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GASTRIC ULCER HEALING EFFECTS OF NIGELLA SATIVA; A COMPARATIVE EXPERIMENTAL STUDY WITH CIMETIDINE

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Introduction: *Nigella sativa seeds 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; the present study was conducted to see the healing effects of Nigella Sativa in experimentally produced gastric ulcers with comparison to Cimetidine.* ***Materials and Methods:*** *It was an experimental study on 40 albino rats, performed in animal house of Postgraduate Medical Institute, Lahore. The animals were given Aspirin (0.2 gm/kg body weight) to produce ulcers. Four animals were killed after 2 weeks to confirm gastric ulcers by histopathology. The remaining animals (36) were equally divided in two groups and the 3 subgroups according to time schedule (2-6 weeks) for the treatment with Nigella Sativa (30 mg/kg body weight) and Cimetidine (15 mg/kg body weight). Rats were anaesthetised and sacrificed at the end of experimental periods and the stomachs were removed, rinsed in lukewarm distilled water. Gross and microscopic examinations were performed to evaluate the results.* ***Results:*** *On gross examination of stomach, 14/18 (78%) albino rats of group "A" (taking Nigella Sativa) did not reveal any abnormality due to complete response to Nigella sativa as compared to 17/18 (94%) of group "B" (taking Cimetidine). On microscopic examination of stomach, 13/18 (72%) albino rats of group "A" (taking Nigella Sativa) revealed complete recovery as compared to 16/18 (89%) of group "B" (taking Cimetidine).* ***Conclusion:*** *We concluded that Nigella sativa is equally effective in healing of gastric ulcer as is Cimitidine therefore we suggest the use of the N-sativa in the therapy of gastric ulcer disease in routine practice.*

Keywords: *Aspirin, gastric ulcer, Cimitidine, Nigella sativa, mucosal injuries.*

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 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.[2-5]

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). NSO produced a concentration dependent inhibition of 5-LO products and 5-hydroxy-eicosa-tetra-enoic (5-HETE) acid production with half maximal effects IC (50) at 25 ± 1 μg/ml, 24 ± 1 μg/ml respectively. Nigellone caused a concentration-related inhibition of 5-HETE production IC (50) at 11.9 ± 0.3 μg/ml. Moreover, thymoquinone, the active principle of NSO inhibited the production of 5-LO products IC (50) at 0.26 ± 0.02 μg/ml and 5-HETE production IC (50) at 0.36 ± 0.02 μg/ml in a similar way. The effects are probably due to an antioxidative action. The data may in part explain the effect of the oil, its derived thymoquinone and nigellone in ameliorating inflammatory diseases.[6]

El-Dakhakhny et al investigated the possible role of Nigella sativa oil (NSO) on gastric secretion and ethanol induced ulcer in rats. Thirty two adult male rats were used in this study and several parameters were determined to assess any degree of protection. It was found that the administration of NSO in rats produced a significant increase in mucin content and glutathione level and a significant decrease in mucosal histamine content. When animals were pretreated with NSO before induction of ulcer, there was a significant increase in glutathione level, mucin content and a significant decrease in gastric mucosal histamine content with a protection ratio of 53.56% as compared to the ethanol group. It can be concluded that NSO imparted a protective action against ethanol induced ulcer in rats.[7]

The protective effect of NSO against ethanol induced ulcer may be explained by different mechanisms. First of all the increase in glutathione level caused a decrease in the ethanol induced gastric

damage. Secondly, glutathione is a cofactor in some steps of PGs synthesis i.e. the conversion of PGG_2 to PGH_2 and the subsequent conversion to PGE_2.[8] Prostaglandin synthetase is nearly incapable of synthesising PGE_2 after depletion of glutathione from the medium.[9,10] This postulation of increased PGE_2 by NSO is in agreement with different other researchers who found that the treatment of normal and sensitised animals with NSO provoked a marked increase in PGE_2 in the perfused guinea pig lung.[6,7]

Prostaglandin have an established role in the protection of gastric mucosa against different types of gastric lesions. Of particular interest is the fact that the anti-secretary prostaglandins can protect the mucosa at non-anti-secretary dosage. Moreover, the non-anti-secretary PGs such as PGF_2 are also protective. The decrease of gastric mucosal histamine level is another explanation of gastric protection. NSO attenuated one of the aggressive factors in the production of peptic ulcer which is the histamine. Finally, an additive protective factor which is gastric mucin is increased by NSO oral administration.[6,7]

Both NS and Thymoquinon (TQ), particularly NS can partly protect gastric mucosa from acute alcohol-induced mucosal injury, and these gastroprotective effects might be induced, at least partly by their radical scavenging activity.[11]

The NS treatment significantly decreased the number of mast cells and reduced the area of gastric erosions. Likewise, thymoquinone (TQ) treatment was also able to reduce the number of mast cells 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, and NS or TQ treatment reversed these increases. 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.[12]

The study was conducted to compare the healing effects of Nigella sativa and Cimetidine in an experimental model.

MATERIALS AND METHODS

This was an experimental study done on 40 male albino rats (weight 180 – 200 gram and 6 – 7 weeks aged). The animals were kept in experimental lab of Postgraduate Medical Institute Lahore. They were obtained from the National Institute of Health, Islamabad. The animals were maintained under optimal atmospheric and hygienic conditions, with food and water available *ad libitum* before the start of the experiment.

After a week of acclimatization and following the ethics of animal experimentation these animals were given Asprin to produce gastric ulcer before starting the treatment of Nigela sativa and Cimetidine. Four of the animals were killed after 3 days to confirm the ulcer histologically. We used the Histopathology as a gold standard for evaluation of changes in gastric mucosa of the animals. After confirmation of lesions with Asprin by two histopathologists the remaining animals were divided into two groups (A and B), comprising of 18 animal, each for further experimentation.

Animal's module

Forty (40) adult Spraque-dawley 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 of Postgraduate Medical Institute, Lahore. In the animal house of PGMI Lahore they were housed in large solid walled iron cages with a maximum of six 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 hour light and 12 hour dark cycle.[13,14]

Preparation of Chemicals

All the chemicals used were of analytical grade and Aspirin powder was obtained from Reckitt Benckiser Karachi Pakistan. The reference anti-ulcer drug was cimetidine salt.[15]

The finely powdered drugs were soaked in water/ methanol (1:1) at 37°C for 24 hours which were shaken occasionally. Then macerates were filtered and the filtrates were evaporated at 37°C. Extracts were collected and weighed, then stored in sealed plastic bags.[16] (For dosing all the test substances were suspended in aqueous 2% gum tragacanth solution.[15]

Gross and Microscopic Examination of Stomach

Processing of the tissue was done in an automatic processor. The paraffin blocks were made in L-shaped moulds. Sections of 3 – 4 μm thickness were cut by rotary microtome. The sections were taken on albuminized slides (Gorden 1990). The rats were sacrificed by an overdose of chloroform vapors and their abdomens were opened through a mid line incision and stomachs were removed. The gastric mucosae were washed with 3 ml of luke warm distilled water. Gross examination was performed before fixing in 10% formalin.

The microscopy was performed after staining the sections with haematoxylin and eosin by an unaware histopathologist in the department of Patho-

logy, King Edward Medical University, Lahore for the confirmation of results.[13]

The results were expressed as Fishers Exact

Table 1: *Schedule of Animal grouping and medication.*

Group	Sub-group	Dose of the chemical	No. of animals	Animals sacrificed after weeks
Total animal given Asprin		0.2 gm/kg body weight	40	4 animals were sacrificed after 3 days
Experimental Groups	A and B		36	
Experimental Group (Nigella sativa) A	AI	30 mg/kg body weight	6	2 weeks
	AII	"	6	4 weeks
	AIII	"	6	6 weeks
ii) Experimental Group (Cimetidine); B	BI	15 mg/kg body weight	6	2 weeks
	BII	"	6	4 weeks
	BIII	"	6	6 weeks

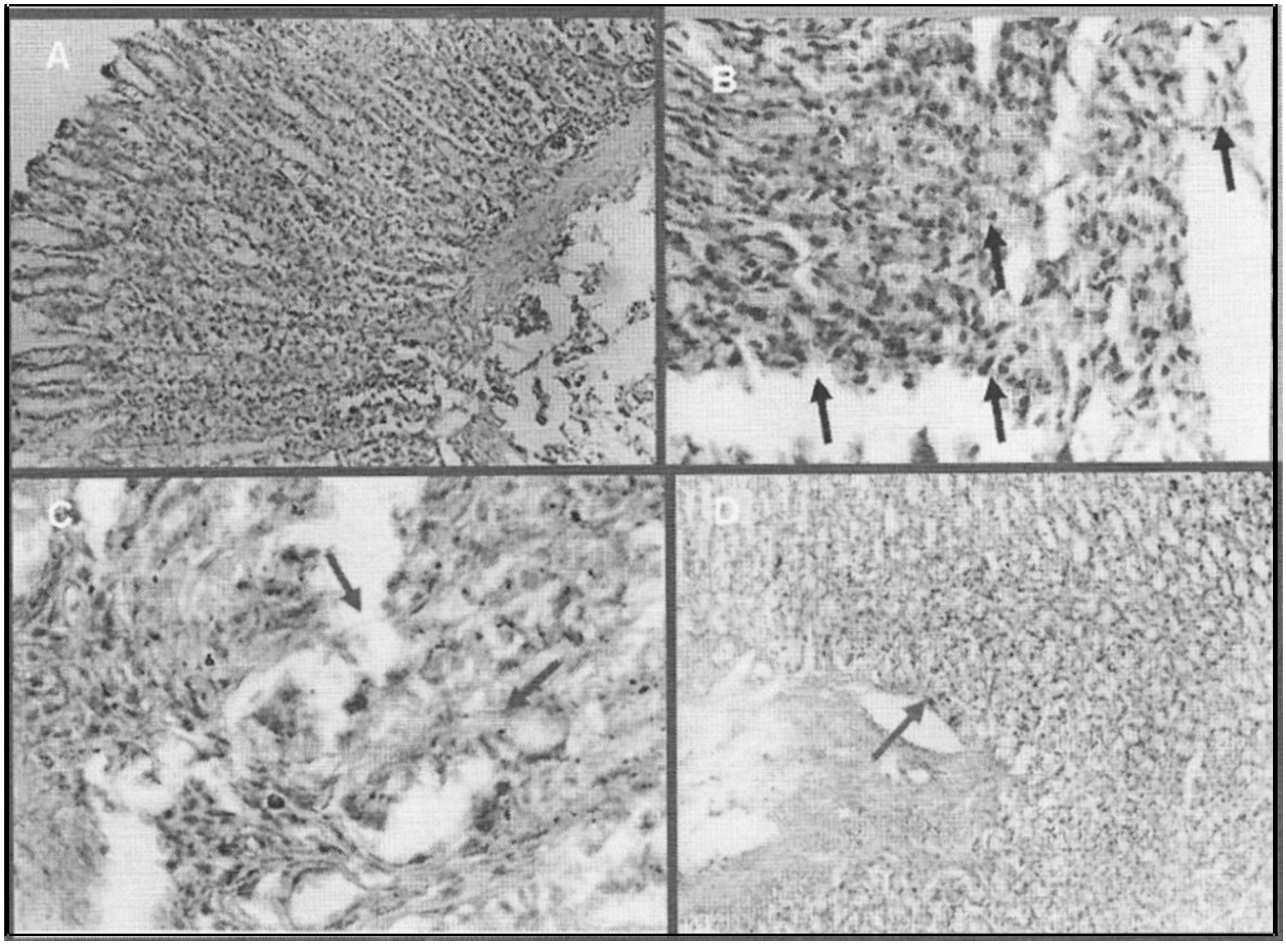

Fig. 1: *Microphotograph A of normal gastric mucosa (Arrow) of Albino rats (H and E 10×). B. gastric mucosa after taking six weeks Aspirin showing multiple erosions (Arrow). (H and E 10×), C. gastric mucosa after taking six weeks of Nigella sativa treatment showing normal mucosa after complete regeneration. (Arrow) (H and E 40×), E. gastric mucosa after taking six weeks Cimetidine showing normal mucosa after complete regeneration. (Arrow) H and E 10×).*

Table 2: *Comparison of Antiulcerogenic healing effects of Nigella Sativa with Cimetidine after two weeks treatment in albino rats.*

Groups (n=6)	Gross Examination of Stomach				Microscopic Examination of Stomath				
	Mucosal appearance	Loss of mucosal Integrity		No. of Animal with lesion	Abnormality of Mucosa			Inflam-mation	No. of animals with lesion
		Erosions	Ulcera-tions		Surface	Glands	Lamina Propria		
A – 1 (n = 6)	1	1	0	1	2	1	2	2	2
B – 1 (n = 6)	0	0	0	0	1	1	1	1	1
A – II	0	1	0	1	0	1	1	1	1
B – II	0	0	1	1	1	0	1	1	1
A – III	1	1	0	1	2	0	2	2	2
B – III	0	0	0	0	0	0	0	0	0

Test. The lesions produced in different groups of animals were compared by Fishers Exact.

Table 3: *Cumulative effect of Nigella Sativa and Cimetidine after 6 weeks of treatment.*

Animal	Gross examination		Microscopic examination		Total animals in each group
	Without apparent lesions	With apparent lesions	With healed lesions	Without healed lesions	
Nigella Sativa	15	3	13	5	18
Cimitidine	17	1	16	2	18

p-value = 0.60 (Gross examination statistical difference is not significant)
p-value = 0.40 (Microscopic examination statistical difference is not significant)

RESULTS

On gross examination of stomach, 14/18 (78%) albino rats of group "A" (taking Nigella Sativa) did not reveal any abnormality due to complete response to Nigella sativa as compared to 17/18 (94%) of group "B" (taking Cimetidine). The statistical difference of both drugs was non significant *(p=0.6)*.

Four animals of groups A retained microscopic surface mucosal injury even after treatment with Nigella Sativa as compared to group B, there were glandular injuries of 2 animals in group A as compared to 1 of group B while 5 animals showed inflammation in the lamina propria of group A as compared to 2 animals of group B (Table 2). On microscopic examination of stomach, 13/18 (72%) albino rats of group "A" (taking Nigella Sativa) revealed complete recovery as compared to 16/18 (89%) of group "B" (taking Cimetidine). The statistical difference of both drugs was non significant *(p=0.4)* (Table 2 and 3 and Fig. 1).

DISCUSSION

Uncontrolled acid secretion and ulceration of gastric mucosa due to several reasons have posed serious problems to the human health all over the world. Many natural products and modern synthetic drugs have been used to treat the peptic ulcer disease but so far a complete cure has not been discovered and exploration of new anti ulcer drugs has remained a field of active research.[17,18]

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 Aspirin induced ulcers. The Aspirin 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 (1989), Eddleston et al (1994) and Shah and Khan (1997).[16,19]

When we observed the experimental group A in which Nigella sativa was given for the treatment of gastric ulcer after ingestion of Aspirin for three days, the results were favourable. Four animals in experimental group A-1 showed normal and intact mucosa with reparative changes in mucosal surface and two animals showed mild acute and chronic inflammation, with infiltration of neutrophils, lymphocytes and macrophages. In group A-II, only one animal showed mild degree of congestion with increased number of glands with fibroblast. There were signs of mild chronic inflammation. In group A-III gross examination of stomachs of albino rats revealed smooth and dull appearance in two animals. On microscopic examination, two animals showed mild degree of congestion with reparative changes. There were signs of chronic inflammation with fibrosis while the remaining four animals had no lesions on gross and microscopic examination.

In experimental group B, Cimitidine was used as a reference drug to compare the effect of Nigella sativa on gastric mucosa. In experimental group B-1 after two weeks of treatment one animal showed mild chronic inflammation with healing ulcer. Fibroblasts were present in the lamina propria. In experimental group B-II, one animal had mild degree of ulceration with signs of mild degree of chronic inflammation. In group B-III all the animals were found normal with no signs of inflammation.

When comparing all the eighteen animals of this group, no animal showed lesion in mucosal appearance and one animal showed lesion in mucosal integrity, on gross examination. On microscopic examination two animals showed lesions in mucosal glands and lamina propria while one animal in mucosal surface. The difference between these lesions was statistically not significant when compared with experimental group B-I and B-II and B-III. The healing results of Nigella Sativa and cimitidine are consistent with that of Shoiab and Munir (1989) and Akhter et al (1998); in which the drug was used as a reference for the treatment of gastric ulcer.[15,16,19]

In **conclusion**, we found that nigella sativa is equally effective in healing of gastric ulcer as is cimitidine therefore we suggest the use of the drug in the therapy of gastric ulcer disease in routine practice.

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