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Research Article

ACUTE AND SUBACUTE TOXICITY STUDIES OF ETHANOLIC EXTRACT OF *Acalypha indica* Linn IN MALE WISTAR ALBINO RATS

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ABSTRACT

The aim of the present study was to evaluate acute and sub acute toxicity of the ethanolic extract of *Acalyphaindica* on male wistar rats at the dose levels of 100,200,300,400, and 500mg/kg b.w.) at a rate of 1.0ml /rat/day to different sets of animal for 30 days Results from the present study have elucidated that treatment of *A.Indica* exerts no significant signs of toxicity at any dose level used in the study. Physical, biochemical as well as hematological parameters was unaltered throughout the study. The results of study have suggested there was no obvious toxicity observed with the treatment of *A.Indica*. It was found to be safe alternative for various severe infections.

Keywords: Acalyphaindica, Acutetoxicity, Sub-acutetoxicity, Haematologicalparameters.

INTRODUCTION

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. (Patwardhan*et al*., 2004). Whenever we administer a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. The types of toxicity tests which are routinely performed by pharmaceutical manufactures in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD50 the dose which has proved to be lethal (causing death) to 50% of the tested group of animals. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. (Akhila *et al*., 2007).

Acute toxicity is produced after administration of a single dose or multiple doses in a period not exceeding 24 hours, up to a limit of 2000 mg/k g. Objective of acute toxicity studies is to identify a dose causing major adverse effects and an estimation of the minimum dose causing lethality (Robinson*et al*., 2007).In recent times there is an increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicines (Steve *et al*., 2009). The major hindrance to the use of traditional herbal preparations is the lack of scientific and clinical data in support of better understanding of the efficacy and safety of the drugs.

Acalyphaindica is a small erect herb up to 60 cm tall or a little more, with a few ascending branches, these angled and pubescent; leaves broadly ovate, subdeltoid, rather coarsely toothed, on petioles as long as or longer than the 3-5 cm long blades; nerves 3-5 from base, thereafter pinnately arranged; stipules minute; flowers sessile on erect axillary spikes longer than the leaf; male flowers minute, crowded distally; stamens 8, female flowers scattered along the inflorescence axis, each subtended by a conspicuous semicupularfoliaceous toothed green bract nearly mm long; capsule hispid, 1 mm broad, 3-locular (Stone and Benjamin,1970).

Collection of the plant material

Acalyphaindica was collected from Maruthamalai hills, Coimbatore district, Tamil Nadu, India during the month of March to May, 2009. The plant was identified and authenticated by Taxonomist Dr.K. Arumugasamy, Lecturer (SG), Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

Preparation of ethanolic plant extract for *in vivo* studies

100g of *A.indica* powder was taken in a conical flask. To this 500ml of 99% ethanol was added. The content of the flask was soaked overnight. This suspension was filtered and residue was resuspended in an equal volume of 99% ethanol for 48hours and filtered again. The two filtrates were pooled and the solvents were dried in an oven at 37ºC and a crude residue was obtained. The yield was 22.8g, and the residue was suspended in water and administered orally to experimental rats.

Selection of animals

For the purpose of sub-acute toxicity and urolithiatic studies, adult male wistar albino rats weighing about 150 to 200 g were collected from animal breeding centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala.

Maintenance of experimental rats

The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. The animals were maintained in 12 hours light and dark cycle at 28^{o}C ± 2^{o} C in a well ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India. All animal experiments were performed according to the ethical guidelines suggested by the institutional animal ethics committee (IAEC). Paddy husk was used as beding material and changed twice a week.

Acute toxicity studies of the ethanolic plant extract

Thirty six male wistar albino rats weighing 150-200g were used for the acute toxicity study. They were randomly distributed into one control group and five treated groups, containing six animals per group and were on standard normal diet provided with water *ad libitum.* They were allowed to acclimatize for seven daysto the laboratory condition before the experiment. The treated group received orally varying doses (5, 50, 300, 500, 2000mg/ Kg b.w) at a rate of 1.0ml /rat/day to different sets of animals for 14days.Control group animals treated with 5% acacia served as control. They were continuously observed for 42hours to detect any changes in autonomic or behavioral responses. Vizalterness, spontaneous activity, irritability, corneal reflex, urination and salivation. Any mortality during the experimentation period of 14 days was also recorded. The percentage in mortality in each group was noted.

Sub-acute toxicity studies of the extract of *Acalyphaindica* L.

a. Experimental setup

To find out the effective dosage of *A.indica*, sub acute toxicity studies were carried out by the method Biswas (1998). The residue was suspended in water administered orally at varying doses (100,200,300,400, and 500mg/kg b.w.) at a rate of 1.0ml /rat/day to different sets of animal for 30 days as .

Table 1: Experimental Design

Group	Experimental design
I	Control rats administered with normal drinking water
II	Plant extract treated rats (100mg/kg b.wt)
III	Plant extract treated rats (200 mg/kg b.wt)
IV	Plant extract treated rats (300mg/kg b.wt)
V	Plant extract treated rats (400 mg/kg b.wt)
VI	Plant extract treated rats (500 mg/kg b.wt)

b. Weekly body weight

The body weight of each rat was assessed using a sensitive balance during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and once on the day of sacrifice.

c. Mortality and clinical signs

During the four-week dosing period, all the animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing and up to 4 hour after dosing(Al-Mamary*et al*.,2002).

On 31st day, the animals were anaesthetized with light chloroform anesthesia, blood was collected by Sino – orbital puncture and centrifuged for 30 min. at 2000rpm to separate serum for biochemical analysis. The liver and kidney were excised immediately and thoroughly washed in ice cold saline and weights were recorded.

d. Relative organ weight

On 31st day, all the animals were by anaesthetized under light chloroform anesthesia. Different organs namely the heart, liver, lungs, spleen, and kidneys were carefully dissected out and weighed in grams. The relative organ weight of each animal was then calculated as follows,

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

e. Hematological assay

On the 31st day the blood samples were collected from external jugular vein under mild chloroform anesthesia for the estimation of hematological parameters like hemoglobin concentration, RBC, WBC, ESR and PCV (Kjeldsberg, 1998; Moura, 1982; Smith, 1995). Blood samples were collected in 10% EDTA/ saline of pH7.2.

f. Preparation of tissue homogenate

10% tissue homogenate was prepared by homogenizing 1.0g of chopped liver or kidney tissue in 10ml of 0.1M trisHCl homogenizing buffer at pH 7.5. The homogenate was used for assaying the enzyme activities and other biochemical parameters.

g. Biochemical parameters assayed

Biochemical parameters such as SGOT (Serum glutamate Oxaloacetate transaminase), SGPT (Serum glutamate pyruvate transaminase), ACP (Acid phosphatase), ALP (Alkaline phosphatase) and LDH in serum; urea, uric acid, creatinine, protein, glucose, calcium and oxalate in serum and urine were assayed.

Collection of urine sample

Before the day of sacrifice the rats were placed in metabolic cages and urine was collected for 24 hours. Urine was freed from faecal contamination. Rats were provided with water but no feed. Urine collected in 50 ml beaker maintained at 0^{o}C in an ice bath.

The collected urine samples were centrifuged for 10 minutes and any sediment present was discarded. The urine was used for further analysis.

Collection of serum sample

After the experimental regimen the animals were sacrificed by cervical decapitation under light ether anesthesia. Blood was collected and centrifuged for 10 min. at 2500 rpm. The serum supernatant was collected and then diluted with water in the ratio of 1:10. Aliquots of the diluted serum were then used for the determination of serum constituents and serum enzymic activities.

Chemicals

All the chemicals used in the present study were of analytical reagent grade.

Statistical analysis

The results of the biochemical estimations were reported as mean ± SD of six animals in each group. Total variations, present in a set of data were estimated by one way Analysis Of Variance (ANOVA) followed by the analysis of level of significance between different groups based on ANOVA using AGRES statistical package (Version 3.1). Difference among means were analyzed by least significant difference (LSD) at 5% level ($p<0.05$).

Results and Discussion

In the acute toxicity study, the rats were treated with different concentration of *Acalypha indica* from the range of 5mg/kgb.wt to 2000mg/kg b.wt which did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.

Table 2: Changes in body weight of rats during the treatment with different doses of *Acalyphaindica*

Dose	Day 7	Day 14	Day 21	Day 28
Group I (Control)	164.34 ±0.21	170.28 ± 0.06	170.18 ± 0.07	172.45 ± 0.15
Group II (100mg/kg b.wt)	164.18±0.04a^{ns}	170.50 ± 0.04a^{ns}	170.20 ± 0.05a^{ns}	172.37 ± 0.11a^{ns}
Group III (200mg/kg b.wt)	165.12±0.09b^{ns}	170.87 ± 0.10b^{ns}	170.47 ± 0.05b^{ns}	172.540. ± 11b^{ns}
Group IV (300mg/kg b.wt)	164.90±0.15c^{ns}	170.89 ± 0.12c^{ns}	170.27± 0.03c^{ns}	172.01 ± 0.09c^{ns}
Group V (400mg/kg b.wt)	164.09±0.05d^{ns}	170.56 ± 0.09d^{ns}	170.26 ± 0.05d^{ns}	172.78 ±0.08d^{ns}
Group VI (500mg/kg b.wt)	164.71± 0.15e^{ns}	170.17 ± 0.11e^{ns}	170.44 ± 0.05e^{ns}	172.74 ± 0.12e^{ns}

Values are expressed as mean ± SD of six animals

The symbol represents statistical significance ns – not significant Comparison between groups

a represents comparison between Group II and I, b represents comparison between Group III and I , c represents comparison between Group IV and I , d represents comparison between Group V and I ,e represents comparison between Group VI and I

In acute toxicity test the extract of *Acalyphaindica* was found to be non toxic at the dose level of 2000mg/ kg body weight. The dose selected for the sub acute toxicity study was 100mg, 200mg, 300mg, 400mg and 500mg /kg b.wt. All the animals were free of intoxicating signs throughout the dosing period of 30 days. No physical changes were observed throughout the dosing period. All rats showed significant increase in body weight compared to their initial values. However there was no significant difference between the different

treatment groups and the control, indicating that it did not have any adverse effects on the body weight, which is used to assess the response to the therapy of drug (Table 1). No mortality was observed during the whole experiment. During the dosing period and in the last day, the quantity of food and water intake by different dose groups was found to be comparable with control group. No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits. The weights of organs recorded did not show any significant differences in the treatment and the control group indicating that *Acalyphaindica* was not toxic to heart, kidney, liver, spleen and brain (Table 2).There was no significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups (Table3).

Table 3: Effects of ethanolic extract of *A.indica* on change in organ weight of control andexperimental rats (sub actute toxicity studies).

Organ weight (g)	Group I	Group II	Group III	Group IV	Group V	Group IV
Haert	1.75 ± 0.03	1.77 ± 0.04 a^{ns}	1.78 ± 0.02b^{ns}	1.75 ± 0.02 c^{ns}	1.75 ± 0.02 d^{ns}	1.77 ± 0.02e^{ns}
Kidny	1.32 ± 0.01	1.32 ± 0.06 a^{ns}	1.36 ± 0.01b^{ns}	1.35 ± 0.01 c^{ns}	1.29 ± 0.05d^{ns}	1.34 ± 0.03e^{ns}
Liver	8.27 ± 0.03	8.35 ± 0.02 a^{ns}	8.29 ± 0.03b^{ns}	8.38 ± 0.03 c^{ns}	8.4 ± 0.04d^{ns}	8.46 ± 0.01e^{ns}
Spleen	1.17 ± 0.02	1.15 ± 0.03 a^{ns}	1.18 ± 0.02 b^{ns}	1.20 ± 0.03 c^{ns}	1.19 ± 0.03d^{ns}	1.20 ± 0.03e^{ns}
Brain	1.85 ± 0.01	1. 88 ± 0.02 a^{ns}	1.86 ± 0.02b^{ns}	1.88 ± 0.03 c^{ns}	1.84 ± 0.02d^{ns}	1.85 ± 0.02e^{ns}

Values are expressed as mean ± SD of six animals
The comparison between groups and the statistical significance are as in table 2.

Table 4: Effects of ethanolic extract on haematological parameters of the control andexperimental rats (sub acute toxicity studies)

Hematological Parameters	Group I	Group II	Group III	Group IV	Group V	Group IV
Hb (g/dl)	14.2 ± 0.04	14.31± 0.09 a^{ns}	14.33 ± 0.07b^{ns}	14.39 ± 0.05 c^{ns}	14.57 ±0.04d^{ns}	14.66 ± 0.04e^{ns}
RBCmillions/ cu. Mm	8.59 ± 0.04	8.51 ± 0.04 a^{ns}	8.61 ± 0.04b^{ns}	8.52 ± 0.04 c^{ns}	8.51 ± 0.03d^{ns}	8.49 ± 0.0e^{ns}
WBC cells/ cu. Mm PCV (%)	6.31 ± 0.06	6.41 ± 0.03 a^{ns}	6.33 ± 0.04b^{ns}	6.40 ± 0.05 c^{ns}	6.49 ± 0.05d^{ns}	6.51 ± 0.04e^{ns}
PCV (%)	35.50 ± 0.04	35.5 ± 0.02 a^{ns}	35.47 ± 0.02b^{ns}	35.48 ± 0.03 c^{ns}	35.47 ± 0.02d^{ns}	35.49 ± 0.02e^{ns}
ESR (mm/1 st hr.)	3.46 ± 0.06	3.48 ± 0.04 a^{ns}	3.44 ± 0.06b^{ns}	3.45 ± 0.09 c^{ns}	3.42 ± 0.09 d^{ns}	3.50 ± 0.04e^{ns}

Values are expressed as mean ± SD of six animals
The comparison between groups and the statistical significance are as in table 2

Table 5: Effect of ethanolic extract of *A.indica* on serum biochemical markers in control and experimental rats (sub acute toxicity studies).

Parameters Group I	Group I	Group II	Group III	Group IV	Group V	Group VI
ACP$^{\$}$	6.29 ± 0.7	63.25 ± 0.68 a^{ns}	6 62.90 ± 0.54 b^{ns}	63.69 ± 0.96 c^{ns}	63.50 ± 0.35d^{ns}	63.56 ± 0.16e^{ns}
ALP$^{\$}$	76.79 ± 0.53	77.01 ± 0.68 a^{ns}	76.66 ± 0.61b^{ns}	78.07 ± 0.68 c^{ns}	78.12 ± 0.55d^{ns}	79.56 ± 1.15e^{ns}
AST$^{\#}$	76.97 ± 0.73	76.74 ± 0.54 a^{ns}	76.81 ± 0.83b^{ns}	77.4 ± 0.61 c^{ns}	77.70 ± 0.61d^{ns}	77.37 ± 1.37e^{ns}
ALT$^{\#}$	33.43 ± 0.92	33.98 ± 0.92 a^{ns}	33.20 ± 0.68b^{ns}	33.73 ± 0.37 c^{ns}	34.09 ± 1.48d^{ns}	35.57 ± 0.10e^{ns}
LDH$^{\#}$	113.10 ±0.94	113.1 ± 0.57 a^{ns}	113.1 ± 0.60b^{ns}	113.1 ± 0.59 c^{ns}	113.19 ± 0.42d^{ns}	115.12 ± 0.87e^{ns}

Values are expressed as mean ± SD of six animals
The comparison between groups and the statistical significance are as in table 2
Units $^{\$}$µ moles of phenol liberated / L , $^{\#}$µ moles of pyruvate liberated / L

Table 6: Effect of alcoholic extract of *A. indica* on serum biochemical parameters in control and experimental rats

Parameters	Group I	Group II	Group III	Group IV	Group V	Group IV
Protein ψ	7.47 ± 0.17	7.52 ± 0.19 a^{ns}	7.47 ± 0.13 b^{ns}	7.61 ± 0.13 c^{ns}	7.44 ± 0.16 d^{ns}	7.66 ± 0.25e^{ns}
Urea★	5.55 ± 0.05	5.56 ± 0.06 a^{ns}	5.55 ± 0.04b^{ns}	5.78 ± 0.15 c^{ns}	5.77 ± 0.10d^{ns}	5.72 ± 0.17e^{ns}
Uric acid★	4.30 ± 0.07	4.44 ± 0.07 a^{ns}	4.33 ± 0.07b^{ns}	4.24 ± 0.07 c^{ns}	4.21 ± 0.08 d^{ns}	4.37 ± 0.09e^{ns}
Creatinine★	0.71 ± 0.05	0.65 ± 0.07 a^{ns}	0.68 ± 0.06 b^{ns}	0.80 ± 0.06 c^{ns}	0.81 ± 0.11d^{ns}	0.91 ± 0.05e^{ns}
Glucose★	110.3 ±0.15	110.32± 0.06 a^{ns}	110.32 ± 0.07b^{ns}	110.35± 0.76 c^{ns}	110.53± 0.03d^{ns}	110.88 ± 0.06e^{ns}
Calcium★	6.91 ± 0.53	7.02 ± 0.43 a^{ns}	6.71 ± 0.45 b^{ns}	7.26 ± 0.51 c^{ns}	6.74 ± 0.45d^{ns}	8.21 ± 0.71e^{ns}
Oxalate ★	2.54 ±0.03	2.45 ± 0.06 a^{ns}	2.45 ± 0.06 b^{ns}	2.45 ± 0.06 c^{ns}	2.4 5 ± 0.06d^{ns}	2.60 ± 0.05e^{ns}

Values are expressed as mean ± SD of six animals
The comparison between groups and the statistical significance are as in table 2
Units ψg/dl, ★mg/dl

Table 7: Effect of ethanolic extract *of A. indica* on urine biochemical parameters in control andexperimental rats.

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Protein ψ	2.47± 0.06	2.61 ± 0.09 a^{ns}	2.72 ± 0.11b^{ns}	2.62 ± 0.09 c^{ns}	2.66 ± 0.08 d^{ns}	2.79 ± 0.10e^{ns}
Urea★	8.3 7± 0.05	8.42 ± 0.07 a^{ns}	8.40 ± 0.06 b^{ns}	8.40 ± 0.05 c^{ns}	8.51 ± 0.04 d^{ns}	8.76 ± 0.03e^{ns}
Uric acid★	3.61 ± 0.04	3.52 ± 0.0 a^{ns}5	3.60 ± 0.05b^{ns}	3.6 8 ± 0.04 c^{ns}	3.61 ± 0.04 d^{ns}	3.67 ± 0.06e^{ns}
Creatinine★	0.70 ± 0.05	0.68 ± 0.03 a^{ns}	0.68 ± 0.0b^{ns}	0.69 ± 0.04 c^{ns}	0.67 ± 0.04d^{ns}	0.81 ± 0.05e^{ns}
Calcium★	0.56 ± 0.10	0.48 ± 0.09 a^{ns}	0.53 ± 0.14b^{ns}	0.43 ± 0.06 c^{ns}	0.40 ± 0.03d^{ns}	0.47 ± 0.05e^{ns}
Oxalate ★	0.64 ± 0.06	0.61 ± 0.04 a^{ns}	0.63 ± 0.03 b^{ns}	0.65 ± 0.03 c^{ns}	0.69 ± 0.03d^{ns}	0.73 ± 0.02e^{ns}

Values are expressed as mean ± SD of six animals
The comparison between groups and the statistical significance are as in table 2
Units ψ g/ 24 hour urine , ★mg/24 hour urine

Results of biochemical studies showed that there was no significant increase in the levels of the parameters at different doses ACP, ALP, AST, ALT and LDH in the different groups of animals treated with (100,200,300,400,500mg/kg b.wt) of the extract compared with control. This implies that the extract at the doses tested had no effects on the liver and kidney tissues (Table4). Table5 and 6 show the effect of the plant extract on protein, urea, uric acid, creatinine, glucose, calcium and oxalate in serum and urine of control and experimental rats respectively. This result showed that the plant extract at different levels tested did not produce considerable change in the levels of the different parameters tested.

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