

Anti-ulcer effects of natural honey against indomethacin induced gastric ulcer in rats

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Abstract

Background and objectives: Non-steroidal anti-inflammatory drugs (NSAIDs) are the leading cause of peptic ulcer disease (PUD). Drug such as proton pump inhibitors or cytoprotective agents used to treat PUD have several adverse effects. Therefore, interest in alternative therapies like honey has increased due to fewer side effects, ease of accessibility and affordability. This study determined the anti-ulcer effect of natural honey against indomethacin induced ulcer in rats.

Materials and Methods: This experimental study was conducted on albino rats. Rats were assigned to four groups (Group1 to 4) and each group consisted of six rats. Gr1 received indomethacin (60 mg/kg) only and Gr2, 3 and 4 were pre-treated with assigned doses of sucralfate, honey, and honey + sucralfate respectively for 7 days. The effects of experimental agents were assessed by ulcer score, ulcer index (UI), percentage protective ratio (PPR). Effect of honey, sucralfate and honey plus sucralfate mixture was compared against high dose indomethacin induced gastric ulcer in rats.

Results: UI significantly ($p < 0.001$) reduced in sucralfate, (0.67 ± 0.82), honey (0.83 ± 0.98) and honey + sucralfate (0.17 ± 0.41) treated group compared to only indomethacin treated group (4 ± 0.63). The PPR of sucralfate, honey and honey + sucralfate was 83.25%, 79.25% and 95.75%, respectively.

Conclusions: The study showed that honey had anti-ulcer properties against the indomethacin-induced gastric ulcers and the effect is potentiated when used with sucralfate. Honey may be used to protect the gastric mucosa against NSAIDs.

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Introduction

Peptic ulcer disease (PUD) is one of the most common gastrointestinal diseases with a worldwide prevalence of nearly 11-14% in men and 8-11% in women [1]. The typical causes include infection with *Helicobacter pylori* (*H. pylori*), consumption of non-steroidal anti-inflammatory drugs (NSAIDs)

and medications like steroids, iron preparations and selective serotonin reuptake inhibitors (SSRIs) [2,3]. Gastric injury following indomethacin ingestion is mediated by interference to prostaglandins production and their physiological actions. Consequently, gastric mucosal blood flow reduces, and there is a drop in mucin levels with

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decreased bicarbonate release with the upturn in leukocyte activation. Additionally, alteration in the production of inflammatory and pro-inflammatory mediators, acid secretion, vasoconstriction and leucocyte adhesion to vascular endothelium gets upper hand, ultimately causing release of free radicals that produce gastric mucosal damage [4].

Misoprostol and sucralfate, used for the treatment of NSAID induced peptic ulcer are associated with several adverse effects. Misoprostol may cause diarrhoea, abdominal pain, headache, uterine cramps, menstrual disorder, fatigue, and muscle cramps [5] while sucralfate may cause constipation, dry mouth, nausea, vomiting, headache, urticaria and rashes [6]. Honey as a medicinal natural product has been studied throughout the last decade. Honey is recognized not only as a sweetener but also as a component of traditional folk medicine around the world. It is the by-product of flower nectar and upper aero-digestive tract secretion of the honeybee, concentrated through a dehydration process inside the beehive. It is principally composed of sugar, water, antioxidant, vitamin, catalase, superoxide dismutase, reduced glutathione, Millard reaction products and peptides, phenolic acids, and flavonoids [7,8]. Honey stimulates the sensory nerve endings of the stomach (capsaicin responding), releases vasodilatory peptides and produce nitric oxide thereby increasing blood supply and protecting the gastric mucosa [9]. It augments levels of non-protein sulfhydryl (NP-SH) groups which prevent oxidative damage, thereby blocking free radical derived self-amplifying inflammatory response [8]. Their anti-inflammatory action reduces the features of inflammation and stimulates the formation of granulation tissue [10]. The objective of this study was to assess the anti-ulcer effects of honey alone and in combination with sucralfate against indomethacin-induced ulcer in rats.

Material and Methods

The study assessed the anti-ulcer effect of honey against experimentally induced gastric ulcers with high dose (60 mg/kg) of indomethacin in albino rats. The study was conducted at the Department of Pharmacology of Dhaka Medical College and was approved by the Institutional Research Review Board.

Experimental animal: Albino rats (150-200 g) of either sex were used. Rats were kept at standard housing condition and fed with standard diet and water during the experiments.

Honey and drugs: The honey used in this study was pure, unprocessed, unboiled and procured from the National Institute of Apiculture, Dhaka, Bangladesh. The dose of honey administered was 1.2 g/kg/day (0.84 ml/kg) body weight. [11]. According to the Density Database Version 2.0 - FAO, 1g honey is equivalent to 0.696 ml. Indomethacin, and sucralfate used in the study were obtained from Beximco Pharmaceutical Ltd, Bangladesh. Indomethacin was used to induce gastric lesion. Sucralfate was used as standard gastro-protective drug to compare with the effects of honey. The dose of indomethacin was 60 mg/kg given once on day 7 while the dose of sucralfate was 250 mg/kg body weight per day [11,15]. Dose was calculated for individual rat according to the body weight and stock solution was prepared just before the daily administration. The entire calculated amount was dissolved in distilled water and administered orally through nasogastric tube at a volume of 1 ml/100 g body weight [16].

Study design: Protective effect of honey alone and in combination with sucralfate was assessed on indomethacin induced gastric ulcer in rats. Anti-ulcer effect of honey alone was also compared with that of sucralfate. Rats were assigned to four groups (Group1 to 4) and each group consisted of six rats and received the treatment as described in Table-1. Group-1 received indomethacin on day 7 only and served as positive control. Group-2, 3 and 4 received pre-treatment with sucralfate, honey and sucralfate plus honey respectively for 7 days and indomethacin on day 7. Thirty minutes after the last (on day 7) daily administration of respective agents, rats of all groups were administered indomethacin (60 mg/kg, orally) suspended in distilled water. Thereafter, all rats were fasted for 24 hours but were given free access to water and were kept in separate cages to prevent coprophagy.

Sacrifice of rats and collection of the stomach: The rats were sacrificed, and stomachs were collected on 8th day. Stomachs were opened along their greater curvature and gently rinsed under running tap water and were spread on paraffin plate.

Table-1: Experimental design: drugs, dose schedule pre-treatment duration and indomethacin treatment

Group	Honey/Drug Dose/day ^a	Pretreatment and duration	Indomethacin treatment	Evaluation
1	Indomethacin (60 mg/kg)	Tap water for 7 days	At day 7	
2	Sucralfate (250mg/kg)	Sucralfate twice daily for 7 days	At day 7 after daily dose	Evaluated by 1. Ulcer score 2. Ulcer index 3. PPR
3	Honey (1.2g/kg)	Honey twice daily for 7 days	At day 7 after daily dose	
4	Honey + sucralfate (0.6g/kg + 125mg/kg)	HS mixture twice daily for 7 days	At day 7 after daily dose	

Note: HS Honey + sucralfate; PPR: Percentage protection ratio. a = dose in per kg body weight.

Measurement of gastric lesions: Lesions were observed with the help of dissecting microscope grossly (10x) with a square grid eyepiece to assess the gastric lesions. Gastric lesion was expressed as ulcer score, ulcer index (UI) and percentage protection ratio (PPR) as described earlier [17].

Macroscopic ulcer score was assessed and scored as 0 = no lesion, 1 = mucosal edema and petechiae, 2 = one to five small lesions (1-2mm), 3 = more than five small lesions or one intermediate lesion (3-4 mm), 4 = two to more intermediate lesions or one gross lesion (>4 mm), and 5 = perforated ulcers. Ulcer index and PPR were calculated by the following formula:

Ulcer index (UI) = Total ulcer score/Number of animals ulcerated.

Percentage protection ration = [(UI of ulcerogen treated group/UI of ulcerogen treated) – (UI of drug pretreated group/ UI of ulcerogen treated)] x100

Statistical analysis: All relevant data for each rat were recorded and analyzed using Statistical Package for the Social Sciences (SPSS).

Results

Table-2 shows the ulcer scores of the Group 1 to 4 of the study groups. Ulcer scores were between 3-5 (score 4 - 66.7% and score 3 and 5 - 16.7% each) of Gr1 rats receiving high dose of indomethacin. Rats of Gr2, Gr3 and Gr4 had ulcer scores from 0 to 2. In Gr4, 83.3% rats pre-treated with mixture of honey and sucralfate had ulcer score of 0. None of the rats in Gr2, 3 and 4 had ulcer score 3-5. Ulcer index of rats receiving sucralfate (Gr2), honey (Gr3) and sucralfate + honey (Gr4) was significantly ($p < 0.001$) less compared to that of indomethacin group (Gr1) (Table-3). However, no significant difference of ulcer index was observed among the rats of Gr2, 3 and 4. PPRs were 83.25%, 79.25% and 95.75%

Table-2: Ulcer score of rat stomachs treated with high dose indomethacin, sucralfate, honey and mixture of honey and sucralfate.

Ulcer score	Number (%) of rats			
	Gr1	Gr2	Gr3	Gr4
Score 0	0 (0.0)	3 (50.0)	3 (50.0)	5 (83.3)
Score 1	0 (0.0)	2 (33.3)	1 (16.7)	1 (16.7)
Score 2	0 (0.0)	1 (16.7)	2 (33.3)	0 (0.0)
Score 3	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
Score 4	4 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)
Score 5	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)

Gr1: Indomethacin, Gr2: Indomethacin + sucralfate, Gr3: Indomethacin + honey and Gr4: Indomethacin + honey + sucralfate.

Table-3: Ulcer index and percentage protection ratio of rats treated with high dose indomethacin, sucralfate, honey and mixture of honey and sucralfate

Group	Ulcer index		PPR ^a
	Mean ± SD	p value by ANOVA	
Gr1	4±0.63	p <0.001 for Gr1 vs. Gr2, 3 and 4; p = 0.97, Gr2 vs. Gr3; p = 0.65, Gr2 vs. Gr4; p=0.42, Gr3 vs. Gr4	-
Gr2	0.67±0.82		83.25
Gr3	0.83±0.98		79.25
Gr4	0.17±0.41		95.75

Note: PPR: Percentage protection ratio, a= p >05, compared among Gr2, 3 and 4.

against high dose indomethacin induced ulcer in sucralfate, honey and sucralfate + honey pre-treated groups respectively. PPRs of different groups were not significantly different from each other (p>0.05).

Discussion

This study demonstrates that honey alone, or in combination with sucralfate is an effective anti-ulcerogenic agent against indomethacin induced gastric lesions. Pre-treatment with sucralfate, honey and combination of sucralfate and honey significantly reduced the UI in rats treated with high dose of indomethacin (p < 0.001). Honey and sucralfate mixture had higher UI lowering ability compared to honey or sucralfate alone. The protection ratio was though maximum in honey sucralfate combination group, but not significantly different from other two groups against high dose indomethacin induced gastric lesions.

In accordance with these results several authors reported that honey possesses a gastro-protective role in NSAIDs induced peptic ulcer disease [18-20]. Several mechanisms for this have been proposed. Stimulation of sensory nerves, release of vasodilatory peptides and nitric oxide, increased blood supply, augmentation of non-protein sulfhydryl (NP-SH) levels, inhibition of free radical derived self-amplifying inflammatory response reduce the feature of inflammation and promote healing of the damaged gastric tissue [8,11]. In conclusion, our study demonstrated that pre-treatment with honey alone or in combination with sucralfate can prevent or reduce mucosal lesions induced by indomethacin. However, the current study was basically a pharmacological study where

both the modern drug and herbal product were used to influence the biological system in a rat model. Biological system is affected by individual variations. Therefore, further study is needed to assess the degree of anti-ulcer effects of natural honey in human.

Comparing the findings observed in different groups of rats, it was obvious that honey had protective ability against indomethacin induced gastric ulcer and the effect was potentiated in combination with sucralfate. Therefore, honey may be used to protect the gastric mucosa against NSAIDs.

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