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RESEARCH ARTICLE

Effects of *Nigella sativa* Aqueous Extracts on Gastric Acid Secretion in Isolated Rat Stomach

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Summary

Nigella sativa (NS) has been shown to have antioxidant, anti-inflammatory and antiulcer activities in different conditions. The present work was done to investigate the possible effects of *Nigella sativa* aqueous extracts (NS) on gastric acid secretion in isolated rat stomach. Gastric acid secretion was measured in the isolated rat stomach preparation. The results obtained *in vitro* experiments showed that doses of 2 mg/100 ml DMSO and 5 mg/100 ml DMSO of NS aqueous extracts reduced gastric acid secretion stimulated by histamine and 5 mg/100 ml DMSO of NS aqueous extracts reduced gastric acid secretion stimulated by bethanechol, but none of the dosis of NS did affect pentagastrin-induced acid secretion. The present results suggest that NS has a gastroprotective effect, probably due to reducing gastric acid secretion.

Keywords: *Nigella sativa, Gastric acid secretion, Stomach*

İzole Edilmiş Sıçan Midesinde Çörek Otu Sulu Ekstraktının Gastric Asit Sekresyonu Üzerine Etkileri

Özet

Çörek otunun farklı şartlarda antioksidant, anti inflamatuar ve anti ülser aktiviteye sahip olduğu gösterilmiştir. Sunulan çalışma izole edilmiş sıçan midesinde çörek otu sulu ekstraktının gastrik asit sekresyonu üzerine etkilerini araştırmak için yapılmıştır. Gastrik asit sekresyonu izole edilmiş sıçan mide preparatında ölçüldü. *İn vitro* şartlarda yapılan deneylerden elde edilen sonuçlar çörek otu sulu ekstraktının 2 mg/100 ml DMSO ve 5 mg/100 ml DMSO dozlarının histamin ile uyarılan ve 5 mg/100 ml DMSO dozunun bethanechol ile uyarılan gastrik asit sekresyonunu azalttığı, ancak deneyde kullanılan hiçbir dozun pentagastrin ile uyarılan asit sekresyonunu etkilemediğini gösterdi. Sunulan çalışma, çörek otunun gastrik asit sekresyonunu azalttığından dolayı gastroprotektif bir etkiye sahip olduğunu destekliyor.

Anahtar sözcükler: *Çörek otu, Gastrik asit sekresyonu, Mide*

INTRODUCTION

Nigella sativa (NS), an annual herbaceous plant of the Ranunculaceae, have been used traditionally in the Middle East, Northern Africa, Far East and Asia for the treatment of various diseases for over 2000 years [1]. It is used in folk medicine as a natural remedy for a number of diseases and conditions such as asthma, hypertension, diabetes, inflammation, bronchitis, headache, fever, dizziness, and gastrointestinal disturbances [2]. Recently many clinical and animal studies have shown that NS have various therapeutic effects such as antioxidant, anti-inflammatory, anticancer [3] antihistaminic [4], antibacterial effects [5]. *N. sativa* seeds contain 36%-38% fixed oils, proteins, alkaloids, saponin and 0.4%-2.5% essential oil [6]. The main compounds are thymoquinone (27.8%-57.0%), ρ-cymene (7.1%-15.5%), carvacrol (5.8%-11.6%), t-anethole (0.25%-2.3%), 4-terpineol (2.0%-6.6%) and longifoline (1.0%-8.0%) [7]. A single report in rats has suggested that the aqueous extract of *N. sativa* seeds was effective in reducing the ulcer index (induced by aspirin) by about 36%. The treatment reduced the peptic activity and acid production, but did not change mucin activity [8]. However, there is little information about the effect of NS and its

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major constituent on the gastrointestinal system.

In this study, the effect of aqueous extracts of *Nigella sativa* on secratogogue-induced gastric acid secretion was investigated using a well-established isolated rat stomach model [9,10].

MATERIAL and METHODS

Animals

Sprague-Dawley rats, weighing 230 and 240 g, were provided by the Experimental Animal Laboratory of Ataturk University. The animals were fed under normal conditions (22°C) before the experiments. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and approved by the local animal care committee of Ataturk University.

Chemicals

Drugs were purchased from Sigma Chemical Co. Ltd., UK unless otherwise indicated. 100 mg *Nigella sativa* aqueous extracts (0.5 mg%, 1 mg%, 2 mg% and 5 mg%) was dissolved in 1 ml DMSO. 0.3 gr histamine 2HCl (10^{-4}M), (H_2 histamine receptor agonist that stimulates gastric acid secretion) was dissolved in 1 ml distilled water. 0.16 gr famotidine (10 µM), (H_2 histamine receptor antagonist) was dissolved in saline containing a small amount of 0.1 N HCl and dilution was made with distilled water. 25 mg pentagastrin (10^{-6}M), (CCK_2 receptor agonist that stimulates gastric acid secretion) was dissolved in 1 ml DMSO. 0.1 gr proglumide (10 µM) (CCK_2 receptor antagonist) was dissolved in 15 ml distilled water. 0.3 gr bethanechol (10^{-5}M) (muscarinic acetylcholine receptor agonist that stimulates gastric acid secretion), was dissolved in 1 ml distilled water. 0.5 gr atropine (10^{-6}M) (muscarinic acetyl-choline receptor antagonist) was dissolved in 1 ml distilled water.

Extraction Method

Air-dried NS were powdered and extracted with water by stirring at room temperature for 24 h. The extract was filtered and evaporated to dryness. The dried extract dissolved in DMSO. 100 mg of the *Nigella sativa* aqueous extracts were dissolved by the initial addition of 1 ml of dimethyl sulfoxide.

Preparation of Isolated Rat Stomach

Rats were fasted for 16 h with free access to water before experiments. Gastric acid secretion was measured in the isolated stomach preparation as previously described for mouse [9], with a slight modification [11,12]. Briefly, animals were killed by cervical dislocation. The stomach was removed and two polythene cannulae (2 mm internal diameter) were inserted and tied in, one into the pylorus via the duodenal bulb and the other into the cardia via the esophagus which the stomach contents was first gently washed out. The stomach was placed in a 20-ml organ bath containing a serosal nutrient solution (mM: NaCl 118, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.14, Na_2HPO_4 15.9, $CaCl_2$ 0.65, glucose 31.6, adjusted to pH 7) kept at 37°C and gassed with 95% O_2 and 5% CO_2. The gastric lumen was perfused (Kendall Kangaroo 924 pump, Turkey) at 1 ml/min with a mucosal nutrient solution (mM: NaCl 135, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 1.3, glucose 31.6, adjusted to pH 5). Drugs were added to the serosal solution in the baths. The pH of the gastric fluid obtained was measured with pH meter (Hanna Instruments, pH 211 micoprocessor). Δ pH was expressed as the difference between pH obtained in the experiment and basal pH.

Experimental Protocol and Measurement of pH

The experimental protocols for each dose and for each active ingredient are shown in appendix 1 *(Table 1)*.

The basal acid output in these experiments was unstable just after the set-up of the preparations, and it took at least 30 min to stabilize basal acid secretion. After the stabilization, different doses of *Nigella sativa* aqueous extracts were added to the serosal solution. After waiting 10 min, the pH was measured in 2 ml fluid taken from duedonal cannula side of stomach and then the mucosal and serosal side of stomach was washed 2 times in five min. Histamine was added to the serosal solution in the presence of proglumide, atropine then pH was measured. After washing the stomach, different doses of *N. sativa* aqueous extracts and histamine was added to the serosal solution in the presence of proglumide, atropine, then pH of gastric fluid was measured. After washing the stomach, histamine and NS was added to the serosal solution in the presence of famotidine, atropine and proglumide then pH was measured. The same protocol is valid for bethanechol and pentagastrin.

Statistics

Statistical analysis was performed with one-way analysis of variance (ANOVA). P value <0.05 was considered statistically significant.

RESULTS

Effect of NS Aqueous Extracts on Basal Acid Secretion

Lower doses of NS aqueous extracts (0.5 mg/100 ml DMSO, 1 mg/100 ml DMSO and 2 mg/100 ml DMSO) had no effect on the basal pH, but at 5 mg/100 ml DMSO dosis level basal acid secretion in isolated rat stomach was decreased (P<0.05) *(Fig. 1)*.

Effect of NS Aqueous Extracts on Histamine-Induced Acid Secretion

Histamine (10^{-4}M/1ml distilled water) produced an increase in gastric acid secretion in the presence of atropine

Table 1. *Experimental protocol and measurement of pH (histamine)*
Tablo 1. *Deneysel protokol ve pH ölçümü (histamin)*

Time	Procedure	Reaction	Test Cycle
2 min	Intragastric infusion	Mucosal solution was given 2 ml with peristaltic pump (1 ml/min)	
10 min	Waited		
2 min	Sample collection	2 ml of the stomach contents were collected	pH measured
5 min	Washed 2 times	Serosal and mucosal side were washed	
2 min	Intragastric infusion	Mucosal solution was given 2 ml with peristaltic pump (1 ml/min)	
10 min	Waited		
2 min	Sample collection	2 ml of the stomach contents were collected	pH measured
5 min	Washed 2 times	Serosal and mucosal side were washed	
2 min	Intragastric infusion	Mucosal solution was given 2 ml with peristaltic pump (1 ml/min)	
10 min	Waited		
2 min	Sample collection	2 ml of the stomach contents were collected	pH measured (Basal pH)
5 min	Washed 2 times	Serosal and mucosal side were washed	
2 min	Intragastric infusion	Mucosal solution was given 2ml with peristaltic pump (1 ml/min)	
	Drug application	NS Aqueous Extract was added to serosal side	
10 min	Waited		
2 min	Sample collection	2 ml of the stomach contents were collected	pH measured
5 min	Washed 2 times	Serosal and mucosal side were washed	
2 min	Intragastric infusion	Mucosal solution was given 2 ml with peristaltic pump (1 ml/min)	
	Drug application	Histamin + Proglumide + Atropine was added to serosal side	
10 min	Waited		
2 min	Sample collection	2 ml of the stomach contents were collected	pH measured
5 min	Washed 2 times	Serosal and mucosal side were washed	
2 min	Intragastric infusion	Mucosal solution was given 2 ml with peristaltic pump (1 ml/min)	
	Drug application	Histamin + Proglumide + Atropine + NS Aqueous Extract was added to serosal side	
10 min	Waited		
2 min	Sample collection	2 ml of the stomach contents were collected	pH measured
5 min	Washed 2 times	Serosal and mucosal side were washed	
2 min	Intragastric infusion	Mucosal solution was given 2 ml with peristaltic pump (1 ml/min)	
	Drug application	Histamin+Famotidin + Proglumide + Atropine was added to serosal side	
10 min	Waited		
2 min	Sample collection	2 ml of the stomach contents were collected	pH measured
5 min	Washed 2 times	Serosal and mucosal side were washed	
2 min	Intragastric infusion	Mucosal solution was given 2 ml with peristaltic pump (1 ml/min)	
	Drug application	Histamin + Famotidin+Proglumide + Atropine + NS Aqueous Extract was added to serosal side	
10 min	Waited		
2 min	Sample collection	2 ml of the stomach contents were collected	pH measured

The same protocol was also performed to pentagastrin and bethanechol

and proglumide in isolated rat stomach preparation. The gastric acid secretion stimulated by histamine was not affected by addition of 0.5 mg/100 ml DMSO, 1 mg/100 ml DMSO NS aqueous extracts into the serosal solution, but the histamine-stimulated gastric acid secretion was reduced by serosal application of doses 2 mg/100 ml DMSO NS and 5 mg/100 ml DMSO NS ($P<0.05$) *(Fig. 2A)*. Also the histamine-stimulated gastric acid secretion was inhibited by serosal application of the histamine-H_2 receptor antagonist famotidine 10 µM in the presence of atropine and proglumide *(Fig. 2B)*.

Effect of NS Aqueous Extracts on Bethanechol-Induced Acid Secretion

Bethanechol (10^{-5}M/1 ml distilled water) produced a small increase in gastric acid secretion in the presence of famotidine and proglumide in isolated rat stomach preparation. NS aqueous extracts at the doses of 0.5 mg/100 ml DMSO, 1 mg/100 ml DMSO and 2 mg/100 ml DMSO did not affect bethanechol-induced acid secretion, but the bethanechol -induced acid output was reduced by treatment with high dosis of NS aqueous extracts (5

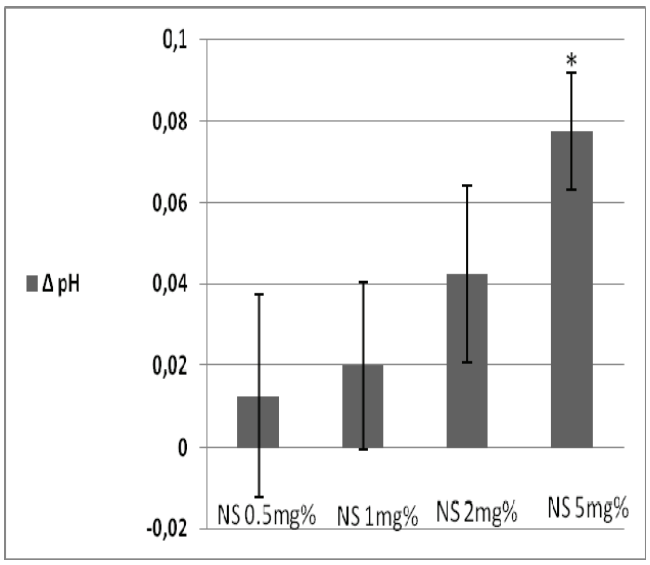

Fig 1. Effect of NS aqueous extracts on basal acid secretion in isolated rat stomach (n=4, * P<0.05)

Şekil 1. İzole edilmiş sıçan midesinde bazal asit sekresyonu üzerine çörek otu sulu ekstraktının etkisi (n=4, * P<0.05)

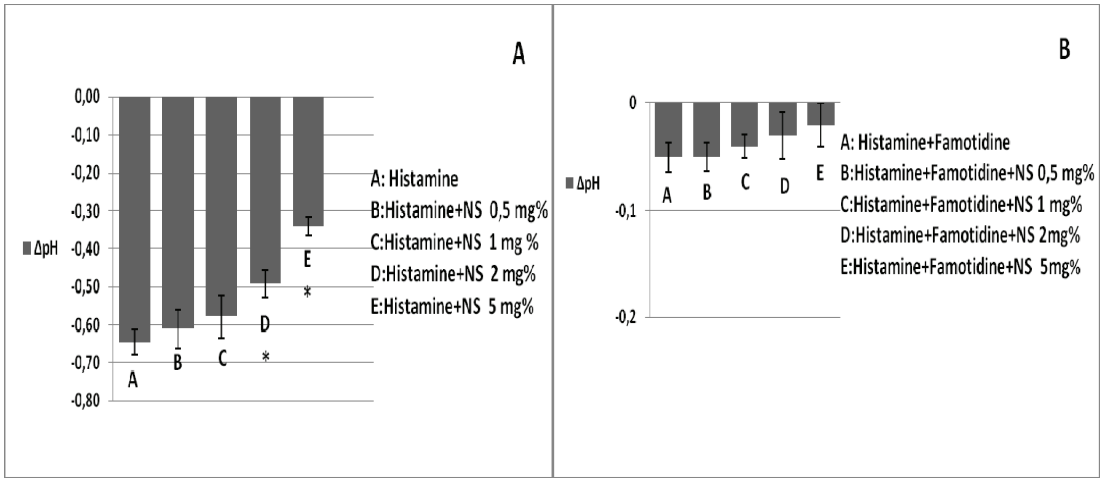

Fig 2. A- Effect of NS aqueous extracts on histamine-induced acid secretion in the presence of atropine and proglumide in isolated rat stomach. There is statistical difference between groups histamine + NS 2 mg% and histamine + NS 5 mg%, with histamine (n=4, * P<0.05). **B-** Effect of NS aqueous extracts on histamine-induced acid secretion in the presence of atropine, proglumide and famotidine in isolated rat stomach. There is no statistical difference between groups (n=4, P>0.05)

Şekil 2. A- İzole edilmiş sıçan midesinde proglumide ve atropine varlığında histamin ile uyarılmış gastrik asit sekresyonu üzerine çörek otu sulu ekstraktının etkisi. Histamine ile histamine + NS 2 mg % ve histamine + NS 5 mg % grupları arasında istatistiksel olarak fark vardır (n=4, * P<0.05). **B-** İzole edilmiş sıçan midesinde proglumide, atropine ve famotidine varlığında histamin ile uyarılmış gastrik asit sekresyonu üzerine çörek otu sulu ekstraktının etkisi. Gruplar arasında istatistiksel olarak fark yoktur (n=4, P>0.05)

mg/100 ml DMSO) (P<0.05) *(Fig. 3A)*. Also the bethanechol-stimulated gastric acid secretion was inhibited by serosal application of atropine 10^{-6}M/1 ml distilled water in the presence of famotidine and proglumide *(Fig. 3B)*.

Effect of NS Aqueous Extracts on Pentagastrin-Induced Acid Secretion

Pentagastrin (10^{-6}M/1ml DMSO) produced a small increase in gastric acid secretion in the presence of atropine and famotidine in isolated rat stomach preparation. NS aqueous extracts at the doses of 0.5 mg/100 ml DMSO, 1 mg/100 ml DMSO, 2 mg/100 ml DMSO and 5 mg/100 ml DMSO did not affect pentagastrin-induced acid secretion (P>0.05) *(Fig. 4A)*. Proglumide, a CCK_2 receptor antagonist (10 µM/1 ml distilled water) inhibited pentagastrin-induced acid secretion in the presence of atropine and famotidine *(Fig. 4B)*.

DISCUSSION

In this study, the effect of NS aqueous extracts on gastric acid secretion stimulated by bethanechol, histamine and pentagastrin in isolated rat stomach was investigated for the first time. Histamine is released from enterochromaffin cells (ECL) of mucous surface [20]. Histamine activates adenylate cyclase in parietal cells, leading to elevation of intracellular cyclic AMP [14]. The increased cyclic AMP results in the activation of H^+, K^+-

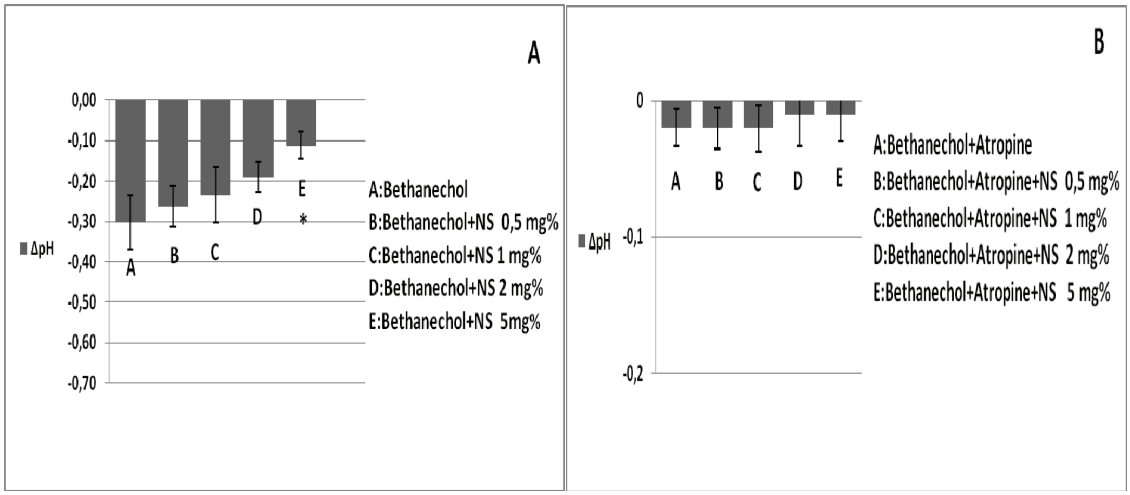

Fig 3. A- Effect of NS aqueous extracts on bethanechol-induced acid secretion in the presence of famotidine and proglumide in isolated rat stomach. There is statistical difference between groups bethanechol and NS 5 mg %, with bethanechol (n=4, * P<0.05). **B-** Effect of NS aqueous extracts on bethanechol-induced acid secretion in the presence of famotidine, proglumide and atropine in isolated rat stomach. There is no statistical difference between groups (n=4, P>0.05)

Şekil 3. A- İzole edilmiş sıçan midesinde famotidine ve proglumide varlığında bethanechol ile uyarılmış gastrik asit sekresyonu üzerine çörek otu sulu ekstraktının etkisi. Bethanechol ile bethanechol + NS %5 mg grupları arasında istatistiksel olarak fark vardır (n=4, * P<0.05). **B-** İzole edilmiş sıçan midesinde famotidine, proglumide ve atropine varlığında bethanechol ile uyarılmış gastrik asit sekresyonu üzerine çörek otu sulu ekstraktının etkisi. Gruplar arasında istatistiksel olarak fark yoktur (n=4, P>0.05)

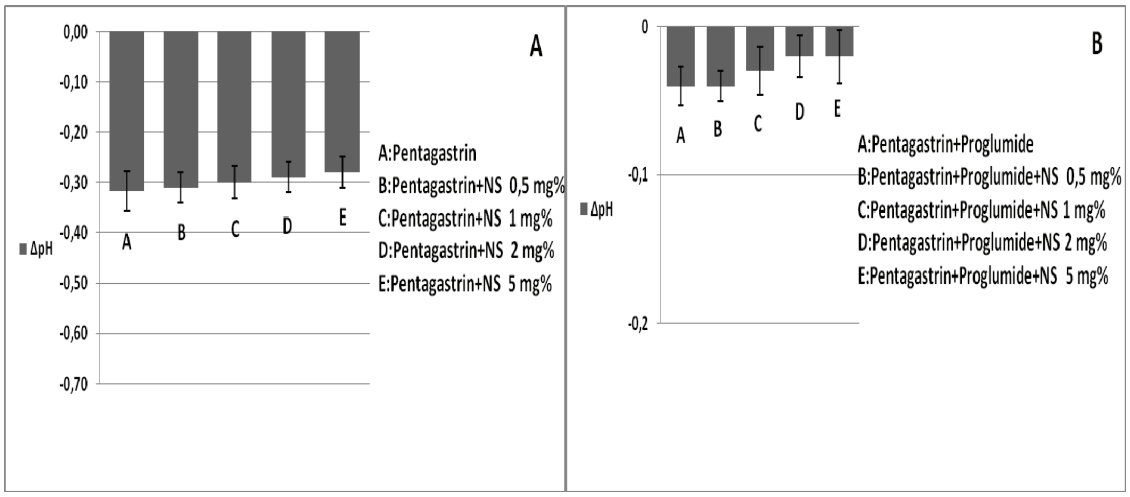

Fig 4. A- Effect of NS aqueous extracts on pentagastrin-induced acid secretion in the presence of famotidine and atropine in isolated rat stomach. There is no difference between groups (n=4, P>0.05). **B-** Effect of NS aqueous extracts on pentagastrin-induced acid secretion in the presence of famotidine, proglumide and atropine in isolated rat stomach. There is no statistical difference between groups (n=4, P>0.05)

Şekil 4. A- İzole edilmiş sıçan midesinde famotidine ve atropine varlığında pentagastrin ile uyarılmış gastrik asit sekresyonu üzerine çörek otu sulu ekstraktının etkisi. Gruplar arasında istatistiksel olarak fark yoktur (n=4, P>0.05). **B-** İzole edilmiş sıçan midesinde famotidine, proglumide ve atropine varlığında pentagastrin ile uyarılmış gastrik asit sekresyonu üzerine çörek otu sulu ekstraktının etkisi. Gruplar arasında istatistiksel olarak fark yoktur (n=4, P>0.05)

ATPase of parietal cells via unidentified pathways of intracellular signaling [15,16] In the present study, our experiment showed that 2 mg/100 ml DMSO and 5 mg/100 ml DMSO doses of NS aqueous extracts reduced gastric acid secretion induced by histamine. Several mechanistic possibilities for this activity are possible, including a direct effect on histamine receptors, an inhibition of histamine release from ECL cells. Other regulating intracellular factors or the H^+, K^+ ATPase cannot be excluded as the target. In our experiment, as M_3 muscarinic and CCK_2 receptors located on ECL cells and parietal cells were blocked with proglumide and atropine *(Fig. 2A)*, NS aqueous extracts might have blocked H_2 receptors by affecting the parietal cells stimulated by histamine. Therefore, NS aqueous extracts appears to have no effect on ECL cells. Also the histamine-stimulated gastric acid secretion was inhibited by famotidine in the presence of NS aqueous extracts, atropine and proglumide *(Fig. 2B)* and there is

no statistical difference between groups. This shows that NS has less effective in the intracellular mechanism. Therefore, NS are thought to suppress the H_2 histamine receptors in the response to histamine.

We investigated the effect of NS aqueous extracts on acid secretion induced by the cholinergic agent bethanechol. Bethanechol-induced acid secretion is mediated by a direct action at muscarinic M_3-receptors on parietal cells [12,13]. Also, bethanechol stimules the release of histamine from ECL cells [9]. Our experiments showed that 5 mg/100 ml DMSO dosis of NS aqueous extract reduced bethanechol-induced gastric acid secretion. The above-mentioned mechanisms might also be applicable to bethanechol-induced gastric acid secretion. There are several mechanisms for this activation including a direct effect on muscarinic M_3-receptors, an inhibition of histamine release from ECL cells, other intracellular regulating factors, an effect H^+, K^+ ATPase. In our experiment, due to CCK_2 receptors located on ECL cells and H_2 receptors on parietal cells were blocked with proglumide and famotidine *(Fig. 3A)*, NS aqueous extracts can block muscarinic M_3-receptors by affecting the parietal cells stimulated by bethanechol. Therefore, NS aqueous extracts appear to have no effect on ECL cells. Also the bethanechol-stimulated gastric acid secretion was inhibited by atropine in the presence of NS aqueous extracts, famotidine and proglumide *(Fig. 3B)* and there is no statistical difference between groups. This shows that NS has less effective in the intracellular mechanism.

Pentagastrin was used as another gastric acid secretion stimulator in our study. Pentagastrin is a pentapeptid similar to gastrin hormone. It exerts its effect via gastrin or cholecystokinin receptor [17,18]. Gastrin receptors related to the regulation of gastric secretions are located on parietal cells, enterochromaffin cells. Therefore gastrin or pentagastrin increase gastric acid secretion via two mechanisms: 1) Directly through exerting effect on receptors on the surface of parietal cells, 2) indirectly through stimulating enterochromaffin cells [17]. In the present study, pentagastrin produced an small increase in gastric acid secretion in the presence of famotidine and atropine in isolated rat stomach preparation, but any doses (0.5 mg/100 ml DMSO, 1 mg/100 ml DMSO, 2 mg/100 ml DMSO and 5 mg/100 ml DMSO) of NS aqueous extracts did not affect pentagastrin-induced acid secretion. Mofleh IAA et al.[19] reported that administration of NS aqueous suspension reduced gastric acid secretion in pyloric-ligated rats *in vivo*. Our results showed that NS aqueous extracts reduced gastric acid secretion stimulated by histamine and bethanechol, but any dose of NS did not affect pentagastrin-induced acid secretion.

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