HEPATOPROTECTIVE ACTIVITY OF *PHYLLANTHUS RETICULATUS*

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ABSTRACT

Two partially purified organic fractions designated by PR1 and PR2 of the fat free ethanol (95%) extract of aerial parts of *Phyllanthus reticulatus* were tested for the hepatoprotective activity in rats against CCl_4-induced liver damage. The rats receiving the fractions showed promising hepatoprotective activity as evident from significant changes of pentobarbital-induced sleeping time, changes in serum levels of sGPT, sGOT, sALP and bilirubin and also from histopathological changes as compared to CCl_4-intoxicated rats.

Keywords: *Phyllanthus reticulatus,* organic fractions, hepatoprotective activity, CCl_4-induced liver damage.

INTRODUCTION

Phyllanthus reticulatus (Bengali name – Panjuli; Family-Euphorbiaceae) is a climbing shrub which grows all over Banlgadesh (Kirtiker *et al*, 1983 and Ghani, 2003). The biological work performed so far on this plant showed hypotensive effects and its folkloric use in gastric complaints including colic, constipation etc. and chemical studies demonstrated the presence of octacosanol, teraxerol acetate, friedeline, teraxerone, betulin, sitosterol etc. (Rav *et al.,* 1964 and Joshi *et al.*, 1991). Although *Phyllanthus reticulatus* has not been studied much for significant chemical as well as biological studies, the plants of this genus were reported to contain lignans, flavonoids, triterpenoids, alkaloids, polyphenolic compounds (Anjenenlu *et al.,* 1973 and Yoshida *et al.,* 1982). These compounds have been shown to possess significant activity against hepatitis B virus responsible for hepatotoxicity causing fatal liver diseases, like liver chirrhosis and hepatocellular carcinoma (Gneti *et al*, 1995 and Kiso *et al*, 1983). The remarkable efficacy was reported in case of *Phyllanthus niruri, Phyllanthus emblica,* and *Phyllanthus urinaria.*

The plant was selected for thorough chemical and biological studies and the present study reports the results of hepatoptotective activity of the two partially purified fractions obtained from the ethanolic extract of the aerial parts of the plant in rat model using functional, biochemical and histopathological parameters.

MATERIALS AND METHODS

Plant material

The aerial parts of *Phyllanthus reticulatus* were collected from Gazipur, Dhaka and was taxonomically identified with the help of Bangladesh National Herbarium.

Extraction and fractionation

Successive hot extraction of the air dried ground powder (500 mg) with petroleum-ether (60°-80°), ethyl acetate and finally with ethanol (95%) were performed for 24 hours. The extract, in all these cases were filtered off and evaporated to dryness in *vacuo*, to get a concentrated gummy mass. The ethanol extract was subjected to vacuum liquid chromatography (VLC) for fractionation over silica gel 60H (VLC grade) using solvents of increasing polarity (first with 100% toluene, then ethyl acetate in various proportions with toluene, then methanol with ethyl acetate in various proportions and finally with 100% methanol). Twenty fractions numbering 1-20 were collected in separate beakers by elution. All of these fractions were tested by TLC. The fractions showing similarity in R_f values of the components and color reaction with vanillin-sulphuric acid spray reagent were bulked together. In this way two partially purified fractions designated as PR1 and PR2 were obtained. The PR1 contained five components (R_f values: 0.83, 0.74, 0.69, 0.55, 0.53) and PR2 contained four components (R_f values: 0.74, 0.69, 0.58, 0.35).

Test for hepatoprotective activity

The organic fractions PR1 and PR2 were investigated for hepatoprotective activity in adult male long Evans rats. The rats were weighed (120-140g) and divided into 4 groups containing 6 rats in each group on the basis of the similar average weight of the rats. These four groups were termed as control, CCl_4-treated, CCl_4+PR1-treated and CCl_4+PR2-treated groups. Aqueous solutions of PR1 and PR2 were administered orally (at the dose level of 200 mg/kg of body weight) by a stomach tube (made of rubber) given once daily for 15 days. Tween 80 was used to dissolve the fraction in water, as the fractions were not freely water soluble. CCl_4 was given intraperitoneally (at the dose level of 0.6 ml/kg of body weight) thrice a week for 2 weeks by a sterile disposable syringe.

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Table 1: *In vivo* effects of PR1 and PR2 on pentobarbital-induced sleeping time in CCl_4-treated rats.

Animal group	Sleeping time (in minute) Mean ± SD
Control group	55.0 ± 10.73 (n=6)
CCl_4-treated group	181.8 ± 20.47* t = 12.52 (n=6)
CCl_4+PR1-treated group	147.2 ± 9.14** t = 3.45 (n=6)
CCl_4+PR2-treated group	150.0 ± 16.5*** t = 2.71 (n=6)

* P<0.001, ** P<0.01, *** P<0.02

Table 2: *In vivo* effects of PR1 and PR2 on CCl_4-induced changes in biochemical parameters in rats.

Biochemical parameter	Control	CCl_4-treated rat		CCl_4+PR1-treated rat		CCl_4+PR2-treated rat	
	$M_1 \pm SD_1$	$M_2 \pm SD_2$	% change & 't' value	$M_2 \pm SD_2$	% change & 't' value	$M_2 \pm SD_2$	% change & 't' value
sGPT (IU/L)	31.5 ± 4.42	52.2 ± 4.39	+ 65.71* t = 7.76	42.8 ± 4.32	-18.01*** t = 3.41	40.4 ± 3.50	-22.60** t = 4.67
sGOT (IU/L)	30.16 ± 3.25	48.6 ± 3.91	+61.14* t = 8.42	43.6 ± 4.21	-10.28## t = 1.95	40.8 ± 2.77	-16.05*** t = 3.64
sALP (IU/L)	212.83 ± 23.96	373.8 ± 110.51	+75.63* t = 5.18	221.8 ± 68.01	-40.72*** t = 3.06	140.6 ± 7.53	-62.38** t = 4.71
Serum bilirubin (μ mol/L)	0.133 ± 0.09	0.38 ± 0.172	+75.93# t = 2.89	0.184 ± 0.065	-51.58## t = 2.38	0.182 ± 0.088	-52.1## t = 2.19
Serum total protein (g/dL)	6.82 ± 0.57	6.13 ± 0.73	-10.12** t = 6.47	6.178 ± 0.741	+0.78## t = 0.103	6.648 ± 0.469	+8.45*** t = 3.44
Serum Albumin (g/dL)	3.79 ± 0.65	3.38 ± 0.39	-10.82# t = 1.29	3.492 ± 0.733	+3.31** t = 0.301	4.142 ± 0.57	+22.54# t = 2.47
Serum Urea (mg/dL)	23.32 ± 3.99	25.58 ± 3.24	+9.69## t = 1.04	25.162 ± 1.952	-1.63## t = 0.247	23.106 ± 2.938	-9.67## t = 1.372

* P<0.001(S), ** P<0.01(S), *** P<0.02(S), # P<0.05(S), ## P>0.05(NS)
sGPT = Serum glutamate pyruvate transaminase, sGOT = Serum glutamate oxaloacetate transaminase, sALP = Serum alkaline phosphatase, S = Significant, NS = Not significant, P = Probability

Hepatoprotective activity was observed by the following studies:

Measurement of pentobarbital-induced sleeping time
On the eighth day of the experiment sodium pentobarbital (in water for injection) at the dose level of 30 mg/kg was injected intraperitoneally to CCl_4-intoxicated, CCl_4+PR1 and CCl_4+PR2 -treated rats. The onset of sleep and total sleeping time were recorded for all the rats of each group.

Estimation of the biochemical parameters
The serum enzyme levels like, serum glutamate pyruvate transaminase (sGPT), serum glutamate oxaloacetate transaminase (sGOT), serum alkaline phosphatase (sALP) were observed. Moreover, serum bilirubin, serum albumin, serum total protein and serum urea were also observed.

Observation of histopathological changes
The histopathological changes were also noted for evaluation of the hepatoprotective activities.

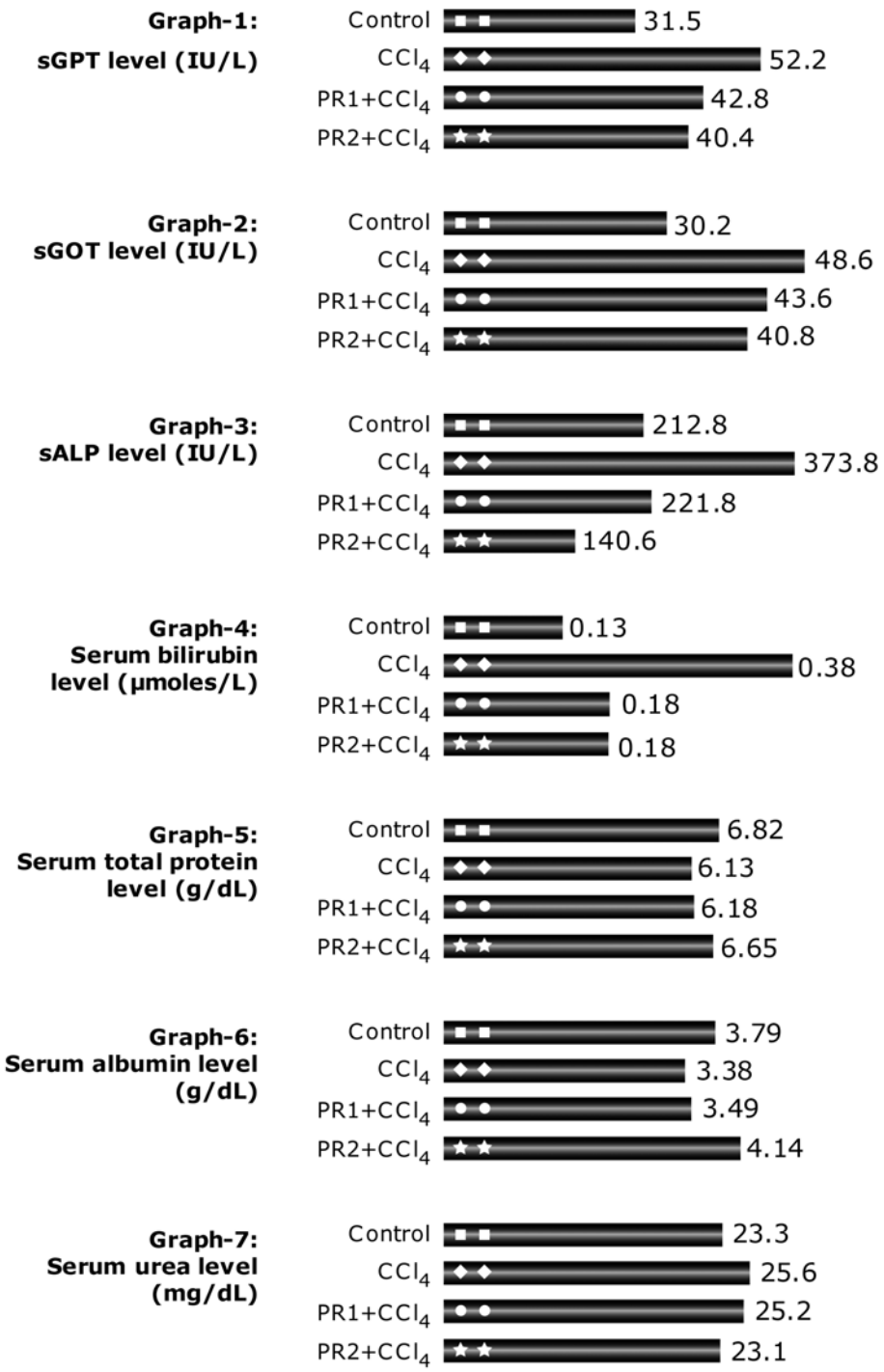
The biochemical parameters for all the groups were estimated by 'enzymatic colorimetric method' at the research division, BIRDEM, Dhaka, using microplate reader. The histopathological studies of the rat livers for all groups were performed in the Department of Histopathology, BSMMU (former IPGMR), Dhaka, Bangladesh, and the micro-photographs of the slides were taken from BCSIR, Dhaka (Hoofnagle *et al*., 1988).

STATISTICAL ANALYSIS

Data were presented as Mean ± SEM (Standard Error of Mean). Student's "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) was applied for the analysis of data. P = 0.05 was taken to be the level of significance.

RESULTS AND DISCUSSION

Measurement of pentobarbital- induced sleeping time
Both the fractions (PR1 and PR2) were found to decrease the sleeping time elevated by CCl_4 due to the decrease of liver functionality (table 1). The action was more prominent in case of PR1 fraction. The decreased sleeping time was due to increased liver function as created by the experimental fractions.



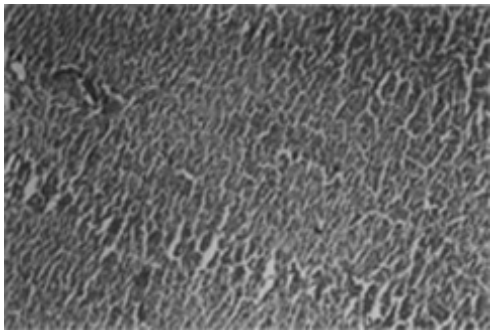
Graph 1-7: Comparison of *in vivo* effects of PR1 and PR2 on CCl_4-induced changes in biochemical parameters in rats.

Measurement of biochemical parameters of blood

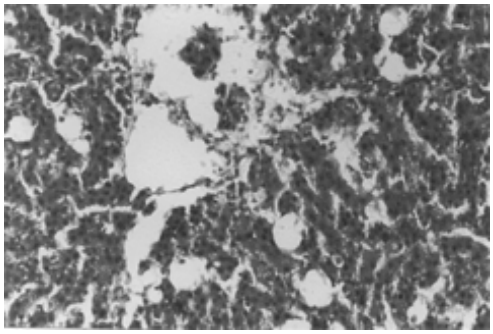
The fractions PR1 and PR2 were found to decrease the levels of serum enzymes like, sGPT, sGOT and sALP in rats with CCl_4-induced liver damage (table 2 and graphs 1-7). The increased serum bilirubin and decreased total protein as induced by the CCl_4 intoxication was reversed nearly to normal values by the experimental fractions. The results indicated that both PR1 and PR2 reversed or prevented the alteration of biochemical parameters which are characteristic to the liver function.

Observation of histopathological changes in the liver

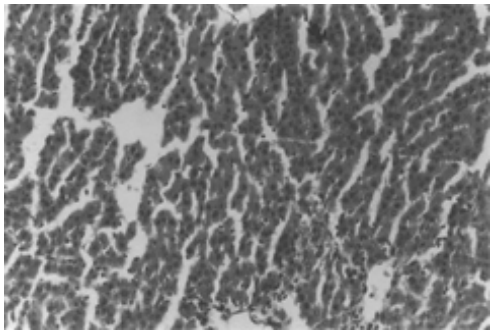
It was found from the histopathological studies that CCl_4-intoxication caused centrilobular hepatocyte necrosis,



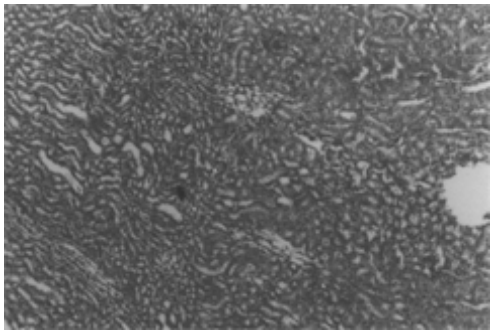
Photograph-1: Photomicrograph of liver tissue of control (normal) rats showing portal triad with normal hepatocytes



Photograph-3: Photomicrograph of rat liver tissue of CCl_4 treated groups showing necrotic cells around the central vein, fatty changes and inflammatory cells



Photograph-2: Photomicrograph of rat liver tissue of CCl_4 and PR1 treated rats showing macrophage infiltration and improvement of histological appearance with less evidence of necrosis.



Photograph-4: Photomicrograph of rat liver tissue of CCl_4 and PR2 treated rats showing regenerated tubular epithelium and healing by fibrosis of the necrotic.

fatty changes, vacuolization and inflammatory changes. Treatment with PR1 and PR2 either reversed or prevented the changes in these histopathological parameters of the liver indicating that the fractions showed remarkable hepatoprotective activity (Photographs 1-4).

The above studies demonstrated that, the fractions PR1 and PR2 possessed significant hepatoprotective activity on CCl_4-induced liver damage in rats.

DISCUSSION

Measurement of pentobarbital-induced sleeping time was used as a monitor for functional integrity of the liver as the drug is completely metabolized by the liver. In our study, intoxication with CCl_4 resulted potentiation of pentobarbital-induced sleeping time, which is evident from the significant differences in sleeping time ($P<0.001$) between the rats of CCl_4-treated group and that of the experimental groups. The increase in pentobarbital-induced sleeping time in CCl_4-intoxicated rats were due to decreased rate of metabolism of pentobarbital which in turn is related to liver dysfunction.

In case of biochemical parameter investigation, significant increases in the serum enzyme levels, like – sGOT, sGPT, sALP etc. have been observed in case of CCl_4-treated rats. Due to the damage of hepatocytes, significant amounts of the enzymes were appeared in the blood.

Serum total protein and albumin were decreased significantly in the CCl_4-intoxicated rats because the chance of plasma protein binding with the components of liver had been minimized. The reverse results of the levels of the serum enzymes and total protein towards normal values indicated the protective activity against damaged liver.

Histopathological changes observed after the treatment of CCl_4-treated rats by the samples reversed hepatic lesions to some extent which indicated the hepatoprotective activity.

CONCLUSION

This study demonstrated the hepatoprotective activity of two semi-purified organic fractions PR1 and PR2 against

CCl_4-induced liver damage which is evident from the changes of functional, biochemical and histopathological parameters. But the prominent hepatoprotective activity was shown by PR2 as compared to PR1. The renoprotective activity study of the fractions is on progress.

REFERENCES

Anjenenlu ASR, Jagonmohon R and Subramanyam, C (1973). Isolation and structural elucidation of three new lignans from the leaves of *Phyllanthus niruri* Linn. *Tetrahedron*, **29**: 1291.

Ghani A (2003). *In:* Medicinal Plants of Bangladesh, Chemical constituents and uses. *Asiatic Society of Bangladesh*, 2nd edition, p.345.

Gneti KR and Agarwal S (1995). Hepatoprotective studies on *Phyllanthus niruri* and quercetin. *J. Exptl. Biol.*, **33**(4): 261-268.

Hoofnagle JH, Peters M, Mulen KD, Jones DB and Rustigi V (1988). Randomized controlled trial of recombinant human α–interferon in patients with chronic hepatitis B. *Gastroenterology.* **95**: 1318-1325.

Joshi KC, Singh P and Mehta A (1991). Crystalline components of the roots of *Phyllanthus reticulatus. J. Ind. Chem. Soc.,* **58**: 102.

Kirtiker KR and Basu BD (1980). *In:* Indian Medicnal Plants, B. Singh and M.P. *Singh Publishers, India,* Vol.1, 2nd edition, p.345.

Kiso Y, Tohkin M and Hikino H (1983). Assay method for antihepatotoxic activity using carbon tetrachloride induced cytotoxicities in primary cultured hepatocytes. *Planta Med.,* **49**: 222-225.

Rav MRR and Siddiqui HH (1964). Screening of Indian plants for Biological activity. *Ind. J. Exp Biol.,* **2**: 49.

Yoshida T, Seno K, Takama Y and Okanda, T (1982). Tannins and related polyphenols of Euphorbiacious plants. *Phytochemistry,* **21**: 1180.