International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.1, pp 63-69, Jan-Mar 2013

Hepatoprotective Activity Of Phyllanthus longiflorus Heyne Ex. Hook. F. Against Acetaminophen Induced Hepatotocxicity In Rats

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Abstract: The present study was conducted to evaluate the hepatoprotective activity of ethanolic extract of *Phyllanthus longiflorus* (EEPL) leaf against acetaminophen induced liver damage in Albino rats. The ethanolic extract of *Phyllanthus longiflorus* **(EEPL)** (200 and 400mg/kg) was administered orally to the animals with hepatotoxicity induced by acetaminophen (835mg/kg). Silymarin (3mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 40% polyethylene glycerol solution. The plant extract was effective in protecting the liver against the injury induced by acetaminophen in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin and total bilirubin. Histopathological studies of the liver showed swelling and necrosis in hepatocytes in acetaminophen treated rats, treatment with different doses of EEPL have significantly reduced the necrosis and swelling of the hepatocytes. It was concluded from the result that the ethanolic extract of *P.longiflorus* possesses hepatoprotective activity against acetaminophen induced hepatotoxicity in rats

Key words: Acetaminophen, Wistar albino rats, Serum Marker enzymes, Histopathology, *Phyllanthus longiflorus.*

Introduction:

Liver diseases remain to be serious health problems and management of liver disease is still a challenge to the modern medicine. The liver occupies the pivotal position in body plays as essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. The role played by this organ in the removal of toxic substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign (xenobiotic) compounds culminating in liver dysfunction[1]. Despite the tremendous studies in modern medicine there is still a need for a drug that stimulates liver function or offers protection to the liver from damage or helps regeneration of hepatic cells[2].While searching for hepatoprotective agents in natural products, highly encouraging results were obtained in our laboratory with *P. longiflorus* Heyne ex Hook.F. (Euphorbiaceae). This is the first report in this plant.

Phyllanthus spp. have been used in Siddha and ayurvedic medicine for the treatment of jaundice, liver disorder, gonorrhea, frequent menstruation and diabetes. However, no systematic attempts have been made to establish the scientific basis of the beneficial effects of *P. longiflorus* leaf extracts. *Phyllanthus amarus* enhanced hepatic recovery after ethanol induced liver injury[3]. Phyllanthin and hypophyllanthin were reported to reduce hepatotoxicity induced by CCl_4 and galactosamine in rats and may be used as marker for

hepatoprotection of *Phyllanthus amarus*[4,5]. Hence, the aim of the present study was to investigate the hepatoprotective acitivity of ethanolic extracts of *P. longiflorus* leaves on acetaminophen induced hepatocellular damage in rats *in vivo*.

Materials and methods:

Plant material:

The aerial part of *Phyllanthus longiflorus* Heyne Ex. Hook.F. was collected from Courtallam hills (Tamil Nadu) at 1000m ht and identified by the Botanical Survey of India, Coimbatore (Fig-5). A voucher specimen was retained in the Centre for Biotechnology and Phytochemistry, Department of Botany, Sri Parasakthi College for Women, Courtallam for further reference.

Preparation of plant extract for phytochemical screening and hepatoprotective studies:

The leaves of the plant were dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to successive extraction in a soxhlet apparatus using ethanol. The extract was subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedure[6]. The ethanolic extracts of the leaves were concentrated in a rotary evaporator. The concentrated ethanol extract were used for hepatoprotective studies.

Chemicals:

Acetaminophen was purchased as tablets from India Pharmaceuticals, Chennai and Silymarin was obtained from Micro laboratories, Bangalore.

EExperimental animal and design:

Thirty pathogen – free male Wistar albino rats (100-200 gm) were obtained from central animal house, K.M College of Pharmacy, Madurai. The rats were kept in a temperature-controlled environment (25±1^{o}c) with relative humidity (55±5%) and with a regular 12 h light / 12 h dark cycle. All animals were fed with a standard rat chow diet and water *ad libitum*. The experiment was conducted according to the method of Parthasarathy *et.al*., (2007)[7]. Acetaminophen was dissolved in 40% polyethylene glycerol 400 for administration.

Acute Toxicity Studies:

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [8]. The animals were kept fasting for overnight and proved only with water, after which the extracts were administered orally at 5 mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100, 2000 mg/kg body weight.

Experimental design: In this investigation, a total of 30 rats (24 acetaminophen hepatotoxicity induced rats and 6 normal rats) were taken and divided in to five groups of 6 rats each

Group-I: Rats received normal saline was served as a normal control.

Group- II: Acetaminophen hepatotoxicity induced control: Rats received 835 mg/kg body weight of acetaminophen for 21 days[9].

Group-III: Liver injured rats received standard drug silymarin at the dose of 3 mg/kg body weight for 21 days.

Group –IV: Liver injured rats received ethanol extract of *Phyllanthus longiflorus* at the dose of 200 mg/kg body weight for 21 days.

Group-V: Liver injured rats received ethanol extract of *Phyllanthus longiflorus* at the dose of 400 mg/kg body weight for 21 days. After 24 h of acetaminophen intoxication, the rats were euthanized and the blood was collected by cardiac puncture in EDTA coated tubes. The liver was immediately dissected out and washed with ice-cold saline. The blood and liver samples were assessed for their biochemical and histological observation.

Biochemical analysis:

The animals were sacrificed at the end of the experimental period of 21 days by decapitation. Blood was collected, sera separated by centrifugation at 3000rpm at 4^{0}C for 10 min. The liver function marker enzymes including Aspartate transaminase (AST), Alanine transaminase (ALT), were measured spectrophotometrically by using the method of Reitman and Frankel[10]. Serum alkaline Phosphatase (ALP) were measured by the method of King and Armstrong[11]. Total bilirubin (TB) were determined as described by Balistrei and Shaw[12], Serum total protein (TP) was estimated by the method of Lowry et.al.,[13].

Histopathological observation in liver:

The rat liver tissues were fixed with 10% formalin buffer solution and embedded in paraffin. The serial

sections were cut with 5 µm thicknesses and stained with haematoxylin-eosin (HE), and then observed for the changes of liver injury by microscope.

Statistical analysis:

All the data are expressed as Mean ±SEM and analyzed using one way analysis of variance (Newman Keul's multiple range test). For the statistical tests *p* values of less than 0.05 and 0.01 was taken as significant.

Results:

The ethanolic extract of leaf of *Phyllanthus longiflorus* subjected for phytochemical study showed the presence of alkaloids, tannins, aminoacid, phenols, reducing sugars, steroid, saponins and flavonoids. The ethanolic extract did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose. The effect of ethanolic extract of *Phyllanthus longiflorus* on serum transaminase (AST and ALT), alkaline phosphatase (ALP), total protein, albumin and total bilirubin in acetaminophen intoxicated rats are summarized in Table-1 and Fig (1-4). There was significant ($p<0.01$) increase in serum AST, ALT and ALP in acetaminophen intoxicated group (Group II) compared to normal control (GroupI). The total protein and albumin levels were significantly ($p<0.01$) decreased to 3.40 mg/dI and 2.10 mg/dI in acetaminophen intoxicated rats from the levels of 6.60mg/dI and 4.80 mg/dI respectively in normal group. Ethanolic extract of *Phyllanthus longiflorus* at the dose of 200 and 400 mg/kg orally administered significantly decreased the elevated serum marker enzyme and restored the altered total protein and albumin to almost near normal level. A significant elevation of total bilirubin in the serum of acetaminophen intoxicated group (Group II) when compared to control group (GroupI). The ethanolic extract of *Phyllanthus longiflorus* at the doses 200 mg/kg and 400mg/kg reduced levels of total bilirubin.

The liver histopathology was studied in the control (GroupI), acetaminophen intoxicated (GroupII), standard drug groups (GroupIII) and EEPL (GroupIV and GroupV). Under the photomicroscope, normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein were observed in the control group . However, acetaminophen intoxicated rats exhibited severe histopathological changes such as centrilobular hepatic necrosis, fatty change, kupffer cell, ballooning degeneration and infiltrating lymphocyte . Pretreatment with EEPL at two doses (200 and 400mg/kg) and silymarin reduced these histopathological changes associated with the hepatotoxicity from acetaminophen intoxicated treatment.

Table 1: Effect of ethanol extracts of *Phyllanthus longiflorus* leaf on ALT, AST, ALP, TP, TB and albumin in acetaminophen intoxicated male albino rats

Treatment group	ALT U/L	AST U/L	ALP U/L	TB U/L	TP mg/dl	Albumin mg/dl
Group-I	31.92±1.70	95.20±9.60	95.10±4.12	0.20±0.019	6.60±0.15	4.80±0.120
Group-II	128.40±5.4[a]	310.40±10.8[a]	210.4±4.80[a]	1.72±0.10[a]	3.40±0.2[a]	2.10±0.04[b]
Group-III	42.90±2.01	106.60±13.21	110.14±1.92	0.26±0.010	5.60±0.19	4.4±0.060
Group-IV	52.81±3.01[b]	116.42±5.01[b]	124±3.91[b]	0.40±0.01[b]	4.42±0.3[b]	3.86±0.0[b]
Group-V	48.40±2.81[b]	112.28±4.08[b]	118.62±4.0[b]	0.32±0.009[b]	5.02±0.2[b]	4.1±0.018[b]

- Values are expressed as Mean ± SEM (n-6)
- a values are significantly different from control at $p<0.01$
- b values are significantly different from control at $p<0.01$

Fig. 1 Effect of ethanolic extracts of different doses of *Phyllanthus longiflorus* on Total Bilirubin against toxic control

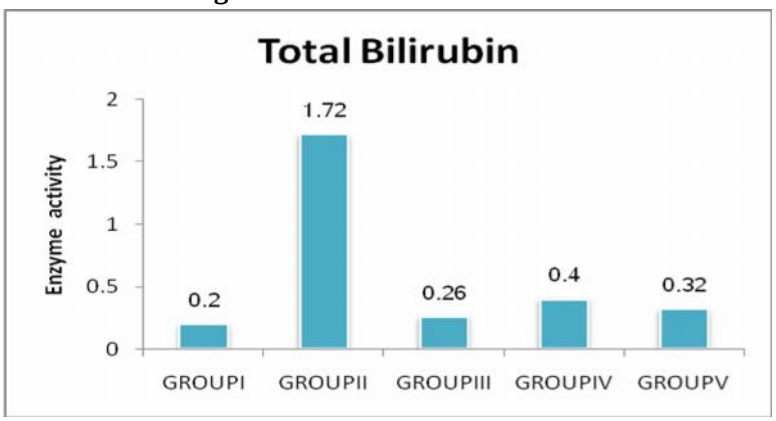


Fig. 2 Effect of ethanolic extracts of different doses of *Phyllanthus longiflorus* on ALP against toxic control

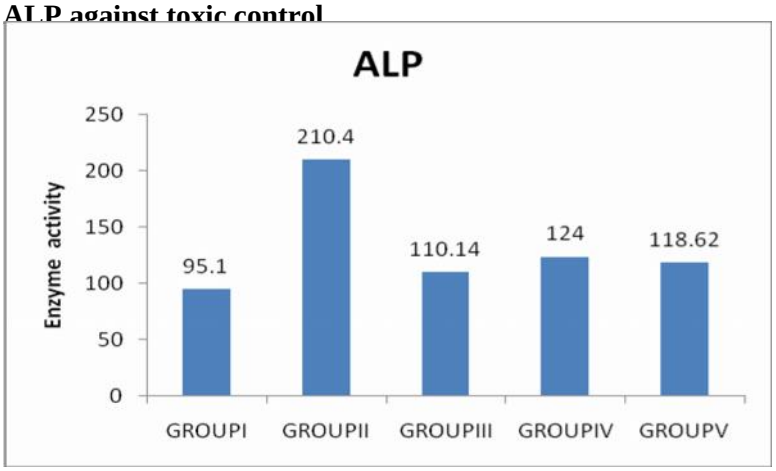


Fig. 3 Effect of ethanolic extracts of different doses of *Phyllanthus longiflorus* on Albumin against toxic control

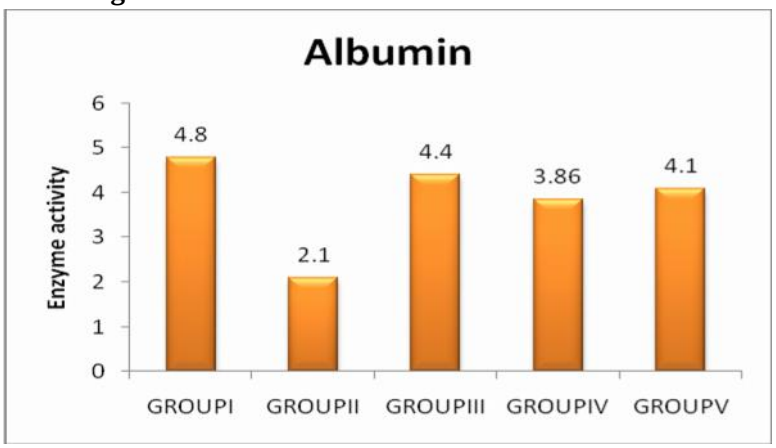
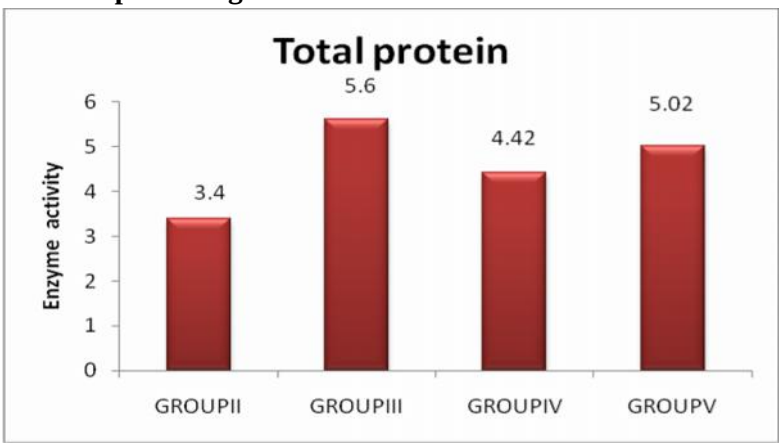


Fig. 4 Effect of ethanolic extracts of different doses of *Phyllanthus longiflorus* on Total protein against toxic control



Discussion:

The liver is the largest and one of the most vital organs in the body which is found to be more susceptible to disease and infection. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years[14.] In the absence of reliable liver protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief[15].

Acetaminophen is the active metabolite of phenacetin, is widely used as an analgesic and antipyretic drug[16]. It is administered in tablet, liquid suspension and suppository, intravenous or intramuscular form. The common adult dose is 500-1000mg. It is considered safe for human use at recommended doses; however acute overdose can lead to potentially lethal liver and in serve cases leads to death [17-20].In general administration of acetaminophen damage hepatocytes as they metabolise the acetaminophen. Rarely acute renal failure also may occur. In spite of this the intake of acetaminophen became inevitable because of to days life. In human adult single dose above 150 mg/kg is found to be toxic [21] . In children acute doses above 200 mg/kg could cause toxicity. Acetaminophen has also been shown to promote hepatocyte apoptosis [22.] And it leads to the formation of lipid peroxidases followed by pathological changes such as depression of protein synthesis, elevation levels of serum markers enzymes such as SGOT,SGPT and ALP, depletion of GPX,GRD,SOD and CAT and increase in lipid peroxidation.

In the present study, it was obtained that, the rats treated with acetaminophen resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the markers levels will reflect in hepatic structural integrity. Ethanol extract of *Phyllanthus longiflorus* at the doses of 200mg/kg and 400mg/kg significantly attenuated the elevated levels of the serum markers. The normalization of serum markers by ethanol extract of *Phyllanthus longiflorus* suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against acetaminophen induced leakage of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes. The alkaline phosphatase is the prototype of these enzymes that reflects the pathological alteration inbiliary flow[23] . The acetaminophen induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubin content. The ethanolic extract of *Phyllanthus longiflorus* induced suppression of the increased ALT activity with the concurrent depletion of raised bilirubins suggests the possibility of the extract to have ability to stabilize biliary dysfunction in rats liver during hepatic injury by acetaminophen. Thus administration of ethanol extract of leaf of *Phyllanthus longiflorus* is against the toxic effect of acetaminophen

The tannins and flavonoids present in *Phyllanthus emblica* fruit extract contain very powerful antioxidant and hepatoprtective properties [24,25.] Pornpen pramyothin *et.al.,* (2007) has reported that the treatment of rats with *P.amarus* (75mg/kg day),p.o) for 7 days after 21 days with ethanol (4g/(kgday),p.o.) enhanced liver cell recovery by bringing the levels of AST,ALT,HTG and TNF-α back to normal. Histopatological observations confirmed the beneficial roles of *P.amarus* and silymarin against ethanol induced liver injury in rats. The present experiment also indicates that animals administered with acetaminophen (2g) alone showed higher hepatotoxicity. The rats administered with

acetaminophen and *Phyllanthus longiflorus* (200 mg/kg and 400mg/kg) showed protective effect in the liver. The pathological changes of rat liver injury induced by acetaminophen were greatly reverted to normal by the treatement of *P.longiflorus* and silymarin. In conclusion, administration of *P.longiflorus* after acetaminophen in rats showed the protective activity quite similar to silymarin with the possible mechanism of antioxidation.

Acknowledgement:

Thanks to Dr. N. Chidambaranathan, Asst. Professor, Department of Pharmacology, K.M College of Pharmacy, Madurai, for their assistance in animal studies.

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