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Full Length Research Paper

Gastroprotective effects of *Stachys Lavandulifolia* extract on experimental gastric ulcer

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Several studies have used *Stachys Lavandulifolia vahl* (*S. lavandulifolia*) as medicinal plant in Iranian folk medicine. The present investigation is designed to elucidate therapeutic and preventive effects of *S. lavandulifolia* extract on gastric acid and pepsin secretions in experimental gastric ulcer. Thirty two Wistar male rats were used to study therapeutic and preventive effects of *S. lavandulifolia* extract on alcohol-induced gastric ulcer. Animals were equally (n=8) divided into 4 groups: (I) Control (II) Alcohol (1 ml/200 g/bw) to induce gastric ulcer (III) Alcohol/Lvandu (100 mg/kg bw/daily, *S. lavandulifolia* extract was given for two weeks post alcohol administration) and (IV) Lvandu/alcohol (*S. lavandulifolia* extract was given for two weeks before alcohol administration). Ulcer index, gastric acid and pepsin secretions was measured. Ulcer index was significantly decreased in alcohol/Lvandu than Alcohol group. Also gastric acid and pepsin secretions, and gastric tissue's NO metabolites level were significantly lower in alcohol and Lvandu/alcohol groups than control (p<0/05). But changes in gastric acid and pepsin secretions have not been noticeable in alcohol/Lvandu group than control. The results of this study show that *S. lavandulifolia* extract protected gastric mucosa from alcohol-induced gastric ulcer. This gastroprotection may mediate via gastric mucosal nitric oxide production.

Key words: *Stachys Lavandulifolia*, acid, pepsin, nitric oxide

INTRODUCTION

Many factors are involved in gastric and duodenal ulcer pathology such as stress, smoking, nutritional deficiencies, alcohol ingestion and nonsteroidal anti-inflammatory drugs (Nash et al., 1994; Basil and Howard, 1995). Protective measures should focus on gastric muco-sa integrity and affect acid-pepsin secretion, parietal cell activity, mucosal barrier, mucus secretion, blood flow, cell regeneration, and endogenous protective agents especially nitric oxide (Singha et al., 2008). Natural products of plant origin are still a major part of global traditional medicine especially in gastric ulcer. Iran's unique meteorological conditions have contributed To the diversity of medicinal plants. There are about 270 species of *stachys* genus widespread throughout the world (Evans, 2002).

Several studies have used *Stachys Lavandulifolia vahl* (*S. lavandulifolia*) as medicinal plant in Iranian folk medicine (Ghasemi-Pirbalouti, 2011; Hajhashemi et al., 2007). This plant is widely distributed in different regions of Iran and popularly known as "chaie koohi" (Hajhashemi et al., 2007). Boiled extract obtained from the aerial parts of *S. lavandulifolia* are used as antipyretic, anti-inflammatory, spasmolytic and sedative medicament (Naghibi et al., 2005). Anti-inflammatory and antibacterial effects, and wound healing activity of S. *lavandulifolia* extract have been shown in several pharmacological studies (Maleki et al., 2001; Khanavi et al., 2005; Skaltsa et al.,

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1999; Skaltsa et al., 2003; Ghasemi-Pirbalouti, 2011). Since gastric acid and pepsin are involved in gastric ulcer pathology and there is no academic study on *S. lavandulifolia* extract effect on this issue, we conducted the present investigation to elucidate therapeutic and preventive effects of *S. lavandulifolia* extract on gastric acid-pepsin secretions together with NO's possible role.

MATERIALS AND METHODS

Animals

Male Wistar rats (250 to 300 g) obtained from Physiology Department of Tehran Medical Sciences University animal room were kept in a temperature-controlled environment on a 12:12 h light/dark cycle with free access to food and water. The procedures were in accordance with the guidelines for the care and use of laboratory animal of Tehran University Medical Science.

Plant material

S. Lavandulifolia was collected from Kordestan Province (west of Iran) and identified by a herbalist. A voucher specimen (1523) was designated for the S. *Lavandulifolia* used in this investigation. Aqueous *S. lavandulifolia* extract prepared from 100 g powder of *S. lavandulifolia vahl* soaked in 1000 ml boiled water was homogenized, stirred, filtered and lyophilized to store at 4°C (Premkumar et al., 2003).

Study design

Thirty two male Wistar rats were used to study therapeutic and preventive effects of *S. lavandulifolia* extract on absolute alcohol-induced gastric ulcer. Animals were equally (n=8) divided into 4 groups: (I) Control, (II) Alcohol (1 ml/200 g/bw) to induce gastric ulcer one hour before each experiment (Hollander et al., 1985; Ozdil et al., 2004), (III) Alcohol/Lvandu (100 mg/kg bw/daily, *S. lavandulifolia* extract was given for two weeks post alcohol administration) and (IV) Lvandu/Alcohol (*S. lavandulifolia* extract was given for two weeks before inducing gastric ulcer). Twenty-four hours before each experiment, animals were deprived of food but free to drink water (Nabavizadeh Rafsanjani and Vahedian, 2004).

Surgical procedure

Animals were first anesthetized with sodium thiopental (50 mg/kg, ip). Then, tracheostomy was performed (Nabavizadeh et al., 2009) and cervical esophagus tied to prevent gastric reflux into the oral cavity. Laparatomy was performed and a polyethylene cannula (3 mm diameter) placed in the stomach via a duodenal incision. Residual gastric secretions were lavaged several times with 1 to 2 ml normal saline (37°C).

Measurement of gastric acid-pepsin secretions and nitric oxide metabolite

In all groups, normal saline (1 ml) was introduced into the stomach. Normal saline was injected again at the same dose 15 min later. Gastric contents were collected using washout technique (Nabavizadeh et al., 2009; Salim, 1988). Basal acid and pepsin secretion were measured with a digital Titrator system (Basic Titrino, Metrohm, 794) and Anson method (Nabavizadeh et al., 2009; Bersted, 1970) respectively. To stimulate acid-pepsin secretion, pentagastrin (25 µg/kg, ip) was used (Nabavizadeh et al., 2009; Kato et al., 1998) and gastric acid-pepsin levels were measured 15 min later.

Gastric tissue's NO metabolites were also measured using Griess micro assay method (Nahrevanian et al., 2006). Also, the number of ulcers was counted. Ulcer scoring was done according to the method of Vogel et al. (1997) as shown below:

The scores were: 0= no ulcer, 1= superficial ulcer, 2= deep ulcer, 3= perforation.

Ulcer index was measured by using following formula (Vogel et al., 1997)

$UI=UN+US+UP\times10-1$

UI= Ulcer Index

UN= Average number of ulcers per animal

US =Average number of severity score

UP=percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

% inhibition of ulceration = (Ulcer index Alcohol-Ulcer index Test) ×100/Ulcer index control. Then gastric and proximal duodenum tissue was removed and kept in fixative solution (formalin 10%) for histological study.

Statistical analysis

Results were expressed as mean±SE. Analysis of variance (ANOVA) and Tukey test were used for comparison among groups. $P<0.05$ considered to be statistically significant.

RESULTS

Effects of *S. lavandulifolia* extract on gastric ulcer parameters

Gastric ulcer measurements showed that *S. lavandulifolia* extract significantly decreased the number of ulcer, ulcer score and index at alcohol/Lvandu than alcohol group ($p<0.05$). But changes have not been noticeable in Lvandu/alcohol group (Table 1).

Effects of *S. lavandulifolia* extract on gastric acid-pepsin

Gastric acid-pepsin levels in alcohol and Lvandu/alcohol groups were significantly lower than control group ($p<0.05$). But changes have not been noticeable in alcohol/Lvandu group compared to control (Table 2).

Effects of *S. lavandulifolia* extract on gastric tissue's NO metabolites

Gastric tissue's NO metabolites level in alcohol and

Table 1. Effects of *S. lavandulifolia* extract on alcohol-induced ulcer of gastric tissue.

	Parameters			
Groups	**Number of ulcers**	**Ulcer score**	**Ulcer index**	**Ulcer inhibition (%)**
Control	-	-	-	-
Alcohol	5.5±0.9	2.5±0.7	20.6±2.4	-
Alcohol/Lvandu	2.1±0.8*	1.0±0.5*	11.0±2.0*	46/9*
Lvandu/alcohol	5±1.1	2.3±0.6	19.5±2.2	11/6

Data were expressed as mean±SE, n=8, Lvandu; *S. lavandulifolia* extract, *p<0.05 compared to alcohol group.

Table 2. Effects of *S. lavandulifolia* extract on gastric acid-pepsin level in experimental gastric ulcer

	Parameters			
Groups	**Basal acid (mmol/ml/15 min)**	**Stimulated acid (mmol/ml/15 min)**	**Basal pepsin (µg/ml/15 min)**	**Stimulated pepsin (µg/ml/15 mi)**
Control	0.57±0.08	1.84±0.06	2.5±0.08	3.9±0.06
Alcohol	0.04±0.02*	0.05±0.02 *	0.31±0.03*	0.33±0.03 *
Lvandu/Alcohol	0.05±0.03*	0.07±0.02 *	0.35±0.03*	0.34±0.04 *
Alcohol/Lvandu	0.62±0.09 #	2.1± 0.08 #	2.7± 0.09 #	4.1± 0.08 #

Data were expressed as mean±SE, n=8, Lvandu; *S. lavandulifolia* extract, *p<0.05 compared to control group, # p<0.05 compared to Alcohol group.

Lvandu/alcohol groups were significantly lower than control (p<0.05). But it did not change in alcohol/Lvandu group compared to control (Figure1).

Effects of *S. lavandulifolia* extract on histopathological findings on gastric tissue

Alcohol caused histopathological lesion including hemorrhage and degeneration of the gastric tissue. Treatment with *S. lavandulifolia* extract offered significant protection against all damages to mucosa in alcohol/Lvandu group (Figure 2).

DISCUSSION

The aim of the present study was to survey *S. lavandulifolia* extract role on protective and treatment of alcohol-induced gastric ulcer in rats. We showed that there is a significant difference in gastric acid-pepsin secretions and gastric tissues's NO metabolites level in alcohol/Lvandu group than control but no significant differences are seen in Lvandu/alcohol group (Table 1, Figure 1). These therapeutic changes may be due to gastric tissue's NO increase followed by *S. lavandulifolia* extract administration.

In the present study, gastric gavage of absolute alcohol created obvious gastric macroscopic and microscopic mucosal injury. The lesions were long, hemorrhic and confined to the glandular portions (Table 1). Alcohol-induced gastric lesions impaired gastric defensive factors such as mucus and mucosa circulation (Kushima et al., 2005). Treatment with *S. lavandulifolia* extract could partly reduce the ulcer index and promote gastric healing, but it could not significantly improve gastric lesions of Lvandu/alcohol group (Table 1). Although ulcer etiology is unknown in most cases, it is generally accepted that an imbalance between acid and pepsin production and mucosal integrity would be causative factor acting via endogenous defense mechanisms (Wallace and Granger, 1996). Hence, it is right to state here that S. *lavandulifolia* extract, which is acted as a therapeutic agent for anti-inflammatory and antibacterial, and wound healing activity (Maleki et al., 2001; Khanavi et al., 2005; Skaltsa et al., 1999; Skaltsa et al., 2003; Ghasemi-Pirbalouti, 2011), has an anti-ulcer potential. Further work for its specific anti-ulcer mechanism is in progress.

In the present study, the gastric tissue's NO metabolites level is significantly less in Lvandu/alcohol group than control. It has been shown that rat parietal cells express neuronal NOS (nNOS). In addition, about 50% of the nerves in the enteric nervous system contain nNOS (Dijkstra et al., 2004). These findings suggest that endogenous NO may participate in the regulation of gastric secretion via an intracellular signaling molecule (Dijkstra et al., 2004). Several studies have demonstrated the importance of endogenous NO in the protection of gastric mucosa (Kim and Kim, 1998; Tanaka et al., 2001; Whittle et al., 1990). Also endothelial NO plays an important role in the modulation of gastric mucosal integrity by interacting with sensory neuropeptides (Whittle et al., 1990; Tepperman and Whittle, 1992), reducing neutrophil adhesion and increasing gastric

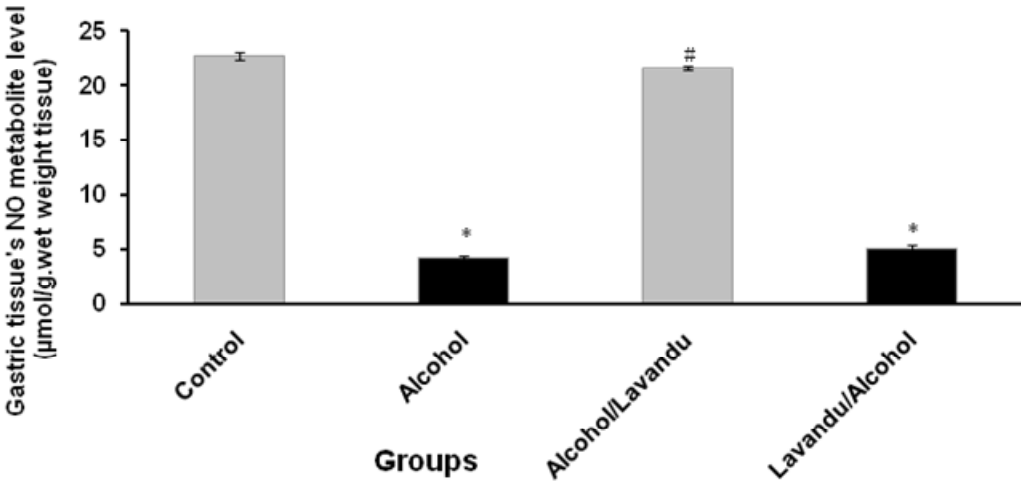

Figure 1. The effect of *S. lavandulifolia* extract on gastric tissue's NO metabolites level. Data were expressed as Mean±SE, n= 8, Lvandu; *S. lavandulifolia* extract, NO; nitric oxide, *p<0.05 compared to control group, #p<0.05 compared to alcohol group.

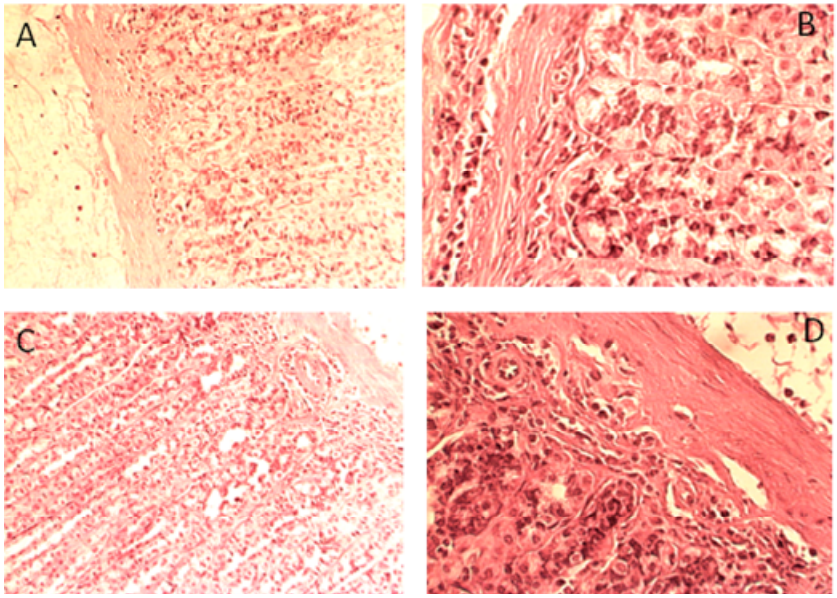

Figure 2. The effect of *S. lavandulifolia* extract on experimental gastric ulcer. A: Control group, B: Alcohol group, C: Alcohol/Lavandu group and D: Lavandu/alcohol group.

blood flow and mucus secretion (Souza et al., 2004). In the present study, alcohol significantly reduced gastric mucosal NO level with increase mucosal injury compared to control group. These findings are in accordance with Trip and Tepperman's study in which they reported decreased NO biosynthesis along with mucosal damage (Tripp and Tepperman, 1995). In present study, it is assumed that NO increase followed by *S. lavandulifolia* extract intake can increase basal and stimulated vagus nerve tone and further acetylcholine effects on gastric muscular cells. On the other hand, it was showed that NO acts presynaptically to facilitate vagal neurotransmission via a pathway that ultimately leads to increased phosphorylation of presynaptic L-type Ca^{+2} channels. This pathway causes increased presynaptic calcium influx and vesicular release of acetylcholine (Herring and Paterson, 2001). Also, NO generated in parasympathetic ganglia may play a modulator role in facilitating the release of acetylcholine and the subsequent tissue response (Herring et al., 2002).

It is also showed that S*. lavandulifolia* mainly comprises germacrene-D, betaphellandrene, beta-pinene, myrcene

and alpha-pinene (Javidnia et al., 2004). Phytochemical and pharmacological studies have suggested that flavonoids presented in ethanolic extract and *stachys* fractions are responsible for the antisecretory and cytoprotective action of *stachys* (Delazar et al., 2005; Karioti et al., 2010). In the present study, although the mechanism underlying the antiulcerogenic effect seen here remains unknown, it may be related to the flavonoid derivatives from luteolin present in S*. lavandulifolia* extract and in active fractions.

Conclusion

The results of this study show that *S. lavandulifolia* extract protected gastric mucosa from alcohol-induced gastric ulcer. This gastroprotection may mediate via gastric mucosal nitric oxide production.

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