



**AN EXPERIMENTAL EVALUATION OF GASTRO PROTECTIVE EFFECT OF
TRICHOSANTHES CUCUMERINA AGAINST CHRONIC ULCERS IN RATS**

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ABSTRACT

Background: The aim of this study was to investigate anti-ulcer activity of a vegetative fruits (*Trichosanthes cucumerina*) in three distinct animal models Model 1: Pylorus ligation + aspirin induced gastric ulcer model, Model 2: Acetic acid induced gastric ulcer, Model 3: Ethanol-hydrochloric acid induced gastric ulcer. **Materials and Methods:** Selected herbo medicinal fruits *Trichosanthes cucumerina* belonging to family: Cucurbitaceae. 50% ethanolic *Trichosanthes cucumerina* fruits extract (TCE) was used. For each experiment, animals were divided in to five groups where each group was consisting of six animals. Group were considered as Normal control, Disease control and test drug treated respectively. 50% ethanolic fruit extract was administered at a dose of 100,200,400 mg/kg/day, orally. Ulcer index, gastric wall mucus content, lipid peroxidation level in stomach tissue and tissue anti-oxidant parameters like superoxide dismutase and catalase enzyme activity were carried out for both experimental studies. Statistical calculations were done by analysis of variance (ANOVA) was performed to compare and analyse the data. **Results:** A dose-response antiulcer study has been done using 100, 200 and 400 mg/kg of TCE against various validated gastric ulcer models like Aspirin + Pylorus ligation induced gastric ulcer, HCl-ethanol induced ulcer and acetic acid induced gastric ulcer, gastric wall mucus content and lipid per oxidation level in comparison to normal control group whereas treatment of 50% ethanolic fruit extract (TCE), showed significant cytoprotection. The result also showed significant increase in level of antioxidant enzymes in comparison of disease control groups. **Conclusion:** On the basis of present data it can be concluded that 50% ethanolic fruit extract (TCE) possesses significant anti-ulcer activity against exposure to a noxious agent like NSAID, ethanol and acetic acid. The observed effect be due to synergistic anti -oxidant property of ingredients of the (TCE).

KEYWORDS: 50% Ethanolic fruit extract (TCE), Ulcer model, anti-ulcer activity, Ranitidine.

INTRODUCTION

Traditional medicine is defined as the sum total of knowledge, skills and practices based on the theories, beliefs and experiences, indigenous to different cultures, that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses (WHO, 2008). In most countries traditional medicine is better known under names like complementary alternative or non-conventional medicine. However, for most developing countries traditional medicine is not an alternative, but the main supply of medicine for the population's primary health care. According to World Health Organization (WHO), 75% of the Malian population depends on traditional medicines and the interest in traditional medicine is growing (Robison & Zhang, 2011). The species snake gourd *T. cucumerina* Family (cucurbitaceae) is a perennial climber widely distributed in the wild across much of South and Southeast Asia, including India, Bangladesh, Nepal,

Pakistan, Sri Lanka, Indonesia, Malaysia, Myanmar (Burma), and southern China. It is also regarded as native in northern Australia. The fruits being an integral part of an average Indian diet are consumed as vegetable. It is a plant with creeping stems and tendrils. Leaves are hairy, 10 - 25 cm in length and 15 cm in diameter (VJ Galani et al., 2010). The pulp of the ripe fruits is used in sauces and is good substitute for tomato paste (Tindall, 1983). The whole plant has been used in the ayurvedic system of medicine for the treatment of hepatic and alimentary canal disorders. Fruits of *T.cucumerina* are used as laxative, purgative, antipyretic, antiulcer agent (Kirtikar KR, 1935). Scientific evidences regarding antidiabetic (Kirana H, 2008), hepatoprotective (Kumar SS, 2009), anti-inflammatory (Kolte et al., 1996), anti fertility (Kage D.N et al., 2009), antioxidant (Adebooye, 2008), antibacterial activities of the plant were reported. The fruits contain ascorbic acid, lycopene, phenols, flavonoids, alkaloids, tannins and saponins. It

would be fruitful to investigate further, the specific active constituents in the plant responsible for its therapeutic actions. Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, frequent use of non-steroidal anti-inflammatory drugs (NSAIDs) hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood (Rao *et al.*, 2000). Nowadays, research has been increased in the treatment of ulcer after the evidences of involvement of *Helicobacter pylori* and other factors in the pathogenesis of ulcer. The antiulcer drugs used in the treatment of gastric ulcers showed various side effects like, nausea, constipation, abdominal pain and diarrhoea. The disease has been also reported for high chances of recurrence and mortality. Thus there is a need for more effective and safe antiulcer agents aiming to relieve pain, heal the ulcer and delay ulcer recurrence. Herbal medicines are considered safer because of the natural ingredients with no side effects. (N.Hamdulay *et al.*, 2012).

MATERIALS AND METHOD

Collection & Authentication of Plant Material

T.cucumerina fruits were collected from the local vegetable Shop. The fruits were identified and taxonomically authenticated by comparison with the departmental herbarium of National Botanical Research Institute, Lucknow, India. The fresh fruits were washed with tap water, shed dried homogenized to fine powder and stored in air tight bottle.

Preparation of Extracts

The air dried powder fruit was exhaustively extracted with 50% ethanol by maceration and concentrated under reduced pressure.

Animals Used

Sprague-Dawley rats (150 - 180 g) and albino mice (15-25 g) were procured from the central animal house of Central Drug Research Institute Lucknow, India. These were kept in the departmental animal house at $26 \pm 2^\circ\text{C}$ 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. And the food was withdrawn 18-24 h before the experiment, though water was allowed ad libitum. The laboratory animals are promulgated according CPCSEA guideline. 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.

Acute toxicity studies

The adult male albino mice selected for acute toxicity study. TCE were taken at various doses levels (100-4000 mg/kg b.wt) dissolved in 1 % carboxy methyl cellulose administered 10 ml/kg b.w. orally to pairs of mice per dose level. The control animals received 1 % carboxy methyl 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. Behaviour (gross behaviour, general motor activity, writhing, convulsion, response to tail pinching, pupil size, fecal output, water intake, feeding behaviour, 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 (OECD, 401).

Pharmacological evaluation

The Albino rats are divided into five groups. Animals were fasted 24 hours. Group I received normal saline 2ml/kg body weight (Control), group II, III, and IV were received extract of TCE 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).TCE (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 with an over dose of ether after 4 h of pylorus ligation. The stomach was opened and the percentage inhibition of ulcer was determined (Shay *et al.*, 1945).

Model 2: Acetic acid- induced chronic ulcers

Induction of chronic gastric lesion was studied according to the methods of (Sairam *et al.*, 2003). 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 or 10 days After induction of ulcer the animals were sacrificed after 18 hr. by with an over dose of ether on 6th or 11th day. The stomach was opened and the percentage inhibition of ulcer was determined and the ulcer index was calculated based upon the product length and width (mm^2/rat) of ulcer.

Model 3: Ethanol-HCl induced ulcer

The experiment was performed as described (P.Malairajan *et al.*, 2006). After 1h all the animals were treat with 0.1 ml of HCl-ethanol mixture p.o. TCE (100,200 and 400mg/kg), (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, 1h after administration of HCl-ethanol mixture and then 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 were calculated. Percentage ulcer inhibition was calculated for each group on comparison with vehicle control group (Paul.V.Tan *et al.*, 2000).

Estimation of oxidative parameters:

Superoxide Dismutase SOD was estimated by following the procedure (Kakkar, 1984)result have been expressed as U of SOD activity/mg protein.Catalase was estimated by following the procedure (Aebi, 1974)and are expressed as U of CAT activity/mg proteinLipid Peroxidation (LPO) was estimated by following the procedure (Ohkawa *et al.* 1979).and is expressed as nmol/mg protein.

Study of gastric secretion:

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 like Total acidity Peptic activity, muco-substances, total hexoses, hexosamine, fucose ,sialic acid and Protein (Lowry *et al.*,1951)

Statistical evaluation

Data expressed as mean \pm 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.

RESULTS AND DISCUSSION**General behavior and acute toxicity studies**

50% ethanolic TCE 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 behaviour etc. during the period of observation.

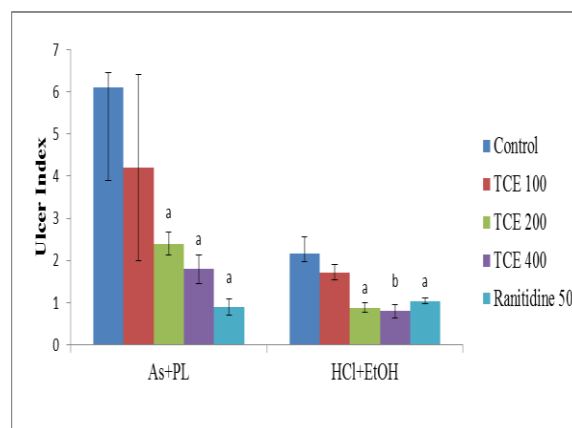
Anti ulcer study**Effect of 50% EtOH fruits extract of *Trichosanthes cucumerina* (TCE) on Aspirin + Pylorus ligation induced ulcer**

The percentage protection of TCE in ulcer induced by aspirin + pylorus model was showed ulcer reduction at different doses respectively (100 mg/kg-31.4%, 200

mg/kg-60.65% and in 400 mg/kg-70.49% while standard drug showed 85.24% (Table3.29). The optimal effect observed was at dose of 400 mg/kg onwards with TCE. So for further studies on other biochemical parameters of gastric secretion or mucosal studies, a dose of 400 mg/kg was selected.

Effect of 50% EtOH fruits extract of *Trichosanthes cucumerina* (TCE) on HCl-ethanol induced ulcer

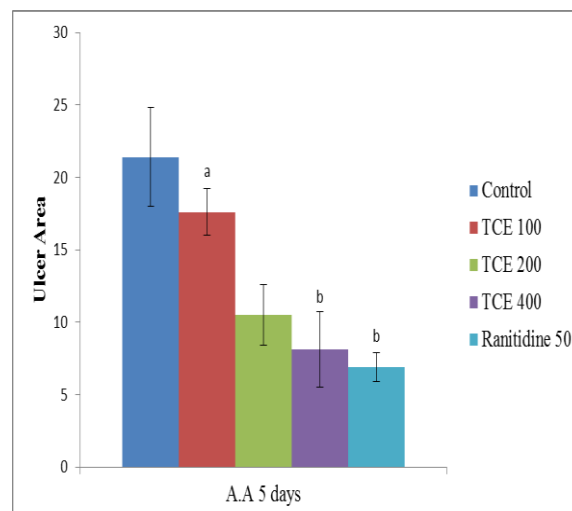
TCE showed significant dose (100, 200 and 400 mg/kg) dependent reduction in ulcer index when compared with control ($P < 0.05$, $P < 0.01$), similarly ranitidine also showed significant reduction in ulcer index. The results are summarized in figure.



Values are mean \pm SEM for 6 rats. ^a $P < 0.01$, compared to respective aspirin + pylorus ligated control group.

Effect of 50% EtOH fruits extract of *Trichosanthes cucumerina* (TCE) on 6thday acetic acid- induced chronic ulcer

A total of 50% acetic acid when applied to the serosal surface of rats gastric mucosal layer in the fundic region produced ulceration. TCE at doses 200 & 400 mg/kg healed ulcer area significantly after 5th day treatment. Data are presented in figure.



Values are mean \pm SEM for 6 rats ^a $P < 0.05$, ^b $P < 0.01$ compared to respective control group.

Gastric secretion study**Effect on volume, acid and pepsin secretion, mucin secretion, mucosal glycoprotein**

The effect of 50% EtOH fruits extract of *Trichosanthes cucumerina* in a dose of 400 mg/kg administered orally, twice daily for 5 days. TCE showed a tendency to decrease in volume, acid-pepsin concentration and output compared with control while reference drug ranitidine (50mg/kg) a known H₂ receptor blocking agent has also caused significant decrease on volume, acid and pepsin concentration and acid output.

Mucoprotein was estimated in the 90% alcoholic precipitate of the gastric juice in aspirin + 4 hrs pylorus

ligation rats treated with TCE. Extract showed tendency to increase the concentration of total carbohydrates with a tendency to decrease protein content while leading to a significant increase in TC: P ratio, indicating an increasing in mucin secretion which was comparable with the effect of ranitidine also.

Gastric mucosal glycoproteins were studied in the 90% alcoholic precipitate of the homogenates of gastric mucosal scraping of the rats treated with TCE. The result indicated a tendency to increase in the concentration of individual carbohydrates and total carbohydrates with a little change in protein level leading to an increase in total carbohydrate: protein ratio.

Table Effect of 50% EtOH fruits extract of *Trichosanthes cucumerina* (TCE) on aspirin + pylorus ligation induced ulcer 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	-	3.65 ± 0.31	61.01 ± 3.1	102.84 ± 3.8	326.3 ± 21.5	629.4 ± 79.3
TCE	400	1.720 ± 0.23 ^a	28.97 ± 1.0	67.09 ± 2.0	268.1 ± 30.6	483.6 ± 69.3
Ranitidine	50	1.52 ± 0.07 ^a	21.13 ± 2.9	51.93 ± 3.5	165.8 ± 23.1	310.2 ± 69.7 ^a

Values are mean ± SEM of 6 rats in each group ^aP < 0.05 compared to respective control group.

Table Effect of 50% EtOH fruits extract of *Trichosanthes cucumerina* (TCE) on gastric secretion in aspirin + pylorus ligation induced ulcer.

Treatment	Dose (mg/kg)	Mucoprotein (μg/ml)					TC (A+B+C+D)	Protein (P)	TC : P
		Total hexose (A)	Hexosamine (B)	Fucose (C)	Sialic acid (D)	TC			
Control	-	230.0 ± 32.3	187.0 ± 27.0	112.3 ± 14.4	22.2 ± 3.8	551.5 ± 50.6	464.4 ± 35.5	1.19 ± 0.18	
TCE	400	330.5 ± 37.5	230.7 ± 30.2 ^a	170.3 ± 21.9	36.4 ± 2.3 ^b	767.9 ± 49.3 ^a	326.1 ± 38.3	2.35 ± 0.25 ^a	
Ranitidine	50	355.3 ± 34.1 ^a	202.5 ± 24.1	130.8 ± 16.8	33.0 ± 2.3 ^b	721.6 ± 36.3 ^a	291.3 ± 46.7	2.53 ± 0.16 ^b	

Values are mean ± SEM of 6 rats in each group, ^aP < 0.05, ^bP < 0.01 compared to respective control group.

Table Effect of 50% EtOH fruits extract of *Trichosanthes cucumerina* (TCE) on gastric mucosal glycoprotein in aspirin + pylorus ligation induced ulcer

Treatment	Dose (mg/kg)	Glycoprotein (μg/100 mg wet tissue)					TC (A+B+C+D)	Protein (P)	TC : P
		Total hexose (A)	Hexosamine (B)	Fucose (C)	Sialic acid (D)	TC			
Control	-	2031 ± 217	1683 ± 196	181 ± 9	83 ± 7	3978 ± 219	4828 ± 287	0.89 ± 0.9	
TCE	400	3134 ± 227 ^a	2039 ± 190 ^a	181 ± 7	112 ± 16 ^a	5466 ± 309 ^b	4210 ± 10	1.30 ± 0.10 ^a	
Ranitidine	50	3202 ± 272 ^a	2580 ± 393	190 ± 27	138 ± 13 ^c	6110 ± 343 ^a	3942 ± 313	1.54 ± 0.13 ^a	

Values are mean ± SEM of 6 rats in each group ^aP < 0.05, ^bP < 0.01, ^cP < 0.001 compared to respective control group.

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

The results of the present study on free radical-mediated Lipid peroxidation, Superoxide dismutase and Catalase revealed that alteration in level of these circulating enzymes that indicate the involvement of these enzymes in ulcer.

Treatment	Dose (mg/kg)	LPO	SOD	CAT
Normal Control	-	3.44±0.66	1.44±0.22	34.04±1.24
Ulcerated Control	-	9.88±0.42	0.82±0.04	30.00±1.24
TCE	100	7.64±0.13 ^c	1.20±0.24 ^{NS}	36.04±0.35 ^b
TCE	200	5.66±0.42 ^c	1.22±0.27 ^{NS}	36.94±0.62 ^c
TCE	400	5.68±0.44 ^c	1.62 ± 0.21 ^a	40.66±0.22 ^c

Values are mean ± SEM for 6 rats. ^aP < 0.05, ^bP < 0.01, ^cP < 0.001 compared to respective control group. NS- Nonsignificant.

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. The 50% ethanolic extract of fruits was 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 and 10days for acetic acid induced gastric ulcer, twice daily. Results of this study provided preliminary data for the fruits of TCE may possess significant dose dependent reduction in ulcer score and gastric secretion also involve damage by reactive oxygen species apart from acid and pepsin related factor. Increased level of LPO is due to increase in generation of reactive species during ulcer leading to oxidative damage. SOD converts the reactive super oxide radicals to H₂O₂, which if not scavenge by CAT can by itself cause lipid peroxidation by generation of hydroxyl radicals. Hence decrease in CAT levels has lead to increase in accumulation of these reactive species and thus has caused increased lipid peroxidation and tissue damage TCE significantly reverses these oxidative changes in ulcer. These observed effects of TCE may be linked with its antioxidant effect due to the presence of bioactive compounds like flavonoids, saponins and tannins in it. Thus the present study confirms the use of TCE fruit 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 TCE extract.

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