STUDY OF HEPATOPROTECTIVE ACTIVITY OF PHYLLANTHUS DEBILIS IN PARACETAMOL-INDUCED HEPATIC INJURY IN EXPERIMENTAL ANIMALS

Aruna Kutani, Chukka Radhika, Anapuram Rakshitha, Kuna Shivatejini, Bayya Sneha, Taj Humera*
Department of Pharmacology, Bojjam Narasimhulu Pharmacy College for Women, Hyderabad, Telangana, India, 500059.

***ABSTRACT:** The present study was conducted to evaluate the Vivo hepatoprotective activity of Phyllanthus devilis. The plant extract was screened using the paracetamol-induced hepatotoxicity model for their hepatoprotective activity. Treatment with the various extracts of Phyllanthus devilis resulted in improvement in the altered biochemical parameters of rats treated with paracetamol. Ethanol extract of Phyllanthus devilis significantly ($p < 0.05$) prevented the elevation in the levels of AST and ALT at 100 and 200 mg/kg dose levels. However, ALP and TB levels were found significantly lower only at higher dose levels (200 mg/kg) of the extracts. In this model, extracts of the selected medicinal plant showed significant hepatoprotection, especially by ethanol extract, possibly because of the higher phenolics and flavonoids content. These polyphenolic compounds are well reputed for their diverse pharmacological activities including hepatoprotective activity. Finally, we conclude that the present study results demonstrate that the plant Phyllanthus devilis, selected based on its traditional and ethnomedical claim, possesses potent hepatoprotective activity.*
***Keywords:** Phyllanthus devilis, Hepatoprotective activity, Silymarin, Paracetamol.*

I. INTRODUCTION

The liver is a central organ in energy metabolism and the biotransformation of xenobiotics. Therefore, frequent exposure to toxic xenobiotics is likely to provoke a liver injury, resulting in cirrhosis, liver cancer, and acute liver failure [1]. Acetaminophen (paracetamol or N-acetyl-para-aminophenol (APAP)) is one of the most widely used analgesics and antipyretic drugs worldwide. Although considered safe at therapeutic doses (up to 4000 mg/day), APAP, at higher doses, can induce centrilobular necrosis which generally leads to a fatal outcome [2]. APAP intoxication would be responsible for about one-half of all cases of acute liver failure in the United States and the United Kingdom [3]. In a recent study in Thailand where 184 patients with APAP overdose were included, 15.6% were reported with mild hepatotoxicity and 6.4% developed severe hepatotoxicity while 3 (1.6%) patients had acute liver failure [4].

APAP hepatotoxicity is initiated by the production of N-acetyl-p-quinone-imine (NAPQI), a reactive metabolite generated by cytochrome P450 enzymes that metabolize the drug when present at high doses. This compound then depletes glutathione stores and binds to several cellular proteins especially mitochondrial proteins, thereby leading to mitochondrial oxidant stress which causes cell death [4]. Moreover, it was shown that the immune system plays a role in the progression of APAP-induced hepatotoxicity. Indeed, activated Kupffer cells produce some proinflammatory and chemotactic cytokines that promote infiltration of neutrophils and macrophages in the liver tissue, causing an exacerbation of liver damage [5]. To block this toxicity, coadministration of N-acetylcysteine (NAC), a cysteine-derived antidote, is often useful, but some side effects, such as hypotension, limit its efficacy [6]. Consequently, the search for novel liver-protective agents is necessary to reinforce the existing therapeutic arsenal.

Phyllanthus debilis is a small, erect, annual herb of the Phyllanthaceae family that grows 30–40 cm in height distributed in Tropical Africa, India, Bhutan, Sri Lanka, and New Guinea. Within India, it is found in North West India, Sikkim, Bihar, Assam, and Peninsular. Āyurveda considers the plant astringent, sour and cooling in action. It destroys aggravations of *pitta* and *prameha*, correcting any obstructions in the urinary flow, quietens the thirst as well as douses any burning sensations. Bhavamishra believes it promotes *vāta* and is beneficial against coughs, *raktapitta* (plethora), vitiations of *Kapha*, and jaundice. The *nighaṇṭu* compiled by Shodala, a later classical scholar of Āyurveda, goes so far as to state that the plant cures poisoning. The (unripe) fruit and plant are acrid and sour. As a drug material, it is also considered astringent, deobstruent (removing obstructions in the passages), stomachic (good for the stomach), diuretic (promoting urine flow), febrifuge (useful at warding off fever), and antiseptic. It is considered good for healing sores. Yunānī physicians consider it to be beneficial

against tubercular ulcers, wounds, sores, unsightly spots, bruises, scabies, and ringworm[7-16].

II. Materials and Methods

2.1 Chemicals and Reagents

Chemicals and reagents used were distilled water (EPHARM, Ethiopia), 2% Tween80 (Oxford Lab Fine Chem LLP, India), absolute methanol (SIGMA-ALDRICH, Germany), n-butanol (SIGMA-ALDRICH, Germany), chloroform (SIGMA-ALDRICH, Germany), Paracetamol (PCM; *Sigma*-Aldrich, USA), silymarin (*Sigma*-Aldrich), 10% formalin (Novochem Engineering, India), ether (Puyer BioPharma Ltd., P.R. China), normal saline (EPHARM, Ethiopia), liquid paraffin (Oxford Lab Fine Chem LLP, India), paraffin wax (Oxford Lab Fine Chem LLP, India), hematoxylin (Santa Cruz Biotechnology, Inc., USA), eosin (Santa Cruz Biotechnology, Inc., USA), xylene (most scient - bioKEMIX GmbH, Germany), 2,2-diphenyl-1-picrylhydrazyl [DPPH] (Chemos GmbH & Co. KG, Germany), the standard drug silymarin (Silybon-140, Micro Lab Limited, India), assay kits for liver chemistry (HUMANA, Germany) and other chemicals and reagents for phytochemical tests. All reagents used were of analytical grade.

2.3 Collection and Authentication of *plant materials*

*Phyllanthus devilis*was collected from the local hill area of Ranga Reddy Dist, Telangana, India. Identified by a taxonomist and deposited in the Department of Pharmacology, of our institution an herbarium specimen of the same was prepared (voucher number APSC 560) was deposited for future use. The collected plant materials were cut into pieces, were gently washed with tap water to remove dirt, and dried under shade for 2 weeks.

Extraction

Three Hundred grams of the air-dried powdered whole plant of *Phyllanthus debilis* was weighed and subjected to cold maceration. It was successively extracted with the solvents like petroleum ether, chloroform, ethanol, and water. Each time the extracts were tested for the constituents and the process continued till they were exhausted. The marc left was air-dried and used for the next maceration process. A rotary flash evaporator was used for distilling off the solvent of each extract, the color, consistency, and yield of each was then noted. The extracts were as follows (PDPEE) (PDCE) (PDEE) (PDAQE)

2.5 Qualitative Phytochemical screening (Preliminary study)

The qualitative phytochemical investigations of the extracts were carried out using standardized tests to identify the presence of secondary metabolites[17].

2.6 Pharmacological Studies

2.6.1 Animals

Wistar albino rats of either sex weighing between 170-200 g were obtained from the Central Animal Research Facility of Manipal University. Before the start of the experiment, animals were acclimatized to the experimental room having a temperature of 23 ± 2 °C, controlled humidity conditions, and a 12 h light/dark cycle. Animals were caged in polypropylene cages with a maximum of three animals per cage. Animals were fed with standard food pellets and water ad libitum. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee of Sree Datta Institute of Pharmacy, Hyderabad (No. IAEC/ KMC/02/2021).

2.6.2 Acute toxicity study

Acute toxicity studies were conducted to determine the safe dose as per OECD 425 guidelines[18] using swiss albino mice. Animals that were fasted for 4 h were treated with different extracts of Phyllanthus devilis at a dose of 2000 mg/kg body weight suspended in 2% gum acacia. For each extract, 3 animals were used. After dosing, animals were observed individually once during the first 30 min., periodically during the first 24 h, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. During this period animals were observed for their behavioral pattern, autonomic pattern, and central nervous system patterns. Based upon maximum tolerated dose 1/10th and 1/20th dose was selected for efficacy studies.

2.6.3 Paracetamol induced hepatotoxicity

The hepatoprotective effect of *Phyllanthus devilis* in rats intoxicated with paracetamol was studied individually as described by Kalaskar and Surana (2011)[19] on 7 groups of six animals each. The animals of group 1 were served as control and treated once daily with vehicle (1% w/v gum acacia), animals of group 2 served as toxic control and given once daily 1% w/v gum acacia. Group 3 was treated with silymarin (100 mg/kg) as standard drug. Group 4 to 7 were treated with chloroform, methanol, and aqueous extracts (100 and 200 mg/kg) orally, for 7 days. On day 5 all the groups, except the normal control group, received paracetamol (3 g/kg) orally (Table 4.2).

Table 1: Experimental protocol for in vivo hepatoprotective activity

Group	Treatment	Dose
1	Normal	1% gum acacia
II	Paracetamol (Toxicant control)	3 g/kg
III	Paracetamol + Silymarin	100 mg/kg
IV	Paracetamol + Phyllanthus devilis Chloroform extract	100 mg/kg
V	Paracetamol + Phyllanthus devilis Chloroform extract	200 mg/kg
VI	Paracetamol + Phyllanthus devilis Ethanol extract 1	100 mg/kg
VII	Paracetamol + Phyllanthus devilis Ethanol extract 1	200 mg/kg
VIII	Paracetamol + Phyllanthus devilis Aqueous extract	100 mg/kg
IX	Paracetamol + Phyllanthus devilis Aqueous extract	200 mg/kg

2.6.4 Collection of blood

At the end of 48 h after the paracetamol treatment, blood was collected by retro-orbital puncture under light ether anesthesia. Blood was allowed to clot and then it was centrifuged at 4000 rpm for 20 min at 4 °C in a cold centrifuge for the separation of serum.

2.6.4.1 Biochemical parameter estimation

Serum biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), direct bilirubin (DB), and total bilirubin (TB) were determined by using Cobas C111 Autoanlyzer (Roche Diagnostics) using biochemical kits obtained from Roche Diagnostics India Pvt. Ltd. Mumbai.

2.7 Statistical analysis

All the results were expressed as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test using Graph Pad Prism version 5.03. A value of $p < 0.05$ was considered statistically significant.

III. RESULTS AND DISCUSSION

3.1 Acute toxicity study

Acute toxicity study on all the extracts of *Phyllanthus devilis* was performed as per the OECD 425 guidelines by performing a limit test at a dose of 2000 mg/kg body weight. Oral administration at this dose level did not show any sign of behavioral toxicity, neurological toxicity, or mortality. Hence 1/10th and 1/20th dose of the maximum tolerated dose was selected for the efficacy studies (Table 2).

Table 2: Doses (mg/kg) selected for acute toxicity study and efficacy study

Extract	Dose	Outcome	Safe dos	Dose 1	Dose 2
Chloroform	2000	00000	>2000	100	200
Ethanol	2000	00000	>2000	100	200
Aqueous	2000	00000	>2000	100	200

3.2 Paracetamol induced hepatotoxicity

3.2.1 Effect of different extracts of Phyllanthus devilis against paracetamol-induced hepatotoxicity

The liver is considered the most important organ in drug toxicity because it is functionally situated between the site of absorption and systemic circulation and is a major site of metabolism and elimination of foreign substances. These features make it a preferred target for drug toxicity[20]. Paracetamol is an analgesic and antipyretic agent. At high doses, it is known to cause hepatotoxicity in humans. It has been used as a successful experimental animal model to evaluate the efficacy of hepatoprotective agents[21,22]. At the therapeutic dose level, a major amount of paracetamol is metabolized in the liver by glucuronyltransferases and sulfotransferases to phenolic glucuronide which is excreted in the urine. About 5 to 10% of paracetamol is metabolized to N-acetyl-p-benzoquinone imine (NAPQI) by cytochrome P450, mainly CYP2E1. NAPQI is a highly reactive, electrophilic molecule that causes harm by the formation of covalent bonds with other intracellular proteins. This is prevented by the reaction between NAPQI and glutathione to generate a water-soluble product that is excreted into the bile. With paracetamol overdose, glucuronyltransferases and sulfotransferases get saturated, which causes the excess production of NAPQI in amounts that lead to depletion of glutathione. This excess NAPQI causes mitochondrial dysfunction and the development of acute hepatic necrosis and it can form covalent bonds with cellular proteins and modify their structure and function[23]. Further, depletion of glutathione enhances the expression of tumor necrosis factor-alpha (TNFα) which stimulates phagocytic NADPH oxidase to enhance the production of oxygen free radicals and contributes to liver damage[24].

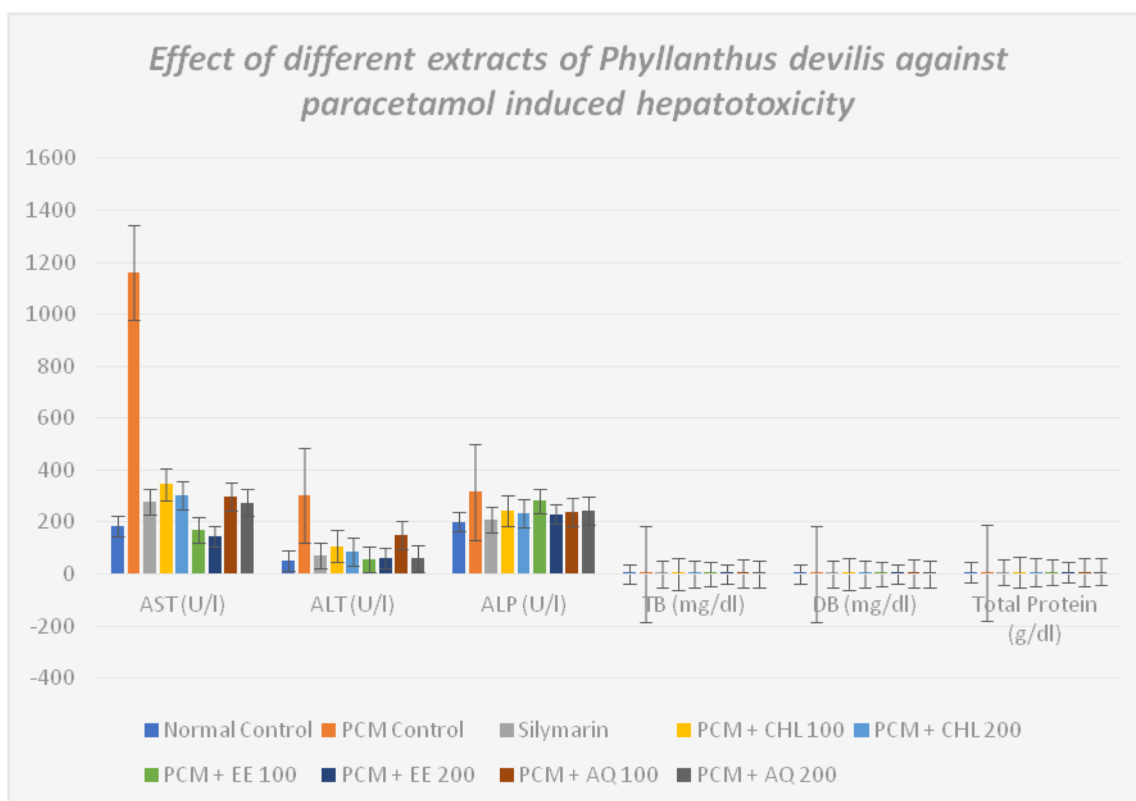
In the present investigation, different extracts of Phyllanthus devilis were investigated for their possible hepatoprotective activity in the paracetamol-induced hepatotoxicity model. Effects of various extracts of Phyllanthus devilis on rat serum parameters are shown in table 3. Oral administration of paracetamol caused significant liver damage as evidenced by altered serum biochemical parameters. Paracetamol administration at a dose of 3 g/kg resulted in a significant ($p < 0.05$) increase in the serum AST, ALT, ALP, TB, and DB levels while there was a significant reduction in TP levels when compared to the normal group. Treatment with standard drug silymarin caused a significant ($p < 0.05$) improvement in the altered serum biochemical parameters at the tested dose level of 100 mg/kg. Silymarin is commonly used as a reference standard for hepatoprotective studies. It is obtained from seeds and fruits of Silybum marianum. The effect of silymarin as hepatoprotective is mainly due to its antioxidant and protein restoring properties[25,26]. Treatment with the various extracts of *Phyllanthus devilis* resulted in improvement in the altered biochemical parameters of rats treated with paracetamol. Ethanol extract of *Phyllanthus devilis* significantly ($p < 0.05$) prevented the elevation in the levels of AST and ALT at 100 and 200 mg/kg dose levels. However, ALP and TB levels were found significantly lower only at higher dose levels (200 mg/kg) of the extracts. The results were found to be comparable with the standard drug silymarin. It is a well-known fact that phenolics and flavonoids are reputed to have good antioxidant properties[27] at the same time plants with antioxidant properties play a very key role in liver protection.

Table 3: Effect of different extracts of Phyllanthus devilis on rat serum parameters after paracetamol administration

Group	AST (U/l)	ALT (U/l)	ALP (U/l)	TB (mg/dl)	DB (mg/dl)	Total Protein (g/dl)
Normal Control	185.3 ± 19.8	51.43 ± 3.86	201.8 ± 6.30	0.12 ± 0.02	0.06 ± 0.01	7.20 ± 0.07
PCM Control	1162.0 ± 125.10 ^{a}	304.3 ± 4.94^{a}	315.9 ± 18.18^{a}	0.22 ± 0.02^{a}	0.20 ± 0.03^{a}	5.52 ± 0.04
Silymarin	279.3 ± 30.42^{b}	70.88 ± 4.36^{b}	209.8 ± 9.51^{b}	0.10 ±0.00^{b}	0.06 ± 0.01^{b}	7.32 ± 0.14^{b}
PCM + CHL 100	345.50 ± 102.6 ^{b}	108.1 ± 40.72 ^{b}	243.7 ± 1.96^{b}	0.20 ± 0.00	0.09 ± 0.01^{b}	7.84 ± 0.10 ^{b}
PCM + CHL 200	302.6 ± 70.73 ^{b}	87.58 ±14.51 ^{b}	232.4 ± 16.68^{b}	0.16 ±0.02	0.12 ± 0.02^{b}	7.47 ± 0.15 ^{b}
PCM + EE 100	170.1 ± 22.37 ^{b}	56.75 ± 4.14 ^{b}	281.7 ± 16.09	0.14 ± 0.02	0.10 ± 0.03^{b}	7.58 ± 0.17 ^{b}
PCM + EE 200	145.3 ± 8.39 ^{b}	60.22 ± 8.04 ^{b}	231.3 ± 13.16^{b}	0.13 ± 0.02^{b}	0.08 ± 0.01^{b}	7.83 ± 0.12 ^{b}
PCM + AQ 100	298.7 ± 76.75 ^{b}	151.8 ± 28.96 ^{b}	239.3 ± 16.24^{b}	0.15 ± 0.02	0.09 ± 0.02^{b}	7.72 ± 0.15 ^{b}
PCM + AQ 200	275.87 ± 7.81 ^{b}	59.87 ± 2.34 ^{b}	244.2 ± 16.51^{b}	0.12 ± 0.02^{b}	0.10 ± 0.04^{b}	7.82 ± 0.15 ^{b}

PCM: Paracetamol, CHL: Chloroform extract, EE: Ethanol extract, AQ: Aqueous extract

Values are expressed as Mean ± SEM, n = 6, PCM = paracetamol, CHL = chloroform extract, EE = Ethanol extract, AQ = aqueous extract, a $p < 0.05$ when compared to normal control, b $p < 0.05$ when compared to paracetamol control.



IV. CONCLUSION

The results of serum biochemical markers studies in the crude extract pre- and post-treated group support the hepatoprotective effect and provide evidence for the traditional use of *Phyllanthus devilis* for the treatment of liver disorders. The larger doses of both the crude extract produced a remarkable hepatoprotective activity, which was comparable to silymarin. These suggest that synergy created between the antioxidant activity and intrinsic protective effects of the plant extract underlie attenuation of paracetamol-induced liver injury. In this model, extracts of the selected medicinal plant showed significant hepatoprotection, especially by ethanol extract, possibly because of the higher phenolics and flavonoids content. These polyphenolic compounds are well reputed for their diverse pharmacological activities including hepatoprotective activity. Finally, we conclude that the present study results demonstrate that the plant Phyllanthus devilis, selected based on its traditional and ethnomedical claim, possesses potent hepatoprotective activity. Further studies are needed to reveal the possible mechanism of action.

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