Anti-inflammatory Activity of *Trichosanthes cucumerina* L. var. *cucumerina* seeds.

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Summary

The *Trichosanthes cucumerina* L. var. *cucumerina* (Cucurbitaceae) is an Ayurvedic, traditional medicinal plant in India. This study was conducted to evaluate the anti-inflammatory activity of chloroform and ethanol extracts of *T. cucumerina* L. var. *cucumerina* seed in carrageenan induced paw oedema in wistar rats at the dose level of 200 and 400mg/kg administrated orally. Both the extracts exhibited significant anti-inflammatory activity, which supports the traditional medicinal utilization of the plant. This study established anti-inflammatory activity of the seed of *T. cucumerina* L. var. *cucumerina.*

Key words: *Trichosanthes. cucumerina* L. var. *cucumerina*, chloroform, ethanol, anti-inflammatory.

Introduction

T. cucumerina L. var. *cucumerina* (Cucurbitaceae) commonly is known by various vernacular names. In English it is known as *Chinese cucumber, wild snake gourd*; Hindi, *Jangali chichonda, kadu padaval*; Chinese, *Gua Ye Gua Lou*; French, *Patol De Malabar*; Kannada, *Bettada padavala, kahi padavala.* It is found distributed in India, Srilanka, Nepal, Australia and North America (1) Nijeria (2) and Pakistan (http://www.EFloras.org). *T. cucumerina* L. var. *cucumerina* is an ayurvedic medicinal plant, whose parts were pharmacologically proven to posses hypoglysimic (http://www.yourproduceman.com/news_august_22_05.html; (5), antibacterial (6) and antiovulatory activity (7), the present study has been made to investigate the anti-inflammatory effects of the *T. cucumerina* L. var. *cucumerina* seed in wistar rats.

Materials and Methods

Plant materials

The fully mature *T. cucumerina* L. var. *cucumerina* seeds were collected in August-September 2006 from Khanapur forest District Bidar, Karnataka. Before conducting any of the investigations on any medicinal plant, their correct botanical identification and authentication forms the most crucial basis. Therefore, the specimen plant material of the species was collected and has been identified with the help of *Flora of The Presidency of Madras* (8), *The Flora of Karnataka* (9) and *The Flora of Gulbarga District* (10). A specimen of the species was deposited in the Herbarium of Botany department, Gulbarga University, Gulbarga (Voucher No. HGUG-804). Also, the plant was confirmed with authenticated herbariums at The Centre for Ecological Studies, IISc, Bangalore, and Botanical Survey of India, Pune.

Preparation of extracts

The *T. cucumerina L. var. cucumerina* fruits were first washed well and pulp was removed from the seeds. Seeds were washed several times with distilled water to remove the traces of pulp from the seeds. The seeds were dried at room temperature and coarsely powdered. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with chloroform and methanol using cold percolation method. The percentage yields were 0.81% in chloroform and 2.37% in ethanol. The phytochemical screening gave positive results for triterpenoids, phenols, flavonoids, saponins and tannins.

Animals

Wistar rats of either sex weighing 200-250g were taken for experimental study. They were acclimated to animal house conditions fed with commercial pellets (Hindustan Lever Ltd., Bangalore, India), and tap water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Preparation of the drug for the experimental study

Extracts and the standard drugs were administered in the form of suspension in water with 1% sodium carboxy methyl cellulose (SCMC) as suspending agent.

Acute toxicity studies

The acute toxicity study was performed according to the method Kattan et al., (11). Adult albino mice (20-25g) of either sex were divided into three groups containing ten animals in each group. Graded doses (200 and 400 mg/kg b.w.) of ethanol extract of *T. cucumerina* L. var. *cucumerina* in SCMC (1%) were administered orally by means of intragastric catheter to mice. Following administration of the extracts, the animals were observed for toxic symptoms continuously for 2h. And then frequently for further 4h, finally overnight. Mortality was recorded. Finally the number of survivors was noted after 24h and these animals were then maintained for further 7 days with observations made daily. Food and water were provided throughout the experiment.

Anti-inflammatory activity

The animals either sex was divided into six groups each composed of six animals.
Group I – Control animals received 1% 10 ml/kg p.o.
Group II – Animals received chloroform extract at the dose of 200 mg/kg p.o.
Group III – Animals received chloroform extract at the dose of 400 mg/kg p.o.
Group IV – Animals received ethanolic extract at the dose of 200 mg/kg p.o.
Group V – Animals received ethanolic extract at the dose of 400 mg/kg p.o.
Group VI- Standard Diclofenac sodium 5 mg/kg, p.o.

Paw oedema was induced injecting 0.1 ml of 1% carrageenan in physiological saline into the sub plantar tissues of the left hind paw of each rat (12). The extracts (chloroform and ethanol) were administered orally 30 min prior to carrageenan administration. The paw volume was measured at intervals of 60, 120, 180 and 240 min by the mercury displacement method using a plethysmograph. The percentage inhibition of paw volume in drug treated group was compared with the carrageenan control group (Group- I). Diclofenac sodium (5 mg/kg/p.o.) was used as reference drug.

Statistical analysis

Data obtained from the experiments are expressed as Mean ± SEM. Difference between the control and the treatments in these experiments were tested for significance using ANOVA followed by Dunnet's *t*–test.

Results and Discussion

The plant extracts did not exhibit any mortality up to the dose level of 1500 mg/kg. So, the extracts safe for long term administration. The chloroform and ethanol extracts of *T. cucumerina L. var. cucumerina* seed at the dose level of 200 and 400 mg/kg decreased the oedema significantly ($p < 0.001$) at 3^{rd} and 4^{th} h after administration of the extract. When compared to the control group. The effect was compared to the activity ($p < 0.001$) produced by standard drug diclofenac sodium at 3^{rd} and 4^{th} h after administration (Table-1). In the present study, the anti-inflammatory activity of the chloroform and ethanol extracts of *T. cucumerina* L. var. *cucumerina* seed has been established. The extracts were found significant to inhibit the carrageenan-induced rat paw oedema, a test which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (13). Carrageenan-induced inflammation is useful in detecting oraly active anti-inflammatory agents (14). Oedema formation due to carrageenan in the rat paw is a biphasic event (15). The initial phase is attributed to the release of histamine and serotonin (16). The extracts of *T. cucumerina* L. var. *cucumerina* seed possessed varying degree of anti-inflammatory activity when tested at various doses of 200 and 400mg/kg. The ethanol extract at the dose of 400mg/kg showed high significant anti-inflammatory activity at 4 h, where it caused 64.7% inhibition, as compared to that of 5 mg/kg of diclofenac sodium.

Table 1. Anti-inflammatory evaluation of *T. cucumerina* L. var. *cucumerina* extracts against carrageenan induced paw oedema in rats.

	Paw oedema volume (ml)			
Group	60 min	120 min	180 min	240 min
I	0.32± 0.14	0.48 ± 0.02	0.63 ± 0.01	0.67 ± 0.01
II	0.26±0.14 ** (20 %)	0.37 ± 0.02* (22.4 %)	0.40 ± 0.02*** (40 %)	0.33±0.02*** (51.7 %)
III	0.23± 0.16 ** (26.6 %)	0.36 ± 0.02* (24.4 %)	0.37 ± 0.02*** (42.7 %)	0.26 ± 0.03*** (55.7 %)
IV	0.29± 0.01 ** (22 %)	0.37 ± 0.03* (24.6 %)	0.37± 0.03*** (43.5 %)	0.32 ± 0.03*** (52.2 %)
V	0.23± 0.02*** (33.4 %)	0.34 ± 0.01** (26.3 %)	0.36 ± 0.02*** (47.1 %)	0.27 ± 0.03*** (66.6 %)
VI	0.22± 0.01*** (39.1 %)	0.36 ± 0.01** (38.6 %)	0.26± 0.02*** (56.8 %)	0.18 ± 0.08*** (72.8 %)

Values are mean ± SEM of 6 animals in each group. Comparisons were made between Group I Vs II, III, IV, V and VI. P- values: *p<0.05, **p<0.01, ***p<0.001 Percentage protection given on Parenthesis.

Conclusion

In conclusion, the results of the present study support to the traditional use of *T. cucumerina L. var. cucumerina* in inflammation. *T. cucumerina L. var. cucumerina* seed extract, possessing significant anti-inflammatory activity. This may be due to the presence of secondary metabolites like triterpenoids, phenols, flavonoids, saponins and tannins. which deserves further studies to establish its therapeutic value as well as its mechanism of action.

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