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Study of Antioxidant, Analgesic and Antiulcer Potential of *Trichosanthes cucumerina* Ethanolic Seeds Extract

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Abstract: The present study was to investigate antioxidant, analgesic and antiulcer effect of ethanolic seeds extract of *Trichosanthes cucumerina*. Extraction was done by cold maceration method for 72 h using ethanol as solvent. The preliminary phytochemical screening showed the presence of triterpenoids, flavanoids and sterols. The antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. The extract showed maximum scavenging activity 86.2% at 150 $\mu\text{g mL}^{-1}$ by DPPH method as compared to standard antioxidant ascorbic acid. Further extract was studied for antiulcer activity by ethanol induced ulcer model and Non steroidal anti-inflammatory drugs induced method and analgesic activity by Eddy's hot plate method. Ethanolic extract of *Trichosanthes cucumerina* cause 72.1 and 57.1% inhibition of ulcer in Non steroidal anti-inflammatory drugs induced model and ethanol induced model at 500 mg kg⁻¹. Result concluded that Ethanolic extract of *Trichosanthes cucumerina* possess high antiulcer activity which is due to high antioxidant activity and extract also showed potential analgesic activity by Eddy's hot plate method.

Key words: 1,1-diphenyl-2-picrylhydrazyl, phytochemical screening, ascorbic acid, non steroidal anti-inflammatory drugs

INTRODUCTION

In food processing and storage conditions lipid per oxidation is a very important deteriorates reaction. It causes lowering food quality also it is reason of many diseases such as arteriosclerosis, cancer, mutation and ageing (Abdullin *et al.*, 2002). Byproducts of biological reaction are singlet oxygen, hydroxyl radical, superoxide radical (Zahin *et al.*, 2009). Active oxygen, either in the form of superoxide ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\bullet) or singlet oxygen ($^1\text{O}_2$), is a product of normal metabolism and attacks biological molecules, leading to cell or tissue injury. Mechanism of antioxidant protection get unbalanced by exogenous factors like organic solvents, smoking, ionizing radiations, pesticides and endogenous factors such as normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages and peroxisomes may occur. Disease associated with oxidative damage is arthritis, cancer, emphysema, cirrhosis and arteriosclerosis (Gulcin *et al.*, 2004). Anthocyanins, flavones, catechins, flavonoids, coumarins, lignans are responsible for antioxidant activity (Aqil *et al.*, 2006). Cancer, stroke, diabetes, Alzheimer's disease can be treated by antioxidant based drug formulation (Khalaf *et al.*, 2008). Examples of antioxidants which are commonly used

are Propyl Gallate (PG) Butylated Hydroxytoluene (BHT) Butylated Hydroxyl Anisole (BHA). Due to side effects of synthetic drugs herbal drugs are used to treat diseases. Development and utilization of naturally occurring antioxidants are desired (Oktay *et al.*, 2003). Various herbal drugs like *Musa sapientum*, *Tectona grandis*, *Rhamnus procumbens*, *Zingiber officinale*, *Curcuma longa* and *Ocimum sanctum* are used to treat oxidative stress (Gill *et al.*, 2011). It is a very well known plant and its fruit is mainly consumed as a vegetable. *Trichosanthes cucumerina* is an annual climber belonging to the family cucurbitaceae. Its common name is snake gourd, viper gourd, snake tomato or long tomato (Sandhya *et al.*, 2010). *Trichosanthes cucumerina* is one among the major constituents of important Ayurvedic preparations like Gulgultiktakam Kasayam, Mahatiktaka ghratam, Vajrakam kashayams and Mahatiktaka kashayam (Kumar *et al.*, 2009).

Trichosanthes cucumerina plant is used in treatment of fever, tumors, alopecia, diarrhoea, skin allergy and malaria. It is also used as cathartic, bronchitis, vermifuge, laxative, emetic and anthelmintic (Reddy *et al.*, 2010). The aim of the present study was to investigate antioxidant activity by using different antioxidant tests including DPPH free radical scavenging, hydrogen peroxide

scavenging. An important goal of this research was to examine antiulcer potential by NSAIDS induced ulcer model and ethanol induced ulcer model and analgesic activity by Eddy's hot plate method of ethanolic extract of *Trichosanthes cucumerina* seeds.

MATERIALS AND METHODS

Plant material: The seeds were purchased from local market of Joginder Nagar, Himachal Pradesh (India) in August 2011. The healthy looking seeds were selected for authentication and deposited in Himachal Pradesh agricultural university, Palampur (India). Seeds were cleaned, washed, dried at room temperature and powdered at low temperature.

Drugs and chemicals: Diclofenac sodium and ranitidine was obtained from Jackson lab. Pvt ltd. Silica gel 60-120, silica gel G, acetone, hydrogen peroxide were obtained from E-merck Ltd., Mumbai, India. Ascorbic acid and ethanol was obtained from central drug house Pvt Ltd., Mumbai. DPPH was obtained from Hi-media Laboratories Pvt. Ltd., India and Hexane was obtained from Loba Chemie Pvt. Ltd., Mumbai.

Experimental animals: The Wistar albino rat and Swiss albino mice of either sex were obtained from Punjab Agriculture University, Ludhiana (Punjab). Institutional animal ethics committee was carried out as per guidelines of committee for purpose of control and supervision of experiments on animals (CPCSEA), Ministry of forest, Government of India.

Extraction: The seeds of *Trichosanthes cucumerina* were cleaned, shade dried, coarsely powdered and extract with ethanol for 72 h at room temperature by process cold maceration. The extract was evaporated and concentrates under reduced pressure in rotary evaporator. Concentrated filtrate was defatted with hexane and filtrate gets partitioned successively. Aqueous layer was separated and concentrate on water bath. The crude extract was further used for various investigations (Uchikoba *et al.*, 1998; Banerjee *et al.*, 2008).

Phytochemical screening: The crude extract were analyzed for alkaloids, tannins, flavanoids, saponins, steroids and phenolic acids using standard procedures of analysis (Hameed *et al.*, 2011).

Antioxidant activity

Free radical scavenging activity

Quantitative evaluation of DPPH free radical scavenging activity: One milliliter of ethanolic extract of

Trichosanthes cucumerina seeds at various concentrations were respective added to 1 mL of 0.005 mM DPPH in ethanol and was made up to 5 mL with ethanol. Then mixture were shaken and allowed to stand in dark for 60 min. Finally absorbance of these mixtures was measured by using spectrophotometer (Shimadzu) at 517 nm using ethanol as blank. Ascorbic acid was used as standard. Control was prepared by diluting 1 mL of 0.05 mM DPPH with 4 mL of ethanol. Capability of scavenging DPPH radical was calculated as following formula:

$$\text{Scavenging (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100 \quad (1)$$

where, A_{control} was absorbance of the control reaction, A_{test} was the absorbance in the presence of the sample of the extracts.

The IC_{50} value was defined as the concentration ($\mu\text{g mL}^{-1}$) of extracts that produced 50% antioxidant effect (Rajan *et al.*, 2011).

Antiulcer activity

Indomethacin induced ulcer: Animals were divided into 5 groups and each comprising 6 rats (Ukwe *et al.*, 2010; Dengiz and Gursan, 2005):

- **Group I:** Administered vehicle (normal saline 0.9% w/v, p.o.) 30 min before indomethacin induced ulcer
- **Group II:** Disease control group administered indomethacin (25 mg kg^{-1} p.o.) for induction of gastric ulcer
- **Group III:** Administered standard (Ranitidine 50 mg kg^{-1} , p.o.) 30 min before indomethacin induced ulcer
- **Group IV:** Administered ethanolic extract (400 mg kg^{-1}) 30 min before indomethacin induced ulcer
- **Group V:** Administered ethanolic extract (500 mg kg^{-1}) 30 min before indomethacin induced ulcer

Four hour after indomethacin administration, animals were killed by decapitation method. Stomachs were removed and open along the grater curvature. Macroscopic examination was carried out with a hand lens and the presence of lesions were scored.

Ethanol induced ulcer model: The ulcer was induced by administering ethanol. All the animals were fasted for 36 h before administration of ethanol. The animals were divided into five groups, each consisting of six rats:

- **Group I:** Represented the control group which received distilled water orally
- **Group II:** Disease group receive ethanol 90% (1 mL/200 g)
- **Group III:** Ranitidine (50 mg kg⁻¹) were administered orally as reference standard drug
- **Group IV:** Received ethanolic extract of *Trichosanthes cucumerina* (400 mg kg⁻¹, p.o.) 15 min before ethanol induced ulcer
- **Group V:** Received ethanolic extract of *Trichosanthes cucumerina* (500 mg kg⁻¹, p.o.) 15 min before ethanol induced ulcer

Orally, after 45 min of ethanolic extract and Omeprazole treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 h latter with anaesthetic ether and stomach was incised along the greater curvature and ulceration will be scored (Okokon *et al.*, 2011; Kore *et al.*, 2011). Percent age ulcer protection was found out by following formula:

$$\text{Ulcer protection (\%)} = \frac{U_c - U_t}{U_c} \times 100$$

where, U_c is the ulcer index of control group, U_t is the ulcer index of disease tested control group (Bhalke *et al.*, 2010)

Analgesic activity

Eddy hot plate method: Animals were divided into 5 groups and each comprising 6 mice:

- **Group 1: Control:** 0.5 mL of distilled water
- **Group 2: Standard:** Diclofenac sodium (5 mg kg⁻¹, p.o.)
- **Group 3: EETC:** Ethanolic extract (300 mg kg⁻¹, p.o.)
- **Group 4: EETC:** Ethanolic extract (400 mg kg⁻¹, p.o.)
- **Group 5: EETC:** Ethanolic extract (500 mg kg⁻¹, p.o.)

EETC indicate ethanolic extract of *Trichosanthes cucumerina*. All the preparations were administered orally. Animals were screened by placing them on a hot plate maintained at 55±2°C with the help of thermostat. The reaction time (pain threshold) was noted at 30, 60 and 90 min. after administration of drugs by oral route (Siddalingappa *et al.*, 2012).

Statistical analysis: All the data were expressed as mean±standard error of means (SEM). Data were analyzed by Tukey’s multiple range comparison tests. A probability value of p<0.05 was considered to be statistically significant.

RESULTS

Phytochemical screening: Ethanolic extract contain maximum amount of flavanoids, saponins and triterpenoids as indicated by double plus (++) and also contains alkaloids in minimum amount indicated by single plus (+) as shown in Table 1.

Antioxidant activity: On comparing ethanolic extract of *Trichosanthes cucumerina* with ascorbic acid as standard the maximum percentage inhibition was 86.2% that was shown at a concentration of 150 µg mL⁻¹ whereas, at 50 µg mL⁻¹ it showed percentage inhibition of 62.9 and at 100 µg mL⁻¹ it showed percentage inhibition 79.9 as shown in Table 2.

Antiulcer activity: *Trichosanthes cucumerina* was further used to evaluate its antiulcer activity by ethanolic induced model and DPPH radical induced model. It was evident from results of present investigation that the

Table 1: Phytochemical screening of EETC

Chemical constituents	Ethanolic extract
Flavonoids	++
Amino acid	-
Triterpenoid	++
Saponins	++
Alkaloids	+
Glycosides	+
Tannins	++
Carbohydrates	-

-: Absence, +: Presence, ++: Max, EETC: Extract of *Trichosanthes cucumerina*

Table 2: Percentage scavenging of DPPH radical

Conc. of extract (µg mL ⁻¹)	DPPH inhibition (%)	
	Ethanolic extract	Ascorbic acid
50	62.9	68.3
100	79.9	80.7
150	86.2	87.2

Values are Mean±SEM of triplicate experiment DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 3: Effect of EETC on NSAIDS induced ulcer (NIU) and ethanolic induced ulcer (EIU) in rats

Group	Treatment	Dose (mg kg ⁻¹)	Ulcer index	Percentageulcer
			Mean±SEM	inhibition (%)
NIU				
I	Normal	0	00.00±0.00	0.0
II	Disease	0	2.500±0.08	0.0
III	Ranitidine	50	0.433±0.03 ^a	82.68
IV	EETC	400	1.000±0.05 ^{ab}	60.1
V	EETC	500	0.700±0.05 ^{ab}	72.1
EIU				
I	Normal	0	00.00±0.00	0.0
II	Disease	0	2.50±0.08	0.0
III	Ranitidine	50	0.80±0.05 ^{7a}	77.1
IV	EETC	400	1.70±0.08 ^{ab}	51.7
V	EETC	500	1.58±0.17 ^{ab}	57.1

Values are mean±SEM of 6 animals in each group, ^ap<0.05 vs. Control, ^bp<0.05 vs. ranitidine, EETC indicates Ethanolic extract of *Trichosanthes cucumerina*

Table 4: Analgesic activity by Eddy's hot plate method

Group	Treatment	Dose (mg kg ⁻¹)	Reaction time (sec)			
			0 min	30 min	60 min	90 min
I	Control	0.5 mL distilled water	3.300±0.08	3.233±0.190	3.403±0.062	3.397±0.07
II	Diclofenac sodium	10	3.300±0.17 ^a	5.770±0.06 ^a	7.453±0.122 ^a	9.450±0.28 ^a
III	EETC	300	3.300±0.08 ^{ab}	4.853±0.04 ^{ab}	6.523±0.18 ^{ab}	7.432±0.23 ^{ab}
IV	EETC	400	3.30±0.08 ^{ab}	4.900±0.05 ^{ab}	6.710±0.16 ^{ab}	7.920±0.02 ^{ab}
V	EETC	500	3.30±0.08 ^{ab}	5.270±0.07 ^{ab}	7.240±0.005 ^{ab}	8.410±0.10 ^{ab}

Values are Mean±SEM of 6 animals in each group, ^ap<0.05 vs. control, ^bp<0.05 vs. diclofenac sodium, EETC: Ethanolic extract of *Trichosanthes cucumerina*

extract showed significant antiulcer activity by DPPH radical induced model and ethanolic induced model. The extract showed reduction in ulcerative index but only at highest dose i.e., 500 mg kg⁻¹ and showed significant reduction in ulcerative index (Table 3). Extract showed 60.1 and 72.1% inhibition of ulcer at dose 400 mg kg⁻¹ (Table 3) and 500 mg kg⁻¹ in 1,1-diphenyl-2-picrylhydrazyl radical induced model and 51.7 and 57.1% inhibition in ethanolic induced model also at same dose as shown in Table 3.

Analgesic activity: Extract was further evaluated for analgesic activity by Eddy's hot plate method. Table 4 depicts analgesic activity shown by extract by Eddy's hot plate. Extract showed dose dependent analgesic activity at medium dose (400 mg kg⁻¹) and high dose (500 mg kg⁻¹) was found to be 7.92±0.8 and 8.4±0.10 at 90 min interval. These doses showed significant difference in analgesic activity when compared with control group. Max analgesic effect was observed 90 min interval. Hence, it was observed that EETC significantly reduce pain sensation in mice at medium and high dose.

DISCUSSION

The present study reports antioxidant, antiulcerogenic and analgesic activity of ethanolic extract of *Trichosanthes cucumerina* seeds. Gastroprotection activity is responsible for reduction of free radical generation, lipid peroxidation and vascular permeability. Presence of phytoconstituents like triterpenoids, saponins may be responsible for these activities. In present study *Trichosanthes cucumerina* was evaluated for antioxidant activity followed by *In vitro* antiulcer activity by DPPH radical induced and ethanolic induced model and also evaluated for its analgesic activity by Eddy hot plate method. Ulcer index parameter was used to evaluate antiulcer activity. Evaluation of *Momordica charantia* ethanolic extract for its antiulcer activity has been carried out already (Alam *et al.*, 2009). Analgesic activity of aqueous and alcoholic extract *Pergularia daemia* has also been carried out already (Nikajoo, 2009). Study of *Cucumis sativus* for its antioxidant and antiulcer activity has been carried out

earlier (Gill *et al.*, 2009). Study of anti-inflammatory activity of hot water extracts of *T. cucumerina* in rats has been done earlier (Arawwawala *et al.*, 2010). Antibacterial activity has been studied on aerial parts of *Trichosanthes cucumerina* (Arawwawala *et al.*, 2011). *Trichosanthes cucumerina* modulates elevated levels of glucose in diabetes mellitus rats (Kirana and Srinivasan, 2008). On carbon tetrachloride induced liver damage in rats hepatoprotective activity of *Trichosanthes cucumerina* has been done already (Kumar *et al.*, 2007). Diuretic activity and anthelmintic activity of *Trichosanthes cucumerina* has been studied earlier (Murthy *et al.*, 2012). In Wistar rats cardioprotective activity of methanolic extract of fruits studied earlier (Sagar *et al.*, 2012).

CONCLUSION

In present study it was concluded that ethanolic extract have antiulcer potential due to its antioxidant action and extract have also potent analgesic activity. So, ethanolic extract of *Trichosanthes cucumerina* can be used for treatment of ulcers and can also use as analgesic to reduce pain.

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