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Research Article

Toxicity Studies on the Extracts of *Amoora rohituka* Roxb. Stem

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ABSTRACT

The present communications describes the toxicity of *Amoora rohituka* Roxb stem on Swiss albino mice. In this study, successively prepared ethyl acetate and dichloromethane extracts from stem bark of *Amoora rohituka* were administered at 20 and 40 mg/kg doses in mice. Dichloromethane extract at 40 mg/kg, significantly ($P<0.05$) altered body weight gain, haemoglobin level, RBC and WBC count but the changes in haematological parameters were not clinically significant. Dichloromethane extract also significantly ($P<0.05$) increased the activity of SGPT, thereby indicating some extent of hepatic cell damage. Ethyl acetate extract did not show any adverse effect at 20 and 40 mg/kg doses.

Keywords: *Amoora rohituka*, Ethyl acetate extract, Dichloromethane extract, Swiss albino mice.

INTRODUCTION

Amoora rohituka Roxb (Benagali: Pithraj) is one of such plants that grows in forests and roadsides of many districts of Bangladesh [1]. The stem bark of *Amoora rohituka* is used in spleen and liver diseases, tumours and abdominal complaints. The seeds have a folkloric reputation to exhibit laxative, anthelmintic and antiulcer properties [2]. The plant contain limonoids [3], triterpenes [4], amooramin (a triterpene acid) [5], alkaloid [6], flavonoid glycosides [7] and sesquiterpenes [8]. In addition, seed oils and plant extracts have been reported to possess multiple therapeutic properties like hepatoprotective [9], antibacterial [10], antiviral [11], and laxative [12] activities. Ethyl acetate extract of the stem bark of *Amoora rohituka* showed anti-tumour activity against Dalton's lymphoma ascites cells (DLA) in mice [13]. Despite these numerous uses and the various chemical constituents reported in *Amoora rohituka*, no data on the toxicity of the plant could be found in the available literature. This work is therefore aimed at establishing the safety or otherwise of *Amoora rohituka* stem with regard to its numerous uses in traditional medicine.

MATERIALS AND METHODS:

Plant materials

Stem bark of *Amoora rohituka* (Family: Meliaceae) were collected in the month of March, 2008 from Rajshahi district of Bangladesh. The plant material was taxonomically identified by Professor A.T.M Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh and a voucher specimen was deposited under the accession number DACB-28927 at the Bangladesh National Herbarium.

Extraction

The shade dried and powdered stem bark of *Amoora rohituka* was successively extracted with ethyl acetate and dichloromethane at room temperature. These two extracts were then filtered through filter papers and filtrates were evaporated under reduced pressure at 40°C using a rotary evaporator to have ethyl acetate and dichloromethane extracts.

Animals

This study was carried out using Swiss albino mice of either sex, 3-4 weeks of age and weighing between 20-25 g. They were collected from the Animal Research Branch of the International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B). The mice were grouped and housed in iron cages with not more than ten animals per cage and maintained under standard laboratory conditions (temperature 25±2 °C; humidity 55±5 %) hours with 12 dark/light cycle. They were allowed free access to standard dry pellet diet (Collected from ICDDR,B) and water *ad libitum*.

Acute Toxicity Study (LD_{50})

The acute toxicity study was conducted by the method of Lorke [14] to determine the LD_{50} value of ethyl acetate and dichloromethane extracts in mice. For each extract, this method was carried out by a single intraperitoneal injection in twenty four animals (4 in each group) at different doses (100, 200, 400, 800, 1600 and 3200 mg/kg body weight). LD_{50} was evaluated by recording mortality after 24 hours.

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Table 1. Effect of *Amoora rohituka* stem extracts on hematological parameters and body weight gain

Hematological Parameters	Treatment (Dose, mg/kg body weight)				
	Normal + 2% DMSO	Normal + Ethyl acetate extract (20)	Normal + Ethyl acetate extract (40)	Normal + Dichloromethane extract (20)	Normal + Dichloromethane extract (40)
Hgb (g/dl)	12.1 ± 0.61	12.2 ± 0.22	12.0 ± 0.30	11.6 ± 0.39	9.45 ± 0.23*
RBC (x10^9 cells/ml)	5.40 ± 0.21	5.17 ± 0.18	5.04 ± 0.06	4.81 ± 0.14	4.46 ± 0.13*
WBC (x10^6 cells/ml)	7.83 ± 1.16	9.66 ± 0.88	10.5 ± 0.88	11.66 ± 1.11	13.16 ± 0.79*
Lymphocytes (%)	73.66 ± 2.02	71.33 ± 1.92	69.66 ± 1.11	68.83 ± 1.95	66.0 ± 2.28
Neutrophils (%)	24.83 ± 1.66	24.80 ± 1.57	26.5 ± 1.33	27.0 ± 1.52	27.5 ± 1.97
Monocytes (%)	1.50 ± 0.42	2.33 ± 0.33	2.30 ± 0.42	2.66 ± 0.33	3.00 ± 0.51
Body weight gain (g)	5.88 ± 0.28	6.21 ± 0.26	6.13 ± 0.28	5.80 ± 0.22	4.38 ± 0.19*

Data are expressed as the mean ± S.E.M. (n = 6). *P<0.05: between normal and extract-treated groups.

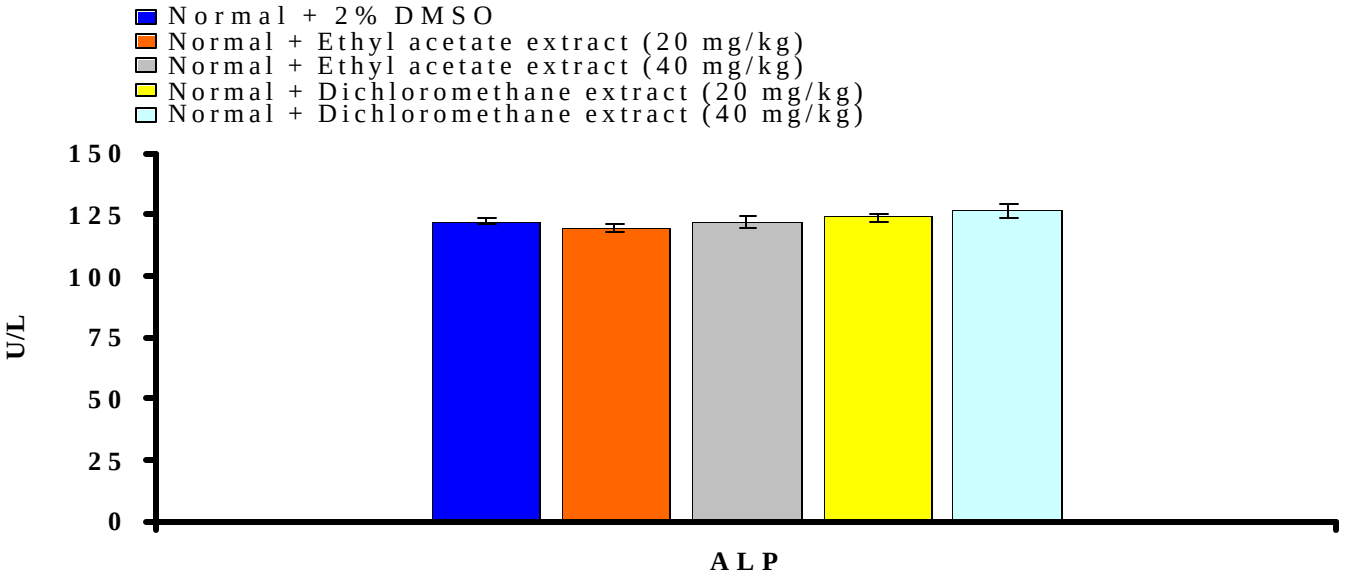

Figure 1: Effect of ethyl acetate and dichloromethane extracts on activity of ALP. Data are expressed as mean ± S.E.M (n = 6). *P<0.05: between normal and extract-treated groups.

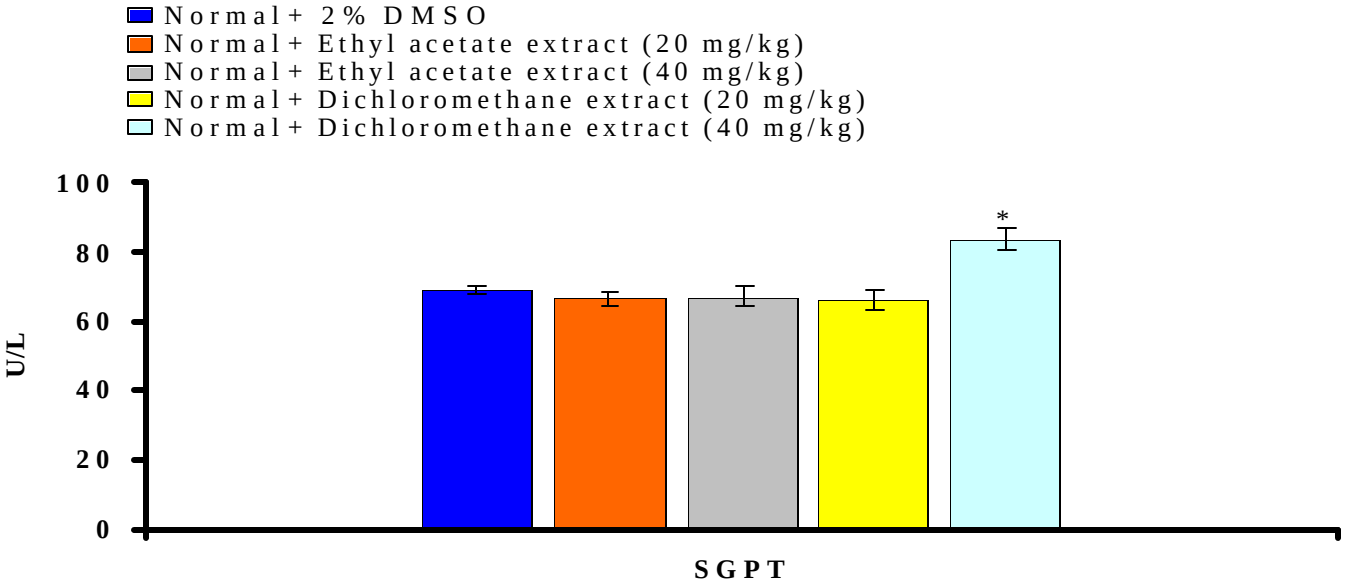

Figure 2. Effect of ethyl acetate and dichloromethane extracts on activity of SGPT. Data are expressed as mean ± S.E.M (n = 6). *P<0.05: between normal and extract-treated groups.

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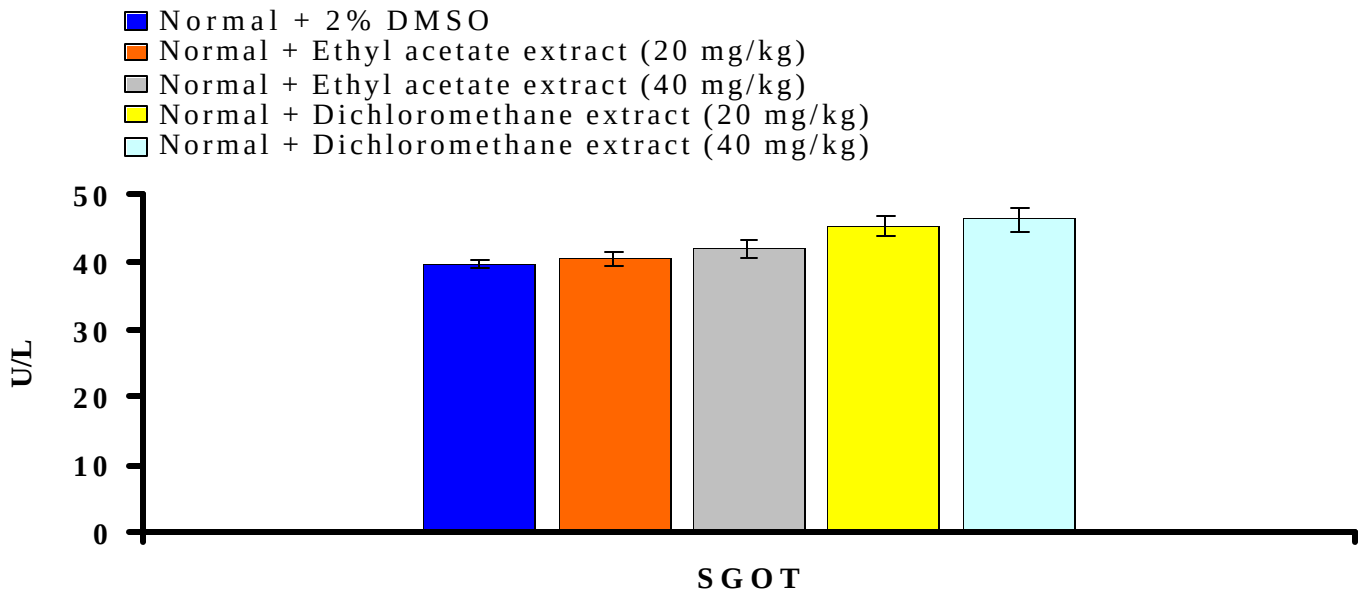

Figure 3. Effect of ethyl acetate and dichloromethane extracts on activity of SGOT. Data are expressed as mean ± S.E.M (n = 6). *P<0.05: between normal and extract-treated groups.

Short-term toxicity

To determine short-term (14 days) toxicity, healthy Swiss albino mice were divided into five groups of 6 animals in each. Group 1 received (i.p.) 2% (v/v) dimethyl sulfoxide (DMSO) at dose 5 ml/kg per mouse per day and group 2 and 3 received (i.p.) ethyl acetate extract at doses 20 and 40 mg/kg per mouse per day, respectively, for 14 days. Similarly dichloromethane extract at doses 20 and 40 mg/kg per mouse per day was administered in groups 4 and 5, respectively. Heamatological parameters (Hemoglobin, RBC, WBC and Differential count of WBC) were measured on 15th day from freely flowing tail vein blood of each mice of each group [15]. Then every mouse was sacrificed by chloroform anesthesia and blood was collected by heart puncture. The blood samples of each animal were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 4000 rpm for 10 minutes and analyzed for alkaline phosphatase (ALP), serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) in an Bioanalyzer (Microlab 200) using commercial kits (Atlas Medica, UK).

Statistical Analysis

All values were expressed as mean ± S.E.M (Standard error of mean). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's 't' test using SPSS statistical software of 10 version. P<0.05 were considered to be statistically significant when compared with control.

RESULTS AND DISCUSSION:

Administration (i.p.) of graded doses of ethyl acetate and dichloromethane extracts to Swiss albino mice, in our toxicity study produced a LD_{50} of 723±0.62 and 563±0.95 mg/kg body weight, respectively. Intraperitoneal administration of ethyl acetate extract at 20 and 40 mg/kg and dichloromethane extract at 20 mg/kg, did not cause any abnormal behavioral changes but treatment with dichloromethane extract at 40 mg/kg, showed slight toxic symptoms, which included inactiveness, loss of appetite and slow movement for some hours. Dichloromethane extract at 40 mg/kg, significantly (P<0.05) increased WBC count and decreased haemoglobin level, RBC count and body weight gain (Table 1).

Figure 1, 2 and 3 shows the effect of ethyl acetate extract (20 and 40 mg/kg) and dichloromethane extract (20 and 40 mg/kg) on the activity of ALP, SGPT and SGOT, respectively. Dichloromethane extract at 40 mg/kg, significantly (P<0.05) increased the activity of SGPT. Both ethyl acetate and dichloromethane extracts did not show any alterations in the activity of ALP and SGOT.

Although dichloromethane extract (at 40 mg/kg) significantly altered haematological parameters (Hb, WBC and RBC) but these changes were not clinically significant. On the other hand, ALP, SGPT and SGOT are important markers for liver function [16]. SGPT is located primarily in the cytosol of hepatocytes and this enzyme is considered a more sensitive marker of hepatocellular damage than SGOT. SGOT is an enzyme found in the cytoplasm and mitochondria in different tissues,

chiefly in the heart and skeletal muscles, liver, kidneys, pancreas and erythrocytes [17]. In the present study, significant increase in SGPT activity indicated that dichloromethane extract (at 40 mg/kg) made damage in some hepatic cells. In conclusion, the overall findings of this toxicity study suggest that ethyl acetate extract is comparatively safe than dichloromethane extract.

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