ORIGINAL ARTICLE

Comparative Gastroprotective Effects of Natural Honey, *Nigella sativa* and Cimetidine Against Acetylsalicylic Acid Induced Gastric Ulcer in Albino Rats

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ABSTRACT

Objective: Natural honey (NH) and *Nigella sativa* (NS) seeds have been in use as a natural remedy for over thousands of years in various parts of the world. The aim of this study was to assess the protective effects of NS *(Nigella sativa)* and NH (natural honey) on acetylsalicylic acid induced gastric ulcer in an experimental model with comparison to Cimetidine (CD).
Study Design: Experimental, case control study.
Place and Duration of Study: Pharmacology and Pathology Department of King Edward Medical University, Lahore, from June to August 2007.
Methodology: The study was conducted on 100 male albino rats, divided into 5 groups, with 20 animals in each group. Group A was used as a control and treated with Gum Tragacanth (GT). Eighty animals of the other groups were given acetylsalicylic acid (0.2 gm/kg body weight for 3 days) to produce ulcers by gavage. Two animals from each group were sacrificed for the detection of gastric ulcers. The remaining 72 animals were equally divided in four groups (B, C, D and E). The rats in group B, C and D were given NS, NH, and CD respectively while those in E were kept as such.
Results: No gastric lesions were seen in control group A while all the animals in group E revealed gastric ulcers. The animals of group B, C and D showed healing effects in 15/18 (83%), 14/18 (78%) and 17/18 (94%) animals grossly; 13/18 (72%), 14/18 (78%) and 16/18 (89%) rats showed recovery on microscopic examination respectively. The healing effects were almost the same in all three groups therefore, the statistical difference was not significant among them (p =0.40 and 0.65) while significant from group E (p=0.0000075, 0.0000016 and 0.0000012 respectively).
Conclusion: NS and NH are equally effective in healing of gastric ulcer similar to cimetidine. Further broad spectrum studies as well as clinical trials should be conducted before the use of these products as routine medicines.

Key words: *Acetylsalicylic acid. Gastric ulcer. Cimetidine. Honey. Nigella sativa.*

INTRODUCTION

The global incidence of peptic ulcer disease has greatly increased during the last decades. The term peptic ulcer refers to an ulcer in the lower oesophagus, stomach, duodenum, in jejunum after surgical anastomosis to the stomach or rarely in ileum adjacent to a Meckel's diverticulum.[1]

Nigella sativa seeds *(Kalonje)* have been in use as a natural remedy for over 4000 years in various parts of the world. These seeds are reported to benefit almost every system of the body.[2-6] *Nigella sativa* oil (NSO), nigellone (polythymoquinone) and derived thymoquinone were studied to evaluate their effect on the formation of 5-lipoxygenase (5-LO) products from polymorphonuclear leukocytes (PMNL). These products inhibit HCl production due to their antioxidative action.[7,8]

The NS treatment significantly decreased the number of mast cells (MC) and reduced the area of gastric erosions. Likewise, thymoquinone (TQ) treatment was also able to reduce the number of MC and the gravity of gastric mucosal lesions, but to lesser extent compared to NS. Gastric tissue histamine levels and myeloperoxidase activities were found to be increased in ethanol treated rats reversed by NS or TQ treatment. Results obtained from this study suggest that both drugs, particularly NS could partly protect gastric mucosa from acute alcohol-induced mucosal injury, and these gastroprotective effects could be due to their antiperoxidative, antioxidant and antihistaminic effects.[7,9]

Honey has been reported to be effective in gastrointestinal disorders in humans, in the healing of wounds, burns and as antimicrobial agents and to have gastric protection against acute and chronic gastric lesions in animals. Furthermore, honey has the ability to inhibit the growth of *Helicobacter pylori (H.pylori) in vitro*. Honey has also been used for stomach pains and problems.

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Received August 23, 2010; accepted February 14, 2011.

Modern research shows that honey is effective when used in the treatment of gastric or peptic stomach ulcers. Research has also revealed that honey is effective in the treatment of various wounds and infections because of its antimicrobial (antibacterial, antiviral and antifungal) properties.[11]

The study was conducted to compare the healing effects of natural honey, *Nigella sativa* and Cimetidine in an experimental rodent model.

METHODOLOGY

An experimental study was carried out at Pharmacology and Pathology Department of King Edward Medical University, Lahore, from June to August 2007.

One hundred (100) adult male albino rats weighing from 200-250 gram were purchased from the animal house of National Institute of Health, Islamabad and kept in the animal house with a maximum of 6 albino rats per cage. Ambient temperature was kept around 25°C. They were acclimatized before starting the experiment under standard conditions of humidity, temperature and light (12 hours light and 12 hour dark cycle).[11,12]

The animals were divided into 5 groups, comprising of 20 each (A-E). The 20 animals of group A were given 2 ml of 2% gum tragacanth (GT). Two of those animals were also sacrificed after 3 days to observe any lesions produced by GT. The remaining 18 animals were assigned control group A and were continued on this treatment parallel to other animals of group B, C, D and E. Animals of group B, C, D and E were given acetylsalicylic acid (0.2 gm/kg body weight) to produce ulcers by gavage. Eight animals (2 from each group) were killed after 3 days to produce gastric ulcers. After confirmation of lesions by two histopathologists, the remaining animals (72) were divided into 12 subgroups with 6 rats in each (B I-III, C I-III, D I-III and E I-III) according to time schedule (2-4-6 weeks) to give NS (30 mg/kg body weight),[13] NH in a dose of 30 mg/kg body weight and CD (15 mg/kg body weight) respectively. The acetylsalicylic acid affected animals of group E were kept with as such without any treatment (Table I).

All the chemicals used were of analytical grade and acetylsalicylic acid powder was obtained from Reckitt Benckiser, Karachi, Pakistan. The reference anti-ulcer drug was cimetidine salt.[14] Finely powdered medicine were soaked in water/methanol (1:1) at 37°C for 24 hours which were shaken occasionally. Macerates were filtered and the filtrates were evaporated at 37°C. Extract were collected and weighed, then stored in sealed plastic bags.[15] For dosing all the test substances were suspended in aqueous 2% gum tragacanth solution.[14]

A natural honey was arranged from the National Agricultural Research Centre, Islamabad and was given to experimental animals of group C I-III, in a dose of 30 mg/kg body weight. The dose of honey was calculated as 30 mg/kg body weight and the calculated amounts were diluted/suspended in distilled water and administered orally in a volume of 0.5 ml/100g body weight (Table I).[16]

After the approval of criteria for animal ethics from Institutional Animal Care and Use Committees,[17] the rats were anaesthetized and sacrificed at the end of experimental periods. Their stomachs were removed and rinsed in lukewarm distilled water. Gross examination was performed before fixing in 10% formalin. The tissues were processed in automatic processor and were embedded in paraffin blocks. After microtomy the sections were stained with haematoxylin and eosin. The microscopy was performed in the Department of Pathology King Edward Medical University, Lahore, for the interpretation of lesion. The microscopy was performed after staining the sections with haematoxylin and eosin.[11]

Data was analyzed by window SPSS 12 and the quantitative difference of lesions between different experimental

Table I: Schedule of animal grouping and postmedication.

Experimental groups	Subgroups	Dose of the chemical	Number of animals	Animals sacrificed after weeks
Experimental group (Control) A	A-I	2 ml of 2% gum tragacanth	6	2 weeks
20 animals	A-II	"	6	4 weeks
2 ml of 2% gum tragacanth	A-III	"	6	6 weeks
Experimental group (*Nigella sativa*) B	B-I	30 mg/kg body weight in water	6	2 weeks
0.2 gm/kg body weight	B-II	"	6	4 weeks
(acetylsalicylic acid)	B-III	"	6	6 weeks
Experimental group (Natural honey); C	C-I	30 mg/kg body weight	6	2 weeks
0.2 gm/kg body weight	C-II	"	6	4 weeks
(acetylsalicylic acid)	C-III	"	6	6 weeks
Experimental group (Cimetidine); D	D-I	15 mg/kg body weight	6	2 weeks
0.2 gm/kg body weight	D-II	"	6	4 weeks
(acetylsalicylic acid)	D-III	"	6	6 weeks
Experimental group (acetylsalicylic acid); E	E-I	15 mg/kg body weight	6	2 weeks
0.2 gm/kg body weight	E-II	"	6	4 weeks
(acetylsalicylic acid)	E-III	"	6	6 weeks

groups of animals were compared by Fishers Exact test for determining significance at p-value < 0.05.

RESULTS

No lesion was seen in any of control animals while almost all animals showed gastric ulcers in group E and the severity was increased with duration. Loss of mucosal integrity, epithelial and glandular distortion with sever acute inflammation was detected in lamina propria of all animals of this group.

On gross examination of stomach, 14/18 (78%) albino rats of group B showed complete healing and did not reveal any abnormality. Complete remission was seen in 17/18 (94%) rats of group D while no remission was observed among animals of group E. The healing capacity of NS and NH was almost same as CD and the difference was found to be non-significant (p=0.6) while significant from group E (p=0.000075 in both the cases, Table II and III).

Out of 18 only 4 animals of groups B retained microscopic surface mucosal injury after treatment with NS as compared to group D, there were glandular injuries in 2 animals in group B as compared to 1 of group D while 5 animals showed inflammation in the lamina propria of group B as compared to 2 animals of group D (Table III).

Fourteen animals of group C showed healing of gastric ulcers treated with NH and the difference was statistically non significant as compared to CD and NS (p = 0.65 and 1 respectively), it was highly significant as compared to group E (p=0.000016). Out of 18 albino rats of group C, 14 (78%) animals showed complete regeneration of inflamed gastric mucosa as compared to 13 in group B and 16 in group D. Two animals retained gastric lesions in group C-I, one animal in group C-II and one animal in group C-III (Table II and III).

On microscopic examination of gastric mucosa, 13/18 (72%) albino rats of group B revealed complete recovery as compared to 14/18 (78%) animals of group C and

Table II: Comparison of antiulcerogenic healing effects of *Nigella sativa* with cimetidine after 2 weeks treatment in albino rats.

Groups (n=6)	Gross examination of stomach				Microscopic examination of stomach				
					Abnoramlity of mucosa				
	Mucosal appearance	Loss of mucosal integrity: Erosions	Loss of mucosal integrity: Ulceration	Number of animals with lesion	Surface	Glands	Lamina propria	Inflammation	Number of animals with lesion
B-1 (n=6)	1	1	0	1	2	1	2	2	2
C-I (n=6)	1	1	0	1	2	1	2	2	2
D-1 (n=6)	0	0	0	0	1	1	1	1	1
E-1 (n=6)	5	5	5	5	5	5	5	5	5
B-II (n=6)	0	1	0	1	0	1	1	1	1
C-II (n=6)	1	1		1		1	2	1	2
D-II (n=6)	0	0	1	1	1	0	1	1	1
E-II (n=6)	6	6	6	6	6	6	6	6	6
B-III (n=6)	1	1	0	1	2	0	2	2	2
C-III (n=6)	1	1	0	1	1	0	1	1	1
D-III (n=6)	0	0	0	0	0	0	0	0	0
E-III (n=6)	6	6	6	6	6	6	6	6	6

Table III: Cumulative effect of *Nigella sativa*, natural honey and cimetidine after 6 weeks of treatment.

Animal	Gross examination		Microscopic examination		Total animals in each group	p-values
	Without apparent lesions	With apparent lesions	With healed lesions	Without healed lesions		
*Nigella sativa	*15	3	13	5	18	*p-value = **0.60 (Gross and microscopic examination statistical difference is not significant) when compared with **NH and **p-value = 0.40 when compared with ***CD and 0.0000075 as compared to ^acetylsalicylic acid group (HS).
**Natural honey	**14	4	14	4	18	**p-value = 0.60 (Gross and microscopic examination statistical difference is not significant) when compared with *NS and CD*** p-value = 0.40 and 0.0000075 as compared to ^acetylsalicylic acid group (HS).
***Cimetidine	*17	1	16	2	18	***p=0.0000012 as compared to ^acetylsalicylic acid
^ Acetylsalicylic acid	*0	18	0	18	18	

Key words: CD = Cimetidine; NS = Nigella sativa; NH = Natural honey; HS = Highly significant.

16/18 (89%) rats of group D and non of group E. The statistical differences were not significant (p=0.4) in groups C and D or B and C (p=0.6) while being highly significant when compared with group E (Tables II and III, Photomicrograph 1).

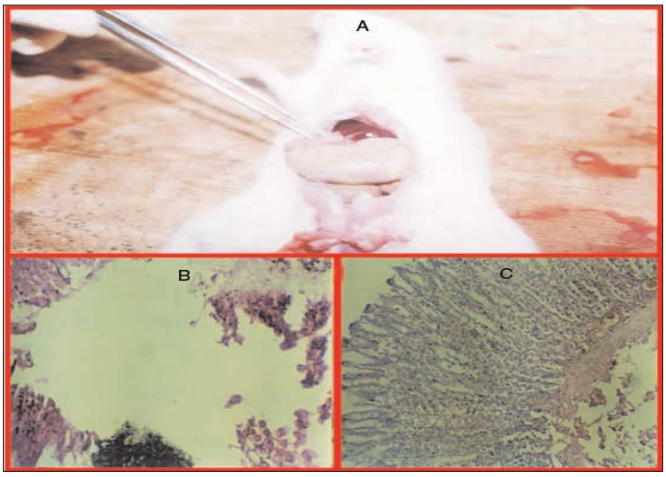

Figure 1 (A, B and C): A. Photograph of stomach of albino rat; **B.** Microphotograph of normal gastric mucosa (arrow) of albino rats after taking 2 weeks gum tragacanth showing no abnormality; **C.** Microphotograph of albino rats gastric mucosa after taking acetylsalicylic acid showing perforation of gastric mucosa after 6 weeks (arrow) (H & E10x).

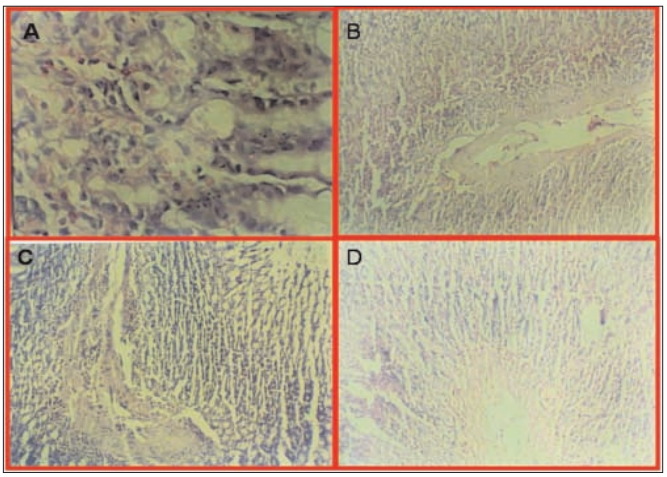

Figure 2 (A, B, C and D): A. Photomicrograph (H&E 40x) of gastric mucosa after taking acetylsalicylic acid showing erosions in albino rats after 2 weeks; **B.** (H & E10x), gastric mucosa after taking 2 weeks of *Nigella sativa* treatment showing regenerative changes; **C.** (H & E10x) gastric mucosa after taking 2 weeks natural honey showing regenerative changes and **D.** (H & E10x gastric mucosa after taking 2 weeks Cimetidine showing regenerative changes.

DISCUSSION

Uncontrolled acid secretion and ulceration of gastric mucosa due to several reasons is a serious problem to human health all over the world. Many natural products and modern synthetic agents have been used to treat peptic ulcer disease but so far a complete cure has not been discovered and exploration of new anti-ulcer agents has remained a field of active research.[18]

Honey has long been recommended in ancient religions and even in Holy Quran.[19] Honey also proved useful in the treatment of burns, wounds, gastroenteritis stomach and skin ulcers because of its antibacterial properties.[20,21]

In an effort to further search, the present study is undertaken to search the curative and safe agents for the treatment of peptic ulcer in our indigenous medicinal plants. The gastroprotective efficacy of *Nigella sativa* extract is determined in albino rats having acetylsalicylic acid induced ulcers. The acetylsalicylic acid model has already been utilized for screening the new compounds for their anti-ulcer effects. Use of this model for the same purpose has been employed for several workers including Akhtar and Munir and Eddleston *et al.*[15,22]

Animals of control group A, taking GT remained healthy throughout the experimental period and no gross or microscopic lesion was seen in their gastric mucosa, while all animals of group E, taken experimental acetylsalicylic acid, showed gastric erosions, ulcers on gross examinations and mucosal injuries on microscopic examinations. The animals of that group were given acetylsalicylic acid only for 3 days and were kept on normal diet for the remaining period of study. These rats did not show healing of gastric mucosa after either 2nd, 4th and even 6th week.

The results were favourable in the gastric mucosa of experimental group B in which NS was given for the treatment of gastric ulcer after 3 days ingestion of acetylsalicylic acid. Four animals in experimental group B-I showed normal and intact mucosa with reparative changes in their mucosal surface and 2 animals showed mild acute and chronic inflammation, with infiltration of neutrophils, lymphocytes and macrophages. In group B-II, only one rat showed mild degree of mucosal congestion on gross inspection while two animal's gastric mucosa showed mild degree of chronic inflammation with increased number of macrophages and fibroblasts. There were signs of mild chronic inflammation. In group B-III gross examination of stomachs of albino rats revealed smooth and dull appearance in 2 animals. On microscopic examination, 2 animals showed mild degree of congestion with reparative changes. There were signs of chronic inflammation with fibrosis while the remaining 4 animals had no lesions on gross and microscopic examination.

Four animals in experimental group C-I showed recovery from gastric ulcers while only 2 animals revealed mild acute and chronic inflammation, with infiltration of neutrophils, lymphocytes and macrophages. In group C-II, only one animal showed mild degree of congestion with increased number of glands with fibroblasts. There were signs of mild chronic inflammation. In group C-III, on gross examination the mucosa were smooth and dull in appearance of one animal. On microscopic examination, only one animal showed mild degree of congestion with reparative changes. There were signs of chronic inflammation with

fibrosis while the remaining 5 animals had no lesions on gross and microscopic examination. In group C 14/18 animals showed complete remission from ulceration, inflammation and erosions produced with acetylsalicylic acid. The difference was statistically non-significant as compared to group B and D.

In the experimental group D, cimetidine was used as a reference medicine to compare the effect of *Nigella sativa* on gastric mucosa. In group D-I after 2 weeks of treatment one animal showed mild chronic inflammation with healing ulcer. Fibroblasts were present in the lamina propia. In experimental group D-II, one animal had mild degree of ulceration with signs of mild degree of chronic inflammation. In group D-III all the animals were found normal with no signs of inflammation. On gross examination, amongst 18 animals of this group, not even a single case showed lesion in mucosal appearance except only one animal with loss of mucosal integrity. On microscopic examination 2 animals showed lesions in mucosal glands and lamina propria while one showed mucosal surface changes. The difference between these lesions was statistically not significant when compared with experimental group B-I and B-II and B-III or C-I, C-II and C-III. The healing results of *Nigella sativa* and cimetidine are consistent with that of Shoiab and Akhter *et al.* (1998); in which the drug was used as a reference for the treatment of gastric ulcer.[14-16,22]

The results obtained from the use of natural honey are also consistent with others.[16,19-23]

CONCLUSION

Nigella sativa and natural honey were equally effective in healing of gastric ulcer in rodents, as was cimetidine. In Pakistan, natural products like NS and NH are not used in the gastric ulcer therapy in routine practice. On the basis of present study, it is suggested that further broad spectrum studies as well as clinical trials should be conducted before the use of these products as routine medicines.

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