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Full Length Research Paper

Saffron (*Crocus sativus*) increases gastric acid and pepsin secretions in rats: Role of nitric oxide (NO)

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This study was designed to investigate the effects of saffron extract on gastric acid and pepsin secretion. In this study wistar rats (n = 8) were divided into 3 groups, N-L-Nitro-L- arginine methyl ester (LNAME) + saffron and control groups. In the saffron group 100 mg/kg saffron extract was administered orally for 5 days. In the LNAME + saffron group, 40 mg/kg LNAME was injected intraperitoneally 1 h before using saffron extract. In the control group normal saline was given orally for 5 days before the experiment. Under general anesthesia with 50 mg/kg intraperitoneal (i.p) sodium thiopental, laparotomy was done and a cannula was inserted into the duodenum. Gastric content was collected by wash out technique. Basal and stimulated acid and pepsin secretions were measured using titration and the Anson method, respectively. In this study, pentagastrin (25 µg/kg, i.p) was used as a stimulator. In the saffron group, basal and stimulated acid and pepsin secretions were significantly more than control group (p = 0.006, p = 0.008). But there were no significant differences in basal and stimulated acid and pepsin secretions in the LNAME + saffron and control groups. Saffron extract increased basal and stimulated gastric secretions. It seems that the saffron extract increases them via NO increment.

Key words: Saffron, gastric acid secretion, pepsin secretion, NO.

INTRODUCTION

Saffron consist of dried stigmas and top of the styles of crocus sativus (Iridaceae) (Evans, 2002). The main active constituents of this plant are picrocrocin, safranal, flavornoid derivatives and crocins (Trantilis et al., 1995). More than 80%of this harvest originates from Iran (Hagh and Keifi, 2006; Betti and Schmidt, 2006). Saffron is as an expensive spice, apart from its traditional value as a food additive and traditional herbal medicine in the world. C. sativus is used in folk medicine as an antispasmodic, eupeptic, gingival sedative, anticatarrhal, nerve sedative, carminative, diaphoretic, expectorant, stimulant, stomachic, aphrodisiac and emmenagogue (Rios et al., 1996). Previous studies have shown different pharmacological effects of this plant. Saffron extract reduces the incidence of artificially induced cancer in vivo and it inhibits tumor growth, also saffron prolongs the life span of the laboratory animals (Nair et al., 1991; Das et al., 2004). The oral administration of saffron extract significantly activated the liver antioxidants superoxide dismutase (SOD) and catalase (CAT) (Premkumar et al., 2003). These results suggest that saffron aqueous extract has potential antimutagenic and antioxidant properties (Premkumar et al., 2003). Oral administration of saffron extract distinctly improved the memory of mice pre-damaged with ethanol (Sugiura et al., 1994). Intraperitoneally applied aqueous and ethanolic extracts of saffron and its constituents, safranal and crocin, were shown to have antidepressant effect in mice (Hosseinzadeh et al., 2004; Karimi et al., 2001). *In vitro* crocin was found to have weak but spe-cial affinity to N-methyl-D-aspartate (NMDA) receptor (Hensel et al., 2006) and to antagonize ethanol-induc-ed depression via NMDA receptor (Abe et al., 1998). According to animal experiments, crocin has lipid lowering properties. It selectively inhibits the activity of pancreatic lipase as a competitive inhibitor (Liang et al., 2006). A blood pressure lowering and relaxant effects on vascular smooth muscle (Fatehi et al., 2003) were also des-

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cribed for this plant. Saffron is commonly used in different parts of Iran and is used as a medical drug to treat numerous diseases. Although the antispasmodic, carminative, stomachic and eupeptic effects of saffron have been mentioned in folk medicine texts, to our knowledge, there is no report in this study regarding its effect on gastric secretions; neither changes of gastric acid and pepsin secretion effect on digestion, peptic ulcer and gastric cancer. Therefore, this study was designed to elucidate the effects of saffron on basal and pentagastrin – stimulated gastric acid and pepsin secretions in rats.

MATERIALS AND METHODS

Animals

This study was carried out in Tehran University of Medical Sciences, Tehran, Iran. In this study, 3 groups (n = 8) of wistar rats weighing 200 – 250 g obtained from animal room of physiology department of Tehran university of medical sciences, were used. The rats were maintained in a temperature controlled environment on a 12:12 h light dark cycle with free access to food and water (Blandazzi et al., 1990). The procedure was in accordance with the guidelines for the care and use of laboratory animal of Tehran University of Medical Sciences. Saffron was collected from Qaenat (east of Iran) and identified by an herbalist. The aqueous saffron extract was prepared, 1 g of saffron was soaked in 100 ml of water. After 2 h it was homogenized in the same water, stirred for 1 h and filtered. This aqueous extract was lyophilized and stored at 4°C before use (Premkumar et al., 2003). In the saffron group 100 mg/kg saffron extract was administered orally for 5 days before the experiment. In the LNAME + saffron group, 40 mg/kg LNAME was injected intraperitoneally 1 h before using saffron extract. In the control group normal saline (as solvent of saffron) was given orally for 5 days before the experiment. 24 h before the experiment, animals were deprived of food, but free to drinking of water (Nabavizadeh et al., 2004; Yang and Tache, 1997). In order to omit circadian rhythms the experiments was started at 8 every morning.

Surgical procedure

The animals were anaesthetized by an intraperitoneal injection of 50 mg/kg sodium thiopental (Nabavizadeh et al., 2004). Tracheostomy was then performed (Nabavizadeh et al., 2004; Mctigue and Rogers, 1995). Cervical esophagus was tied in order to prevent gastric reflux into the oral cavity. Laparotomy was done and a polyethylene cannula 3 mm in diameter was placed in the stomach via a duodenal incision. Residual gastric secretions were removed by performing lavage several times with 1 - 2 ml normal saline at 37°C and allowed to reach steady state in 30 min (Nabavizadeh and Vahedian, 2004). After 30 min of recovery time, gastric acid and pepsin secretions were measured in 3 groups.

Measurement of gastric acid and pepsin secretions

In all groups, 1 ml normal saline was introduced into the stomach. After 15 min, another 1 ml normal saline was injected into the stomach. All the gastric contents were collected by the wash out technique (Salim, 1988) for measuring basal acid and pepsin secretions. Acid and pepsin secretions were measured using automatic titrator (DIN, Germany) and Anson method, (Berstade, 1970) respectively. In order to measure stimulated acid and pepsin secretions, pentagastrin 25 µg/kg intraperitoneally was used (Kato et al., 1998). After 15 min stimulated acid and pepsin secretions were measured.

Statistical analysis

Results were expressed as mean ± SE. Analysis of variance (ANOVA) and Turkey tests were used for comparison between the groups. For comparison of basal and stimulated gastric acid and pepsin secretions in 3 groups, paired t-test was used. $P < 0.05$ was considered to be statistically significant.

RESULTS

Basal and pentagastrin-stimulated acid output were significantly higher in the saffron group in comparison with control group (9.99 ± 0.78, 13.92 ± 0.88, 2.3 ± 0.27, 6.12 ± 0.79 µm/15 min, respectively) ($p = 0.006$, $p = 0.008$) (Figure 1). There is no significant difference in the basal and pentagastrin-stimulated acid secretions in the LNAME+ saffron and control groups(3.5 ± 0.26, 7.3 ± 0.65, 2.3 ± 0.11, 6.12 ± 0.79 µm/15 min, respective-ly) (Figure 1).The pepsin secretion was significantly greater in the basal and pentagastrin-stimulated states in the saffron group than in control group(1.65 ± 0.3, 3.25 ± 0.26, $p = 0.007$ vs. 0.38 ± 0.28, 1.47 ± 0.43, $p = 0.01$, µg/15 min) (Figure 2) while there were no significant difference between basal and pentagastrin-stimulated pepsin secretions in the LNAME + saffron and control groups(0.88 ± 0.08, 2.83 ± 0.48, 0.38 ± 0.28, 1.47 ± 0.43 µg/15 min, respectively) (Figure 2). Also, pentagastrin - stimulated acid secretions in the control, saffron and LNAME+ saffron groups (6.12 ± 0.79, 13.92 ± 1.88, 7.3 ± 0.65 µm/ 15 min) were significantly higher than the basal state in all groups (2.3 ± 0.11, 9.99 ± .78, 3.5 ± 0.26, µm/15 min) ($p < 0.05$) (Figure 1). Furthermore, pentagastrin-stimulated pepsin output in control, saffron and LNAME+ saffron groups (1.47 ± 0.43, 3.25 ± 0.26, 2.83 ± 0.48 µg/15 min) were significantly greater in the basal state in all groups (0.38 ± 0.28, 1.65 ± 0.3, 0.88 ± 0.08 µg/15 min) ($p < 0.05$) (Figure 2).

DISCUSSION

In this study, both basal and pentagastrin-stimulated acid and pepsin output were significantly higher in the saffron group in comparison with the control group. While there were no significant difference in the basal and pentagastrin-stimulated acid and pepsin secretions in the LNAME (NO synthethase inhibitor) + saffron and control groups. LNAME is an inhibitor of NO synthethase. It seems saffron components might work via activation of NO synthethase. It has been shown that NO enhances histamine release from gastric histamine containing cells, probably entrochoromafin-like cells (ECLs). This effect is due to an increase in intracellular cGMP concentration induced by NO (Hasebe et al., 2001, 2003, 2005). Therefore histamine increases gastric acid and pepsin secretions via

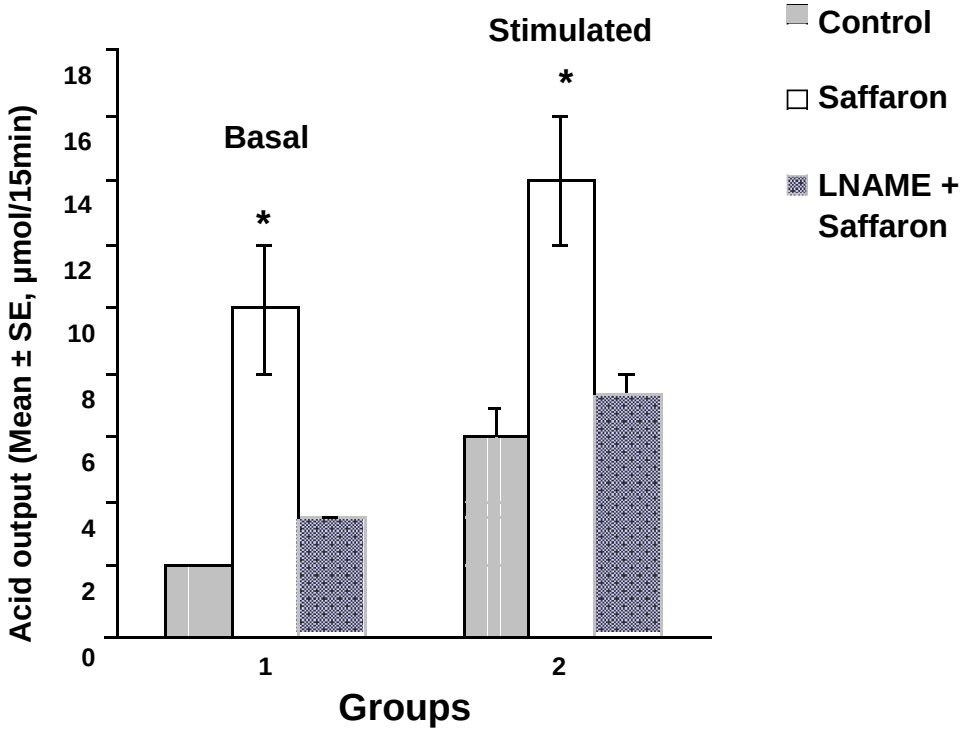

Figure 1. Acid output in Control, Saffron and LNAME+Saffron groups in basal and stimulated states *(P<0.05).

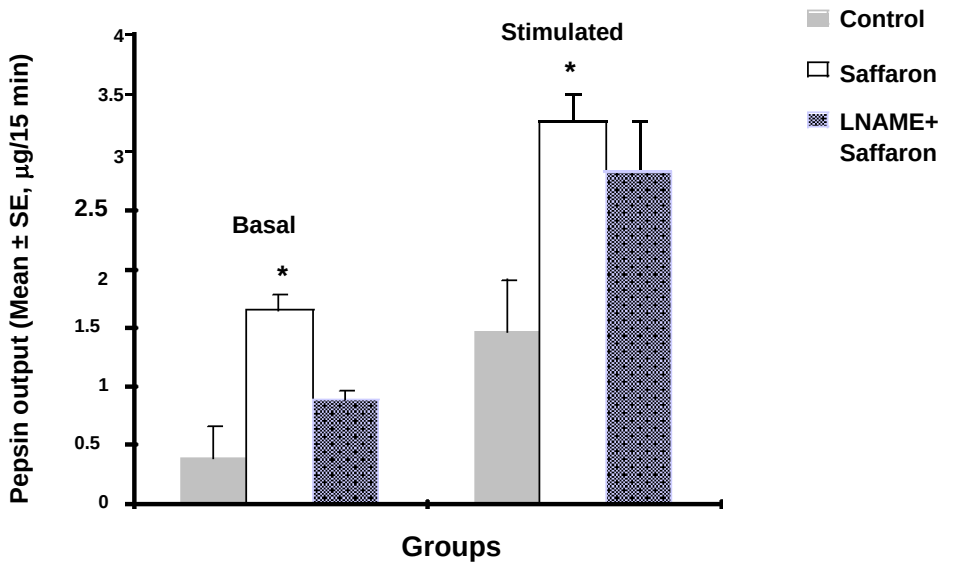

Figure 2. Pepsin output in Control, Saffron and LNAME+Saffron groups in basal and stimulated states * (P<0.05).

activation of parietal and chief cells. It is shown that rat parietal cells express the enzyme NO- synthethase, neuronal isoform (nNOs). This finding suggests that endogenous NO acting as an intracellular signaling moleculue may participate in the regulation of gastric acid secretion (Premaratne et al., 2001). Also about 50% of the nerves in the enteric nervous system contain nNOs (Bertaccine and Coruzzi, 1998), therefore NO might release from enteric nervous system in the stomach and this endogenous NO increases acid and pepsin secretion.

Also it is probable that saffron components increase the number of parietal and chief cells or enhances their activation. Further studies are needed to evaluate these probable mechanisms. In this study, pentagastrin - stimulated acid and pepsin secretion in the control, saffron and saffron + LNAME groups were significantly higher than basal state. Pentagastrin is a gastrin-like pentapeptide that binds to gastrin receptors (CCK- B) and activates parietal and entrochromaffin cells (Bertaccine and Coruzzi, 1998; Hills et al., 1996). Stimulation of acid secretion by gastrin has been shown to be mostly mediated by histamine release from entrochromaffin cells in the rat. Pentagastrin has increased pepsin secretion in comparison with the basal state in all groups. Pentagastrin activates Ca^{+2} channels in the chief cells and increases intracellular Ca^{+2} ion concentration. Ca^{+2} ion has a pivotal role in pepsin exocytosis from chief cells (Johnson, 2001).

Conclusion

Saffron extract, probably via NO increment increases basal and pentagastrin-stimulated acid and pepsin secretions.

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