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## Evaluation of Free Radical Scavenging and Antiulcer Potential of Methanolic Extract of *Benincasa hispida* Seeds

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### ABSTRACT

In the present study free radical scavenging and antiulcer potential of *Benincasa hispida* seeds was evaluated. The powdered seeds were extracted with methanol. The extract was evaluated for its free radical scavenging activity by DPPH method followed by antiulcer activity using pyloric ligation, water immersion stress and NSAID (indomethacin) induced gastric ulcer model (NIU) in rats. The parameters assessed were gastric volume, free acidity, total acidity, ulcer index and percentage ulcer protection. Ranitidine was used as the reference antiulcer drug. The methanolic extract showed concentration dependent DPPH radical scavenging activity. The methanolic extract of *Benincasa hispida* seeds (MEBH) inhibited gastric ulceration by decreasing the gastric volume, free and total acidity. The high dose (300 mg kg<sup>-1</sup>) showed significant reduction in the above parameters which was comparable to the standard drug ranitidine (p<0.05). The MEBH caused 52.7, 67.4 and 61.2% inhibition of ulcers in pyloric ligation, water immersion stress and NSAID induced ulcer models, respectively at 300 mg kg<sup>-1</sup>. Thus it can be concluded that the seeds of *Benincasa hispida* possess potent antiulcer activity. The antiulcer action may be exerted through free radical scavenging mechanism.

**Key words:** *Benincasa hispida*, pyloric ligation, water immersion stress, NSAID, ulcer index

### INTRODUCTION

Gastric hyperacidity and ulceration of the stomach mucosa are serious health problems of global concern. The various causes of gastric ulcers include age, inheritance, cigarette smoking and diet habits (Malysenko *et al.*, 2005). Other common causes are physical or physiological stress, use of Non-steroidal Anti-inflammatory drugs and bacterial infection (Caso *et al.*, 2008; Kim, 2008; Ernst and Gold, 2000). Gastric lesions occur due to the loss delicate balance between gastro-protective (bicarbonate ions, mucin and prostaglandins) and aggressive factors (*Helicobacter pylori*, acid and pepsin) (Desai *et al.*, 1997). Therefore, treatment with antioxidants and synthetic drugs such as H<sup>+</sup>K<sup>+</sup> ATPase pump inhibitors, histamine H<sub>2</sub>-receptor blockers can decrease gastric mucosal damage (Salim, 1994; Waldum *et al.*, 2005). But these synthetic drugs have various side effects such as diarrhoea, headache, drowsiness, fatigue, and muscular pain (Zimmerman, 1984). Hence these days' natural compounds are being explored so that they could replace these synthetic drugs. The

treatment of peptic ulcers with plant products and the protection of induced gastric ulcers in laboratory animals using medicinal plants have been reported.

Many researchers have paid attention towards the Cucurbitaceae family. Various plants of the family such as *Momordica charantia*, *Cucumis sativum*, *Trichosanthes cucumerina*, *Wilbrandia ebracteata*, *Cucumis melo* have been reported to possess antiulcer potential (Alam *et al.*, 2009; Gill *et al.*, 2009; Arawwawala *et al.*, 2010; Gonzalez and Di-Stasi, 2002; Gill *et al.*, 2011). Plants such as *Sechium edule* and *Lagenaria siceraria* have been studied for their medicinal properties (Dire *et al.*, 2007; Fard *et al.*, 2008). The seeds of *Cucumis melo* var. *utilissimus* are reported to possess antioxidant activity (Gill *et al.*, 2010a). *Benincasa hispida* is an important plant of the family. The extract of the fruit is reported to possess anti-ulcer, anti-angiogenic and antihistaminic activities (Grover *et al.*, 2001; Lee *et al.*, 2005; Kumar and Ramu, 2002). The seeds of the plant have been found to possess analgesic and anti-inflammatory potential (Gill *et al.*, 2010b). These seeds have been used traditionally in Ayurveda for treatment of various disorders such as peptic ulcer and as vermifuge (Warrier *et al.*, 1994). So the present study was carried out to evaluate the antiulcer potential of *Benincasa hispida* seeds.

## **MATERIALS AND METHODS**

**Plant material:** The seeds were purchased from the Khari Baoli (spice market) Delhi (India) in August 2009. The healthy looking seeds were selected for authentication and voucher specimen number 0389 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar (India). The seeds were cleaned, washed, dried at low temperature and powdered.

**Drugs and chemicals:** Ranitidine and indomethacine was obtained as a free sample from Jackson Laboratories Amritsar. Phenobarbitone (Neon pharmaceuticals), methanol, hexane and sodium hydroxide were of analytical grade and purchased from SD fine chemicals, Merk and Loba chemicals.

**Animals:** The wistar albino rats (200-250 g) of either sex were obtained from NIPER Mohali. They were kept at standard laboratory diet, environmental temperature and humidity. A 12 h light and dark cycle was maintained throughout the experimental protocol. The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. 874/ac/05/CPCSEA).

**Extraction:** The powdered seeds were extracted for 72 h with methanol at room temperature. The solvent was filtered off and residue macerated again with the fresh solvent. Both solvents were combined and concentrated under reduced pressure on a rotary evaporator (Heidolph) at 40°C. The concentrated filtrate was suspended in distilled water and partitioned successively with hexane. The aqueous layer was separated and concentrated on water bath. The crude extracts were used for further investigation.

**Phytochemical screening:** The crude extract was studied for the presence of phytochemicals such as alkaloids, tannins, saponins, flavonoids, steroids, triterpenoids, carbohydrates, proteins and amino acids using standard procedures (Harborne, 1973).

**DPPH radical scavenging activity:** The free radical scavenging activity of MEBH was determined by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method. Briefly, 0.05 mM solution of DPPH in methanol was prepared and 1.5 mL of this solution was added to 0.5 mL of extract solution in methanol at different concentrations (100-300  $\mu\text{g mL}^{-1}$ ) (Sreejavan and Rao, 1997; Sood *et al.*, 2009). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV-1700 Pharma spec). A blank without DPPH was used to remove the influence of the color of the samples. A methanolic solution of DPPH was used as negative control. Ascorbic acid was used as a reference drug. All measures were carried out in triplicate. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The DPPH radical scavenging activity was calculated using the equation:

$$\text{Percentage scavenging of DPPH radical} = 100 \times (A_o - A_s) / A_o$$

where,  $A_o$  is absorbance of the negative control  $A_s$  is the absorbance of the sample.

**Experimental design for pyloric ligation induced gastric ulcer (PL):** Animals were divided into 6 groups, each comprising of 6 rats.

- **Group I:** Administered vehicle (normal saline 0.9% w/v, p.o.) 1 h before pyloric ligation on the day of experiment
- **Group II:** Sham control group subjected to surgical procedure without pyloric ligation
- **Group III:** Disease control group subjected to pyloric ligation for the induction of ulcer
- **Group IV:** Administered standard (ranitidine 50 mg  $\text{kg}^{-1}$ , p.o.) 1 h before pyloric ligation on the day of experiment
- **Group V:** Administered methanolic extract (150 mg  $\text{kg}^{-1}$ , p.o.) 1 h before pyloric ligation on the day of experiment
- **Group VI:** Administered methanolic extract (300 mg  $\text{kg}^{-1}$ , p.o.) 1 h before pyloric ligation on the day of experiment

The pylorus was ligated according to the method of Shay *et al.* (1945). Mean ulcer score for each animal was expressed as ulcerative index and the percentage ulcer protection was also calculated (Bose *et al.*, 2003).

**Experimental design for water immersion stress induced gastric ulcer (WIS):** Animals were divided into 5 groups, each comprising of 6 rats.

- **Group I:** Administered vehicle (normal saline 0.9% w/v, p.o.) 1 h before water immersion stress
- **Group II:** Disease control group subjected to water immersion stress for the induction of gastric ulcers
- **Group III:** Administered standard (ranitidine 50 mg  $\text{kg}^{-1}$ , p.o.) 1 h before water immersion stress
- **Group IV:** Administered methanolic extract (150 mg  $\text{kg}^{-1}$ , p.o.) 1 h before water immersion stress
- **Group V:** Administered methanolic extract (300 mg  $\text{kg}^{-1}$ , p.o.) 1 h before water immersion stress

The rats were fasted 24 h prior to experiment and test samples were administered 1 h before stress induction. Rats were immobilized in a stress cage and then immersed to the level of the xiphoid in a water bath at  $23\pm 0.2^{\circ}\text{C}$  for 4 h (Hayase and Takeuchi, 1986). After 4 h animals were removed and sacrificed. The stomach of each animal was removed and cut open along the greater curvature and pinned on wooden board after washing it with running tap water. Then ulcerative index and percentage ulcer protection were calculated.

**Experimental design for NSAID (indomethacin) induced ulcer model (NIU):** Animals were divided into 5 groups, each comprising of 6 rats.

- **Group I:** Administered vehicle (normal saline 0.9% w/v, p.o.) 30 min before indomethacin induced ulcers
- **Group II:** Disease control group administered indomethacin ( $25\text{ mg kg}^{-1}$ , p.o.) for the induction of gastric ulcers
- **Group III:** Administered standard (ranitidine  $50\text{ mg kg}^{-1}$ , p.o.) 30 min before indomethacin induced ulcers
- **Group IV:** Administered methanolic extract ( $150\text{ mg kg}^{-1}$ , p.o.) 30 min before indomethacin induced ulcers
- **Group V:** Administered methanolic extract ( $300\text{ mg kg}^{-1}$ , p.o.) 30 min before indomethacin induced ulcers

Normal saline, ranitidine, extract were given orally and 30 min later indomethacin was administered to all the groups. Six hours later, the animals were killed by decapitation. The stomachs were removed, opened along the great curvature and washed with tap water to remove gastric contents and examined under a dissecting microscope with square-grid eyepiece to assess the formation of ulcers. For each stomach, ulcerated and total areas were measured as  $\text{mm}^2$  and the ulcer indexes for each stomach were calculated (Dengiz and Gursan, 2005).

**Estimation of gastric volume and free and total activity changes in PL mode:** Four hour after ligation, stomachs were dissected out and contents were collected into measuring cylinder to measure the volume of gastric contents. The gastric contents were centrifuged and subjected to titration for estimation of free and total acidity. One milliliter of the supernatant liquid was pipette out and diluted to 10 mL with distilled water. The solution was titrated against 0.01 N NaOH using Topfer's reagent as indicator (Raj Kapoor *et al.*, 2002) to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued by adding 1% solution of phenolphthalein till the solution gained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. The sum of the two titrations was total acidity (Raj Kapoor *et al.*, 2002) acidity was expressed as:

$$\text{Acidity (mEq/L/100 g)} = \text{Volume of sodium hydroxide} \times \text{normality} \times 100/0.1$$

**Estimation of gastric ulcerative index changes in PL, WIS and NIU model:** Ulcerative index was measured by method of Takagi *et al.* (1969).

**Statistical analysis :** All the results were expressed as Mean±Standard error of means (SEM). The data was statistically analyzed by one way Analysis of Variance (ANOVA) followed by Tukey's multiple range tests by using Sigmastat Version-2.0 Software. The p<0.05 was considered to be statistically significant.

## RESULTS

Preliminary phytochemical screening of methanolic extract of *Benincasa hispida* seeds (MEBH) showed the presence of sterols, triterpenes, carbohydrates, tannins, protein and amino acids (Table 1). MEBH was further used to evaluate its antiulcerogenic potential in various ulcer models.

DPPH reacts with antioxidants and gets converted into 1,1-diphenyl-2-picrylhydrazine by accepting a hydrogen atom and hence shows decrease in absorbance. The MEBH showed concentration dependent DPPH radical scavenging activity. The highest radical scavenging activity of MEBH was found to be 79.8% at concentration of 300 µg mL<sup>-1</sup> as shown in Table 2.

In pyloric ligated rats, there was an increase in the gastric volume, free and total acidity and ulcerative index as compared to the sham control group. MEBH showed reduction in gastric secretion, free and total acidity and ulcerative index. But only highest dose i.e., 300 gm kg<sup>-1</sup> showed significant reduction in the above parameters which was comparable to the standard drug ranitidine (Table 3, 4). The MEBH caused 40.6 and 57.2% inhibition of ulcers at the dose of 150 and 300 mg kg<sup>-1</sup>, respectively in PL model.

In WIS induced ulcer model and NSAID (indomethacin) induced ulcer model the MEBH showed reduction in ulcerative index, but only highest dose i.e., 300 mg kg<sup>-1</sup> showed significant reduction in the above parameter which was comparable to the standard drug ranitidine (Table 4). The MEBH caused 52.6 and 67.4% inhibition of ulcers at the dose of 150 and 300 mg kg<sup>-1</sup>, respectively in WIS model.

Table 1: Phytochemical chemical screening of methanolic extract of *Benincasa hispida*

Chemical constituents	Result
Alkaloids	-
Flavonoids	-
Protein and amino acid	+
Triterpenoids	+
Saponin	-
Carbohydrates	+
Phytosterols	+
Tannins	+

+ = Presence of chemical constituent, - = Absence of chemical constituent

Table 2: Percentage scavenging of DPPH (1,1-diphenyl-2 picryl-hydrazyl) radical

Conc. of extract (µg mL <sup>-1</sup> )	Percentage scavenging of DPPH radical	
	Methanol extract	Ascorbic acid
100	54.4±0.37	62.07±0.79
200	66.5±0.52	71.20±0.64
300	79.8±0.49	85.30±0.67

Values are the average of triplicate experiments and represented as Mean± SEM

Table 3: Effect of MEBH on gastric secretion, free acidity and total acidity in pylorus ligation induced gastric ulcer in rats

Group	Dose (mg kg <sup>-1</sup> )	Gastric volume	Free acidity	Total acidity (mEq/L/100 g)
		(mL/100 g)	(mEq/L/100 g)	
I		1.16±0.21	24.62±0.29	59.72±1.04
II		1.27±0.44	25.12±0.32	57.43±0.52
III		3.14±0.36 <sup>a</sup>	64.34±0.57 <sup>a</sup>	103.26±1.12 <sup>a</sup>
IV	50	1.32±0.78 <sup>b</sup>	26.86±0.48 <sup>b</sup>	62.58±0.32 <sup>b</sup>
V	150	2.47±0.25 <sup>ac</sup>	46.08±0.67 <sup>ac</sup>	80.32±0.81 <sup>ac</sup>
VI	300	1.84±0.58 <sup>b</sup>	34.53±0.38 <sup>b</sup>	75.63±0.22 <sup>b</sup>

Values are Mean±SEM, n = 6 animals in each group; <sup>a</sup>p<0.05 as compared with sham control group. <sup>b</sup>p<0.05 compared with disease control groups <sup>c</sup>p<0.05 compared with ranitidine treated group

Table 4: Effect of MEBH on ulcerative index and percentage inhibition in PL, WIS and NSAID induced gastric ulcer in rats

Group	Dose (mg kg <sup>-1</sup> )	Ulcerative index			Percentage inhibition		
		PL	WIS	NIU	PL	WIS	NIU
I		00±0.00	00±0.00	00±0.00	0.0	0.0	0.0
II		00±0.00	00±0.00	00±0.00	--	--	--
III		5.47±0.01 <sup>a</sup>	5.96±0.01 <sup>a</sup>	6.33±0.01 <sup>a</sup>	0.0	0.0	0.0
IV	50	1.86±0.01 <sup>b</sup>	1.38±0.01 <sup>b</sup>	1.84±0.01 <sup>b</sup>	65.9	76.8	70.9
V	150	3.25±0.01 <sup>ac</sup>	2.82±0.01 <sup>ac</sup>	3.17±0.01 <sup>ac</sup>	40.6	52.6	49.9
VI	300	2.34±0.01 <sup>b</sup>	1.94±0.01 <sup>b</sup>	2.45±0.01 <sup>b</sup>	57.2	67.4	61.2

Values are Mean±SEM, n = 6 animals in each group; <sup>a</sup>p<0.05 compared with sham control group, <sup>b</sup>p<0.05 compared with PL and WIS groups respective columns, <sup>c</sup>p<0.05 compared with ranitidine treated group. NIU: NSAID (indomethacin) induced ulcer model, PL: Pyloric Ligation Induced Gastric Ulcer, WIS: Water Immersion Stress Induced Gastric Ulcer

In NSAID (indomethacin) induced ulcer model the MEBH showed reduction in ulcerative index but only highest dose i.e., 300 mg kg<sup>-1</sup> showed significant reduction in the above parameter which was comparable to the standard drug ranitidine (Table 4). The MEBH caused 49.9 and 61.2% inhibition of ulcers at the dose of 150 and 300 mg kg<sup>-1</sup>, respectively in NSAID (indomethacin) induced ulcer model.

## DISCUSSION

In the present study, the methanolic extract of *Benincasa hispida* seeds was evaluated for its free radical scavenging activity followed by *in vivo* antiulcer activity in pyloric ligation, water immersion stress and NSAID induced ulcer models. Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity (Khayum *et al.*, 2009). The ulcer formation in each of these models occurs by different mechanisms. Hence, it is not possible to propose a single mechanism for antiulcer effect of a particular drug. Pylorus ligation induces gastric ulcers due to accumulation of gastric secretion in the stomach (Shay *et al.*, 1945). The reflex or neurogenic effect has also play an important role in the formation of gastric ulcer in this model (Bose *et al.*, 2003; Goswani *et al.*, 1997). In water immersion stress induced ulcer model ulcers are formed as a result of disturbance of gastric secretion, alteration in microcirculation of gastric mucosa and abnormal gastric motility (Bose *et al.*, 2003; Kitagawa *et al.*, 1979). It has been suggested that active oxygen species may be involved in the pathogenesis of gastric mucosal injuries (Szelenyi and Brune, 1988). The MEBH showed dose dependent antiulcer effect in pyloric ligation, water immersion stress and

indomethacin induced ulcer models. The MEBH significantly decreased the total acidity, free acidity and ulcer index. Various plants of the family such as *Momordica charantia*, *Cucumis sativum* Cucurbitaceae have shown to possess significant antiulcer potential (Alam *et al.*, 2009; Gill *et al.*, 2009). The induction of stress generates free radicals which cause mucosal damage and change in antioxidant enzymes (Das and Banerjee, 1993). Consequently, some radical scavengers have shown to possess a protective effect against the mucosal injuries induced by active oxygen species (Oka *et al.*, 1991). The decrease in ulcerative index suggests the ability of extract to protect the gastric mucosa against free radical mediated tissue injury. Thus the action of extract may be through free radical scavenging mechanism.

## CONCLUSION

In the present study it may be concluded that the methanolic extract of the *Benincasa hispida* seeds possessed anti-ulcer effect due to its free radical scavenging potential and can be used as a future natural antiulcerogenic agent.

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