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TOXICITY STUDY OF ALCOHOLIC EXTRACT OF THE AERIAL PARTS OF *ARISTOLOCHIA INDICA* L.

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ABSTRACT
Aristolochia indica L. (Family Aristolochiaceae) has been used for the treatment of various ailments from the ancient time. The present study was conducted to investigate the effects of ingestion of the alcoholic extract of the plant on liver and kidney functions in Wistar albino rats. Acute oral toxicity test was performed to determine the LD_{50}, sub chronic toxicity was then carried out by the oral administration of different doses of the extract on daily basis to different groups of rats for 28 days. The animals were sacrificed and biochemical study was done. The acute oral toxicity result revealed that LD_{50} of the alcoholic extract of *A. indica* was more than 7500mg/kg body weight but less than 10,000 mg/kg body weight. The result of sub chronic toxicity revealed that liver and kidney function of different groups receiving different doses of *A. indica* was affected as the SGOT, SGPT, total protein, urea nitrogen and creatinine was significantly elevated and uric acid level was significantly declined as compared to control group.
Keywords: LD_{50}, acute toxicity, sub chronic toxicity, *Aristolochia indica*

INTRODUCTION

Herbal drugs constitute a major part in all the traditional systems of medicine. There are approximately 1250 Indian medicinal plants, which are used in formulating therapeutic preparation according to Ayurveda and the other traditional system of medicine[1]. Herbs and species are generally considered safe and proved to be effective against certain ailments, while literature has documented severe toxic reactions from the use of herbs on many occasions still the potential toxicity of herbs has not been recognized by the general public or by professionals groups of traditional medicine[2,3].

Aristolochia indica L. (Family Aristolochiaceae) commonly known as Ishwari, Nakuli or Gandhanakuli possesses good medicinal value in traditional system of medicine. It is used as appetizer, aphrodisiac and anthelmintic. This has been used in skin diseases and fresh juices are antidote to snake poison. It possesses antioestrogenic, antifertility, abortifacient and interceptive activity. It is used ethanomedicinally as an antitumor, anti-inflammatory, antibacterial, antioxidant and antimicrobial[4-11].

MATERIAL AND METHODS

Collection of the plant material

The leaves were collected in the month of February 2011 from the Jhansi, Uttar Pradesh, India. The plant was identified and authenticated by Dr. Tariq Husain, National Botanical research Institute, Lucknow. The specimen was deposited in LWG herbarium with Accession No. 97858 for further reference.

Processing of the plant material

The leaves were collected, shade dried, coarsely powdered and passed through the mesh no. 40. It was stored in an air tight container.

Extraction procedure

The powder of aerial part was defatted with the petroleum ether and followed by the extraction with alcohol by soxhlet apparatus.

Animals

Swiss albino male mice (25-35gm) and Wistar albino rats (150-200g) of both sex were obtained from the animal house of the college of pharmacy, Institute of Foreign Trade and Management, Moradabad were used for the study. The animals were housed in spacious polypropylene cages. They were maintained for 12 hr in light and dark cycle at 28±2ºC in a well ventilated house, with free access to food and water *ad libitum*. The mice and rats were acclimatized to laboratory conditions for 5 days prior to commencement of the experiment. All the animal experiment was performed according to the ethical committee (IAEC No.837/ac/04/CPCSEA).

Acute oral toxicity/LD_{50} Determination

The acute oral toxicity was performed according to the OPPTS (office of prevention, pesticides and toxic substance) guidelines following Up and Down method[15,18]. Swiss albino male mice (25-30) were fastened overnight, food but not water was withheld. Animals were weighed; limit and main test were performed. The limit test is carried out first at 1000mg/kg body weight for one animal and if animal dies, main test is performed. If the animal survive two more animals are dosed, if both survives the test should be terminated. The main test is performed with an initial dose of 1000mg/kg body weight. The sequence followed is 1000, 2000, 5000, 7500 and 10000 mg/kg body weights. First one animal is dosed with 1000mg/kg body weight. If the animal dies a much lower dose is tested. If animal survives then two more animals are dosed, after 48 hours observation of the first animal. If survive, then the main test should be terminated. If the animal dies, two more animals are dosed and observed. The dosing is stopped when once the following stopping criteria are met.

1) 3 consecutive animals survive at the upper end.
2) 5 reversals occur in any 6 consecutive animals tested.
3) At least 4 animals have followed the first reversals and the specified likelihood ratios exceed the critical value.

The control rats received vehicle (Tween 80, 2% p.o) only

Sub chronic toxicity in rats

Healthy Wistar albino rats of both serves were randomly assigned to control and treatment groups. The Aristolochia indica alcoholic extract was suspended in 2% Tween 80 and administered orally on a daily basis for 28 days at doses of 1500 mg/kg body weight. All the rats were anesthetized under chloroform inhalation at the end of 28 days. Blood samples were collected for and centrifuged at 2000 rpm for 10 minutes. The serum was separated and stored at -70ºC until use for biochemical analysis[17].

Measurement of serum Liver Enzymes

SGOT, SGPT and total protein were measured to assess the damage to the hepatic parenchyma on 29th days after AEAI administration using a semi autoanalyser[13,14,16,19].

Measurement of renal function test

Blood urea nitrogen, creatinine and uric acid levels in serum were measured to assess the nephrotoxicity on 29th day after Aristolochia

indica aqueous extract administration using a semi autoanalyser[13,14,16,19].

Statistical analysis

The results are expressed as Mean±SEM and the difference between experimental groups were compared by one way analysis of variance followed by Dunnett's test. Dunnett's test were considered statistically significant when P<0.01.

RESULT

In the acute oral toxicity study, there was no mortality in control group of mice upto the dose of 10,000 mg/kg body weight but in the treated group mortality was observed at the dose of 10,000mg/kg body weight. The results are shown in table 1. Hence, 1/5th of the dose 7500mg/kg body weight was selected for sub chronic activity.

In the sub chronic toxicity study, the food consumption was equal in the treated groups. All the biochemical parameters SGOT, SGPT, total protein, creatinine and urea were found more significant compared to control group than the treated groups except uric acid level which was more in control group than the treated groups. It is also observed that females are more affected by the AI as compared to male. The result are tabulated in table 2 and represented by graph 1.

DISCUSSION

Acute toxicity investigation is the first step in the toxicological investigation of an unknown substance. The index of acute toxicity is the LD_{50}.

The result of acute oral toxicity (LD_{50}) of AEAI was found to be greater than 7500mg/kg body weight as no mortality was recorded in any group of experimental rats but less than 10,000mg/kg body weight as mortality was observed at this dose.

In sub chronic toxicity, SGOT, SGPT, Total protein, creatinine and blood urea nitrogen shows statistically significantly (p>0.01) levels when compared to control group and uric acid compared to control shows statistically significant decreased level. Clinical data shows that SGOT and SGPT level rise in the case of cirrhosis, metastatic carcinoma, obstructive jaundice and viral hepatitis while the increased urea, creatinine are associated with decreased renal function and increased tissue breakdown . Uric acid aids in diagnosis of Gout and mycloproliferation disorders.

In conclusion, results of serum biochemical analysis revealed that treatment with dose of AEAI (1500mg/kg body weight respectively) significantly increased blood levels of AST, ALT and Total Protein, indicating liver toxicity and increased blood urea nitrogen and creatinine and significantly decreased level of uric acid as compared to control group showing that kidney function and liver were affected. Female rats are more affected as compared to the male rats.

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Table 1: Result of acute toxicity study of AEAI

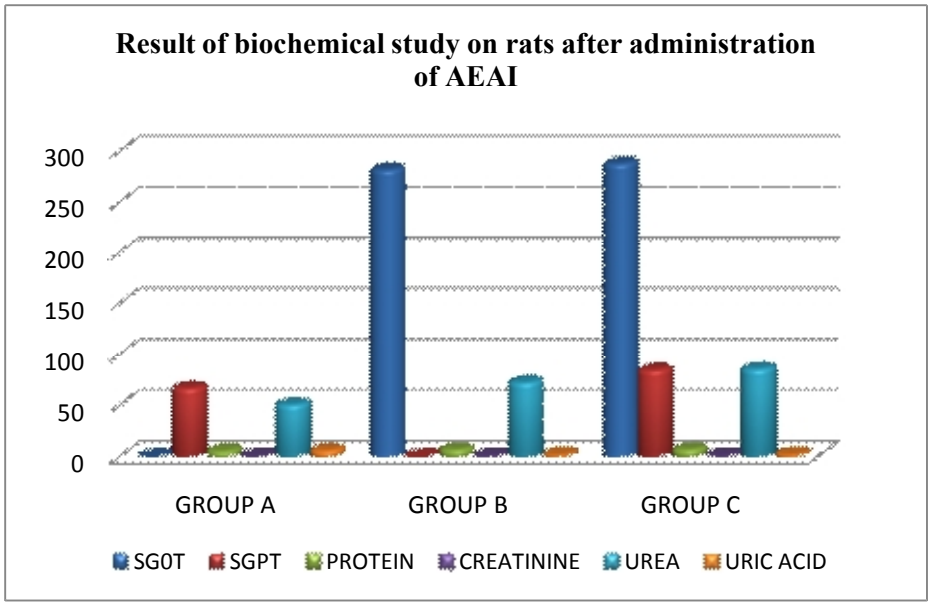
S.No.	Treatment	Dose mg/kg body weight					Inference
		1000	2000	5000	7500	10,000	
1	Control Tween 80 (2% p.o)	+	+	+	+	+	Stop dosing
2	AEAI 1500mg/kg	+	+	+	+	-	Stop dosing

Where (+) =Survival and (-) = Death

Table 2: Serum chemistry and liver and kidney function test after treatment with AEAI

Parameters	Group A control	Group B(Male) 1500 mg/kg	Group C(Female) 1500mg/kg
SGPT(U/L)	67.16 ±0.00	77.15±0.00	84.86±0.01*
SGOT(U/L)	233.25±0.43	282.82±0.04	288.31±0.08*
Protein(g/dl)	6.03±0.00	7.24±0.02	7.38±0.05*
Creatinine(mg/dl)	0.72±0.00	0.93±0.00	0.98±0.00*
Urea(mg/dl)	51.14±0.00	72.68±0.00	85.76±0.00*
Uric acid(mg/dl)	6.43±0.00	2.61±0.00*	2.76±0.00*

Results are presented as Mean±SEM, n=6, *P>0.01



Graph 1: Biochemical result of study on rats for renal and hepatic toxicity after administration of AEAI.

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