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Original Article

ACUTE AND SUB-CHRONIC TOXICOLOGICAL STUDIES ON METHANOLIC STEM EXTRACT OF *ACALYPHA INDICA* LINN IN ALBINO WISTAR RATS

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ABSTRACT

Objective: The present study was aimed to investigate safety evaluation studies of methanolic stem extract of *A. indica* (AIS-ME) through acute and sub-chronic toxicity studies in albino wistar rats.

Methods: Acute toxicity test was carried out in albino wistar rats by single dosage administration of 500, 1000 and 1500mg/kg body weight of AIS-ME and observed for mortality and behavioural changes for a period of 24h. Sub-chronic toxicity studies were conducted by an oral administration of 100mg/kg body weight AIS-ME in albino wistar rats for a period of 30 consecutive days. Rats in sub-chronic toxicity studies were checked for body weight once in a week for 30 days and at the end of the experimental period, blood samples were collected for hematological and biochemical parameters. Organs (Liver and Kidney) were harvested and histological sections were made to examine for any organ damage after the treatment of AIS-ME.

Results: Acute toxicity study resulted in no mortality and behavioural changes even at the post oral dosage of 1500mg/kg body weight of AIS-ME. Sub-chronic toxicity revealed no significant changes in body weight, hematological and biochemical parameters of treated groups in comparision with the control groups. Histological sections of treated groups resulted in similar architecture as that of nomal groups suggesting no morphological damages of the internal organs.

Conclusion: This study concluded that AIS-ME is non toxic as per the results of acute and sub-chronic toxicity studies. Thus, it could be considered as a safe phytomedicine for oral administration in experimental rats.

Keywords: *A. indica*, Safety evaluation studies, Albino wistar rats.

INTRODUCTION

Acalypha indica is commonly known as Indian copper leaf belongs to the family Euphorbiaceae is an erect annual weed plant growing to a height of 60 cm. It grows extensively in most of the regions of Asian continent including India, Pakistan, Yemen, southern districts of China and South Africa [1,2]. Stems are longitudinally ribbed and pubescent. Leaves are ovate with toothed margins except near the base. Flowers are unisexual with axillary spikes. Fruits are tuberculate, three lobed and pubescent. The whole plant of *A. indica* is used in traditional medicine system to treat various diseases. In Ayurveda, *A. indica* used as a laxative, antihelmintic, emetic, expectorant and also used to treat scabies, earache, syphilitic ulcers and snake bites [3]. In Siddha medicine, *A. indica* is used to treat diseases associated with teeth and gums, stomach ache, irritations, burns, wheezing and respiratory diseases [4].

A. indica has been studied for various pharmacological activities in experimental animals namely, antidiabetic [5], wound healing [6], postcoital antifertility [7], anti snake venom [8], analgesic and antipyretic activities [9]. Many phytochemical compounds have been isolated from *A. indica.* The whole plant of *A. indica* contains saponins and alkaloids [10]. Acalyphamide was isolated from the roots of *A. indica* [11]. Cyanogenic and noncyanogenic glycosides were isolated from the leaves and twigs of *A. indica* [12].

Kaempferol glycosides (mauritianin, clitorin, nicrotiflorin, biorobin) were isolated from the leaves of *A. indica* [13]. These plants as a whole or its parts were used widely as phytomedicine in tropical and subtropical countries. Toxicity evaluation studies are most important for the safe consumption of herbal medicine at selected dosages by initial testing of the herbal extracts in experimental rats with many trials considering the biosafety of humans. However, to the best of our knowledge, there is no report for *in vivo* pharmacological activities as well as safety evaluation studies on the methanolic stem extract of *A. indica* (AIS-ME). Hence, the present study was aimed to investigate acute and sub-chronic toxicity studies of methanolic stem extract of *A. indica*.

Fig. 1: Photograph of medicinal plant *Acalypha indica* Linn.

MATERIALS AND METHODS

Plant material and method of extraction

Acalypha indica stems were collected in fresh condition from Seshachalam forest area (40°35′02″N79°43′19″N), Chittoor district, Andhra Pradesh, India. The herbarium of the plant was authenticated by Dr. P. Jayaraman, Director, Institute of herbal botany, Plant Anatomy Research Centre (PARC), Chennai with reference no. PARe/2013/2146. Stems were shade dried, pulverized into powder and were extracted using methanol in Soxhlet apparatus. Thus obtained methanolic stem extract of *A. indica* (AIS-ME) was concentrated in the rotary evaporator at 40°C under reduced pressure (72 mbar) to completely evaporate methanol from the extract, lyophilized and stored in air tight container at 4°C in a refrigerator.

Animals used for the study

Adult albino wistar rats of either sex (150-200 g) were acquired from Animal house, Centre for Biomedical Research, SBST, VIT University, Vellore. Animals were maintained in a room at 20°C with 12 h light to dark cycle and fed with standard rodent diet obtained from Hindustan Lever Ltd (Mumbai, India) and had access to water ad libitum. The experimental protocols were approved by the ethical committee in accordance with institutional animal ethical committee, 1333/C/10/CPCSEA.

Acute toxicity studies

Acute toxicity test was performed as per the protocol recommended by the World Health Organization for the evaluation of safety and efficacy of herbal medicines [14]. Twenty albino wistar rats were divided into four groups of five animals in each group. Group 1 served as a normal control. Group 2, 3 and 4 served as treated groups and received 500, 1000 and 1500 mg/kg body weight of AIS-ME respectively. Behavioral changes, symptoms of toxicity and mortality were observed after 24 h of oral administration of the AIS-ME.

Sub-chronic toxicity studies

Sub-chronic toxicity test was performed as per the guidelines of the World Health Organization for the evaluation of safety and efficacy of herbal medicines [14]. Twenty albino wistar rats were divided into four groups of five animals in each group. Group 1 and group 3 received saline and considered as control males and control females. Group 2 and group 4 were orally administered with 100mg/kg body weight of AIS-ME was considered as test males and test females. Each animal in the four groups received oral administration everyday for a period of 30 days.

Clinical observations

Each animal in the four groups was observed for the abnormalities, physical appearance and mortality.

Body weight

Body weight of each animal in the four groups was measured using an electronic weighing balance at the time of acclimatization, once before starting the experiment, weekly once during the experimental period and at the sacrifice after fasting the animals.

Clinical pathology

Animals fasted overnight were euthanized on 30th day. Blood samples were collected from each group by cardiac puncture and transferred to heparinised and non heparinised centrifuge tubes. Heparinised blood samples of the animals in each group were tested for hematological parameters such as red blood cell count, white blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, Platelet distribution width and plateletcrit. Non-heparinised blood samples of the animals in each group were tested for biochemical parameters such as total cholesterol, high density lipoproteins, low density lipoproteins, very low density lipoproteins, triglyceride, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, alkaline phosphatase, total bilirubin and creatinine. Hematological and biochemical analysis was carried out using hematological and biological semi automatic analyzer (3000 Evolution, Tulip Group, India).

Histopathology

Animals in each group were sacrificed, kidneys and liver were dissected, washed using cold phosphate buffered saline and fixed in 10% neutral buffered formalin for further histological analysis.

RESULTS

Acute toxicity study

Acute toxicity study was initially conducted with a series of dosages (500, 1000 and 1500mg/kg body weight) to select the proper and safe dosage for sub-chronic toxicity study. In an acute toxicity study, there were no signs of mortality and behavioural changes even after the oral administration of 1500mg/kg body weight. The result of acute toxicity is tabulated (Table 1).

Table 1: Effect of AIS-ME on mortality and behavioral changes on the rats in acute toxicity test

Dose (mg/kg b. wt.)	Observations*	
	Mortality (%)	Behavioral changes
500	0.0	NAD*
1000	0.0	NAD*
1500	0.0	NAD*

Observations were recorded after 24 h of extract administration and NAD: no abnormality detected.

Sub-chronic toxicity study

Sub-chronic toxicity studies were performed to check the overall toxicity of AIS-ME. During the period of experiment, there was no mortality in the treated as well as the control groups.

Body weight

Checking the body weight of the animals in the experimental period is an indicator of their normal growth because healthy animals cannot reduce 10% of their initial body weight [15]. AIS-ME did not exhibit any significant changes in body weight of test males and test females in comparision with the control males and control females (Fig. 1).

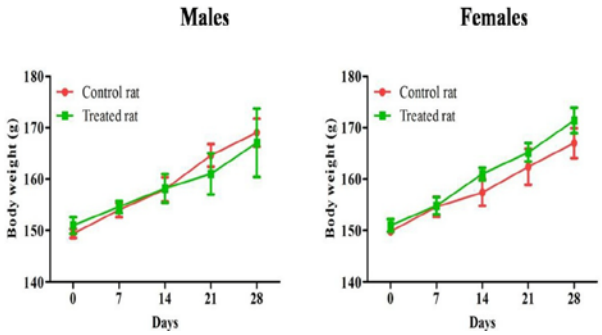

Fig. 2: Effect of AIS-ME on body weight in albino wistar rats. Values were expressed as Mean±SD of five animals (n=5)

Hematological parameters

There were no abnormalities in hematological parameters of tested groups of males and females to that of control males and control females (Table 2).

Biochemical parameters

Both AIS-ME treated groups and control groups exhibited normal levels of the biochemical parameters. Result of biochemical parameters is tabulated (Table 3).

Histopathological analysis

There was no inflammation, necrosis, hemorrhage or cellular abnormalities (deposits, degeneration, vacuoles etc.) in the test males and test females which were compared with the control males and control females (Fig. 2).

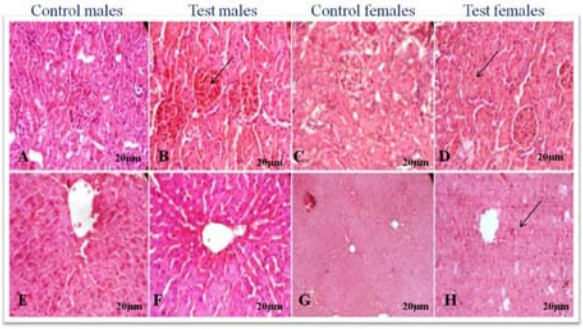

Fig. 3: Effect of AIS-ME on Histopathological analysis

A & E - sections of kidney and liver from Male control group
B & F - sections of kidney and liver from Male treated group
C & G - sections of kidney and liver from Female control group
D & H - sections of kidney and liver from Female treated group

Table 2: Effect of AIS-ME on hematological parameters in albino wistar rats

Test Performed	Control males	Test males	Control females	Test females
RBC ($\times10^6$/µl)	7.346±0.13	7.406±0.04	7.234±0.21	7.092±0.16
WBC ($\times10^3$/µl)	7.78±0.61	7.71±0.79	8.494±1.01	9.294±0.28
Plt ($\times10^3$/µl)	455.8±14.1	444±10.13	433±20.77	437±15.85
HGB (g/dl)	12.6±0.71	12.4±0.92	11.84±0.49	11.66±0.82
HCT (%)	35.1±2.08	32.6±0.81	33.12±0.64	33.38±1.82
MCV (fL)	46.2±2.09	45.3±0.87	42.62±2.70	42.5±2.78
MCHC (g/dl)	35.1±0.89	36.1±0.21	34.52±2.25	32.16±1.62
PDW (%)	14.9±0.10	14.5±0.16	14.56±0.46	14.9±0.27
PCT (%)	0.371±0.06	0.311±0.09	0.423±0.013	0.323±0.08

RBC: Red blood cell, WBC: white blood cell, Plt: platelets, HGB: haemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular haemoglobin concentration, PDW: Platelet distribution width, PCT: plateletcrit; Values were expressed as Mean±SD of five animals (n=5).

Table 3: Effect of AIS-ME on biochemical parameters in albino wistar rats

Test Performed	Control males	Test males	Control females	Test females
TC (mg/dl)	71±3.87	68±4.13	74.6±6.65	70.4±6.65
HDL (mg/dl)	62.8±5.11	60±6.19	58.6±7.70	63.2±5.58
LDL (mg/dl)	23.2±2.01	19.1±1.20	19.68±1.33	17.46±1.76
VLDL (mg/dl)	33.2±1.18	30.2±1.14	29.06±2.03	30.74±2.55
TGL (mg/dl)	163±6.02	154.6±3.04	172±2.54	159.8±10.61
SGPT (U/L)	47.8±2.23	39.2±2.79	44.4±4.84	39.02±1.48
SGOT (U/L)	75.2±2.36	71.5±2.74	67.6±3.50	64.46±3.95
ALP (U/L)	107.8±3.72	107.1±3.11	104±4.30	101±1.58
TB (mg/dl)	1.11±0.18	1.03±0.15	1.08±0.08	0.942±0.04
Creatinine (mg/ml)	1.01±0.09	0.95±0.09	1.054±0.07	0.972±0.05

TC: total cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, VLDL: very low density lipoproteins, TGL: triglyceride, SGPT: serum glutamic pyruvic transaminase, SGOT: serum glutamic oxaloacetic transaminase, ALP: alkaline phosphatase, TB: total bilirubin; Values were expressed as Mean±SD of five animals (n=5).

DISCUSSION

Plant derived drugs became popular worldwide for the prevention and treatment of various diseases. Globally, one hundred and nineteen secondary metabolites isolated from medicinal plants were used as phytomedicine in recent times. In India and other Asian countries, herbal medicine became a part for the regular management of their health [16]. Hence, safety evaluation studies are at most important which are carried out using experimental animals and thus providing guidelines to select safe dosages for human consumption as a phytomedicine. *Acalypha indica* gained popularity in both conventional and Western medicine because of having valuable phytochemical compounds within the herb [17]. In our previous study, AIS-ME was found to be rich in polyphenolic content and has shown promising antidiabetic and antioxidant activities [2]. Therefore this study was undertaken to evaluate the safety of AIS-ME through acute and sub-chronic toxicity studies.

In acute toxicity study, there were no signs of mortality and behavioural changes even after the oral administration of 1500mg/kg body weight which is the highest dosage than the regular doses used in the pharmacological experiments of *A. indica* such as antidiabetic [18], antiarthritic [19], wound healing activity [20], post-coital antifertility [7], analgesic and antipyretic activities [9]. Since there was no toxicity detected at 1500mg/kg body weight, LD_{50} value for *A. indica* is beyond 1500 mg/kg body weight, hence this study revealed the safety of AIS-ME upon acute administration for the pharmacological studies.

Sub-chronic toxicity studies were performed to check the overall toxicity of AIS-ME. Treatment dosage was selected based on the previous study on whole plant ethanolic extract of *A. indica* [21]. During the period of experiment, there was no mortality in the treated as well as control groups. Change in hematological parameters causes anemia which is an accompaniment of bone marrow toxicity [22]. There were no abnormalities detected in test males and test females in comparision with the control males and control females.

Biochemical toxicity evaluation resulted in normal levels of cholesterol, triglycerides, high density, low density and very low density lipoproteins in male and female rats treated with AIS-ME as that of control groups. SGOT and SGPT act as biomarkers for the prediction of toxicity. Their levels are in normal range in the AIS-ME treated groups and also in the control groups. Normal level of creatinine indicates good renal function [23]. Raise in creatinine levels creates functional damage to nephrons. In the present study AIS-ME has shown no abnormalities in the biochemical parameters in comparision with the control groups.

The organs harvested for histological analysis did not show any color and texture changes and were similar to that of control groups. Histopathological assessment of tissues collected from control and treated groups was carried for the evaluation of any systemic damage. In comparision with the control group, there were no abnormalities in the liver and kidney sections of male and female rats treated with AIS-ME.

CONCLUSION

In the light of the above findings, it can be concluded that the methanolic extract of *A. indica* is safe since it has not shown mortality and behavioural changes even after the oral administration of higher dosages in acute toxicity study. There was no reduction in the body weight of the treated animals as well as normal range of hematological and biochemical parameters throughout the sub-chronic toxicity evaluation study. Histological analysis of liver and kidney sections has shown normal architecture. Therefore, the methanolic extract of *A. indica* could be considered as safe for animal model experiments. Further, mutagenicity, carcinogenicity and teratogenecity studies have to be conducted for safe consumption of the extract by the human beings.

CONFLICT OF INTERESTS

Declared None

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