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PROTECTIVE EFFECTS OF *PHYLLANTHUS POLYPHYLLUS* EXTRACT ON CARBON TETRACHLORIDE - INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The study was aimed to investigate the hepatoprotective potential of *Phyllanthus polyphyllus* (whole plant) leaf methanol extract (PPLME) on CCl_4-induced hepatotoxicity in male Wistar rats. CCl_4 injection induced liver damage by a significant rise in serum marker enzymes. The hepatoprotection was assessed in terms reduction in histological damage, changes in serum enzymes (SGOT, SGPT, ALP) and metabolites bilirubin (BL). Pre-treatment of rats with different doses of plant extract (200, 300 and 400 mg/kg) significantly lowered SGOT, SGPT, ALP and BL levels against CCl_4-induced rats. Histopathological examinations showed extensive liver injuries, characterized by extensive hepatocellular degeneration/necrosis, inflammatory cell infiltration, congestion, and sinusoidal dilatation. Oral administration of the leaf extract at a dose of 200, 300 and 400 mg/kg body weight significantly reduced the toxic effects of CCl_4. The activity of leaf extract at the dose of 300 and 400 mg/kg was comparable to the standard drug, silymarin. Based on these results, it was observed that *P. polyphyllus* extract protects liver from hepatotoxicity induced by CCl_4 and thus helps in evaluation of traditional claim on this plant.

Keywords: ethnomedicine, *Phyllanthus polyphyllus,* hepatoprotective activity, carbon tetrachloride, silymarin

INTRODUCTION

Phyllanthus polyphyllus Willd. (Euphorbiaceae) is a small tree widely distributed in tropical and subtropical regions in India and Sri Lanka (Gamble, 1935). *P. polyphyllus* is one of the predominant elements found in abundance in the semiarid belts of Eastern and Western Ghats of South India. It used for the treatment of jaundice and locally called Keelkainelli (Viswanathan et al., 2005). A plant paste (15g) is consumed either with cow milk or Coconut milk once a day for 2 to 3 days for jaundice. The patient should take a bath daily. Consumption of salt, Pepper, fish and dry fish is prohibited. It is also used as an anti-inflammatory drug in folk medicine (Rao *et al*., 2006). The traditional healers (Nattu vaidhyas) of Kolli hills claim this plant to have certain important medicinal properties i.e., a potential antidiabetic and antidepressant drug.

Increase in scientific investigations into indigenous wealth of herbal medicines gives ample evidence of medicinal plants as sources of drugs (Bannerman, 1980). Liver damage in animal models can be induced by CCl_4, from which free radical derivatives are biotransformed and lead to increasing lipid peroxidation as well as cell death. Therefore, a large amount of transaminases leakage in the blood can be detected, which is often associated with hepatonecrosis. Herein, we report the hepatoprotective properties of the leaves of *P. polyphyllus* on CCl_4 - induced liver damage in Wistar rats.

MATERIALS AND METHODS

Plant Material

Phyllanthus polyphyllus Willd. was collected from the Kolli hills (Eastern Ghats) of Namakkal district of Tamil Nadu, India, during the month of March 2003, identified and authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre (PARC), Tambaram - 600 045, Tamil Nadu, India. The voucher specimen (XCH 24909) has been deposited in the herbarium of St. Xavier's College Herbarium (XCH) of the St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India for reference.

Extraction

The shade-dried and powdered whole plant (2 kg) was extracted with methanol using a Soxhlet apparatus. The extract was filtered through Whatman No. 1 filter paper, concentrated on a water bath and obtained syrupy mass weighing 35.47 g after removing the last traces of solvents *in vacuo*.

Preliminary Phytochemical Screening

The methanol extract was tested for preliminary phytochemical screening (Brindha *et al*., 1982).

Animals

Healthy Male Wistar rats (150-175 g) were procured from Tamil Nadu Veterinary College, Chennai, India. They were kept in the Departmental Animal House, Department of Pharmacy, Vel's College of Pharmacy, Pallavaram, Chennai- 600 117, at 26±2 °C and relative humidity 44 – 55%, 10-h light: 14-h dark cycles for one week before the experiment. Animals were provided with rodent diet (Lipton India Ltd., Bombay) and water *ad libitum.*

Drugs and Chemicals

All the drugs and chemicals were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). All solvents were of analytical grade and were obtained from S.D. Fine Chemicals, Mumbai, India.

Evaluation of Hepatoprotective Activity

Animals were divided into six groups of six animals in each group. Normal control group I animals were administered a single dose of liquid paraffin daily (1 ml/kg body weight, p.o.). Negative control group II received carbon tetrachloride (1.25 ml/kg body weight, i.p.). Dosage of carbon tetrachloride was administered as 30% solution in liquid paraffin for every 72 h. Positive control group III received silymarin at a dose of 100 mg/kg, p.o., along with carbon tetrachloride. Test groups IV, V and VI were administered orally 100, 200 and 300 mg/kg body weight of methanol extracts along with carbon tetrachloride respectively, in the form of aqueous suspension once a day. On 16th day, blood samples (48 h after the last injection) were collected, allowed to clot and serum were separated and analysed for various biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) Reitman and Frankel (1957), alkaline phosphatase (ALP) Kind and King (1954) and total bilirubin Mallay and Evelyn (1937).

Histopathoglogical Examination

On 16th day, after withdrawal of the blood, the animals were sacrificed and liver were dissected out from the animals and washed separately with normal saline for examined grossly and weighed, processed for dehydration, infiltration and embedding. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. They were infiltered and embedded with

paraffin. The microtome sections were taken at 5 µ thickness, processed in alcohol–xylene series, stained with alum haematoxylene and eosin and examined under microscope for the evaluation of histopathological changes.

Statistical Analysis

The values were expressed as mean ± SEM. Data were analysed by one way analysis of variance (ANOVA) followed by Dunnett test. $P < 0.05$ value were considered to be significant.

RESULTS AND DISCUSSION

The level of various biochemical enzymes and liver weight in normal, CCl_4 control and treated groups were represented in Table 1 & 2. The activities of SGOT, SGPT, ALP and BL were significantly increased in CCl_4 control compared to normal control. The levels of the above enzymes were significantly reversed on treatment with PPLME in a dose-dependent manner. The activity of the leaf extract at the dose of 300 and 400 mg/kg was comparable to that of the reference drug silymarin. The liver protection (Table 1) in terms of % protection the methanol extract treated group with reference to SGOT was 62.14%, 73.79% and 80.58% for 200, 300 and 400mg dose of extract per kg body weight, respectively. The percent protection for SGPT was 75.37%, 85.96% and 89.14%, ALP was 67.71%, 84.68% and 87.43% and BL was 83.57%, 97.14% and 101.43%.

The histopathological studies of the liver showed (Fig. 1) fatty changes, swelling and necrosis with loss of hepatocytes in CCl_4 control rats in comparison with normal control. The treated groups of PPME showed regeneration of hepatocytes, normalization of fatty changes and necrosis of the liver. The silymarin treated group showed almost normalization of fatty accumulation and necrosis. The maximum protection against hepatic damage was achieved with the PPLME at dose of 400 mg/kg.

Carbon tetrachloride is well-known hepatotoxic agent in liver diseases. The biochemical mechanism of CCl_4 toxicity is based on mitochondrial damage that leads to an accumulation of fat within 60 min, damage of endoplasmic reticulum within 30 min (Christie and Judah, 1954) and damage of lysosomes leading to the death of hepatocytes (Judah, 1969). Liver microsomal oxidizing systems are connected with cytochrome P_{450} system in the endoplasmic reticulum producing trichloromethyl free radicals (CCl_3^{-})

which then bind covalently to neighboring proteins and lipids in the presence of oxygen to form a trichloromethyl peroxyl radicals, which initiate lipid peroxidation, and finally result in the death of cells (De Groot and Noll, 1986; Clawson, 1989; Reckengel et al., 1989). *Phyllanthus* species reported to have potent hepatoprotecive property inhibiting various hepatotoxins (Gulati *et al.* 1995; Prakash *et al.* 1995; Unander *et al.* 1995; Asha *et al.* 2004; Harish and Shivanandappa, 2006; and Pramyothin *et al.* 2006). In the present investigation, the rats treated with an overdose of CCl_4 developed significant hepatic damage, which was observed through a substantial increase of serum enzymes. Treatment of the rats with PPLME at 200, 300 and 400mg/kg for 15 days resulted in a significant protection of CCl_4-induced by the elevation of serum marker enzymes. The hepatoprotective effect of PPLME against CCl_4-hepatotoxicity is attributed to the presence of flavonoids, triterpenoids and steroids which are known to be hepatoprotective and antioxidants (Banskota et al., 2000; DeFeudis et al., 2003) that act as free radical scavengers for the lipoperoxidants. However, the preliminary phytochemical studies reveal the presence of these secondary metabolites in PPLME. Hence, the possible mechanism of hepatoprotective effect of PPLME may be due to its flavonoid content. On the basis of the study, the present results conclude that of PPLME exhibited significant hepatoprotective activity on dose dependent manner. Further studies are needed to isolate the active principle of *P. polyphyllus* and establish chemical nature, which are responsible for their hepatoprotective properties.

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Table 1: Effect of PPLME on biochemical parameters in CCl_4 - induced rats

Treatments	SGPT (U/L)	% protection	SGOT (U/L)	% protection	ALP (U/L)	% protection	BL (mg%)	% protection
Normal control	138.50		45.00		176.60		0.70	
CCl_4 (1.25 ml/kg, b.w.)	327.30		148.00		486.00		2.10	
PPME (200 mg)+CCl_4	185.00^c	75.37	84.00^b	62.14	276.50^c	67.71	0.93^c	83.57
PPME (300 mg)+CCl_4	165.00^c	85.96	72.00^c	73.79	224.00^c	84.68	0.74^c	97.14
PPME (400 mg)+CCl_4	159.00^c	89.14	65.00^c	80.58	215.50^c	87.43	0.68^c	101.43
Silymarin (50 mg)+CCl_4	178.00^c	79.08	59.00^c	86.41	198.00^c	93.08	0.80^c	92.86

The values represent the mean ± S. E. M. for six rats per group. $^a p<0.05$; $^b p<0.05$; $^c p<0.05$ with respect to disease control (One way ANOVA followed by Dunnet *t*-test)

Table 2: Effect of PPLME on liver weight variation in CCl_4-induced rats

Treatments (mg/kg)	Liver wt/100 g body weight
Normal control	3.4±0.10
CCl_4 (1.25 ml/kg, b.w.)	6.8±0.28
PPME (200 mg)+CCl_4	4.9±0.05^b
PPME (300 mg)+CCl_4	4.5±0.04^c
PPME (400 mg)+CCl_4	4.1±0.06^c
Silymarin (50 mg)+CCl_4	3.8±0.26^c