Asian Journal of Chemistry Vol. 21, No. 8 (2009), 5973-5978

Evaluation of Acute and Subacute Toxicity of Alcoholic Extract of Whole Plant of *Phyllanthus rheedii*

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Present studies reports the acute and subacute toxicity of alcoholic (95 %) extract of whole plant of *Phyllanthus rheedii* in Swiss mice and Wister albino rats. The mice were divided into 5 groups of 10 animals and each group received once 100, 500, 1000, 2000 and 3000 mg/kg dose of extract by intra-gastric gavages for 1 d. For the sub acute toxicity, four groups of 6 rats (3 males and 3 females) were received distilled water (control), 125, 250 or 500 mg/kg of extract every 24 h orally for 28 d. The results indicated that the LD_{50} of the extract was about 2588 mg/kg of body weight. No significant variation ($p < 0.05$) in the body and organ weights between the control and the treated group was observed after 28 days of treatment. Hematological analysis and clinical blood chemistry revealed no toxicity effects of the extract. Pathologically, neither gross abnormalities nor histopathological changes were observed. No mortality was recorded in 28 days. *Phyllanthus rheedii* extract was found to be fairly nontoxic.

Key Words: *Phyllanthus rheedii*, Acute toxicity, Subacute toxicity.

INTRODUCTION

Phyllanthus rheedii Linn (Family: Euphorbiaceae) a slender branching erect herb, the calyx-lobes usually white margined, found through out in India. It is used as an oriental folk medicine in diabetes mellitus[1]. Antihyperglycemic, antihyperlipidemic and antioxidant effects of *Phyllanthus rheedii* were evaluated on streptozotocin induced diabetic rats[2]. Despite the popular use of this plant by the rural communities to treat several diseases, present study is aimed to obtain data on the safety of the crude extract. The acute and subacute toxicity of the alcohol (95 %) extract of *P. rheedii* in mice and rats were assessed with the hope that the results would provide information on the safety of the methanolic extract prior to the evaluation of its therapeutic efficacy in humans. In subacute toxicity study the effect on biochemical, hematological and histopathological parameters were investigated.

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EXPERIMENTAL

The whole plant of *Phyllanthus rheedii* were collected near the Yercaud hills, India in the month of October 2004 and were authenticated by the Botanist, Botanical Survey of India, Coimbatore, India. A voucher specimen has been stored and maintained in our laboratory (ET-30). The plants dried in shade and powdered. The powder was extracted with ethanol (95 % v/v) using Soxhlet apparatus. The extract was dried under reduced pressure and stored in a desiccator. The yield of extract was 3.5 % w/w.

Experimental animals: Swiss albino mice (20-25 g) and male Wister rats (150-200 g) were purchased from Perundurai Medical College, Perundurai, Tamilnadu and housed in polypropylene cages at room temperature (22 ± 2 ºC) with proper ventilation. Prior to the experiments, animals were fed with standard diet for 1 week in order to adapt to laboratory conditions. They were fasted but allowed free access to water 16-18 h prior to administration of the test drug. The study was conducted after obtaining clearance from Institutional animal ethical committee (Ph.Chem/3/2005).

Acute toxicity: The bioassays were conducted according to the World Health Organization guideline for the evaluation of the safety and efficiency of herbal medicine[3]. For the study albino mice were divided into six groups of 10 animals. Each group were given single oral doses of 100, 500, 1000, 2000 doses at 3000 mg/kg body weight of the extract. Control received vehicle at the same volume. Observations were made and recorded systemically 1, 2, 4 and 24 h. After substance administration the visual observation included skin changes, morbidity, aggressively, sensitivity of sound and pain, as well as respiratory movement. The number of survivors was noted after 24 h. The LD_{50} was then determined at the end of the experiment based on Miller and Tainter method.

Subacute toxicity: Four groups of 6 rats (3 males and 3 females) received by intra gastric gavages the plant extract at the dose of 125, 250 and 500 mg/kg body weight every 24 h for 28 d and control received vehicle at the same volume. The toxic manifestation such as body weight, mortality, food and water intake was monitored. After 28 d all surviving animals were fasted overnight. Rats were anesthetized with ether on day 28. The heparinized blood samples were collected for determining hematological parameters and the serum from non-heparinized blood was carefully collected for determining clinical blood chemistry. Animals were sacrificed after blood collection the internal organs were weighed to determine relative organs weights and observed for gross lesions. The internal organs were preserved in 10 % buffered formaldehyde solution for histological examination.

Biochemical estimations: Blood collected into non-heparinized tubes were then centrifuged at 3000 rpm for 10 min. The serum separates was analyzed to evaluate the liver enzymes. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed using the method of Reitman *et al.*[4], alkaline phosphatase (ALP) was analyzed by the method of Kind *et al.*[5] and cholesterol by the

method of Hawk *et al.*[6]. Urea and blood urea nitrogen (BUN) was estimated by the method of Webster[7]. Blood glucose was estimated by the method of Sasaki *et al.*[8]. γ-glutamyl transferase (γ-GT) was estimated by the method of Rosalki *et al.*[9].

Hematological assay: Blood sample collected in the heparinized tubes were used to investigate white blood cells (WBC), red blood cell (RBC), hemoglobin (Hb)[10] and clotting time using the visual method[11].

Histopathological study: Histopathological investigation of the organs was done according to the method described by Lamb[12]. The organ pieces (3-5 µm thick) were fixed in 10 % formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an autotechnicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50 °C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

Statistical analysis: The values were expressed as mean ± SEM. Statistical analysis were performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison test. P value < 0.05 were considered as significant.

RESULTS AND DISCUSSION

In the acute toxicity study oral administration of the alcohol (95 %) extract of *P. rheedii* doses from 100 to 2400 mg/kg did not produce significant changes in behaviours, breathing, cutaneous effects, sensory, nervous systems, responses and gastroinstinal effects in mice. These effects were observed during the experimental period 24 h. Death of mice was noted at 2600 and 3000 mg/kg. The LD_{50} value obtained was 2588 mg/kg body weight.

The rats in subacute toxicity study received the alcohol (95 %) extract of *P. rheedii* at dose of 125, 250 and 500 mg/kg orally for every 24 h for 28 days did not result in death of the animal. No sign of observable toxicity was detected during the experimental period according to the OECD guideline.

Generally, the reduction in body weight gain and internal organ weights is a simple and sensitive index of toxicity after exposure to toxic substances[13,14]. In subacute toxicity study rats treated with 125, 250 and 500 mg/kg doses of alcohol extract of *P. rheedii* had a progressive weight in body and organ gained. The increase in weight was not significantly different from that of the control. The progressive increase in body weight and organ weight at dose of 125, 250 and 500 mg/kg of rats during 28 days of administration of alcohol extract of *P. rheedii* may indicate the improvement the nutritional state of the animal. The growth response effect could be as a result of increased food and water intake. The calculated relative weight of the control and treated animal groups varied from one organ to other, no significant differences were noted in the relative weight of other organs (liver, heart, lung, spleen, kidney). However there was no correlation between relative weight of the organs and the various doses of the extract of *P. rheedii* administered (Table-1).

TABLE-1
EFFECT OF ORAL ADMINISTRATION OF *Phyllanthus rheedii* EXTRACT ON BODY AND ORGANS WEIGHT

Organs	Control	125 mg/kg	250 mg/kg	500 mg/kg
Liver (g)	6.61 ± 0.02	6.64 ± 0.01	6.67 ± 0.04	6.70 ± 0.03
Heart (g)	0.76 ± 0.02	0.78 ± 0.01	0.81 ± 0.01	0.80 ± 0.01
Lung (g)	1.85 ± 0.01	1.87 ± 0.01	1.88 ± 0.01	1.86 ± 0.02
Spleen (g)	0.84 ± 0.02	0.81 ± 0.01	0.86 ± 0.01	0.85 ± 0.01
Kidney (g)	0.63 ± 0.01	0.64 ± 0.01	0.66 ± 0.01	0.65 ± 0.01
Body weight (g)	199.32 ± 16.8	199.88 ± 18.7	200.87 ± 17.8	201.11 ± 18.3

Values are expressed as mean ± SEM of 6 rats in each group. $p < 0.05$ were considered significant. No significant difference was observed in any parameter.

The hematological status (Table-2) after 28 d of oral administration of alcoholic extract *P. rheedii* was also assessed. In general the results showed that the values for the RBC and WBC were slightly increased in groups compared to the control. However no significant variation for RBC, WBC, Hb and clotting time were observed. The small transient of values observed in blood haematology did not show any dose responsiveness. Nevertheless, all values lay within the normal limits[15]. *P. rheedii* showed significant reduction of blood glucose on dose 500 mg/kg due to its hypoglycemic property.

TABLE-2
HEMATOLOGICAL PARAMETERS AFTER 28 DAYS TREATMENT WITH THE *Phyllanthus rheedii* EXTRACT

Parameter	Control	125 mg/kg	250 mg/kg	500 mg/kg
Hb (g%)	12.45 ± 0.29	12.26 ± 0.12	12.63 ± 0.29	12.33 ± 0.33
RBC (10^6/Cu.Mm)	3.60 ± 0.13	3.70 ± 1.10	3.40 ± 0.90	3.50 ± 1.30
Total WBC (10^3/Cu. Mm)	7.050 ± 0.76	6.93 ± 0.68	7.18 ± 1.68	7.21 ± 1.79
Clotting time (s)	111.16 ± 0.90	110.83 ± 1.80	110.66 ± 2.30	112.16 ± 1.90

Values are expressed as mean ± SEM of 6 rats in each group. $p < 0.05$ were considered significant. No significant difference was observed in any parameter.

The alcoholic extract of *P. rheedii* did not induce any damage to the kidney, liver and pancreas as examined by clinical blood chemistry (Table-3). ALT and AST are two liver enzymes that are associated to the hepatocellular damage. Although both AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, only ALT is remarkably specific for liver function since AST is mostly present in the myocardium, skeletal muscle, brain and kidneys[16]. A slight but not significant change was noted on ALP activities in the rat. No significant changes in ALT, AST and ALP activities in the serum of rats. In other parameters like total cholesterol, urea and blood urea nitrogen there was no significant changes observed. In general with liver disease serum levels of AST and ALT rise and fall at the same time[17]. A mild elevation of AST level has been shown to be associated with liver injury or myocardial infarction. The higher the activity of AST has been

observed in larger infarction size[18]. A typical myocardial infarction gives an AST/ALT ratio greater than 1 while an AST/ALT ratio less than 1 is as a result of release of ALT from the affected liver[18]. AST / ALT of more than 2 indicates alcoholic hepatitis or cirrhosis[18]. These results indicated that the alcohol extract of *P. rheedii* when taken for long periods of time might not cause liver disease. The γ-GT level increase gradually at doses of 125, 250 and 500 mg/kg body weight compared to the control after 28 d of administration. This increased level of γ-GT is not statistically significant. These values are within the normal range[19]. Furthermore, gross examination of internal organs of all the rats revealed no detectable abnormalities. Thus, it can be concluded that the alcohol extract of *P. rheedii* is virtually non-toxic.

TABLE-3
EFFECT OF TREATMENT WITH *Phyllanthus rheedii*
EXTRACT ON BIOCHEMICAL PARAMETERS

Parameter	Control	125 mg/kg	250 mg/kg	500 mg/kg
Cholesterol (mg %)	77.00 ± 2.12	75.68 ± 1.930	74.83 ± 1.24	72.50 ± 0.76
ALT (U/L	185.00 ± 2.70	181.33 ± 2.290	179.83 ± 2.48	178.43 ± 2.62
AST (U/L)	202.00 ± 2.26	198.50 ± 0.880	203.33 ± 1.68	201.63 ± 0.90
γ-GT (U/L)	260.50 ± 2.23	261.83 ± 1.790	262.43 ± 1.76	264.50 ± 1.66
ALP (U/L)	379.50 ± 0.88	382.66 ± 2.440	378.33 ± 0.88	383.41 ± 1.60
Glucose (mg %)	73.88 ± 1.79	71.50 ± 0.940	69.66 ± 1.22	67.42 ± 0.82*
Urea (mg %)	49.00 ± 0.81	51.50 ± 1.250	48.33 ± 0.98	50.10 ± 1.64
Bun (mg %)	21.16 ± 0.74	22.66 ± 0.800	20.33 ± 1.20	20.16 ± 0.60

Values are expressed as mean ± SEM of 6 rats in each group. $p < 0.05$ were considered significant. No significant difference was observed in any parameter. *$p < 0.01$.

In conclusion, this study presents strong evidence of the non-toxic effect of the alcoholic extract of *Phyllanthus rheedii*. These results showed that the use of extract of *Phyllanthus rheedii* is safe and explained the extensive utilization of the plant in traditional medicine.

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(*Received*: 23 August 2008; *Accepted*: 21 May 2009) AJC-7568