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Ameliorative Effects of *Allium sativum* in Pyloric Ligation Induced Peptic Ulcer in Rat

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ABSTRACT

Background: Peptic ulcer is very common disorder in developing countries. The treatment for peptic ulcer with conventional therapeutic agents remains to be a challenge due to their multiple pathological mechanism and significant development of adverse effects. **Methods:** The present study was designed to evaluate the effect of aqueous extract of *Allium sativum* L. (*AqAs*) in Shay model (i.e., pyloric ligation) of peptic ulcer. **Results:** The administration of *AqAs* (200, 300 and 400 mg kg^{-1} p.o.) for 10 consecutive days reduced the gastric volume, total and free acidity, ulcerative index, total calcium, thiobarbituric acid reactive substances (TBARS), myeloperoxidase (MPO) levels and increase in the levels of total protein and reduced glutathione (GSH) levels in a dose dependent manner. The significant ($p<0.05$) ulcer protective effects of *AqAs* was observed at 400 mg kg^{-1} on treated group and was compared with ranitidine treated group. **Conclusion:** The ulcer protective effect of *AqAs* may be due to its anti-oxidative, anti-inflammatory, immunosuppressive, anti-secretary action. Hence, it was concluded that, the *AqAs* may serve as a newer herbal candidate from plant origin for the management of gastro-inflammatory disorder along with improving the quality of life.

Key words: *Allium sativum*, gastric volume, myeloperoxidase, pyloric ligation, reduced glutathione

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INTRODUCTION

Peptic ulcer is a serious gastrointestinal inflammatory disorder which is due to an imbalance between the offensive factors i.e., acid, pepsin and *H. pylori* and defensive factors i.e., mucin, prostaglandin, bicarbonate, nitric oxide and growth factors (Prabha *et al*., 2011; Kalra *et al*., 2011). The treatment and management of peptic ulcer involves two main approaches; (1) Reducing the production of gastric acid and (2) Re-enforcing the gastric mucosal protection (Tang and Chan, 2012). Commonly used drugs for peptic ulcer is histamine receptor-2 (H_2) blockers i.e., ranitidine, roxatidine and famotidine; muscuranic receptor-1 (M_1) blockers i.e., pirenzepine and telenzepine; proton pump inhibitors i.e., omeprazole, lansaprazole and pantoprazole; acid neutralizing agents i.e., sodium-bi-carbonate and aluminum hydroxide; ulcer protective and ulcer healing drugs i.e., sucralfate and carbenoxolone to promote mucosal defense systems (Den Hollander and Kuipers, 2012). Although, these drugs have brought the remarkable changes in ulcer therapy, the efficacy of these drugs is still debatable. Clinical evaluation of these drugs have shown tolerance, incidence of relapses, rebound acid secretion, stomach distention and drug interactions which make their efficacy arguable (Haruma *et al*., 2012; Chubineh and Birk, 2012). Hence, there is a rapid progress in exploring herbal medicines and plant extracts for their use in peptic ulcer as they are considered to be safer because of natural ingredient with no side effects (Zayachkivska *et al*., 2005; Sumbul *et al*., 2011; Gadekar *et al*., 2010).

Allium sativum Linn. is commonly known as garlic growing wild in South India that belongs to the family Liliaceae. Garlic contains carbohydrate, protein, fat, mucilage and volatile oil. The volatile oil is the chief active constituent; it contains allyl propyl disuphide and diallyl disulphyl, alliin and allicin (Khosla *et al*., 2004; Lee *et al*., 2008). Allicin is the most effective substance found in the garlic. Aqueous garlic extract contains primarily S-allyl-L-cysteines derived from -glutamyl-S-allyl-L-cysteines. Garlic extracts are reported to prevent cardiovascular disease, liver damage and aging (Lanzotti, 2006). It is believed in the traditional medicine that its consumption can cure stomach problems. *H. pylori* are gram -ve organism which is one of the major

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risk factor of 70% peptic ulcer cases. Garlic is known to inhibit the growth of *H. pylori* and can prevent peptic ulcers (Muhammad *et al*., 2012). Garlic oil is also reported to possess antiulcer effect in ethanol induced gastric damage and this effect was found to be due to antioxidant property (Khosla *et al*., 2004). Literature shows that ulcer development involve various biochemical changes like free radical generation, decrease in antioxidant enzyme, immune cell activation, toxic protein and mast cell activation (Muthuraman *et al*., 2011a; Muthuraman and Sood, 2010; Sood and Muthuraman, 2009; Sood *et al*., 2010). *Allium sativum* is known to possess the potential to the management of various ailments i.e., Alzheimer's disease, cancer, cardiotoxicity, hepatic necrosis, hyperlipidemia, diabetes mellitus including mucosal injury due to its free radical scavenging, anti-inflammatory, immunosuppressive, cell cycle regulatory actions (Alkreathy *et al*., 2012; Ashraf *et al*., 2005; Shukla and Kalra, 2007; Flora *et al*., 2009; Bagga *et al*., 2008; Ray *et al*., 2011). Based on these literature reports, the present study was undertaken to evaluate the ameliorative effect of *AqAs* on pyloric ligation induced peptic ulcer in rats.

MATERIALS AND METHODS

Animals: Male Wistar rats weighing between 200-250 g were used. They were kept at standard laboratory diet, environmental temperature and humidity. A 12 h light-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).

Chemicals: Folin-Ciocalteus phenol reagent (Merck Limited, Mumbai), 5,5-dithio, bis-2-nitro benzoic acid (DTNB), reduced glutathione, bovine serum albumin, (Sisco Research Laboratories Pvt. Ltd., Mumbai), thio-barbituric acid (Loba Chem. Mumbai), ranitidine (Gift sample from Ranbaxy Pvt. Ltd., Gurgaon) were procured for the present study. All other reagents used in this study are analytical grade and obtained from SD Fine chemicals, Mumbai, India.

Plant material and preparation of aqueous extract of *Allium sativum* (*AqAs*): Raw garlic bulbs were purchased from local market. The plant material was identified and authenticated in the P.G. Department of Horticulture, Khalsa College, Amritsar (Voucher No. HD-1112). Peeled garlic bulbs were subjected to maceration with water to obtain the aqueous extract of *Allium sativum*. The crude extract was filtered and concentrated under vacuum on a rotary evaporator at 40°C and stored in a refrigerator for further pharmacological evaluation.

Induction of peptic ulcer in rat (Shay ligation method): Ulcer study was performed by pyloric ligation process in rats as per described method of Shay model (Shay *et al*., 1945). Animals were fasted for 24 h before Pylorus Ligation (PL) with water *ad libitum*. Normal saline (1 mL rat^{-1}, p.o.) was administered twice daily to all the animals. Under light ether anesthesia, the abdomen was opened by midline incision; below the xiphoid process. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. On the day of experiment, the aqueous garlic extract, ranitidine or normal saline was administered orally before 1 h of pylorus ligation. The stomach was placed back carefully and the abdominal wall was closed with sutures. Animals were sacrificed 6 h after pylorus ligation and gastric content and isolated tissues were subjected to further studies.

Estimation of total and free acidity: The gastric juice was collected and its volume was measured. Further, the gastric juice was centrifuged and the clear supernatant was analyzed for total and free acidity (Hawk, 1965). Briefly, 1 mL of supernatant liquid was pipetted out and diluted to 10 mL with distilled water. The solution was titrated against 0.01 N sodium hydroxide using Topfer's reagent as indicator. The end point was determined when the solution turned to orange colour. The volume of sodium hydroxide consumption was noted which corresponds to free acidity. Further it was titrated till the solution regains pink color. The total volume of sodium hydroxide consumption was noted which corresponds to the total acidity.

Measurement of ulcerative index: Ulcerative index was measured by area calculation method (Takagi *et al*., 1969). Briefly, the stomach was opened and washed with running tab water. Then it was placed on a flat glass plate to count the ulcerative area. Standardization was made with a 10×10cm squared glass plate. Opened stomach, overlaid squared flat glass plate, exposing the mucous, showing the counting methodology of the injuries per square mm. The ulcer index was determined by using the equation:

$$\text{Ulcer index} = \frac{10}{X}$$

where:

$$X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

Biochemical estimation: Tissue homogenate was prepared with 10 volume of 0.1 M Tris-HCL buffer (pH 7.4) and supernatant of homogenate was employed to estimate total calcium, Thiobarbituric Acid Reactive Substance (TBARS), reduced glutathione (GSH), myeloperoxidase (MPO) and total protein content.

Estimation of tissue total calcium: Total calcium level was estimated atomic emission spectroscopic (flame photometric) method (Severenghaus and Ferrebee, 1950) with slight modification (Muthuraman *et al*., 2008a,b). Briefly, tissue homogenate was mixed with 1 mL of trichloroacetic acid (4%) in ice cold condition and centrifuged at 2500 rpm for 10 min at 4°C. The clear supernatant was used for the estimation of total calcium by atomic emission spectroscopy at λ^{556} nm. The calcium chloride was used as the standard. The concentration of tissue total calcium was expressed as ppm per mg of proteins.

Estimation of TBARS: Lipid peroxidation products i.e., malondialdehyde (MDA, thiobarbituric acid reactive substances) was estimated by spectrophotometric method (Ohkawa *et al*., 1979). The absorbance was determined spectrophotometrically at λ^{532} nm. The concentration of thiobarbituric reactive substances was expressed in terms of nmol of TBARS per mg of protein.

Estimation of reduced glutathione: Reduced glutathione levels were estimated by spectrophotometric method (Ellman, 1959). The absorbance was taken at λ^{412} nm within 15 min. The reduced glutathione was used as the standard. The concentration of reduced glutathione was expressed as μmol g^{-1} of protein.

Estimation of myeloperoxidase (MPO) activity: MPO, an enzyme of activated polymorphonuclear leukocytes, is used as an indication of tissue neutrophil accumulation. MPO activity levels were estimated by spectrophotometric method (Hillefass *et al*., 1990). The presence of MPO was measured at λ^{460} nm for 3 min. MPO activity was expressed as U g^{-1} tissue. One unit of MPO activity was defined as that degrading 1 μmol peroxide min^{-1} at 25°C.

Estimation of protein content: Protein concentration was estimated by spectrophotometric method (Lowry *et al*., 1951). The absorbance was determined spectrophotometrically at λ^{750} nm. The bovine serum albumin was used as the standard. The concentration of total protein was expressed as mg of protein per gram of tissue.

Experimental design: Seven groups, each comprising of six rats, were included in the antiulcer studies.

Group I (Normal control group): Rats were subjected to administration of 1 mL normal saline (p.o.) for 10 days.

Group II (Sham control group): Rats were subjected to surgical procedure without pyloric ligation process.

Group III (Negative control group): Rats were subjected to pyloric ligation for induction of ulcer.

Group IV (Positive control group): Rats were subjected to administration of ranitidine (50 mg kg^{-1} p.o.) for 10 consecutive days.

Group V-VII (*AqAs* treated groups): Rats were subjected to administration of *AqAs* (200, 300 and 400 mg kg^{-1}, p.o.) for 10 consecutive days.

Statistical analysis: All the results were expressed as Mean±Standard Deviation (SD). 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-value <0.05 was considered to be statistically significant.

RESULTS

Effect of *AqAs* on gastric volume, acid content and ulcerative index: Pyloric Ligation (PL) induces the changes of gastric volume, acid content and ulcerative index was increased significantly when compared to the sham control group. Pretreatment with *AqAs* (200, 300 and 400 mg kg^{-1}; p.o.) for 10 consecutive days have shown to reduced the above parameters when compared to the PL control groups in a dose dependent manner. The pretreatment of 200 and 300 mg kg^{-1} of *AqAs* showed insignificant ($p<0.05$) reduction in PL induced changes of above parameters. However, the higher dose (400 mg kg^{-1}) showed significant ($p<0.05$) changes of gastric volume ($F_{6,35} = 1082.065$), total acidity ($F_{6,35} = 504.248$), free acidity ($F_{6,35} = 659.544$) and ulcerative index ($F_{6,35} = 6258.112$) which is similar to that of ranitidine (50 mg kg^{-1}) treated group (Fig. 1- 3).

Effect of *AqAs* on gastric tissue biomarker changes: PL induces the changes of tissue biomarker changes i.e., increased the levels of total calcium, TBARS, MPO activity and decreased levels of GSH, total protein when

Table 1: Effect of *AqAs* on pyloric ligation induced biochemical changes

Groups	Total calcium (ppm mg^{-1} protein)	TBARS (nmol g^{-1} protein)	GSH (μmol g^{-1} protein)	MPO (U g^{-1} protein)	Total protein (mg g^{-1} tissue)
Normal	3.47±1.90	3.33±0.35	1.33±0.15	0.42±0.09	41.10±2.35
Sham	3.89±1.11	3.23±0.29	1.30±0.36	0.47±0.06	40.87±2.39
Pyloric ligation	14.95±2.36[a]	4.87±0.41[a]	0.78±0.22[a]	1.18±0.16[a]	7.31±1.61[a]
Ranitidine (50 mg kg^{-1})	3.89±1.94[b]	3.32±0.09[b]	1.30±0.15[b]	0.49±0.14[b]	37.51±2.09[b]
AqAs (200 mg kg^{-1})	12.78±2.03[a]	4.40±0.15[a]	0.87±0.29[a]	1.08±0.23[a]	16.04±1.54[a]
AqAs (300 mg kg^{-1})	10.07±1.56[a]	4.11±0.61[a]	1.04±0.35[a]	0.89±0.21[a]	23.86±1.94[a]
AqAs (400 mg kg^{-1})	4.69±0.95[b]	3.41±0.11[b]	1.28±0.16[b]	0.53±0.14[b]	35.99±1.16[b]

AqAs: Aqueous extract of *Allium sativum*, TBARS: Thiobarbituric acid reactive substances, GSH: Reduced glutathione, MPO: Myeloperoxidase, values are Mean±SD of 6 animals, [a]p<0.05, as compared to sham control group, [b]p<0.05, as compared to pyloric ligation control group

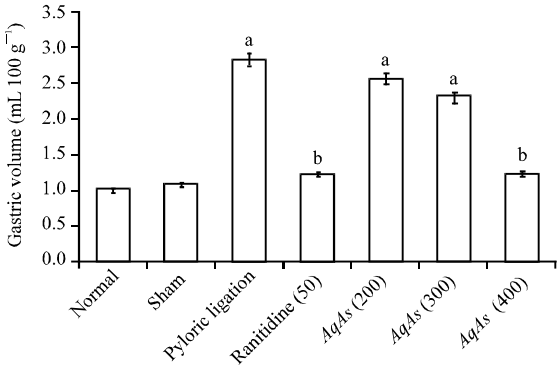

Fig. 1: Effect of *AqAs* on pyloric ligation induced changes of gastric volume. Data in parenthesis indicates mg kg^{-1}. *AqAs*: Aqueous extract of *Allium sativum*. Values are Mean±SD of 6 animals, a: p<0.05, as compared to sham control group, b: p<0.05, as compared to pyloric ligation control group

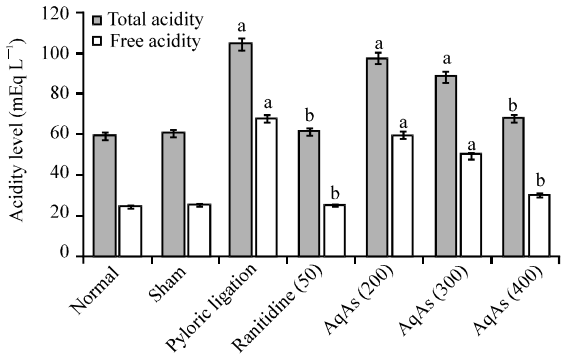

Fig. 2: Effect of *AqAs* on pyloric ligation induced changes of total and free acidity. Data in parenthesis indicates mg kg^{-1}. *AqAs*: Aqueous extract of *Allium sativum*. Values are Mean±SD of 6 animals, a: p<0.05, as compared to sham control group, b: p<0.05, as compared to pyloric ligation control group ligation control group

compared to sham control group. Pretreatment with *AqAs* (200, 300 and 400 mg kg^{-1}; p.o.) for 10 consecutive

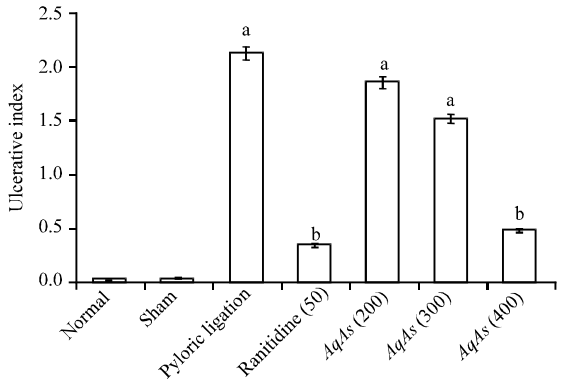

Fig. 3: Effect of *AqAs* on pyloric ligation induced changes of ulcerative index. Data in parenthesis indicates mg kg^{-1}. *AqAs*: Aqueous extract of *Allium sativum*. Values are Mean±SD of 6 animals, a: p<0.05, as compared to sham control group, b: p<0.05, as compared to pyloric ligation control group

days have shown to reduced the above parameters when compared to the PL control groups in a dose dependent manner. The pretreatment of 200 and 300 mg kg^{-1} of *AqAs* showed insignificant (p<0.05) reduction in PL induced changes of above parameters. However, the higher dose (400 mg kg^{-1}) showed significant (p<0.05) changes of total calcium ($F_{6,35}$ = 307.284), TBARS ($F_{6,35}$ = 26.074), MPO ($F_{6,35}$ = 517.150) total protein ($F_{6,35}$ = 511.959), GSH ($F_{6,35}$ = 205.670) which is similar to that of ranitidine (50 mg kg^{-1}) treated group (Table 1).

DISCUSSION

In the present study, the ligation of pyloric sphincter potentially increase the levels of gastric volume, acid content, ulcerative index, total calcium, TBARS, MPO activity and decrease the levels of GSH, total protein. Pretreatment of aqueous extract of *Allium sativum* (*AqAs* 400 mg kg^{-1}, p.o. for 10 consecutive days) significantly attenuates the pyloric ligation induced alteration of hypersecretory, oxidative stress and inflammatory parameters. Literature report has also evident that *Allium sativum* has shown the

anti-secretory, anti-oxidative and immunomodulatory action (Arhan *et al*., 2009; Shukla and Kalra, 2007; Ray *et al*., 2011). The pathogenesis of peptic ulcer by pylorus ligation is mainly involved the enhancement of gastric acid secretion by activation of proton pump (PP, H^+-K^+-ATPase) (Singh *et al*., 2012). The activation of PP is noted in the condition of peptic ulcer due to the abnormal changes of endogenous bio-molecule i.e., histamine, serotonin, calcium, gastrin, acetylcholine etc (Onasanwo *et al*., 2010; Yang *et al*., 2011) which causes the gastric mucosal damage leads to develop the peptic ulcer. *Allium sativum* is traditionally used as food ingredients as well as medicaments for various ailments. Numerous experimental evidences documented that, *Allium sativum* has therapeutic effect in gastrointestinal disease in rodent as well as in human due to its free radical scavenging, anti-inflammation and immunomodulatory action (Abel-Salam, 2012; Hodge *et al*., 2002). *Allium sativum* has shown the potential anti-ulcer effect in ethanol induced gastric damage due to its anti-oxidant property (Khosla *et al*., 2004). In our experimentalm, data, also showed that, *AqAs* alter the level of oxidative stress marker (i.e., decreases the TBARS and increases the level of reduced glutathione as an endogenous anti-oxidant molecule), inflammatory markers (i.e., myeloperoxidase) and tissue total calcium. The excess formation of free radicals is known to produce the calcium accumulation in the intracellular space (Muthuraman *et al*., 2011a, b).

In physiological condition, calcium ion plays a buffering action in cytosolic region whereas in pathological situation its play "calcium sparks" action with free radicals (Muthuraman *et al*., 2011b; Nivala *et al*., 2012). The alterations of cellular calcium levels play a key role in the subsequent alteration of acid secretion and development of gastric ulceration (Wong *et al*., 1991). In addition, free radical and calcium accumulation are contributed to the activation of immune like cells particularly mast cells (Suzuki *et al*., 2012). In the pathogenesis of peptic ulcer condition, mast cells are known to be a major key role (Hampton and Hale, 2011). Activated mast cells release various biochemical mediators like cytokins, chemokines, histamine, serotonin, eicosanoids and myloperoxidase enzymes (Costanza *et al*., 2012; Gan *et al*., 2012) which is also known mediators in the peptic ulcer progress. Histamine plays a critical role in the hyperacidity of peptic ulcer disease (Adami *et al*., 2012). Further, ranitidine (histamine receptor-2 blocker) has anti-ulcerative and anti-secreatory actions in human as well as in rodents (Pimple *et al*., 2012; Gashi *et al*., 2012).

Cardinal feature of activated mast cell indicates that, increase the activity of myeloperoxidase (MPO) and neutrophils infiltration which are the major inflammatory markers and it causes the major degree of mucosal damage (Gan *et al*., 2012; Arakawa *et al*., 2012). In the present study, the pretreatment of *AqAs* has also significantly reduced the MPO levels in gastric tissue of pyloric ligated rats.

CONCLUSION

Hence, it may be conclude that the pretreatment of aqueous extract of *Allium sativum* ameliorate the pyloric ligation induced peptic ulcer due to its anti-oxidative, anti-inflammatory, immune cell modulatory and calcium modulatory actions. Therefore, aqueous extract of *Allium sativum* may be a potent herbal candidate for the treatment of peptic ulcers. However, the more elaborative studies are required to explore the responsible phytoconstituents and possible pharmacodynamic actions in peptic ulcer disease.

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