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. 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. 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, antiinflammatory, antioxidant and antinfectious and antimicrobial.

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±2°C 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/LD₅₀ Determination
The acute oral toxicity was performed according to the OPPTS (office of prevention, pesticides and toxic substance) guidelines following Up and Down method. 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 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 sexes 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 70°C until use for biochemical analysis.

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. 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.

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\textsuperscript{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, 1/5\textsuperscript{th} 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\textsubscript{50}. The result of acute oral toxicity (LD\textsubscript{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 myeloproliferation 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.

**REFERENCES**

2. Deng JF: Clinical Toxicity of Herbal Medicine in Taiwan. 7th International Conference on Health Problems Related to the Chinese. 1994.

**Table 1: Result of acute toxicity study of AEAI**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Dose mg/kg body weight</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td></td>
<td>Stop dosing</td>
</tr>
<tr>
<td></td>
<td>Tween 80 (2% p.o)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>AEAI</td>
<td>+</td>
<td>Stop dosing</td>
</tr>
<tr>
<td></td>
<td>1500mg/kg</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A control</th>
<th>Group B(Male) 1500 mg/kg</th>
<th>Group C(Female) 1500mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT( U/L)</td>
<td>67.16±0.00</td>
<td>77.15±0.00</td>
<td>84.86±0.01*</td>
</tr>
<tr>
<td>SGOT(U/L)</td>
<td>233.23±0.43</td>
<td>282.82±0.04</td>
<td>238.31±0.08*</td>
</tr>
<tr>
<td>Protein(g/dl)</td>
<td>6.03±0.00</td>
<td>7.24±0.02</td>
<td>7.38±0.05*</td>
</tr>
<tr>
<td>Creatinine(mg/dl)</td>
<td>0.72±0.00</td>
<td>0.93±0.00</td>
<td>0.98±0.00*</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>51.14±0.00</td>
<td>72.68±0.00</td>
<td>85.76±0.00*</td>
</tr>
<tr>
<td>Uric acid(mg/dl)</td>
<td>6.43±0.00</td>
<td>2.61±0.00*</td>
<td>2.76±0.00*</td>
</tr>
</tbody>
</table>

Results are presented as Mean±SEM, n=6, *P>0.01
Result of biochemical study on rats after administration of AEAI

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

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