

Original
Article

Biochemistry

Evaluation of Acute and Sub Acute Toxicity of Ethanol Extracts of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana*

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ABSTRACT [ENGLISH/ANGLAIS]

The acute and sub acute toxicity of ethanol extracts of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana* were evaluated in Wistar rats. The acute toxicity studies were conducted as per the OECD guidelines 420 where the limit dose of 2000 mg/kg body weight was used. Observations were made and recorded systematically 1, 2, 4 and 24 h after dose administration for skin changes, morbidity, aggressivity, sensitivity of the sound and pain as well as respiratory movement. For sub acute toxicity there were 5 groups (each made up by 6 rats) respectively treated as follows: distilled water (control), 250, 500, 1000, 2000mg/kg of extracts every 24 h for 28 days. No sign of observable toxicity was detected during the experimental period. 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. Haematological analysis and clinical chemistry revealed no toxic effects of the extract. No significant histopathological change in the liver was observed. Mortality was not recorded in 28 days.

Keywords: *Entada pursaetha*, *Toddalia aculeata*, *Ziziphus mauritiana*, acute, subacute, Histopathology, Haematology, biochemical parameters

RÉSUMÉ [FRANÇAIS/FRENCH]

La toxicité aiguë et subaiguë des extraits de l'éthanol *pursaetha Entada*, *aculeata Toddalia*, et *Ziziphus mauritiana* ont été évalués chez le rat Wistar. Les études de toxicité aiguë ont été menées conformément aux lignes directrices de l'OCDE 420, où la dose limite de 2000 mg / kg de poids corporel a été utilisé. Les observations ont été notées systématiquement 1, 2, 4 et 24 h après administration d'une dose de modifications de la peau, la morbidité, l'agressivité, la sensibilité du son et de la douleur ainsi que les mouvements respiratoires. Pour les sous toxicité aiguë, il y avait 5 groupes (chacun composé de 6 rats) respectivement traités comme suit: eau distillée (témoin), 250, 500, 1000, 2000 mg / kg d'extraits toutes les 24 heures pendant 28 jours. Aucun signe de toxicité observable a été détecté au cours de la période expérimentale. Aucune variation significative ($p < 0,05$) dans le poids du corps et d'organes entre le contrôle et le groupe traité a été observée après 28 jours de traitement. Analyse hématologiques et de chimie clinique n'a révélé aucun effet toxique de l'extrait. Pas de changement significatif histopathologiques dans le foie a été observée. La mortalité n'a pas été enregistrée dans les 28 jours.

Mots-clés: *Pursaetha Entada*, *aculeata Toddalia*, *Ziziphus mauritiana*, aiguë, subaiguë, histopathologie, l'hématologie, les paramètres biochimiques

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Accepted/Accepté: May, 2011

Full Citation:
Suriyavadhana M, Pakutharivu T. Evaluation of acute and sub acute toxicity of ethanol extracts of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana*. World Journal of Life Sciences and Medical Research 2011;(2):43-47.

INTRODUCTION

Plant derived products have been used for medicinal purposes for centuries. Herbs and spices are generally considered safe and proved to be effective against certain ailments, the potential toxicity of herbs has not been recognized by the general public or by groups of traditional medicine [1, 2]. Our study was aimed to obtain data on the safety of the extract of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana*. *Entada pursaetha* is a gigantic creeper with giant pods among

legumes and is an endangered species belonging to the family Fabaceae. It can be used as a narcotic or as a tonic or used in curing liver troubles, allaying body pains, in warding of cold, curing eye diseases, arthritis and paralysis [3]. *Toddalia aculeata* is a thorny large shrub belongs to the family Rutaceae. It has been used by traditional health practitioners in East Africa for management of diseases [4]. *Ziziphus mauritiana* is a tropical fruit tree species belonging to the family Rhamnaceae. The fruits are sweet, cooling, anodyne

purgative, mucilaginous, pectoral. Styptic, aphrodisiac, invigorative, depurative, appetizer and tonic[4]. In the present study the acute and sub acute toxicity of ethanol extracts of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana* in albino rats were assessed with the hope that the result would provide information on the safety of this extract prior to the evaluation of its therapeutic efficacy in humans. In sub acute toxicity study the effect on biochemical, hematological and histopathological parameters were investigated.

MATERIALS AND METHODS

Extraction of plant materials

The seeds of *Entada pursaetha*, the stems of *Toddalia aculeata* and the fruits of *Ziziphus mauritiana* were collected from Kolli hills, Namakkal, Salem District, Tamilnadu, India. The samples were air dried in shade at room temperature and then ground to a fine powder in a mechanic grinder. 10 g of the mixed powdered plant material was extracted with 400ml of ethanol in a soxhlet extractor for 24hrs. The resultant crude ethanolic extract was evaporated to dryness and then stored in the freezer until ready for use.

Experimental animals

The male Wister rats are housed in poly propylene cages at room temperature (22±2°C) with proper ventilation. Prior to the experiments, animals were fed with standard diet for one week in order to adapt laboratory conditions. They were fasted but allowed free access to water 16-18h prior to administration of the test dose.

Acute toxicity

The acute toxicity studies were conducted as per the OECD guidelines 420 (OECD, 2001) where the limit dose of 2000 mg/kg body weight used [5, 6]. Observations were made and recorded systematically 1, 2, 4 and 24 h after dose administration for skin changes, morbidity, aggressivity, sensitivity of the sound and pain as well as respiratory movement.

Sub acute toxicity

The plant extract at the dose of 250, 500, 1000, 2000 mg/kg body weight were administered orally to 5 groups of 6 rats respectively to every 24 h for 28 days and control group received distilled water at the same volume. The toxic manifestation such as body weight, mortality, food

and water intake was monitored. After 28 days all surviving animals were fasted overnight and anaesthetized with ether. 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 and the internal organs were removed and weighed to determine the relative organ weights and observed for gross lesions. The internal organs were preserved in 10% buffered formaldehyde solution for histological examination

Biochemical estimations

Blood collected in non-heparinized tubes were then centrifuged at 3000 rpm for 10 min. The serum separated was analyzed for various parameters such as AST, ALT [7], ALP [8]. Blood glucose was determined by glucose oxidase method [9], Total cholesterol and triglycerides were estimated by modified enzymatic method [10]. Total protein concentration was determined by Biuret method [11], albumin was determined based on its reaction with bromocresol green (Binding method) [12]. Urea was determined according to Urease-Berthelot method [13], and plasma creatinine was estimated using Jaffe reaction [14].

Haematological assay

Blood sample collected in the heparinized tubes were used to estimate white blood cells (WBC), red blood cells (RBC), hemoglobin content (Hb) [15] and clotting time [16].

Histopathological study

Histopathological investigation of the organs was done according to the method described by Lamb [17]. The organ pieces (3-5 micro meters thick) were fixed in 10% buffered formalin for 24 h and washed in running water. Samples were dehydrated with serial ethanol cycles (70% to absolute), followed by clarification in xylol and then embedded in paraffin. Embedding was carried out with a paraffin embedding station. Duplicate slides of each block were obtained. Slices of 5 µm were produced with a rotation microtome. Deparaffination was performed with the following protocol. Xylol 4 min; 100% ethanol 2min; 90% ethanol 2min; 70% ethanol 2min; Afterwards slices were stained with Mayer hematoxylin and eosin and mounted with mounting medium (DPX). Sections were then screened under the light microscope at low (x5) and high (x40) magnifications for histopathological study.

Statistical analysis

The values were expressed as mean \pm SD. Statistical analysis was performed by one way analysis of variance (ANOVA). p values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

The acute toxicity studies were conducted as per the OECD guidelines 420 [18], where the limit test dose of 2000mg/kg was used. No test substance related mortality was observed at 2000 mg/kg and the compound was practically non toxic [19]. Toxicity evaluation was further carried out by observing both body weight and internal organ weight which is presented in Table 1.

TABLE 1

Table 1 shows the effect of oral administration of ethanol extract of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana* on body weight (g) and organs (g per 100g body weight) of rats

	Body Weight	Liver	Heart	Lung	Spleen	Kidney
Control	201.34 \pm 3.21	7.53 \pm 0.35	0.74 \pm 0.04	1.87 \pm 0.02	0.83 \pm 0.03	0.62 \pm 0.02
250 mg	203.00 \pm 7.22	7.83 \pm 0.15	0.77 \pm 0.02	1.84 \pm 0.03	0.84 \pm 0.02	0.63 \pm 0.04
500 mg	210.00 \pm 5.00	8.20 \pm 0.26	0.78 \pm 0.02	1.87 \pm 0.02	0.87 \pm 0.02	0.62 \pm 0.02
1000 mg	214.00 \pm 4.58	8.43 \pm 0.35	0.82 \pm 0.02	1.87 \pm 0.02	0.88 \pm 0.03	0.64 \pm 0.03
2000 mg	216.34 \pm 3.51	7.60 \pm 0.26	0.85 \pm 0.03	1.85 \pm 0.01	0.83 \pm 0.02	0.64 \pm 0.02

Values are expressed as mean \pm SD of 6 rats in each group. P values < 0.05 were considered as significant. No significant difference was observed in any organ.

TABLE 3

Table 3 shows the effect of oral administration of ethanol extract of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana* on biochemical parameters

	Glucose (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Total bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	117.33 \pm 1.53	6.30 \pm 0.20	3.43 \pm 0.20	69.00 \pm 1.00	70.33 \pm 1.52	0.17 \pm 0.02	43.00 \pm 1.00	1.24 \pm 0.04
250 mg	122.67 \pm 2.08	6.37 \pm 0.32	3.64 \pm 0.15	65.67 \pm 0.57	72.00 \pm 1.00	0.15 \pm 0.03	37.00 \pm 1.00	1.05 \pm 0.04
500 mg	119.67 \pm 1.52	7.10 \pm 0.10	3.57 \pm 0.35	69.00 \pm 1.00	66.68 \pm 1.52	0.17 \pm 0.02	36.67 \pm 1.52	1.24 \pm 0.04
1000 mg	115.00 \pm 2.00	6.37 \pm 0.11	3.47 \pm 0.35	67.00 \pm 2.00	67.33 \pm 2.08	0.19 \pm 0.02	42.00 \pm 1.00	1.34 \pm 0.04
2000 mg	103.00 \pm 2.00	6.40 \pm 0.20	3.74 \pm 0.15	65.67 \pm 1.52	66.33 \pm 1.52	0.19 \pm 0.02	49.00 \pm 1.00	1.36 \pm 0.02

Values are expressed as mean \pm SD of 6 rats in each group. P values < 0.05 were considered as significant. No significant difference was observed in any parameter.

TABLE 2

Table 2 shows the haematological parameters after 28 days oral treatment with ethanol extract of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana*

	Hb g/dl	RBC (*10 ⁶ /μl)	Total WBC (*10 ³ /μl)	Clotting time(S)
Control	12.43 \pm 0.20	6.20 \pm 0.26	7.73 \pm 0.25	112.00 \pm 1.00
250 mg	11.00 \pm 1.00	6.30 \pm 0.10	8.23 \pm 0.25	110.67 \pm 2.08
500 mg	12.00 \pm 1.00	7.13 \pm 0.15	8.26 \pm 0.25	111.00 \pm 1.00
1000 mg	13.50 \pm 0.50	6.30 \pm 0.26	9.00 \pm 0.50	112.00 \pm 1.00
2000 mg	11.50 \pm 0.50	6.20 \pm 0.20	9.73 \pm 0.25	112.67 \pm 2.51

Values are expressed as mean \pm SD of 6 rats in each group. P values < 0.05 were considered as significant. No significant difference was observed in any parameter.

No significant difference was found with the control group. The gross examination of the internal organs of treated rats revealed no pathological abnormality as compared with those of the control. According to reports, reduction in body and internal organ weights are considered sensitive indices of toxicity after exposure to toxic substance [20, 21, 22]. From the evaluation of its sub acute toxicity at the doses of 250, 500, 1000 and 2000 mg/kg/day for 28 days presented no signs of behavior changes and toxic signs.

The effects of extracts on haematological parameters of the animals are shown in Table 2. The values did not record appreciable difference with respect to the control. The results of biochemical parameters are presented in Table 3. In a toxic environment the blood level of AST and ALT are known to significantly increase [23, 24].

These two classical enzymes are reliable indices of liver toxicity [25]. Since in this study the enzymes showed no appreciable increase in the treated animals, it implied that the extract has no hepato-toxic effect. The histopathological examination of liver was performed to further confirm whether or not the tissue had been damaged and is shown in Figure 1. No significant histopathological change in the liver was observed.

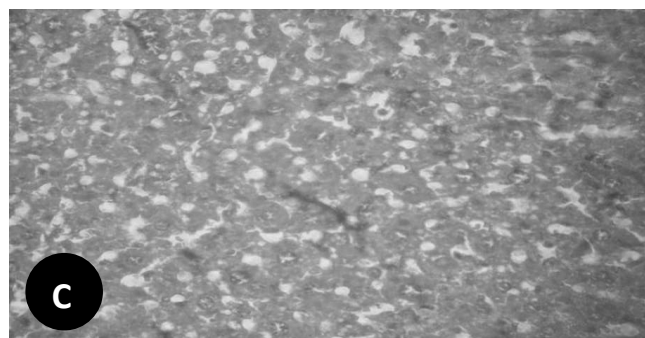
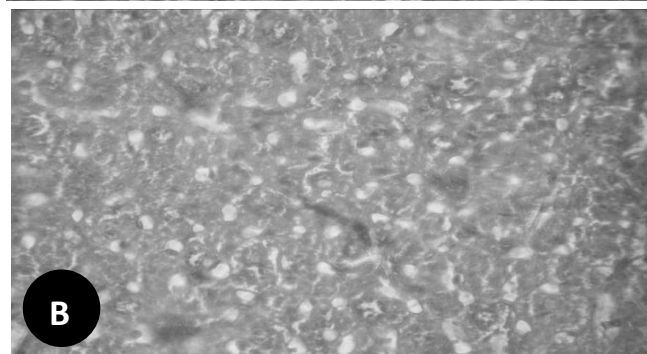
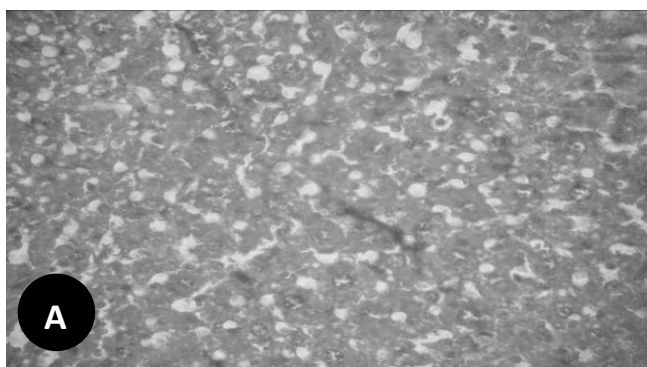
In summary the extract administered orally did not cause acute and sub acute toxicities in the albino rats. A chronic toxicity study should be further carried out to assess the long term safety of the extract.

.TABLE 4

Table 4 shows the effect of oral administration of ethanol extract of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana* on liver marker enzymes

	Aspartate Aminotransfer (U/L)	Alanine Aminotransfer (U/L)	Alkline Phosphatase (U/L)
Control	46.34±1.52	52.00±2.00	147.33±2.08
250mg	45.34±1.52	53.34±3.05	148.34±2.08
500mg	46.67±1.52	55.00±3.00	149.00±1.00
1000mg	48.68±1.52	53.00±3.00	151.00±1.00
2000mg	49.00±2.00	57.34±2.08	153.00±2.00

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



Photomicrograph of Liver histology of treated and untreated albino rats with ethanol extract of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana*. [A]control group, [B] ethanolic extract treated rats [1000mg/kg body weight], [C] ethanolic extract treated rats [2000mg/kg body weight]. Liver sections stained with haematoxylin and eosin (100X).

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ACKNOWLEDGEMENT / SOURCE(S) OF SUPPORT

Nil

CONFLICT OF INTEREST

Nil

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