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Research Article

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF THE AERIAL PARTS OF *PHYLLANTHUS RETICULATES* AGAINST PARACETAMOL INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

Objective: The present study deals with evaluation of hepatoprotective effect against paracetamol induced hepatitis in rats and in-vitro antioxidant activity of aerial parts of ethanolic extract (70%) of Phyllanthus reticulates.

Methods: Alteration in the levels of biochemical markers of hepatic damage like AST, ALT, ALP and total Bilirubin were tested in both treated and untreated groups.

Results: Treatment with ethanolic extract of Phyllanthus reticulates (400 mg/kg) has brought back the altered levels of biochemical markers to the near normal level which is comparable with the standard drug Silymarin.

Conclusion: Further the in-vitro antioxidant activity and total phenolic content showed significant result which in turn encourages further evaluation of this plant in future.

Keywords: *Phyllanthus*, Hepatitis, Biochemical markers, Antioxidant activity, Total phenolic content.

INTRODUCTION

Phyllanthus reticulates (Euphorbiaceae) is dense deciduous shrub or small tree, usually 1-5 m high, or a small twiggy tree that grows up to 8 m in height [1,2]. The plant is found throughout tropical India, Ceylon, tropical Africa, China, and Malay Islands. Traditionally, *P. reticulates* is uses as diuretic and cooling agent; to treat inflammation, diarrhea, sore in eyes and sores, burns, suppurations, and chafing of the skin [3,4]. It is widely used in India for treatment hepatitis [5].

Liver is one of the largest organs in human body and the chief site for metabolism and excretion. It has a surprising role in the maintenance, performance, and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision, and reproduction [6]. Liver performs many important physiological functions such as carbohydrate, protein and fat metabolism, detoxification, secretion of bile, and storage of vitamin. Thus, liver health is a crucial factor for overall health of the body. Exposure to environmental toxins, generation of free radical reactions inside the system, poor food habits, alcohol abuse and prescribed, and over-the-counter drug, etc., can eventually lead to various liver ailments like hepatitis, cirrhosis, and fatty liver disease [7,8]. At present, liver diseases are one of the most serious ailments posing a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and there is not much drug available for the treatment of liver disorders [9,10]. It is chiefly the plant based preparations are employed for the treatment of liver disorder. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations [11-13]. However, most of these plants or formulations have not been scientifically tested for safety and efficacy. In present, a research trend has been generated to evaluate folk remedies from plant origin for their potential hepatoprotective activity and antioxidant potential [14].

In the present study, we have evaluated the hepatoprotective activity of this plant against paracetamol overdose - induced hepatotoxicity in rats and *in-vitro* antioxidant activity to prove its folklore claim.

METHODS

Plant material

The aerial parts of the young maturated plants were collected from the rural belt of Salipur, Odisha, India during September 2009. The plant materials were authenticated by the Taxonomist, Regional Plant Resource Centre, Bhubaneswar, India. A voucher specimen has been kept in our research laboratory for future reference. The plant material was washed under running tap water, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain a coarse powder. The powder was extracted with 70% ethanol in distilled water using soxhlet apparatus. The solvent was removed under reduced pressure and kept in a desiccator for future use.

Animals

Male adult male Wistar rats (150-180 g) were housed in standard environmental conditions and fed with standard rodent diet and water ad libitum. All experiments were carried out according to the guidelines of Institutional Animal Ethics Committee (Regd. No-1053/ac/07/ Committee for the Purpose of Control and Supervision of Experiments on Animals) of Institute of Pharmacy and Technoloy, Salipur.

Preliminary phytochemical screening

The extracts of *P. reticulates* were subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids, etc., [15,16].

Evaluation of hepatoprotective activity

In-vivo hepatoprotective activity was evaluated on the basis of the model described previously [17,18] was employed with some modification [19]. Group I served as control and fed orally with normal

saline 5 ml/kg daily for 7 days and Group II - serve as toxic control group and received distilled water (10 ml/kg) daily for 7 days and then received paracetamol (1 g/kg, p.o.) diluted with sucrose solution (40% w/v) on day 7, 30 minutes after administration of distilled water. Group III was treated with ethanolic extract 400 mg/kg/day orally for 7 days, while Group IV was fed silymarin 25 mg/kg as standard daily for 7 days. Paracetamol was administered to the animal Group of III and IV in a single dose of 1 g/kg p.o. diluted with sucrose solution as described previously. 24 hrs after administration of paracetamol, the rats were sacrificed on day 8th day under light ether anesthesia. The blood was collected from all groups by cardiac puncture and serum was separated by centrifugation at 3500 rpm (Eppendorf 5403) at 4°C for 15 minutes and analyzes for various biochemical parameters. The serum parameters for liver function test such as aspartate aminotransferase (AST) [20], alanine aminotransferase (ALT) [21], alkaline phosphate (ALP) [22], and total bilirubin were estimated [23]. For histopathological studies, sections of liver of each rat were immersed immediately in 250 ml of neutralized 10% (v/v) formalin. The tissues were kept in the fixative for 12 hrs, dehydrated with serial ethanol cycles (70% to absolute), and then embedded in paraffin. The paraffin-embedded tissue was cut into 5 µm sections. The tissue sections were deparaffinized and stained with hematoxylin-eosin. Microscopic examinations were done at the magnification of ×400 [24].

Evaluation of antioxidant activities

Determination of total phenolic content

Total phenolic contents in the extract were determined by the modified Folin–Ciocalteu method [25]. An aliquot extracts of were mixed with 5 ml Folin–Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vertexed for 15 seconds and allowed to stand for 30 minutes at 40°C color development. Absorbance was measured at 765 nm using the Jasco ultraviolet-visible spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1 mg/ml. Total phenolic content were expressed as mg/g gallic acid equivalent using the following equation based on the calibration curve: y = 0.0672x − 0.0318, R^2 = 0.998, where x was the absorbance and y was the gallic acid equivalent (mg/g).

1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

The free radical scavenging activity was estimated using the method of Liyana-Pathirana and Shahidi [26]. A solution of DPPH (0.135 mM) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of varying concentration of the plant extract. The reaction mixture was vortex thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm using ascorbic acid as references. The ability to scavenge DPPH radical was calculated as (Table 1).

$$(\%) \text{ of DPPH scavenging activity} = ([Abs_{control} - Abs_{sample}]/[Abs_{control}]) \times 100$$

Where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract/standard.

Statistical calculations

The data are represented as mean ± standard error of mean. Student's t-test is used for statistical analysis and $P < 0.05$ is considered significant.

Table 1: Antioxidant activities of hydroalcoholic extract of *P. reticulatus*

Treatment	Total phenolic content	EC50 DPPH scavenging activity (µg/ml)
Hydro alcoholic extract	948.33±11.36	34.12±9.31
Standard	-	40.8 (ascobic acid)

P. reticulates: Phyllanthus reticulatus, DPPH: 1,1-diphenyl-2-picrylhydrazyl

RESULTS

The preliminary phytochemical screening of the ethanolic extract reveals the presence of phytoconstituents is as follows: Steroids, lipids, terpenoids, flavonoids, saponins, etc.

The results of acute oral toxicity studies in rats indicated that no visible toxic effect up to 2000 mg/kg for the ethanol extract [27]. The effect of ethanolic extract of *P. reticulates* on ALT, AST, ALP and total bilirubin were observed with the administration of the paracetamol at a dose of (1 g/kg), which was depicted in the Table 2. There was a significant ($p < 0.001$) increase in serum ALT, AST, ALP, and total bilirubin in paracetamol intoxicated group compared to the normal control group. Ethanolic extract of *P. reticulates* at the dose of 400 mg/kg orally significantly decreased the elevated serum marker enzymes to almost normal level. The extract also reduced the level of bilirubin compared to the untreated group.

The histological appearance of the hepatocyte reflects their damage conditions [28]. Normal histological appearance was observed in untreated control Group I (Fig. 1a) whereas the hepatocytes of rat treated with a single dose of paracetamol (Group II), showed centrilobular necrosis and extensive fatty change was observed on the midzonal or entire lobe at 24 hrs after treatment (Fig. 1b). Histological changes in the liver of rats treated with 400 mg/kg of ethanolic extract and paracetamol showed a significant recovery except cytoplasmic vascular degenerations around portal tracts, mild inflammation, and foci of lobular inflammation (Fig. 1c). However, liver tissues of rats treated with paracetamol and silymarin showed no necrosis or fatty deposition but had only minimal portal inflammation (Fig. 1d) reflecting good protection of the known hepatoprotective drug silymarin.

A significant increase in inhibition of DPPH radical due to the scavenging ability of the ethanolic extract which can be compared to that of standard Ascorbic acid was observed (Table 2). The extract was also found to have high phenolic content as per Folin–Ciocalteu test.

DISCUSSION

Paracetamol (acetaminophen) is a widely used antipyretic and analgesic, produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in the liver to excretable glucuronide

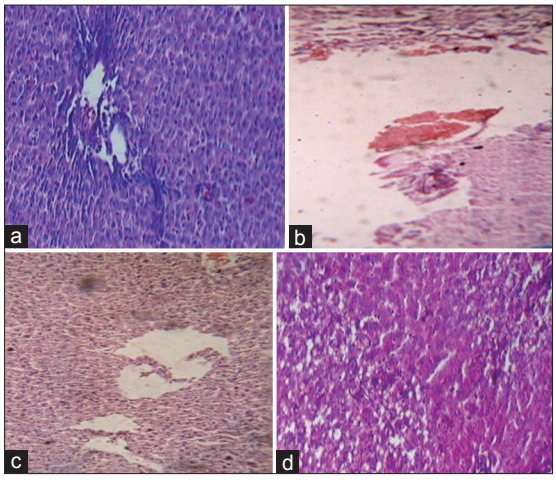

Fig 1: (a) Normal cells, (b) liver cells of rats treated with paracetamol showed centrilobular necrosis and extensive fatty change was observed on the midzonal or entire lobe at 24 hrs after treatment, (c) liver cells of rats treated with paracetamol and ethanolic extract showed a significant recognization, (d) liver cells of rats treated with paracetamol and silymarin showed no necrosis or fatty deposition but had only minimal portal inflammation

Table 2: Effect of ethanolic extract of *P. reticulates* on some biochemical parameters of paracetamol intoxicated rats

Parameters	Normal control	Paracetamol control	EATE (400 mg/kg)	Silymarin (25 mg/kg)
AST (U/L)	32.7±4.3	148.3±8.8[a]	58.7±6.2***	45.3±5.7***
ALT (U/L)	13.2±1.2	70.5±5.9[a]	24.5±2.1**	17.08±1.8**
ALP (U/L)	133.21±3.82	210.67±4.04[a]	157.22±2.58***	163±10.9***
Bilirubin (mg/dl)	0.10±0.04	0.44±0.07[a]	0.22±0.05*	0.14±0.04*

Values are mean±SEM, (n=6), [a]p<0.01: Paracetamol control group compared with normal control group, *p<0.01: Experimental groups compared with paracetamol control group, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphate, SEM: Standard error of mean, *P. reticulates: Phyllanthus reticulatus*

and sulfate conjugates [29]. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450, to a highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or sulfhydryl group of protein and alters the homeostasis of calcium after depleting GSH [30].

In assessment of liver damage by paracetamol hepatotoxin, the determination of enzyme levels ALT and AST is more specific to the liver and a better parameter for detecting liver damage. Necrosis and liver damage release the enzyme in to circulation; therefore it can be measured in serum. High levels of serum ALT indicate liver damage, due to viral hepatitis as well as cardiac infarction and muscle injury. AST catalyzes the conversion of alanine to pyruvate and glutamate and is released in similar manner. Therefore, AST is more specific to liver and a better parameter for detecting liver injury [31].

Serum ALP and bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis, in the presence of increasing biliary pressure [32,33]. The site-specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis. Hyperalbunemia is most frequent in the presence of advanced chronic liver diseases. Hence, decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the generation process and the production of liver cells. Treatment with paracetamol increases the levels of total lipids, total triacylglycerols, and total cholesterol in liver [34].

The widest spread secondary metabolite polyphenols in plant kingdom have received much attention as a potential natural antioxidant in term of their ability to act as both radical scavenger and hepatoprotective [35]. The DPPH assay is based on the inhibition of the absorbance of the DPPH radical cation.

Phytoconstituents such as flavonoids, terpenoids, and steroids, etc., have received considerable attention in recent years due to their diverse pharmacological properties including hepatoprotective and antioxidant activity [23]. There has been growing interest in the analysis of certain flavonoids, terpenoids, and steroids stimulated by intense research into their potential benefits to human health. Presence of high polyphenols contents in *P. reticulates* reported previously may act as effective DPPH scavenger to be responsible for the observed hepatoprotective activity of this plant *P. reticulates.*

CONCLUSION

Ethanolic extract of the plant *P. reticulates* showed a protective effect against paracetamol-induced hepatotoxicity which may attribute to its antioxidant potential and phenolic content.

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