TOXICOLOGICAL SCREENING OF *TRICHOSANTHUS ANGUINA* LINN ROOTS IN EXPERIMENTAL ANIMALS

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ABSTRACT

*Trichosanthus anguina* Linn (*T. anguina*) an important medicinal plant in Indian traditional system of medicine and is widely used in many countries by different tribal. Despite the wide use of *T. anguina* in folk medicine, no study has been published in the scientific literature about its toxicological profile. In present study various extract of *T. anguina* (Cucurbitaceae) roots was evaluated for the acute and subacute toxicity. In the acute toxicity test, oral administration of 2 g/kg of *T. anguina* roots extracts produced neither mortality nor changes in behavior or any other physiological activities in rats. In subacute toxicity studies, no mortality was observed when the two doses of 1 or 2 g/kg day of methanolic extract of *T. anguina* roots were administered p.o. for a period of 28 days in rats. There were no significant changes occurred in the body weight, organ weight and behaviour changes in both sexes of animals. There were no significant differences in the body and organ weights between controls and treated animals of both sexes. Pathologically, neither gross abnormalities nor histopathological changes were observed. Methanolic extract *T. anguina* roots was found safe in acute and subacute toxicities while chronic toxicity studies are further required for the support of the safe and sound use of this traditional plant.

KEYWORDS: *Trichosanthus anguina*, Cucurbitaceae, Traditional medicine, Acute and subacute toxicology.
INTRODUCTION

Trichosanthes anguina Linn is an annual, dioecious climber belonging to the family Cucurbitaceae.\(^1\) The whole plant including roots, leaves, fruits, seeds have medicinal properties. The root is used as a cure for bronchitis, headache and boils. The fruit is used as an anthelmintic in French Guiana. Both the root and fruit are considered to be cathartic. Externally, the leaf juice is rubbed over the liver to relieve liver congestion. The seeds are used for stomach disorders in Malabar Coast and are also considered antifebrile and anthelmintic. The aerial parts of Trichosanthes anguina Linn are used along with other plant materials for indigestion, bilious fevers, boils, sores, skin eruptions such as eczema, dermatitis, psoriasis, ulcers and diabetes.\(^2,3\) The plant, represented by Trichosanthes anguina Linn. is medicinally important plant and traditionally reported liver-protective herb of the Cucurbitaceae family commonly known as ‘Padaval’ is a commonly found on the Kokan, Western Ghats and Western coasts of India.\(^4\) Roots of this plant having yellowish brown color with 3-6 cm long, wavy shape.\(^5\) In the folk medicine, the roots of this plant has been known since ancient times for treatment of jaundice and is curative properties and has been utilized for treatments of various ailments such as purgative and tonic.\(^5,6\) The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active.\(^7\) Pharmacological review of the selected plant shows that anti inflammatory, cytotoxic activity, larvicidal activity, antidiabetic activity, anti fertility activity, gastroprotective activity and anti bacterial activity.\(^8\) In the ethnobotanical claims, the roots of this plant are used for the treatment of jaundice and other hepatic diseases by the folk tribes of Trimbakeshwar Hills, Maharashtra state, India. Despite the wide use of T. anguina in folk medicine, no study has been published in the scientific literature about its toxicological profile.\(^21\) The present study was designed to determine the acute and subacute oral toxicity of the methanolic extract of roots of T. anguina on experimental animals.\(^8,9\)

MATERIALS AND METHODS

Plant Material

The plant, T. anguina were collected in Trimbakeshwar Hills, Nashik District (Maharashtra) in June 2012. The plant was authenticated and herbarium deposited in Botanical Survey of India (Ref. No. BSI/WC/Tech/2012/79). The roots of the plant was dried, powdered and passed through 40 mesh sieve and stored in an airtight container for further use.\(^9\)
Preparation of Extract
The air-dried roots of *T. anguina* were made into a coarse powder. The powdered material was defatted with petroleum ether. The defatted material was extracted with methanol using a Soxhlet extractor. Then the extract was filtered through muslin and the filtrate was evaporated under reduced pressure and vacuum-dried. All the extracts were administered to the animals as a suspension in gum acacia.

Procurement of Animals
Mice of either sex (three females and three males, weight: 25-35 g, age: 6-8 weeks) for acute toxicity study and Adult Wistar rats (weight: 150–250 g; age: 6–8 weeks old) for subacute toxicity study of either sex were obtained from the National Institute of Biosience, Pune, Maharashtra, India. The rats were maintained under controlled temperature, 12 h light/12 h dark conditions for 1 week before the start of the experiments to acclimatize to laboratory conditions. They were allowed to feed standard rodent pellet diet and water ad libitum. The study protocol was approved by the IAEC (Institutional animal ethics committee of CPCSEA, Govt. of India, Registration Number 1367/AC/10/ CPCSEA.).

Toxicological Screening
Acute toxicity studies
The acute toxicity of the methanolic extract of the *T. anguina* evaluated in mice using the up and down procedure of OECD (The Organization of Economic Co-operation Development) guideline for testing of chemical: 425 for acute oral toxicity test. Mice of either sex (three females and three males, weight: 25-35 g, age: 6-8 weeks) received various extract of the *T. anguina* starting at 2000 mg/kg orally by gavage. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. Finally, the number of survivors was noted after 24 h and these animals were then maintained for further 13 days with observations made daily.

Subacute toxicity
Eighteen rats (weight: 150–250 g; age: 6–8 weeks old) were randomly assigned into three groups (*n* = 6), were housed in each group. Treatments were administered orally by oral gavage daily once a day for 28 days. The first group of rats, serving as control, received gum acacia (5 mg/kg); the second and third group received the methanolic extract of *T. anguina* at doses of 1000 mg/kg and 2000 mg/kg, respectively. All animals were supplied with standard
food and tap water *ad libitum* during the testing periods. All rats were observed daily for physiological and behavioral changes.\[^{13}\]

**Observation and examination methods**

Clinical signs were observed at least once a day through the 28 days of dosing. Body weight and weight gain were measured once a week.

**Statistical analysis**

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnette’s multiple comparison test. The values are expressed as mean ± SEM and P<0.05, P<0.01 was considered significant.

**RESULT**

**Acute Toxicity**

There was no mortality or any sign of behavioral changes or toxicity observed after oral administration of various extract of *T. anguina* upto the doses level of 2000 mg/kg. During the observation period animals did not produce any variations in the general appearance.

**Subacute toxicity**

During this study, no death were observed, no significant clinically relevant changes were observed in general behavior and other physiological activities in the present study. All parameters are within acceptable limit.

**Body weight**

No significant differences either in the control or treated group were noticed in various parameters like, body weight, organ weight & etc.

**Effect on serum level of enzyme and biochemical parameters**

The levels of serum serum glutamic oxaloactate transminase (SGOT)\[^{14}\], serum glutamic pyruvate transaminase (SGPT)\[^{14}\], serum alkaline phosphatase (SALP)\[^{15}\], total bilirubin (TB)\[^{16}\], total proteins (TP)\[^{17}\], total cholesterol (TC)\[^{18}\] and total triglyceride (TG)\[^{19}\] were no significantly varied in the animals groups treated with methanolic extracts against control group. Treatment with methanolic extract showed highly safer and no any significant changes in serum level with no toxicity symptoms. So, the methanol extract treated group is safer, non-toxic and superior to its effectiveness for therapeutic action.
Histopathological examination

There were no any abnormality or alteration was detectable in histopathological examinations of the organs in both groups.

Table 1: Acute Toxicity Study of extracts of methanolic extracts of *T. anguina* roots.

<table>
<thead>
<tr>
<th>Design of treatment</th>
<th>Dose (mg/kg)</th>
<th>Mice Sex</th>
<th>Mortality D/T</th>
<th>Mortality latency (h)</th>
<th>Symptoms of Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic Extract</td>
<td>2000</td>
<td>Male</td>
<td>0/3</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>0/3</td>
<td>-</td>
<td>None</td>
</tr>
</tbody>
</table>

D/T, number of dead/number of treated mice; (-) no mortality observed during the period of observation

Table 2: Effects of subacute toxicity study of methanolic extract of *T. anguina* roots.

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of treatment</th>
<th>Dose (mg/kg)</th>
<th>Rats Mortality D/T</th>
<th>Mortality latency (h)</th>
<th>Symptoms of Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>0/6</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>II</td>
<td>Methanolic extracts</td>
<td>1000</td>
<td>0/6</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>III</td>
<td>Methanolic extracts</td>
<td>2000</td>
<td>0/6</td>
<td>-</td>
<td>None</td>
</tr>
</tbody>
</table>

D/T, number of dead/number of treated rats;

(-) no mortality observed during the period of observation

Table 3: Body and organ weights (in grams) of the rats following a 28-days subacute toxicity of oral treatment with methanolic extracts of *T. anguina* roots.

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of treatment</th>
<th>Dose (mg/kg)</th>
<th>Body Weight (gm)</th>
<th>Liver (gm)</th>
<th>Kidney (gm)</th>
<th>Brain (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>151.33 ± 0.8433</td>
<td>4.145±0.09025</td>
<td>1.277± 0.004216</td>
<td>0.6500±0.004472</td>
</tr>
<tr>
<td>II</td>
<td>Methanolic extracts</td>
<td>1000</td>
<td>153.33±1.022 ns</td>
<td>4.242±0.06375 ns</td>
<td>1.287± 0.004216 ns</td>
<td>0.6483±0.01138 ns</td>
</tr>
<tr>
<td>III</td>
<td>Methanolic extracts</td>
<td>2000</td>
<td>154.00±0.3651 ns</td>
<td>4.125±0.07473 ns</td>
<td>1.258±0.007032 ns</td>
<td>0.6683±0.003073 ns</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6.

*P< 0.05, **P<0.01 as compared with control

Data were analyzed by using One way ANOVA followed by Dunnett’s Multiple Comparison test.
Table 4: Effect of methanolic extracts of *T. anguina* roots on enzyme serum levels in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of treatment</th>
<th>Dose (mg/kg)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>SALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>49.91 ± 2.11**</td>
<td>69.48 ± 1.41**</td>
<td>142.60 ± 8.07**</td>
</tr>
<tr>
<td>II</td>
<td>Methanolic extracts</td>
<td>1000</td>
<td>68.31 ± 2.02**</td>
<td>89.48 ± 1.41**</td>
<td>284.50 ± 8.52*</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>2000</td>
<td>71.98 ± 19.01**</td>
<td>96.07 ± 1.37**</td>
<td>144.90 ± 6.52*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6.

*P< 0.05, **P<0.01 as compared with control

Data were analyzed by using One way ANOVA followed by Dunnett’s Multiple Comparison test.

Table 5: Effect of methanolic extracts of *T. anguina* roots on biochemical parameters in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>TB (mg/dl)#</th>
<th>TP (mg/dl)#</th>
<th>TC (mg/dl)#</th>
<th>TG (mg/dl)#</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>0.72 ± 0.07**</td>
<td>6.43 ± 0.01**</td>
<td>81.90 ± 0.30**</td>
<td>177.10 ± 11.75**</td>
</tr>
<tr>
<td>II</td>
<td>Methanolic extracts</td>
<td>1000</td>
<td>0.78 ± 0.05**</td>
<td>6.48 ± 0.60**</td>
<td>86.31 ± 2.39*</td>
<td>178.93 ± 1.01**</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>2000</td>
<td>0.82 ± 0.04*</td>
<td>6.57 ± 0.01**</td>
<td>89.11 ± 0.53*</td>
<td>181.77 ± 0.97**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6.

*P< 0.05, **P<0.01 as compared with control

Data were analyzed by using One way ANOVA followed by Dunnett’s Multiple Comparison test.
Figure 1: Effects of subacute toxicity study of methanolic extract of *T. lampas* stems on histopathological changes in rats.

1A: Liver sections of normal control rats, showing normal hepatic cells with well-preserved cytoplasm;
1B-1C: Liver section of methanolic extract treated rats showing no any massive fatty changes and the loss of cellular boundaries;
2A: Kidney section of normal control rats, showing normal tubular cells
2B-2C: Kidney section of methanolic extract treated rats showing no any alteration in the normal cells and the loss of cellular boundaries.
3A: Brain section of normal control rats, showing normal cells.
3B-3C: Brain section of methanolic extract treated rats showing no any alteration in the normal cells and the loss of cellular boundaries.

CONCLUSION

The plant, *Trichosanthus anguina* L. is reported to be useful in traditional medicine for the treatment of some ailments, it was planned to evaluate it for its ethno medicinal uses and traditional claim for hepatoprotective as a folk medicine. Thus, it can be concluded that, present study gives some scientific evidences on effect of *T. anguina* roots various extract having better efficacy and significant hepatoprotective and antioxidant activity against liver toxicity.\[^9\] The acute oral toxicity test of *Trichosanthes anguina* roots extracts as per OECD guidelines 425 using up and down procedure showed methanolic extract is safer and nontoxic at dose level of 2000 mg/ kg and its LD 50 is greater than 2000 mg/kg. So, acute toxicity test concluded that, methanolic extract of *Trichosanthes anguina* roots are safer and nontoxic. Also, the sub acute oral toxicity test of *Trichosanthes anguina* roots extracts as per OECD guidelines 425 using up and down procedure showed methanolic extract is safer and nontoxic.
at dose level of 2000 mg/kg and its LD 50 is greater than 2000 mg/kg. So, sub acute toxicity test concluded that, methanolic extract of Trichosanthes anguina roots are safer and nontoxic.

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REFERENCES

CITATION OF THIS ARTICLE