
SHORT COMMUNICATION

**AMELIORATIVE POTENTIAL OF *PHYLLANTHUS AMARUS* AGAINST
CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY**

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Ahmedabad – 380 009, India**Keywords:** biochemical changes, carbon tetrachloride, liver, *Phyllanthus amarus* extract, serum

World is endowed with a rich wealth of medicinal plants. Herbs are becoming popular as they alleviate human sufferings. Nowadays, science is making such strides that new horizons are opening up for phytotherapy. Current research into new drugs is focused on agents that will prevent, slow down and/or halt the progress of the disease process.

The plants of the genus *Phyllanthus* are commonly known as bhuiamlki in India (synonym: *Phyllanthus niruri* L. of Hooker, family Euphorbiaceae), and these were later distributed into three species namely *Phyllanthus amarus* Schum & Thonn, *Phyllanthus fraternus* Webster and *Phyllanthus debilis* Klein ex Wild (1). As a folk medicine, the bhuiamlki have been reported to be a potent remedy for jaundice. It is used to treat dropsy urinogenital problems, dysentery, dyspepsia, colic and fever (2).

Phyllanthus amarus extract has been reported to possess a broad spectrum of biological activities mainly attributed to its antioxidative capacity (3). This plant has been reported to possess hepatoprotective activity against ethanol-induced oxidative stress (4).

Carbon tetrachloride (CCl₄) toxicity is the model commonly used to study liver injury (5). It is being used extensively to screen drugs with hepatoprotective activity on various experimental animals (6). The current investigation was undertaken to study the effect of 50% hydroalcoholic extract of *P. amarus* in reducing hepatotoxicity induced by CCl₄ in mice.

MATERIALS AND METHODS**Chemicals**

Carbon tetrachloride was procured from Merck, Mumbai, India. All other chemicals were obtained from Himedia, Mumbai, India and were of analytical grade.

Preparation of extract

Phyllanthus amarus Schum & Thonn was collected in the month of August and September 2007 from the herbal garden of Gujarat University, Ahmedabad, India. Herbarium specimen was authenticated by National Institute of Science Communication and Information Resources (Ref: NISCAIR/RHMD/consult/-2007-08/938/122); New Delhi, India. The preparation of extract was made according to WHO protocol CG – 04 (7). The aerial parts of the plant were shade dried, powdered and 5 g of plant powder was extracted in a Soxhlet apparatus using 100 mL of 50% aqueous – alcoholic solvent (50% distilled water and 50% ethanol) for 3 h. The extract was twice filtered through Whatman filter paper no. 1. It was then concentrated in water bath at 37°C and dried, with a resulting yield of 8.5%.

Animals

Young inbred, Swiss strain female albino mice (*Mus musculus*), weighing approximately 32 – 35 g, were obtained from Zydus Research Centre, Ahmedabad, India. They were provided normal food pellet (Amrut feeds, Mumbai, India) and water *ad libitum* and maintained under laboratory conditions. The research protocols were reviewed and approved by “The Committee for the Purpose of Control and Supervision of Experiment on Animals” (Reg – 167/1999/CPCSEA), New Delhi, India.

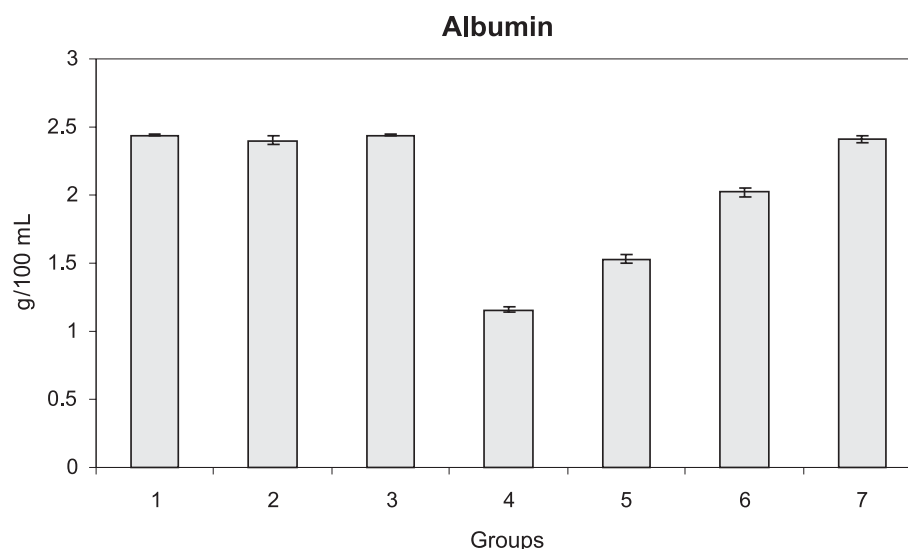
Experimental Design**Antihepatotoxic potential of *Phyllanthus amarus***

Seventy animals were divided into seven groups (n = 10) and caged separately. The experimental protocol is shown in Table 1. Group 1 ani-

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Table 1. Experimental protocol

Groups	Treatments	Number of animals	Duration of treatment (Days)	Day of autopsy
1	Untreated control	10	30	31 st
2	Vehicle contro 1 (0.2 mL olive oil /animal/day)			
3	<i>Phyllanthus amarus</i> control (300 mg/ kg body weight/day) in 0.2 mL of distilled water			
4	Carbon tetrachloride (xenobiotic treated group) 826 mg/kg body weight/day in 0.2 mL of olive oil			
5	CCl ₄ along with 100 mg/kg body weight/day of <i>P. amarus</i> extract dissolved in 0.2 mL of distilled water			
6	CCl ₄ along with 200 mg/kg body weight/day of <i>P. amarus</i> extract dissolved in 0.2 mL of distilled water			
7	CCl ₄ along with 300 mg/kg body weight/day of <i>P. amarus</i> extract dissolved in 0.2 mL of distilled water			

Figure 1. Effect of *P. amarus* on carbon tetrachloride-induced alteration in serum albumin content of mice

mals (untreated control) were given free access to food and drinking water. Group 2 received olive oil (0.2 mL/animal/day). Olive oil was used as a vehicle to dissolve CCl₄. Animals of group 3 were given *Phyllanthus amarus* extract (300 mg/kg body weight/day) in 0.2 mL of distilled water, which served as plant control group. Group 4 received CCl₄ (826 mg/kg body weight/day). The dosage of carbon tetrachloride administered (corresponding to 1/10th of LD₅₀ value of CCl₄) was based on our earlier study (8). Groups 5, 6 and 7 animals were orally treated with CCl₄ along with 100, 200 and 300 mg/kg body weight/day of *Phyllanthus amarus* extract dissolved in 0.2 mL of distilled water for 30 days.

On the 31st day, blood was collected by cardiac puncture and serum was separated by centrifugation at 1000 × g for 10 min. The animals were then sacrificed by cervical dislocation. The liver was dissected out quickly and blotted free of blood. A 10% homogenate of the liver tissue was prepared in 5% TCA for estimation of DNA and RNA, while a 10% homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4) and utilized for the estimation of protein, SDH and ATPase activities.

Biochemical analysis

The estimation of DNA and RNA in the liver was carried out by the method of Giles and Meyer (9) and Schneider (10), respectively. The protein content

Table 2. Effect of *P. amarus* on carbon tetrachloride-induced biochemical changes in liver of mice

Parameters	Untreated control group 1	Vehicle control group 2	<i>P. amarus</i> control group 3	CCl ₄ group 4	CCl ₄ + <i>P. amarus</i> 100 mg	CCl ₄ + <i>PP. amarus</i> 200 mg	CCl ₄ + <i>PP. amarus</i> 300 mg
Deoxyribonucleic acid (µmoles/100 mg tissue weight)	482.61 ± 0.61	483.01 ± 0.16	483.44 ± 0.55	353.90 ± 0.89 ^{a***}	393.21 ± 0.91 ^{b***}	439.8 ± 0.22 ^{b***}	483.48 ± 0.77 ^{b***}
Ribonucleic acid (µmoles/ 100 mg tissue weight)	325.70 ± 0.71	326.90 ± 0.80	326.00 ± 0.72	254.69 ± 0.33 ^{a***}	280.90 ± 0.12 ^{b***}	300.82 ± 0.90 ^{b***}	326.20 ± 0.54 ^{b***}
Protein (mg/100 mg tissue weight)	22.57 ± 0.42	23.01 ± 0.44	22.95 ± 0.17	15.79 ± 0.36 ^{a***}	20.62 ± 0.06 ^{b***}	21.80 ± 0.09 ^{b***}	22.99 ± 0.12 ^{b***}
Succinate dehydrogenase activity(µg formazan formed/ mg protein/15 min)	61.93 ± 0.41	61.09 ± 0.30	61.17 ± 0.44	35.77 ± 0.70 ^{a***}	43.24 ± 0.30 ^{b*}	49.55 ± 0.55 ^{b***}	60.67 ± 0.21 ^{b***}
Adenosine triphosphatase activity (µ moles <i>i.p.</i> released/mg protein/30 min)	0.34 ± 0.05	0.33 ± 0.01	0.34 ± 0.08	0.21 ± 0.07 ^{a***}	0.25 ± 0.08 ^{b**}	0.29 ± 0.09 ^{b***}	0.34 ± 0.10 ^{b***}

Results are expressed as the mean ± SEM., n = 10

No significance difference was noted between groups 1, 2 and 3

^a as compared between vehicle control (group 2) and toxin treated (group 4)

^b as compared between toxin treated (group 4) and toxin + antidote treated (groups 5, 6, 7)

Level of significance * p < 0.05., ** p < 0.01., *** p < 0.001

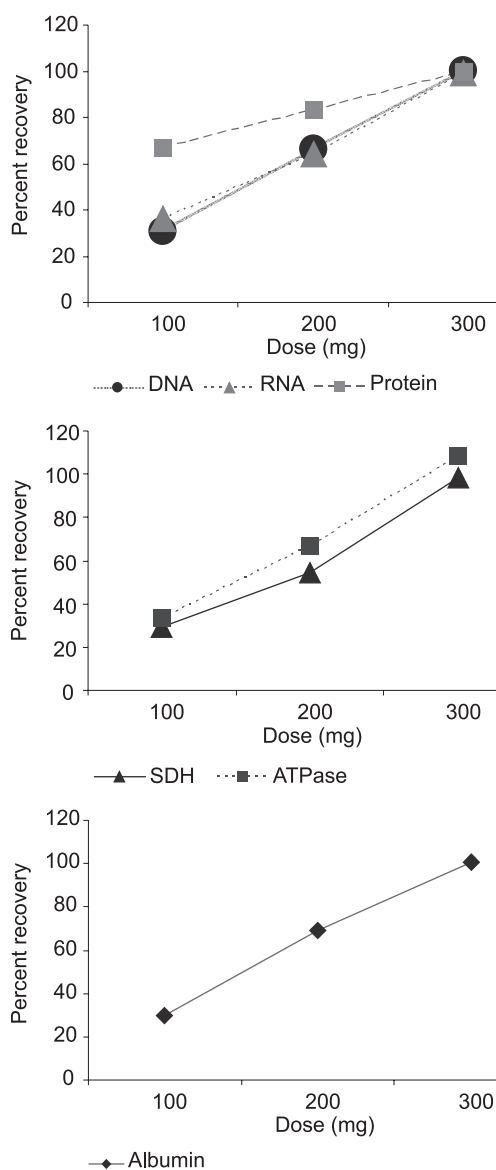


Figure 2. Percent recovery of liver and serum parameters by *Phyllanthus amarus* extract

was measured by the method of Lowry et al. (11) using bovine serum albumin as a standard. The SDH activity and ATPase activity in the liver were measured by the method of Beatty et al. (12) and Quinn and White (13), respectively. Serum albumin was determined using the method of Miyada et al. (14).

The recovery after *Phyllanthus amarus* (*Pa*) treatment in three different doses: 100 (Group 5), 200 (Group 6) and 300 (Group 7) mg/kg body weight/day for 30 days along with CCl_4 , was calculated from the mean value of the respective groups in the form of percent recovery (15) using the formula:

$$\text{Percent recovery} = \frac{\text{Xenobiotic group} - \text{group treated with } Pa}{\text{Xenobiotic group} - \text{control group}} \times 100$$

Statistical analysis

The data were statistically analyzed using SPSS statistical software, version 10. The results were expressed as the means \pm SEM. Hypothesis testing methods included one-way Analysis of Variance (ANOVA) followed by the least significant difference (LSD) multiple comparison test. The level of significance was accepted with * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

RESULTS AND DISCUSSION

The results obtained are presented in Table 2 (liver parameters) and Figure 1 (serum albumin). No significant difference was noted between untreated and vehicle control groups (Group 1 and 2) in all parameters.

Carbon tetrachloride is accumulated in hepatic parenchyma cells and it is metabolically activated by cytochrome P450-dependent monooxygenases to form a trichloromethyl radical ($\text{CCl}_3\cdot$). The $\text{CCl}_3\cdot$ radical reacts with cellular proteins and other macromolecules like DNA and RNA with a simultaneous attack on polyunsaturated fatty acids leading to liver damage (16).

The results obtained indicated that oral administration of CCl_4 (826 mg/kg body weight/day) caused significant reduction ($p < 0.001$) in DNA, RNA and protein contents in liver with a concurrent decrease in the serum albumin concentration. Omar et al. (17) have reported that CCl_4 administration causes disturbances in cellular functioning. They have demonstrated that the xenobiotic diminished the levels of DNA in liver by using Feulgen histochemical staining technique. Voronova et al. (18) have reported alterations in RNA metabolism after CCl_4 administration followed by a decrease in the tissue RNA levels, subsequently changing the nucleo-cytoplasmic ratio in the affected liver tissue. The metabolism of CCl_4 yield products that cause fragmentation of endoplasmic reticulum and disruption of ribosomes into subunits. The capacity of liver microsomes to incorporate amino acids is depressed, causing a generalized inhibition of protein synthesis. These changes result in rapid loss of the ability of liver to synthesize albumin (19) and other proteins causing an overall significant decrease in the protein and serum albumin content. The activities of SDH and ATPase were significantly ($p < 0.001$) reduced after CCl_4 administration in

the liver as compared to the vehicle control group (Group 2). According to Jadon et al. (20), uncoupling of oxidative phosphorylation by CCl_4 causes a fall in the activity of ATPase while reduction in SDH activity may be due to the structural and functional disorganization of the mitochondrial assembly. Thus, when mitochondria are damaged, energy generation in them is inevitably inhibited which contributes to the overall loss in the energy production (21).

The results shown in Table 2 and Figure 1 clearly indicate that oral administration of hydroalcoholic extract of *Phyllanthus amarus* (300 mg/kg body weight) alone did not cause any significant effect in all parameters as compared to vehicle control.

However, oral administration of the *P. amarus* (100, 200 and 300 mg/kg body weight/day) along with CCl_4 for 30 days significantly ($p = 0.001$) increased DNA, RNA and protein content in the liver and albumin in serum as compared to CCl_4 alone treated mice, probably by enhancing the synthesizing function of the liver. Previous studies have shown that *P. amarus* extract increased DNA (22) and RNA (23) contents in the liver tissue.

The CCl_4 provoked significant loss of liver SDH and ATPase enzyme activities were subdued by the administration of *P. amarus* extract for 30 days in a dose-dependent manner, indicating restoration of energy status of the cell and mitochondrial re-organization.

Administration of *Phyllanthus amarus* extract attenuated the toxic effects of CCl_4 and caused a subsequent recovery towards normalization. The recovery process was dose-dependent and has been presented in the form of percent recovery (Figure 2). The recovery was much faster in protein than that of DNA and RNA at lower doses. Administration of *P. amarus* at 300 mg/kg body weight for 30 days offered maximum recovery (98 – 100%) against CCl_4 toxicity.

Thus, from the results obtained we can conclude that hydroalcoholic extract of *Phyllanthus amarus* effectively mitigated the toxic effects of carbon tetrachloride in a dose-dependent manner and offered significant protection to the liver thus proving its antihepatotoxic potential.

Acknowledgment

The financial assistance to Krithika R. from Birla Smarak Kosh in the form of Junior Research Fellowship is acknowledged with thanks.

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Received: 15. 02. 2009