ACUTE AND SUB-ACUTE TOXICITY STUDY OF *Acronychia pedunculata* LEAVES

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Introduction

Acronychia pedunculata ("Ankenda" in Sinhala, Family: Rutaceae) is a small evergreen tree found in Sri Lanka and the leaves, stems, roots and fruits have been used for centuries in folk medicine for the treatment of various diseases. Our previous studies have shown that 70 % ethanol extract of leaves of this plant has significant ($p < 0.05$) acute and chronic anti-inflammatory activity on carrageenaninduced rat hind paw oedema modal and adjuvant induced arthritis rat model respectively. Further it was found that it has significant ($p < 0.05$) anti-histamine, anti-nociceptive, *in-vivo* and *in-vitro* anti-oxidant, nitric oxide scavenging activities as well as prostaglandin E_2 inhibitory activity. Although, it shows different biological actions no scientific data are available regarding its potential adverse effects. The lack of information regarding the toxicity of this plant limits the possible long-term use in chronic disease conditions. Hence, in the present study an attempt has been made to evaluate the acute and sub-acute toxicity of *A. Pedunculata* leaves.

Material and Methods

Plant materials

Fresh *A. pedunculata* leaves were collected from Kottawa area in the district of Colombo. It was authenticated and a voucher specimen (KMR002) was deposited at National Herbarium, Department of National Botanic Garden, Peradeniya, Sri Lanka.

Ethical clearance

The protocol for animal experiments was approved by the Ethics Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka (No. 35/15).

Animals

Healthy adult female, Wistar rats weighing 150-200 g were purchased from Medical Research Institute, Colombo 8, Sri Lanka. Rats were housed under standard conditions with a natural light-dark cycle and fed with standard diet and water ad libitum. The animals were acclimatized for at least one week to the laboratory conditions prior to the experiment.

Preparation of 70% ethanol extract of A. pedunculate leaves (EEAL)

One hundred grams of fresh leaves were refluxed with 500 mL of 70% ethanol for two hours. The extract was filtered and the filtrate was evaporated under reduced pressure to dryness. The residue was collected and dissolved in 0.5 % carboxymethyl cellulose (CMC) for oral administration to rats.

Toxicity study of A. pedunculate leaves

To evaluate the safety of the 70 % ethanol extract of *A. pedunculata* leaves, a limited dose acute oral toxicity study and sub-acute oral toxicity study (28 days) were carried out in compliance with the Organization for Economic Co-operation and Development (OECD) guidelines. In each assay, healthy control was administered with 1 mL of distilled water and the negative control group was administered with 1 mL of 0.5 % CMC. In the limited dose test, treated group received 5000 mg/kg b. w. of EEAL. The sub-acute toxicity study was done for the therapeutic effective dose of EEAL as found in anti-inflammatory assays (200 mg/kg b. w.) and doses which are lesser and higher than this dose, i.e.100 mg/kg b. w. and 2000 mg/kg b. w. respectively for 28 days. In each assay, assessment of mortality and the behavior of the animals were carried out by the general observations of each animal twice daily from the stage of dosing to the end of the study. Further, changes in the body weight, water and food consumption were compared with the control group. In addition to this haematological and biochemical parameters i.e. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GT), urea, creatinine, glucose, cholesterol, calcium and bilirubin levels, were measured to evaluate the safety of the plant extract. All the results were expressed as mean ± standard error of mean (SEM). Data was analyzed using one-way analysis of variance test (ANOVA) to determine the significance of the differences between the healthy control and test groups as well as in between the test groups. The *p*- values < 0.05 were considered as statistically significant. Further relative organ index was calculated to assess the safety of EEAL on different organs. Histopathological studies were also done.

Results and Discussion

During the entire period of all studies, there were no mortalities found following administration of EEAL. As mortality is the main criteria in assessing the acute toxicity of any drug, the absence of mortality by EEAL is an indicator of the safety. Further absence of any changes or abnormalities in the condition of fur, urine color, faeces or signs such as diarrhoea, salivation and breathing abnormalities, damaged skin, subcutaneous swelling or lumps, wetness or soiling of perineum in treated group in comparison to the control group indicated that EEAL in acute and sub-acute dosing is safe.

As the body weight is also an important factor to monitor the health of an animal, the OECD guidelines of toxicity testing place considerable emphasis on reporting on changes in weight gain of each animal. Loss of body weight is frequently the first indicator of the onset of an adverse effect. A dose which causes 10 % or more reduction in the body weight, is considered to be a toxic dose [2]. As EEAL treated groups in each assay have not shown the body weight reduction throughout the study periods, it provides the evidence for safety of usage of EEAL. Further, the results showed that, there were no significant ($p > 0.05$) difference in food and water consumption in treated groups as compared to negative control as well as in between three test groups.

When considering the serum clinical profiles, all tested parameters in the rats treated with EEAL comparable with those of the control rats of each study. The results on sub-chronic study are given in Table 1. As AST, ALT, ALP and γ- GT are good enzymatic indicators of hepatic diseases, the absence of any significant difference ($p > 0.05$) of those markers in EEAL treated group indicates lack of toxicity

on liver. Further, absence of any significant difference ($p > 0.05$) in serum urea and creatinine levels in EEAL treated rat group as compared to the control group indicate lack of toxicity on kidney. The absence of any difference in relative weights of vital organs in treated as compared to the control group also provides scientific evidence for the safety of the test extract.

In addition to clinical chemistry profile, haematological parameters are also good indices of physiological and pathological status in humans as well as the animals. Hence, haematological parameters were also measured and any level of abnormal haematological parameters was not found in EEAL treated groups. The results on sub-chronic study are given in Table 2.

As histological assessment is also important in toxicology studies, the collected tissues were subjected for histopathology observations including leukocyte infiltration, haemorrhages in kidneys, necrosis in liver and several other features in each toxicity study. The results have shown that there were no any morphological changes in microscopic examination of tissues. Hence, it also provides strong evidence for the non-toxicity of EEAL.

Table 1. Clinical chemistry data of Wistar rats in the sub-acute oral toxicity study of *A. pedunculate* leaves

Clinical chemistry parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Albumin (mg/dL)	4.3 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1
ALP (IU/L)	117 ± 3.9	116.5± 3.6	116.9 ± 3.2	122.2 ± 3.5	116.7 ± 2.6
ALT (IU/L)	59 ± 10.4	43.6 ± 0.8	46.7 ± 2.4	48.3 ± 2.7	46.0 ± 2.2
AST (IU/L)	75.2 ± 2.2	72.0 ± 2.4	76.6 ± 3.1	77.8 ± 2.3	75.0 ± 2.0
Calcium (mg/dL)	12.1 ± 0.5	11.6 ± 0.4	11.6 ± 0.3	11.6 ± 0.2	11.1 ± 0.4
Cholesterol (mg/dL)	72.6 ± 3.5	74.4 ± 3.1	73.0 ± 3.6	75.8 ± 2.4	71.1 ± 3.4
Creatinine (mg/dL)	0.7 ± 0.05	0.6 ± 0.01	0.7 ± 0.02	0.7 ± 0.05	0.6 ± 0.06
γ – GT (IU/ L)	24.0 ± 1.1	23.8 ± 0.6	22.4 ± 1.1	22.8 ± 0.7	22.2 ± 1.7
Glucose (mg/dL)	84.0 ± 1.5	87.0 ± 1.1	80.1 ± 2.4	81.7 ± 1.2	83.2 ± 1.1
Phosphorous(mg/dL)	21.1 ± 2.3	19.5 ± 2.3	18.7 ± 2.4	19.6 ± 0.5	18.1 ± 0.7
Total bilirubin (mg/dL)	2.8 ± 0.3	3.0 ± 0.2	3.0 ± 0.2	3.5 ± 0.2	3.3 ± 0.4
Urea (mg/dL)	4.1 ± 0.4	3.6 ± 0.2	4.4 ± 0.3	3.2 ± 0.1	3.6 ± 0.3

Values for clinical chemistry parameters are expressed as mean ± SEM

Group 1: Healthy control group (DW), Group 2: Negative control group (0.5% w/v CMC), Group 3: Treated group (100 mg/kg b. w., EEAL in 0.5% w/v CMC), Group 4: Treated group (200 mg/kg b. w., EEAL in 0.5% w/v CMC), Group 5: Treated r group (2000 mg/kg b. w., EEAL in 0.5% w/v CMC)

Table 2. Haematological parameters of Wistar rats in the sub-acute oral toxicity study of *A. pedunculate* leaves

parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Haemoglobin (g/dL)	15.0 ± 0.4	15.0 ± 0.3	15.5 ± 0.5	15.4 ± 0.2	15.1 ± 0.4
RBC (x 10^9 / L)	7.7 ± 0.2	7.8 ± 0.2	8.0 ± 0.2	8.1 ± 0.2	8.0 ± 0.3
PCV (%)	43.7 ± 0.9	43.6 ± 0.8	44.7 ± 1.4	44.5 ± 0.7	44.2 ± 1.3
Platelet count (x10^9/L)	847 ± 67	831 ± 67	883 ± 27	866 ± 60	840 ± 74
MCV (fL)	114 ± 0.8	95.8 ± 1.7	111.7 ± 1.9	92.7 ± 1.7	111.5 ± 1.6
MCH (pg)	38.9 ± 0.3	38.6 ± 0.1	38.7 ± 0.7	38.1 ± 0.6	38.1 ± 0.7
MCHC (g/ dL)	68.9 ± 0.7	68.9 ± 0.4	69.6 ± 0.5	69.4 ± 0.6	68.5 ± 0.3
WBC (x 10^9 / L)	8.5 ± 0.6	8.4 ± 0.5	7.0 ± 0.4	7.6 ± 0.6	7.5 ± 0.3
Granules (x 10^9 / L)	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.3	0.6 ± 0.1	0.8 ± 0.1
Lymphocytes(x10^9/ L)	7.1 ± 0.2	6.9 ± 0.4	5.6 ± 0.5	6.2 ± 0.5	5.9 ± 0.2
Monocytes(x 10^9 / L)	0.8 ± 0.2	0.9 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1

Values for haematological parameters are expressed as mean ± SEM, (n=6/group)
Group 1: Healthy control group (DW), Group 2: Negative control group (0.5% w/v CMC), Group 3: Treated group (100 mg/kg b. w., EEAL in 0.5% w/v CMC), Group 4: Treated group (200 mg/kg b. w., EEAL in 0.5% w/v CMC), Group 5: Treated r group (2000 mg/kg b. w., EEAL in 0.5% w/v CMC)

Conclusion

The results of this study when considered along with the ethnomedical usage of *Achronia pedunculata* and its reported anti-inflammatory properties, suggests that the 70% ethanolic extract of the plant has the potential to be developed as a safe and effective treatment for chronic inflammatory conditions such as arthritis.

Acknowledgement

Financial assistance by World Class University Project (WCUP) under the research grant number Ph. D. /15/ 2012 is gratefully acknowledged.

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