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# *In-vitro* antiulcer activities of petal extract of *Crocus sativus* var. *cashmerianus*

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## ABSTRACT

Medicinal plants have been known for millennia as a rich source of traditional therapeutic agents for the prevention of diseases and ailments. The aim of the present study was performed to evaluate the antiulcer activities of hydro-alcoholic extracts of petals of *Crocus sativus* var. *Cashmerianus* by *in-vitro* methods viz. acid neutralizing capacity and H<sup>+</sup>/K<sup>+</sup> - ATPase inhibition activity. In acid neutralizing capacity method, the petals extract significantly reduced acidity to 6.10 at a concentration of 1000 mg/ml as compared to 11.90 with standard 500 mg/ml of Aluminium hydroxide + Magnesium hydroxide combination. However, H<sup>+</sup>/K<sup>+</sup> - ATPase inhibition activity method, petals extract showed maximum percentage inhibition of 70.31 % at the concentration 400µg/ml as compared to 73.82 % with a similar dose of standard Omeprazole. The IC<sub>50</sub> value of petals extract of *C. sativus* var. *cashmerianus* is shown 100 µg/ml in comparison with standard omeprazole of 82.5 µg/ml. The study reveals that the petals extract of *C. sativus* var. *cashmerianus* may contain compounds possessing acid neutralize and enzyme inhibition activities, thus it can be used as an alternative medicine for gastrointestinal disorders.

**KEYWORDS:** *C. sativus*, Acid Neutralizing capacity, H<sup>+</sup>/K<sup>+</sup> - ATPase inhibition activity, Omeprazole

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## INTRODUCTION

Peptic ulcer is formed by an imbalance between gastro duodenal mucosal defence mechanisms and the aggressive factors, particularly gastric acid and pepsin. Ulceration is reported for high chances of recurrence and mortality [1]. Gastric acid is an important factor for the genesis of ulceration in stomach. The activation of the vagus-vagal reflexes by stimulation of pressure receptors in the antral gastric mucosa is believed to increase gastric acid secretion [2]. Phytoconstituents like flavonoids i.e. quercetin, catechin seems to play a very important role in promoting mucus secretion for prevention and treatment of peptic ulcer [3]. In addition to this, quercetin has reported to inhibit the growth of *H. pylori* bacterium *in-vitro* studies. Catechin interferes with the formation of histamine in gastric mucosa and hence produces the protective effect [4]. Presently, a dire need of most effective and safer anti-ulcer agents aiming to relieve pain, heal the ulcer and delay ulcer recurrence. Therefore herbal medicines are considered safer alternatives because of natural ingredients with no side effects. Petals extracts of *Crocus sativus* has reported for their antioxidants, anti-inflammatory and antibacterial activities. The present study to evaluate antiulcer activity of hydro-alcoholic extract of

petals of *Crocus sativus* var. *Cashmerianus* by *in-vitro* methods viz. acid neutralizing capacity and H<sup>+</sup>/K<sup>+</sup> - ATPase inhibition activity. Therefore, an attempt had been made to validate its traditional claim as anti ulcer agent by these selected methods.

## MATERIAL AND METHODS

### Plant Material

The petals of *Crocus sativus* “Cashmerianus” were collected from Pampore area of Kashmir (J&K, India). The petals were collected, shade dried and powdered in coarse form. Hydro-alcoholic extract of petals was prepared, dried and used for further research work.

### *In-vitro* Evaluation of Antiulcer Activity

#### *Acid neutralizing capacity*

The acid neutralizing capacity (ANC) value for hydro-alcoholic extract of petals of *C. sativus* “cashmerianus in different concentrations (100 mg/ml, 200mg/ml, 500mg/ml, 1000mg/ml)

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were compared with the standard antacid AHMH (aluminum hydroxide + magnesium hydroxide -500 mg/ml). To the 5ml quantity of each extract individually, water was added and mixed well to make up the total volume up to 70 ml. Then 30 ml of 1N HCl was added into standard and test preparation and stirred for 15 minutes, 2-3 drops of phenolphthalein solution was added and mixed. The excess HCl was immediately titrated with 0.5N Sodium hydroxide solution drop wise until a pink color is appeared [5].

The moles of acid neutralized is calculated by,

$$\text{Moles of acid neutralized} = (\text{vol. of HCl} \times \text{Normality of HCl}) - (\text{vol. Of NaOH} \times \text{Normality of NaOH})$$

Acidneutralizingcapacity (ANC)

$$\text{per gram of antacid} = \frac{\text{Moles of HCl neutralized}}{\text{Grams of Antacid / Extract}}$$

## Determination of H<sup>+</sup>/K<sup>+</sup> ATPase inhibition

### Preparation of H<sup>+</sup>/K<sup>+</sup> - ATPase enzyme

Preparation of H<sup>+</sup>/K<sup>+</sup> - ATPase Enzyme: To prepare H<sup>+</sup>/K<sup>+</sup> - ATPase enzyme sample, fresh sheep stomach was obtained from a local slaughterhouse of Moga city market. The sheep stomach was cut opened, washed, entire mucosa at gastric fundus was cut-off, and the inner layer was scraped out for parietal cells. Then parietal cells were homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton X-100 and centrifuged at 5000xg for 10 min. The supernatant (enzyme extract) was separated and used to determine the H<sup>+</sup>/K<sup>+</sup>- ATPase inhibition activity. The protein concentration in the supernatant was determined with bovine serum albumin used as standard reagent. The parietal cell extract was then used to determine H<sup>+</sup>/K<sup>+</sup> ATPase activity.

### Assessment of H<sup>+</sup>/K<sup>+</sup> ATPase inhibition

The reaction mixture containing 0.1 ml of H<sup>+</sup>/K<sup>+</sup> - ATPase Enzyme (300 µg/ml) and petals extract at different concentrations (25 µg, 50 µg, 100 µg, 200 µg, 400 µg) was pre-incubated for 60 min at 37°C. The reaction was initiated by adding substrate 2 mM Adenosine triphosphate (200 µL), in addition to this 2mM MgCl<sub>2</sub> (200 µL) and 10mM KCl (200 µL) was added. After 30 min of incubation at 37°C, the reaction was stopped by the addition of assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid followed by centrifugation at 2000xg for 10 min and inorganic phosphate released was measured UV spectrophotometer at 660 nm. Further, inorganic phosphate was determined by Fiske-Subbarow method [4,6]. The enzyme source was also treated similarly with the standard drug omeprazole and the enzyme activity was measured.

The percent enzyme inhibition was calculated using the formula:

$$\text{Percentage of inhibition} = \frac{[\text{Activity (control)} - \text{Activity (test)}] / \text{Activity (control)}}{\times 100}$$

## RESULT AND DISCUSSION

### In-vitro Acid Neutralizing Capacity

The *in-vitro* acid neutralizing effects of hydro-alcoholic extract of petals of *C. sativus* "cashmerianus in different concentrations (100 mg, 200mg, 500mg, and 1000 mg per ml) were compared with the standard antacid AHMH- 500 mg/ml. The results showed concentration dependent reduction in acid neutralizing capacity per gm of antacid was found as 115.7, 42.17, 10.22 and 6.10 respectively. As Similar fashion, AHMH (500 mg) which is found ANC value 11.90 quite similar concentration of test drug. Whereas, test drug concentration 1000 mg was found double to neutralize acid more significantly as compared to standard. The results are tabulated in Table 1.

### In-vitro H<sup>+</sup>/K<sup>+</sup> - ATPase Inhibition Activity

*In-vitro* H<sup>+</sup>/K<sup>+</sup> - ATPase inhibition activity of hydro-alcoholic extract of petals of *Crocus sativus* "cashmerianus" in different concentrations (25 µg, 50 µg, 100 µg, 200 µg and 400 µg per ml) were compared with the standard drug omeprazole in similar concentrations. The results of test and standard drugs shows concentration dependent inhibition in H<sup>+</sup>/K<sup>+</sup> - ATPase activity, concentration more than 100µg were found effective i.e., 47.52, 56.46 and 70.31 % with test drug and 52.22, 62.47 and 73.82 % inhibitions with 100 µg/ml, 200 µg/ml and 400 µg/ml of concentration respectively. The extract at concentration more than 400 µg/ml was found more than 70% inhibition in H<sup>+</sup>/K<sup>+</sup> - ATPase activity. The results are tabulated in Table 2.

**Table 1: Acid Neutralizing Capacity (ANC) of hydro-alcoholic extract of petals of *Crocus sativus* "cashmerianus" by *in-vitro* method.**

Concentration (mg/ml)	Volume of NaOH consumed (ml)	mEq of Acid Consumed	ANC per gram of Antacid
HECS*- 100mg	34.8	12.25	115.7
HECS*- 200mg	28.1	17.15	42.17
HECS*- 500mg	43.2	9.68	10.22
HECS*- 1000mg	37.6	12.1	6.10
AHMH*- 500mg	47.2	7.75	11.90

\*HECS- hydro-alcoholic extract of petals of *Crocus sativus* cashmerianus; AHMH- aluminium hydroxide + magnesium hydroxide

**Table 2: *In-vitro* H<sup>+</sup>/K<sup>+</sup> - ATPase inhibition activity of hydro-alcoholic extract of petals of *Crocus sativus* "cashmerianus".**

Concentration (µg/ml)	Percentage Inhibition (%) (Mean ± SEM)	
	Standard Omeprazole	HECS
25µg	-42.55 ± 2.83	-20.21 ± 1.93
50µg	-28.54 ± 1.55	-13.37 ± 1.21
100µg	52.22 ± 1.69	47.52 ± 1.42 <sup>b</sup>
200µg	62.47 ± 2.71 <sup>a</sup>	56.46 ± 2.18 <sup>a</sup>
400µg	73.82 ± 2.52 <sup>a</sup>	70.31 ± 1.43 <sup>a</sup>

\*HECS- hydro-alcoholic extract of petals of *Crocus sativus* cashmerianus; Values are expressed as mean ± SD for six animals in each group. Statistically significant difference is expressed as <sup>a</sup>p<0.01, <sup>b</sup>P<0.05

## CONCLUSION

The causes of peptic ulcer is unclear in most of the patients, generally accepted that it may results of imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous protection system [7]. Acidity is a common gastrointestinal problem due to excessive secretion of gastric acid or stomach acid inflames the stomach lining mucosa and produces ulceration [8]. Antacids act by neutralizing gastric acid and thereby reduce the gastric pH [9]. The acid neutralizing capacity of an antacid is the amount of per gm of acid that it can neutralize [5, 10]. In ANC, results showed concentration dependent reduction was found in acid neutralizing capacity vs. per gm of antacid used. Hyperchlorhydria is another gastric problem characterized by hypersecretion of hydrochloric acid from parietal cells of gastric mucosa through proton pump.  $H^+/K^+ - ATPase$  is a key enzyme of parietal cells that inducing acidity. In  $H^+/K^+ - ATPase$  inhibition activity, the hydroalcoholic extract showed concentration dependent as similar like. It can be concluded that hydro-alcoholic extract of petals of *Crocus sativus* “cashmerianus” possess potent antiulcer activity.

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