Vol 7|Issue 1| 2017 | 1-6.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

AN EXPERIMENTAL EVALUATION OF ULCERPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF TRICHOSANTHES CUCUMERINA IN DIFFERENT MODELS IN RATS

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ABSTRACT

The aim of the present study was to investigate the Gastro protective effect of selected Indian medicinal plant *Trichosanthes cucumerina* belonging to family: Cucurbitaceae. 50% ethanolic extact of *Trichosanthes cucumerina* was used for gastroprotective effects on experimental models in rats.All the drug solutions were prepared in 1% CMC solution and administered twice daily for five days drug treatment. Three distinct animal models were taken for efficient estimation of ulcerogenic activity. Model 1: *Pylorus ligation + aspirin induced gastric ulcer model, Model* 2: *Acetic acid induced gastric ulcer*, Model 3: *Ethanol-hydrochloric acid induced gastric ulcer*. After drug treatment the rats were sacrificed for measurement of ulcer index and chemical parameters. Result: The fruit extract showed a dose dependent effect on Anti ulcer activity in rats. Conclusion: our result showed that TCE possess significant gastro protective activity, which might be due to gastric defence factor.

Keywords: *Trichosanthes cucumerina* fruits, Ethanolic extract, Aspirin, Ulcer models.

INTRODUCTION

Disease and sickness have been a part of mankind's progress through the ages. It comes as no surprise that a substantial portion of the intellectual efforts of every historical age has been directed at understanding disease and finding cures. It is but natural that each of the formally organized and structured attempts has to depend on the knowledge base and paradigms that were available at the particular period of time when the system evolved and matured. As our view of the world changed, largely due to the efforts of science, newer systems of medicine emerged and older ones fell out of favour or even discarded. Over the last century or less, keeping pace with the advances made by modern science, the Western or allopathic medical system has been the dominant influence on the health care scene.

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There is no denying its contribution to our well-being. Yet, there remains the lingering reality that no single system of health care delivery is able to provide all the answers to health and disease. Traditional medicines have been the starting point for the discovery of many for more than a century, peptic ulcer disease has been a major cause of morbidity and mortality. In clinical practice, Peptic ulcers are a common disorder of the entire gastrointestinal tract (GIT) that occurs mainly in the stomach and the proximal duodenum. Generally ulcers develop due to imbalance between aggressive factors such as hydrochloric acid (HCL), pepsin, refluxed bile, leukotrienes (LTs), reactive oxygen species (ROS) and defensive factors such as mucus-bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood flow, cell renewal and migration. The success of commercially available antiulcer drugs in the treatment of gastric ulcer is usually overshadowed by various side effects. At present, approximately 25% of drugs in modern pharmacopoeia were derived from plants (phytomedicines) and many

others were synthetic analogues built on the prototype compounds isolated from plants. Indian folk medicine comprises of numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diarrhoea, scabies, venereal diseases, ulcers, snake bite etc.

Trichosanthes cucumerina fruit is known in English "snake gourd", in hindi "chichinda and is widely grown throughout India. It is a grown in gardens and road sides for shades The leaves of this plant have also been used in traditional system of medicine for overcoming problems like skin infection, wound healing [1].

MATERIALS AND METHOD

Collection & Authentification of Plant Material

The *Trichosanthes cucumerina* Roxb. fruits were collected from the subji mandi of basant vihar, kanpur. The fruits was identified and taxonomically authenticated by comparison with the herbarium.in the departmental herbarium of National Botanical Research Institute, Lucknow, India. The fresh fruits were washed with tap water, air dried homogenized to fine powder and stored in air tight bottle.

Preparation of Extracts

The air dried powder fruit (1000g) was exhaustively extracted with 50% ethanol (3x10L) and concentrated under reduced pressure Ethanolic extract of *Trichosanthes cucumerina* fruits were subjected to preliminary phytochemical screening for the detection of various plants constituents.

Animals Used

Sprague-Dawley rats (150 - 180 g) and albino mice (15 – 18 g) were procured from the central animal house of Central Drug Research Institute Lucknow, India. These were kept in the departmental animal house at 26 ± 2^{o}C and relative humidity 44 –56 %, light and dark cycles of 10 and 14 h respectively for 1 week before and during the experiment for acclimatization. The animals were provided with standard rodent pellet diet (Dayal, India) and the food was withdrawn 18-24 h before the experiment, though water was allowed *ad libitum*. The laboratory animals are promulgated by the Institutional Animal Ethical Committee. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration in experimental animals.

Phytochemical screening: Preliminary phytochemical screening of *Trichosanthes cucumerina* extract was performed for the presence of alkaloids, tannins, saponins, tannins, carbohydrates, triterpenoids, flavonoids and steroids

Acute toxicity studies

The adult male albino mice selected for acute toxicity study. Before the actual LD_{50} determination, a pilot study was made on a small group of mice mainly to select the dose ranges for the subsequent study. The fruit extract of *Trichosanthes cucumerina* were taken at various doses levels (100, 200, 400, 800, 1000, 2000, 4000 mg/kg b.wt) dissolved in 1 % carboxymethyl cellulose administered 10 ml/kg b.w. orally to pairs of mice per dose level. The control animals received 1 % carboxymethyl cellulose in distilled water (10 ml/kg) orally. The animals were observed continuously for two hour and then occasionally for further four hours and finally any mortality. Behavior (gross behavior, general motor activity, writhing, convulsion, response to tail pinching, pupil size, fecal output, water intake, feeding behavior, sedation *etc*.) of the animals and any other toxic symptoms also observed for 72 hours and the animals were kept under observation up to 14 days.

Pharmacological evaluation

The Albino rats are divided into five groups of five animals each. Animals were fasted 24 hours. Group I received normal saline 2ml/kg body weight (Control), group II, III, and IV were received extract of *Trichosanthes cucumerina* fruits in the dose of 100,200,400mg/kg body weight respectively by oral route. While the V group received Ranitidine 50mg/kg body weight by oral route. Three distinct models were employed for the study:

Model 1: Aspirin-pylorus ligation-induced gastric ulcer in rats

Ulceration in rats will be induced by administration of aspirin (a dose of 200 mg/kg orally) as described by Goel et al. (1985). *Trichosanthes cucumerina* extract (100,200 and 400 mg/kg), CMC (1ml/kg) as control and ranitidine (50 mg/kg) as positive control were administered 30 min before each aspirin treatment. On the fifth day pylorus part was ligated following 36 h fasting. The animals were sacrificed by with an over dose of ether after 4 h of pylorus ligation.. The stomach was opened and the percentage inhibition of ulcer was determined. The animals were sacrificed 4 hour later and stomach was then opened to calculate the ulcer indexby adding the total no.of ulcer per stomach and the total severity of ulcer per stomach [2].

Model 2: Acetic acid- induced chronic ulcers

Induction of chronic gastric lesion was studied according to the methods of sairam et al (2003) [2]. Animal were anaesthetized using pentobarbitone (35mg/ kg,i.p).Abdomen was opened and solution of 0.06 ml 50% acetic acid was instilled into the glass tube of 6 mm in diameter and allowed to remain 60s on the anterior serosal surface of the glandular portion of stomach 1 cm away from the pyloric end. TCE was given in the dose of 100,200,

400mg/kg on day 1, orally, twice daily, 4h after application of acetic acid and continued up to 5 days. After induction of ulcer The animals were sacrificed after 18 hr. by with an over dose of ether. On 6th day the stomach was opened and the percentage inhibition of ulcer and to calculate the ulcer index was calculated based upon the length and width of ulcer

Model 3: Ethanol-HCl induced ulcer

The experiment was performed as described by Rao et al. (2000) [3]. After 1 h all the animals were treat with 0.1 ml of HCl–ethanol mixture p.o. *Trichosanthes cucumerina* extract (100,200 and 400mg/kg), CMC (1ml/kg) and ranitidine (50mg/kg) were administered orally prior to ethanol/HCl treatment. (0.3 M hydrochloric acid and ethanol 60%) to induce gastric ulcer. Animals were sacrificed with anaesthesia, 1 h after administration of HCl–ethanol mixture and the stomach was excised. The length in mm of each lesion was measured, and the lesion index was expressed as sum of the length of all lesions. Mean lesion index for each group as calculate. Percentage ulcer inhibition was calculated for each group on comparison with vehicle control group.

Estimation of oxidative parameters:

Superoxide Dismutase [SOD, Catalase, Lipid Peroxidation (LPO) were performed [4].

Collection of gastric juice and mucosal scrapings

The gastric juice was collected 4 h after pylorus ligation and centrifuged for 5 min at 2000 rpm and the volume of the supernatant was expressed as ml/100g body weight. The mucosal scrapings were taken from the glandular portion of the stomach and were homogenized in distilled water (10 mg/ml) to be used for various biochemical estimation.

Total acidity Peptic activity Estimation of muco-substances Estimation of total hexoses Estimation of hexosamine Estimation of fucose Estimation of sialic acid Estimation of Protein [5]

Statistical evaluation

Data expressed as mean ± SEM (standard error of mean) for five rats. The difference among means has been analysed by unpaired student's t-test Analysis of variance (ANOVA) was performed to compare and analyse the data followed by post hoc test. Results were considered significant when $P \leq 0.001$ and $P \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening of 50% ethanolic extracts of *Trichosanthes cucumerina*

The phytoconstituents were identified by chemical test which showed the various phytoconstituents in the ethanolic extract. Alkaloids, carbohydrates, flavonodes, glycosides, phenolic compounds, gums and tannins.

General behavior and acute toxicity studies

50% ethanolic extracts of *Trichosanthes cucumerina* up to 4000 mg/kg did not cause any mortality in mice. None of the doses tested produced any gross apparent effect on general motor activity, muscular weakness, fecal output, feeding behavior etc. during the period of observation.

Anti ulcer study

A dose-response antiulcer study has been done using 100, 200 and 400 mg/kg of ethnol extract of *Trichosanthes cucumerina* against various validated gastric ulcer models like Aspirin + Pylorus ligation induced gastric ulcer, HCl–ethanol induced ulcer and acetic acid induced gastric ulcer. The EtOH extract were administered to various groups, orally, twice daily for five days for (Asp+ Pylorus, HCl–ethanol)and experiment were carried out on 18-36 h fasted rats on 5th day.. The result indicated a dose-dependent antiulcerogenic activity for our further subsequent studies on other parameters of gastric secretion or mucosal studies, a dose of 400 mg/kg was selected.

Gastric secretion study

Effect on volume, acid secretion

The effect of EtOH extract of *Trichosanthes cucumerina* (400 mg/kg) when administered orally, twice daily for 5 days was studied for their effect on volume, acid secretion in 4 h pylorus ligation rats. *Trichosanthes cucumerina* showed a tendency to decrease in volume, acid concentration and output. However, reference drug ranitidine a known H2-receptor antagonism agent has little effect on volume, acid concentration and acid output.

Antioxidant study

Effect on lipid peroxidation (LPO), Catalase (CAT) and Superoxide dismutase (SOD) activities

A growing body of experimental and clinical evidence have shown that gastric mucosal damage by acetic acid [6], ethanol-HCL [7], NSAIDs+pylorus [8] is mediated through the involvement of reactive oxygen species. The present investigation of herbal drug was done to study their antioxidant and free radical scavenging activities.

Thus, the results of the present study on free radical-mediated Lipid peroxidation and alteration in circulating enzymatic antioxidants, CAT and SOD, indicate the involvement of these enzymes in ulcer.

Table 1. Effect *Trichosanthes cucumerina* extract (twice daily for five days) on aspirin + Pylorus ligation induced gastric ulcer model

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	Percent protection
I	control	-	16.3 ± 2.8	-
II	*Trichosanthes cucumerina* extract	100	7.2 ± 2.2	55.82
III	*Trichosanthes cucumerina* extract	200	4.2 ± 2.0	74.23
IV	*Trichosanthes cucumerina* extract	400	2.0 ± 1.6	87.73
V	Ranitidine	50	2.5 ± 1.2	84.66

Values are mean ± SEM for 5 rats. Statistical analysis was done by one-way ANOVA followed by post hoc test. a $P < 0.001$ as compared to control; b $P < 0.01$ as compared to control; c $P < 0.05$ as compared to control

Table 2. Effect *Trichosanthes cucumerina* extract (twice daily for five days) on HCL- Ethanol acid gastric ulcers.

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	Percent protection
I	control	-	22.9 ± 2.9	-
II	*Trichosanthes cucumerina* extract	100	16.3 ± 2.1	28.82
III	*Trichosanthes cucumerina* extract	200	11.9 ± 2.4	48.03
IV	*Trichosanthes cucumerina* extract	400	5.4 ± 2.1	76.41
V	Ranitidine	50	6.9 ± 2.3	69.86

Values are mean ± SEM for 5 rats. Statistical analysis was done by one-way ANOVA followed by post hoc test. a $P < 0.001$ as compared to control; b $P < 0.01$ as compared to control; c $P < 0.05$ as compared to control

Table 3. Effect *Trichosanthes cucumerina* extract (twice daily for five days) on acetic acid induced chronic gastric ulcers.

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	Percent incidence of perforation
I	control	-	25.6 ± 3.9	-
II	*Trichosanthes cucumerina* extract	100	19.7 ± 2.8	31.56
III	*Trichosanthes cucumerina* extract	200	15.3 ± 2.4^a	25.04
IV	*Trichosanthes cucumerina*	400	12.2 ± 1.6^b	16.7
V	Ranitidine	50	11.2 ± 1.7^b	9.1

Values are mean ± SEM for 5 rats. Statistical analysis was done by one-way ANOVA followed by post hoc test. a $P < 0.001$ as compared to control; b $P < 0.01$ as compared to control; c $P < 0.05$ as compared to control

Table 4. Effect *Trichosanthes cucumerina* extract (twice daily for five days) on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in aspirin + Pylorus ligation induced gastric ulcer model.

Treatment	Dose (mg/kg)	LPO	SOD	CAT
Control	-	0.54 ± 0.02	98.9 ± 9.8	36.2 ± 2.2
Aspirin +Pylorus ligation	-	0.72 ± 0.02	225.3 ± 12.3	21.3 ± 2.0
Trichosanthes cucumerina extract	100	0.61 ± 0.01	181.2 ± 6.2	27.8 ± 2.1
Trichosanthes cucumerina extract	200	0.45 ± 0.01	147.0 ± 4.4	33.2 ± 2.8
Trichosanthes cucumerina extract	400	0.40 ± 0.02	130.2 ± 4.8	33.0 ± 2.8

Values are mean ± SEM for 5 rats. Statistical analysis was done by one-way ANOVA followed by post hoc test. a $P < 0.001$ as compared to control; b $P < 0.01$ as compared to control; c $P < 0.05$ as compared to control

Table 5. Effect *Trichosanthes cucumerina* extract (twice daily for five days) on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in HCL-Ethanol induced gastric ulcers.

Treatment	Dose (mg/kg)	LPO	SOD	CAT
Control	-	0.40 ± 0.02	106 ± 3.8	32.6 ± 2.5
HCL-Ethanol	-	0.60 ± 0.0	136 ± 7.3	15.5 ± 0.6
Trichosanthes cucumerina extract	100	0.51 ± 0.01	117 ± 4.9	23.5 ± 2.7
Trichosanthes cucumerina extract	200	0.45 ± 0.01	101 ± 4.0	28.0 ± 0.7

Trichosanthes cucumerina extract	400	0.44 ± 0.01	103 ± 3.1	27.3 ± 2.0

Values are mean ± SEM for 5 rats. Statistical analysis was done by one-way ANOVA followed by post hoc test. a $P < 0.001$ as compared to control; b $P < 0.01$ as compared to control; c $P < 0.05$ as compared to control

Table 6. Effect *Trichosanthes cucumerina* extract (twice daily for ten days) on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in acetic acid induced chronic gastric ulcers.

Treatment	Dose (mg/kg)	LPO	SOD	CAT
Control	-	0.38 ± 0.01	104.0 ± 10.7	30.1 ± 1.9
acetic acid	-	0.54 ± 0.02	147.6 ± 5.3	21.2 ± 1.6
Trichosanthes cucumerina extract	100	0.47 ± 0.02	123.4 ± 3.9	26.7 ± 1.8
Trichosanthes cucumerina extract	200	0.28 ± 0.01	117.8 ± 2.3	29.3 ± 1.9
Trichosanthes cucumerina extract	400	0.25 ± 0.01	116.8 ± 3.0	28.4 ± 0.9

Values are mean ± SEM for 5 rats. Statistical analysis was done by one-way ANOVA followed by post hoc test. a $P < 0.001$ as compared to control; b $P < 0.01$ as compared to control; c $P < 0.05$ as compared to control

Table 7. Effect of ethanolic extract of *Trichosanthes cucumerina extract* on gastric secretion in 4 h PL rats: effect on volume, acid and pepsin.

Treatment	Dose (mg/kg)	Volume (ml/100g)	Acid		Peptic	
			Concentration (μEq/ml)	Output (μEq/4 h)	Concentration (μmol/ml)	Output (μmol/4 h)
Control	-	2.47 ± 0.17	98.1 ± 15.5	284.4 ± 38.9	295.3 ± 31.5	729.4 ± 89.3
Trichosanthes cucumerina extract	400	2.04 ± 0.28	70.6 ± 11.9	144.0 ± 30.7	286.1 ± 33.6	383.6 ± 79.3
Ranitidine	50	2.11 ± 0.19	86.0 ± 8.1	181.5 ± 29.3	204.8 ± 29.6	453.2 ± 69.7

Table 8. Effect of ethanolic extract of *Trichosanthes cucumerina extract* on gastric mucosal glycoprotein in 4 h PL – rats

Treatment	Dose (mg/kg)	Mucoprotein (μg/ml)						TC : P
		Total hexose (A)	Hexosamine (B)	Fucose (C)	Sialic acid (D)	TC (A+B+C+D)	Protein (P)	
Control	-	260.8 ± 39.3	170.6 ± 17.8	62.3 ± 4.4	26.2 ± 3.8	519.9 ± 53.6	534.4 ± 51.5	1.05 ± 0.11
Trichosanthes cucumerina extract	400	343.3 ± 29.5	184.7 ± 14.2	70.3 ± 5.9	35.2 ± 2.3	633.5 ± 49.3	450.1 ± 41.3	1.48 ± 0.15
Ranitidine	50	304.3 ± 24.1	180.9 ± 17.1	69.8 ± 6.8	36.0 ± 2.3[a]	519.0 ± 36.3	391.3 ± 46.7	1.53 ± 0.16

CONCLUSION

Ulcer formation is currently viewed as an interactive process resulting from an imbalance of aggressive gastric juice and defensive mucosal factors, causing a break in the line of gastrointestinal mucosa. Because of multifactorial, etio-pathogenesis of mucosal damage and there is now an evidence that the gastric mucosa can increase its resistance to injury when challenged repeatedly with many agents, including ethanol, acid, alkali, and nonsteroidal anti-inflammatory drugs and over a periods of time ranging from a few minutes to several weeks. The 50% ethanolic extract of fruitswas studied in different gastric ulcer model in rats at a dose of 100, 200, and 400mg/Kg body weight p.o. for 5 days (Aspirin + Pylorus ligation induced gastric ulcer, HCl–ethanol induced ulcer) and 5 days for acetic acid induced gastric ulcer, twice daily. Results of this study provided preliminary data for the first time that the fruits of *Trichosanthes cucumerina* may possess significant antiulcer activity. In acute study, observed reduction in ulcer score and gastric secretion may be attributed to its antisecretory activity. In chronic study, reduction in ulcer score may be due to its antiulcer and partly cytoprotective activity. These observed effects of *Trichosanthes cucumerina* may be linked with its antioxidant and anti-inflammatory effect due to the presence of bioactive compounds like flavonoids, saponins and tannins in it. Thus the present study confirms the use of *Trichosanthes cucumerina* Roxb leaves in the traditional management of peptic ulcer disease. Hence, further studies are required to

confirm the exact mechanism underlining the ulcer healing and ulcer protecting property of the *Trichosanthes cucumerina* extract and to identify the chemical constituents responsible for it.

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