

### The effect of garlic methanol extract on gastric acid and pepsin in basic and stimulated conditions by electrical stimulus of vagus nerve in rats

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**Abstract:** This study aims to determine the digestive effect of garlic (*Allium sativum* L.) on acid secretion and stomach pepsin. In this study two groups of 12 wistar rats were tracheostomized, laparatomized, gastroduodenostomized, and then 100mg/kg of garlic methanol extract dissolved in 9% saline was introduced into their stomach. The stomach secretions then washed out in first base, second base, vagus-stimulated conditions and reverse to base. The vagus nerves of both side of the neck released from carotid sheath and stimulated with 15 millivolts, frequency of 4 Hz and 1 millisecond wide. The gastric acid amounts were measured by titrimetry and gastric pepsin amounts were measured by Anson technique. The study showed that garlic extract increased the level of acid secretion in all stages and pepsin secretion in first base, vagus stimulated conditions, and reverse to base significantly compared to the control group ( $P<0.05$ ). The vagus-stimulated conditions caused a significant increase in gastric acid and pepsin secreting compared to the first base ( $P<0.05$ ). The level of gastric acid and pepsin secretion did not show a relationship between the rats and their gender. In conclusion using this plant and its other products is forbidden for those patients suffering from indigestion due to acid or pepsin increase. So patients suffering from gastritis, duodenal and ulcer are not allowed to use this plant in their daily diet.

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#### Introduction:

Digestive problems are growing widely within human society and more often appear as ulcer, duodenal, gastritis, indigestion, etc. All these diseases are due to some disorder in acid or pepsin secreting.(1)

Garlic (*Allium sativum* L.) has been cultivating for ages as a medicinal plant and spice and nowadays is used as a popular medicinal plant. Its importance is growing widely. (2)

Alien is one of the most important components of garlic. Other chemical components such as allicin, ajoene, allylpropyl, disulfide, alliinase, peroxides, and fibronase and elements such as selenium, germanium, tellurium, etc have been found in the garlic sprout but compared to alien they are not considerable(3,4). Garlic extract has various effects such as antibacterial(5,6), antiviral(7), antifungal(8,9), lipid and serum cholesterol lowering (10,11), blood sugar lowering(12), and anti-ligation(13). Eating the fresh garlic sprout, garlic extracts, or garlic oil can cause nausea, vomiting and diarrhea, because of adenosine that acts as a diarrhea central stimulator (14).

This study tried to determine the digestive effect of this plant in order to present an independent study about the garlic methanol extract function mechanism and its effect on acid secretion and stomach pepsin. In order to stimulate acid secretion and stomach pepsin and to figure out function mechanism of the elements found in the garlic, we used vagus nerve electronic stimulator.

#### Materials and Methods:

The present experimental study was conducted on 24 (12 male and 12 female) wistar rats weighing 200-250 gr. They were kept under 12 h light: darkness in Medical Plants Research Center (Shahrekord University of Medical Science, Shahrekord, Iran), divided into two equal (6 male and 6 female) groups: control and garlic. The rats had access to food and water freely 24h before the experiment. 12h before the experiment, the rats were transferred to a cage where they were deprived of food but had access to water. The cage was designed in a way to prevent the animal from eating feces in the case of hunger. Eliminating the effect of light/ day rhythms, we started the experiment regularly every morning at 8 am. By

administering sodium thiopental (in 50 mg/kg) intraperitoneally, each animal was anaesthetized (1). To prevent mouth secretions from entering into esophagus, tracheostomy (opening tracheal out surgically) was performed (15, 16). Before being disconnected, the vagus nerves unkept parts were held by a string and then delivered to the researcher to stimulate it. Preventing from electrical connection to rat muscles and neck, under stimulus electrode, we put a paraffin sheet and treated it with paraffin to be insulated (17). Then, we Laparotomized the rats (opened the animals abdomen out surgically), introduced a cannula into duodenum and moved it forward to stomach. The dried garlic extract was made soluble using a physiology serum 9% in mg/1kg body weight introduced into body via gastroduodenostomy (a tube which opens stomach and duodenum out after surgery). The gastric juice was extracted by washing out stomach secretion. In the control group, only 1ml of physiology serum 9% was introduced into stomach. To eliminate the stress effect of surgery and reach stability, the anesthetized animal was left for 30 min (18). All gastric secretions were extracted during this 30 min (stress elimination step). The first sample for experiment was lavaged 15 min after stress elimination step (stomach secretion elicitation) (the first basal) the second basal, electrical stimulation of vagus nerve, and return to basal were extracted and experimented, respectively 30 minutes, 45 minutes, and 60 minutes after stress elimination step. To stimulate the vagus nerve, we use a stimulator (15 mlv, 4 Hz frequency, 1 ms width). Methanol was used as soluble in this study and the extract was elicited using percolation. The secretion was dried before the experiment in order to eliminate the probable remaining ethanol. To measure acid secretion in basal state in the control group 2 ml of physiology serum (divided into two 1ml doses with a 15 min interval) was injected into the stomach and the stomach was emptied. 1 ml of physiology serum was also used to measure the stomach acid by an acid titrator (Iran) according to titrimetry using sodium hydroxide 0.01 normal (1), and another 1ml to measure stomach pepsin according to Anson method.

#### ***Stomach acid measurement:***

To measure stomach acid, acid titrator device was used. To calculate gastric acid secretion  $N1V1 = N2V2$  equation was employed, where N1 was normality of gastric acid and undetermined in this equation, V1 is the volume of gastric milk, N2 is normality of consumed NaOH, and V2 is the volume of

consumed NaOH. Considering that we knew the value of V1, V2 and N2, we calculated N1 in 15min.

#### ***Stomach pepsin measurement:***

To do this we used Anson method (19). In this method 0.3 normal trichloroacetic acid (TCA) solution, 25 gr/l hemoglobin of bovine, 30 mg/l standard pepsin and HCl 0.01 and 0.3 normal are applied. Initially the standard curve for pepsin was developed and the value of stomach pepsin reception was reported in 15min according to this curve.

After 10 min 5ml of TCA 0.3 normal was introduced and the reaction between pepsin and hemoglobin was stopped at this point. The only difference between blank pipe and other pipes is that 5ml TCA was introduced into blank pipe after adding hemoglobin and HCl 0.3 normal while in other pipes standard pepsin and HCl were introduced after adding hemoglobin and 5cc TCA (0.3 normal) was introduced 10 min after this reaction. Finally, all samples were filtered using filter papers and the amount of light absorption by the filtrated liquid which contained amino acids obtained from standard pepsin effect on hemoglobin, was read by spectrophotometer with wavelength of 280nm (UV), and standard pepsin curve was developed. All samples pepsin was reported according to this curve.

Measuring the pepsin of the samples obtained from gastric juice was per the same procedure. The only difference was that 0.1 ml of the obtained gastric juice was diluted by 9.9 ml of physiology serum (0.9%), and of the 10 ml of this solution 0.5 ml was each time introduced into the case sample pipe instead of the standard pepsin employed for developing the standard pepsin curve. In other words, initially 2 ml hemoglobin 25gr/1000cc, then 0.5 ml HCl 0.3 normal and finally 0.5 ml of the diluted gastric juice was introduced in to the pipe per the above mentioned procedure, and 5 ml of TCA (0.3 normal) was added into the pipe to end the reaction. After filtering the solution, the amount of absorbed UV with the wavelength of 280nm was measured by filtered light using spectrophotometer UV and the pepsin in each basal was calculated according to the developed standard curve.

The data were analyzed by using independent t-test and  $P < 0.05$  was considered as significant.

#### ***Results:***

this study showed that garlic extract increased the level of acid secretion in the first base ( $P < 0.001$ ), second base ( $P < 0.001$ ) vagus stimulated conditions ( $P < 0.05$ ), and reverse to base ( $P < 0.001$ ) significantly compare to the

control group (Table 1). Garlic extract increased pepsin secretion in the first base ( $P<0.05$ ), vagus stimulated conditions ( $P<0.05$ ), and reverse to base ( $P<0.05$ ) significantly compared to the control group (Table 1).

The vagus-stimulated conditions caused a significant increase in acid ( $P<0.001$ ) and pepsin ( $P<0.005$ ) secreting compared to the first base (table 1). The level of acid and pepsin secretion did not show an association between the rats and their gender.

Table 1: Comparison of mean ( $\pm$ SD) of the acid and pepsin secretion between the control and garlic groups

Variables	groups	first base (15 min)	second base (30 min)	vagus stimulated conditions	reverse to base	P-value (between the first base and vagus stimulated conditions)
acid secretion	control	4.88 $\pm$ 1.1	3.98 $\pm$ 1.05	10.68 $\pm$ 4.61	5.03 $\pm$ 1.61	0.048
	garlic	16.07 $\pm$ 0.59	11.35 $\pm$ 6.46	17.04 $\pm$ 5.93	12.28 $\pm$ 5	0.000
	Pvalue	0.000	0.001	0.022	0.000	
pepsin secretion	control	5.26 $\pm$ 1.36	5.48 $\pm$ 1.39	7.86 $\pm$ 2.5	6.15 $\pm$ 1.43	0.039
	garlic	7.1 $\pm$ 1.48	6.04 $\pm$ 1.15	10.28 $\pm$ 2.16	8.36 $\pm$ 2.55	0.039
	Pvalue	0.019	0.297	0.019	0.015	

(n=12 in each group)

Table 1: Comparison of mean ( $\pm$ SD) of the acid and pepsin secretion between two genders in control and garlic groups

Variables	groups	gender	first base (15 min)	second base (30 min)	vagus stimulated conditions	reverse to base
acid secretion	control	male	4.86 $\pm$ 1.2	4.28 $\pm$ 1.16	14.66 $\pm$ 11.6	4.8 $\pm$ 1.96
		female	4.9 $\pm$ 1.1	3.68 $\pm$ 0.93	9.13 $\pm$ 4.3	5.26 $\pm$ 1.33
	garlic	male	9.31 $\pm$ 4.63	12.35 $\pm$ 8.55	17.11 $\pm$ 7.39	12.58 $\pm$ 4.7
		female	16.58 $\pm$ 5.01	10.36 $\pm$ 4.06	17.78 $\pm$ 4.74	11.98 $\pm$ 5.72
pepsin secretion	control	male	5.43 $\pm$ 0.96	5.15 $\pm$ 0.78	6.03 $\pm$ 0.76	6.02 $\pm$ 1.76
		female	5.81 $\pm$ 1.76	5.81 $\pm$ 1.84	9.68 $\pm$ 2.27	6.08 $\pm$ 1.98
	garlic	male	6.86 $\pm$ 0.96	5.88 $\pm$ 0.55	9.83 $\pm$ 1.86	6.84 $\pm$ 0.93
		female	7.33 $\pm$ 1.95	6.2 $\pm$ 1.59	10.73 $\pm$ 2.51	9.86 $\pm$ 2.83

$p>0.05$  between two genders in all Variables

#### Discussion:

The results showed that the level of pepsin and acid in the rats which received the methanol extract of garlic increased significantly both in the first and second 15 min compared to the control group. Because the pepsin controlling mechanism is related to some factors such as neural, secretion, chemical, etc., one of these categories or a combination of them must have

involved. A study about the rats showed that in the stomach step of acid secreting was due to gastric secretion which happened due to stimulating the stomach chemical receptors. (20)

Gastric had the most important effect on parietal cells and enterochromaffin cells in mouse and gastrin affected stomach pepsin and acid secretion in 2 ways: 1) Directly through

affecting their receptors in parietal cells and main cell. 2) Directly through stimulating antrochromahin cell and histamine release. (21,22) In addition gastrin and Asetilkolin increased the level of acid and pepsin secretion via its receptors and increased intracellular calcium, but histamine affected pepsin and acid secreting through H2 receptor and cAMP(21). Another study showed that gastrin both affected parietal cells and directly increased the acid through stimulating the histamine release. It was shown in a research that increasing acid secretion and pepsin through calcium release from the intracellular storage in a research; gastrin used extracellular calcium (23, 24). Because the garlic methanol extract increased acid in rats, all or some part of the above mentioned interaction must have been stimulated because stomach pH increase caused gastrin stimulating and releasing. Some component(s) existing in the garlic could cause increase in pH, gastric secretion and then increase in the acid, or caused gastric receptors activities. However, to prove these issues we need a precise study and approval of gastrin receptor activities. We should also consider somatostatin which had a great effect on controlling acid, and garlic cause a decrease in D-cell activities in antral mucosa in the stomach and resulted in decrease in somatostatin secretion. Approval of this issue also needs further study. Having compared control and garlic group in table 1, we observed that the extract increased the pepsin secretion significantly. There are so many factors involved in secreting pepsinate by the main cell and finally producing the pepsin. Among two neural and secreting systems in pepsinate secreting there has been a great emphasis on neural system. Acetylcholine is the most powerful neurotransmitter stimulator in pepsinogen secreting (25). So, the vagus nerve causes secretion of a large amount of pepsinogen (26, 16). In secretion system gastrin was the pepsin secretion stimulator. Gastrin, in the dogs, caused acid secreting and a mechanism sensitive to acid was active for pepsinogen secreting. Our study showed that garlic extract increased the pepsin through secreting or otherwise except for stimulating the cholinergic neural system.

If the extract components had acted through occupying the receptor cholinergic. After stimulating the vagus nerve there should not have been a difference between acid secreting and pepsin. Another probability was that if the existing components had acted through occupying the cholinergic receptors, we could say that the extract component could not have occupied all the cholinergic receptor in the

stomach and there would have still been a time for stimulating cholinergic system to increase acid secretion and pepsin. This can be proved using cholinergic receptors or the plant extract. The result showed that there was not a significant difference between acid and pepsin secreting in both male and female rats. The basal acid secretion in both male and female rats was the same but the stimulated secretion in male rats was higher than that in female. If stimulating had occurred during the day and at the night when acid secreting would be the same in both male and female rats (27). Besides, acid secreting in responding to the stimulation by histamine in the dogs was the same in both male and female rats (28). A group of researchers showed that basal acid secreting and accumulation of parietal cells in male rats was less than female (21). Researchers showed that male rat abortion caused a decrease in the amount of parietal cells and acid secreting (29). They also showed that the density of enterochromaffin cells in female rats was more than that in male while the amount of parietal cells was less in female (30,31). Considering the above mentioned about androgen secretion effect on acid decreasing and stomach pepsin.

The similarity between acid secreting and basal pepsin in both genders in this research was due to enterochromaffin cells and histamine increase, and an accumulation of parietal cells decrease in female rats compared to male. On one hand enterochromaffin cells and histamine increase in female rats caused the increase in acid secreting and pepsin and on the other hand, parietal cell decrease in female rats caused a reduction in acid secreting compared to male. Probably these factors caused the similarity between the acid secreting and pepsin in both genders.

#### **Conclusion:**

Using garlic in daily diet has a useful effect on digestion. Using this plant for patients suffering from indigestion is very useful. Using this plant and all its products is not recommended for those patients suffering from indigestion due to acid or pepsin increase. So, patients suffering from gastritis, duodecimal, and ulcer should not be allowed to use to this plant in their daily diet.

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