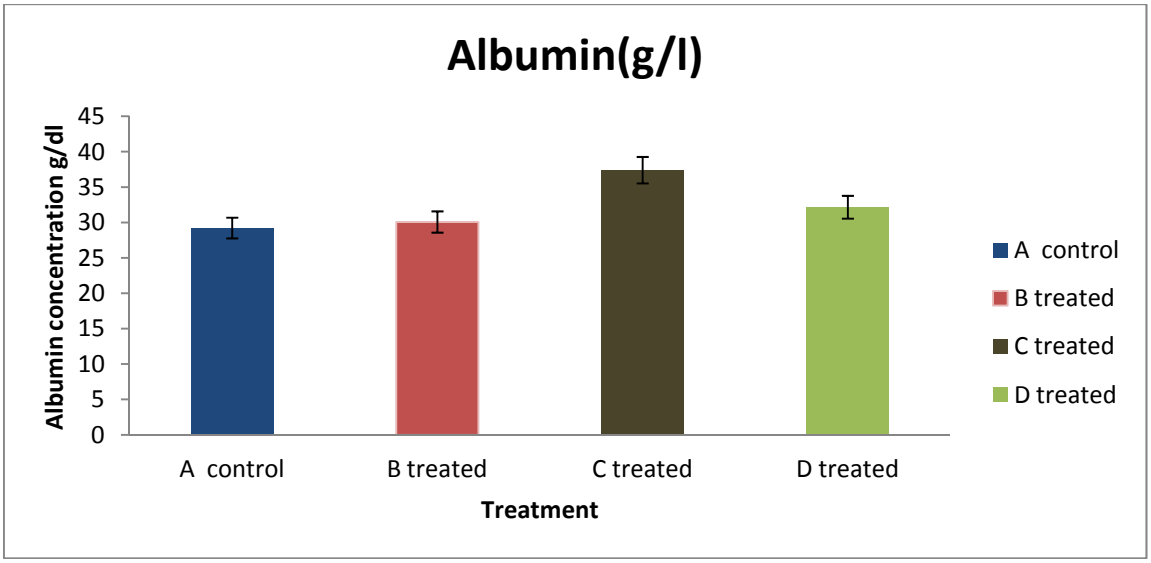

Fig 1: Effect of ethanol leaf extract of *P. thonningii* and Pefloxacin on serum albumin concentration

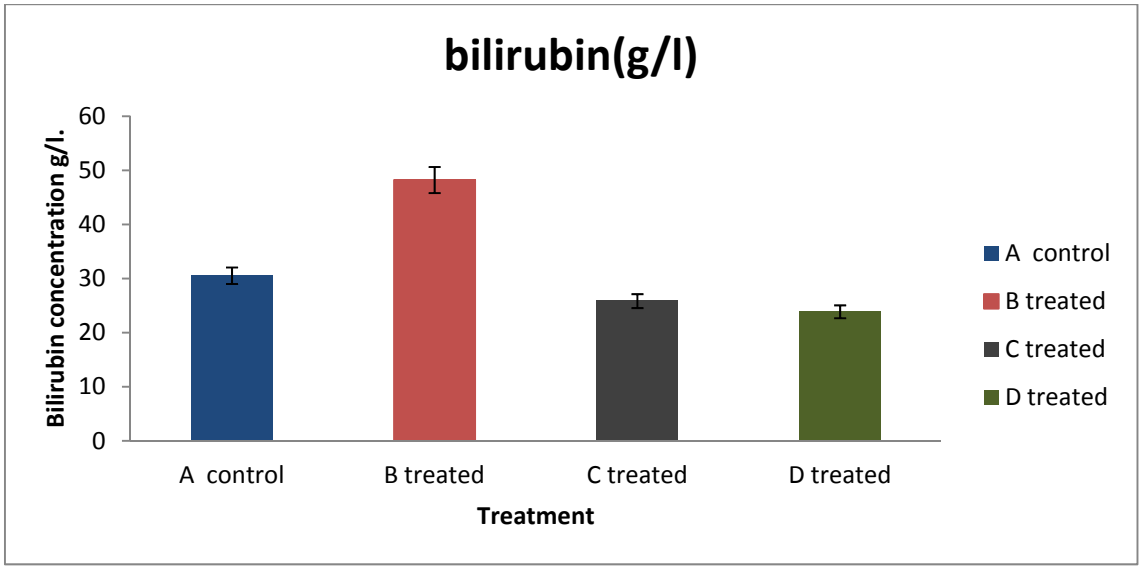

Fig 2: Effect of ethanol leaf extract of *P. thonningii* and Pefloxacin on serum bilirubin concentration

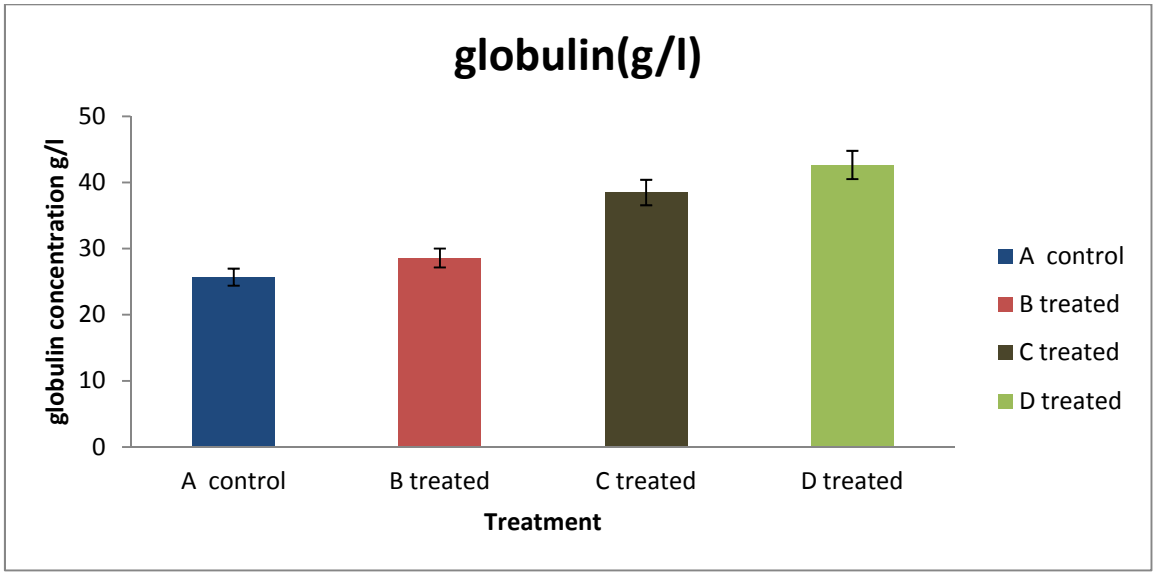

Fig 3: Effect of ethanol leaf extract of *P. thonningii* and Pefloxacin on serum globulin concentration

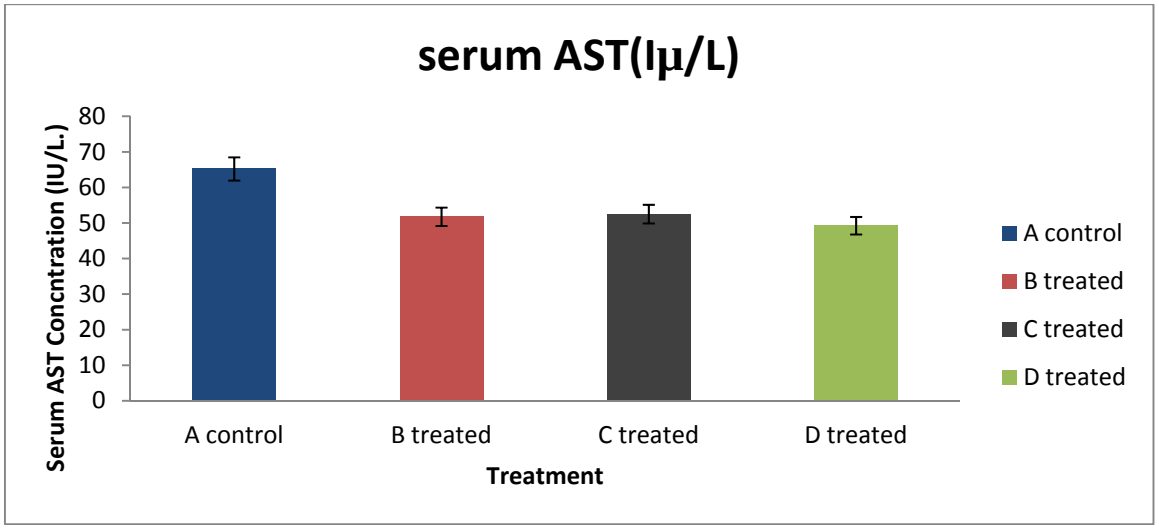

Fig 4 : Effect of ethanol leaf extract of *P. thonningii* and Pefloxacin on serum AST concentration

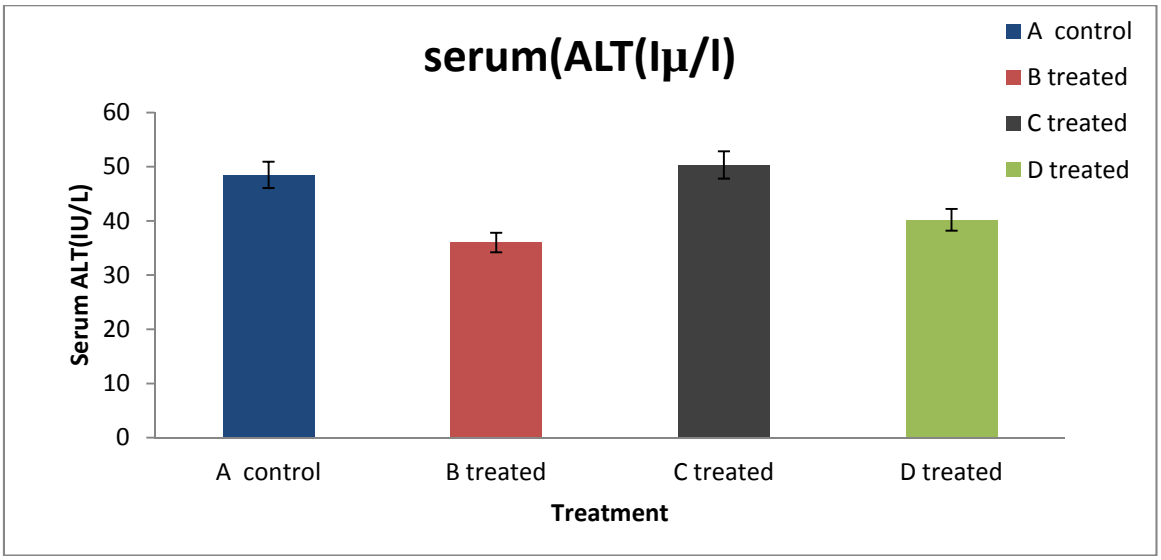

Fig 5 :Effect of ethanol leaf extract of *P. thonningii* and Pefloxacin on serum ALT concentration

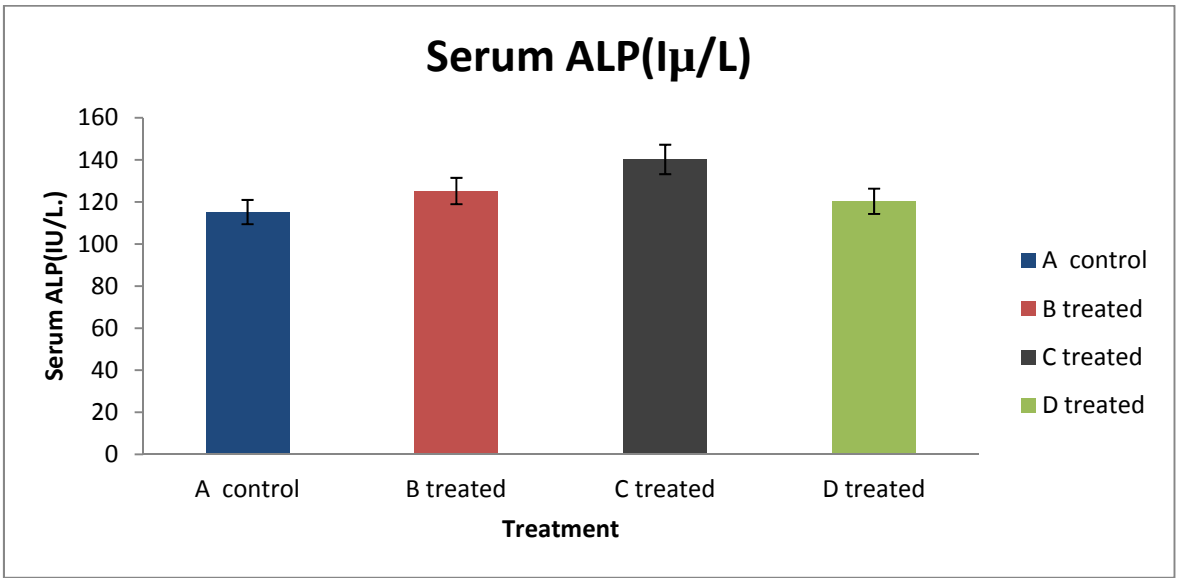

Fig 6: Effect of ethanol leaf extracts of *P. thonningii* and Pefloxacin on serum ALP concentration

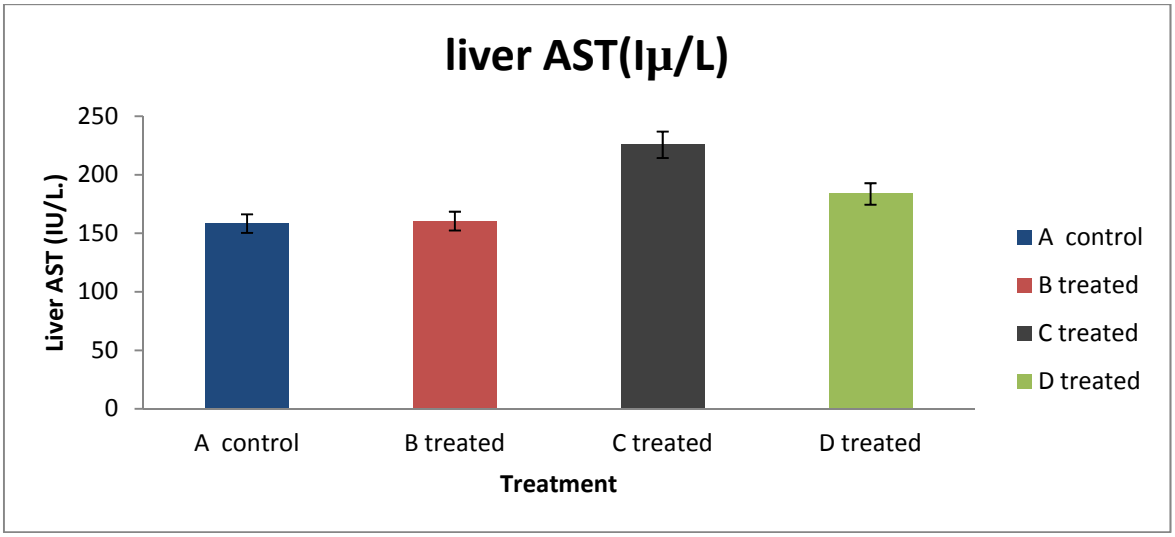

Fig 7: effect of ethanol leaf extract of *P. thonningii* and Pefloxacin on liver AST concentration

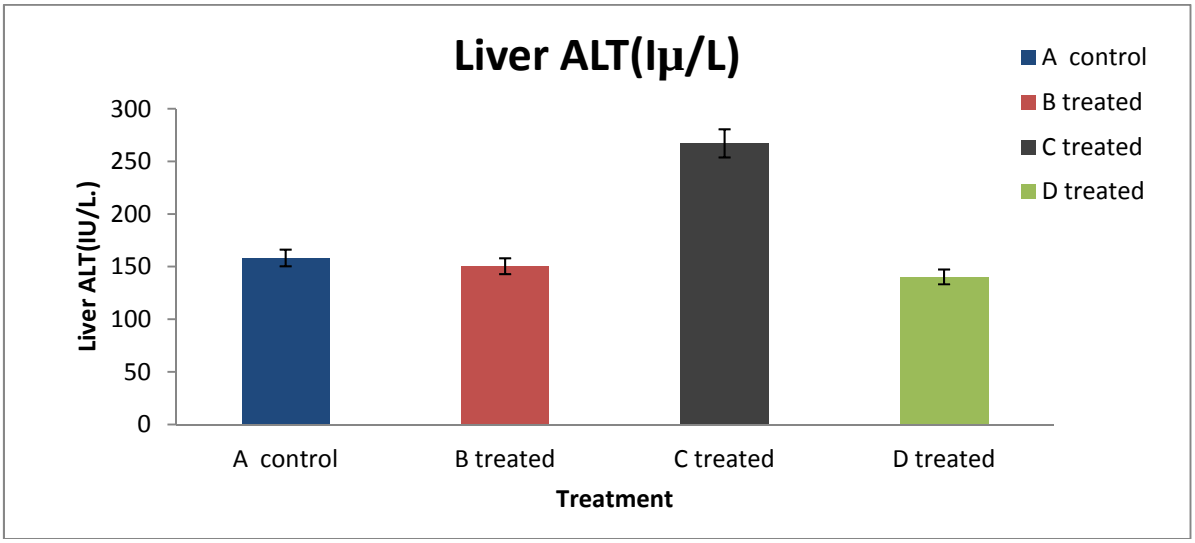

Fig 8: Effect of ethanol leaf extract of *P. thonningii* and Pefloxacin on liver ALT concentration

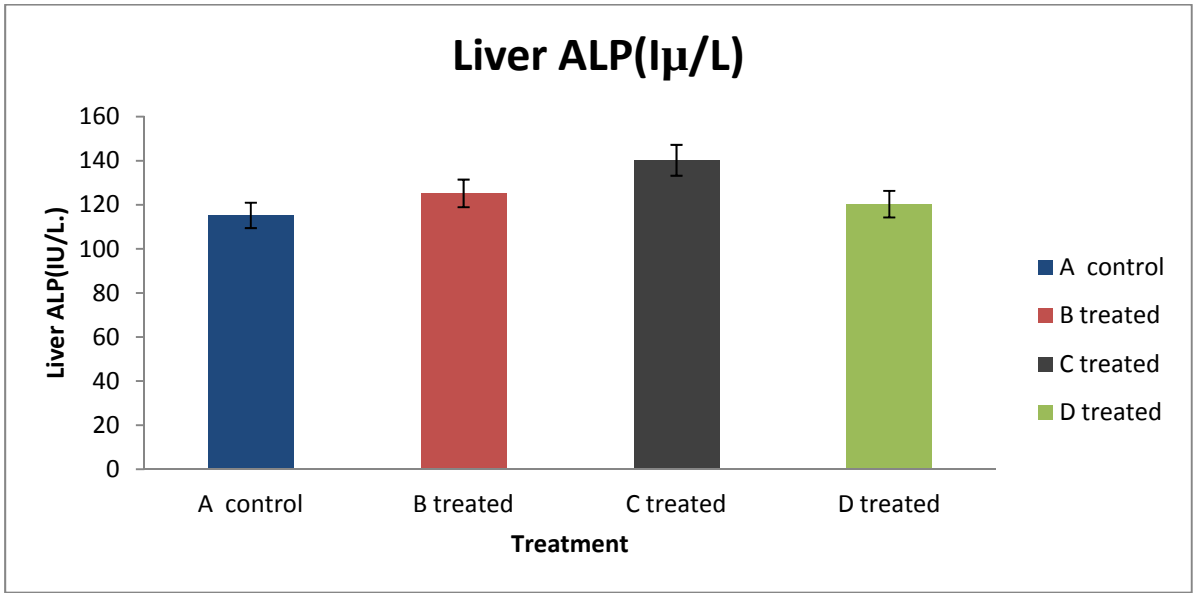

Fig 9: Effect of ethanol leaf extract of *P. thonningii* and Pefloxacin on liver ALP concentration