

THE INTERACTION BETWEEN *NIGELLA SATIVA* FIXED OIL AND RANITIDINE ON STRESS-ETHANOL INDUCED GASTRIC MUCOSAL DAMAGE IN RABBIT: A POTENTIAL HERB-DRUG INTERACTION

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ABSTRACT

Background: The use of herbal preparations has increased dramatically, making drug interactions with these preparations a major health concern, especially as herbal medications are usually not subjected to the same regulations as prescription drugs

Aim: as a potential drug-herb interaction is possible, this study was designed to investigate the interaction between *Nigella sativa* (NS) and ranitidine (R) on absolute ethanol induced gastric mucosal damage in rabbit

Materials and Methods: Five groups of rabbits (6 each) were used. Acute gastric ulcerations were induced by ethanol through a stomach tube. The oil of NS was given orally, ranitidine by (IM), combination of NS+R or normal saline were given 1 hour before ethanol. Ulcer index, serum and stomach tissue MDA, gastric volume and pH, and histopathology were evaluated.

Results: Monotherapy of NS or R reduced the mean ulcer index from 91.7 ± 19.4 mm in the control group to 43.3 ± 8.7 and 22.5 ± 9.4 mm for NS and R treatment respectively. There were significant reductions in serum and stomach tissue MDA and in gastric secretion. When NS and R were given in combination the anti-ulcer effect of both disappeared. This was associated with increased MDA levels in stomach tissue, but not serum. The pH of stomach content was also changed toward ethanol treated values.

Conclusion: These findings document the gastro-protective potential of NS against ethanol-induced gastric ulcer. There was a significant NS-R interaction manifested as failure of the combination to inhibit ulcers formation. Until further wider studies are available to confirm such interaction, the simultaneous use of *Nigella sativa* and ranitidine should be discouraged.

INTRODUCTION

The likelihood of herb-drug interactions could be higher than drug-drug interactions, because drugs usually contain single chemical entities, while almost all herbal medications (even a single herb) contain mixtures of pharmacologically active constituents^[1] and usually the herbal supplements are not subject to the same regulations as prescription drugs. *Nigella sativa*, a frequently used herbal preparation and as food supplement has a number of pharmacologic effects such as immunomodulative,^[2] antibacterial,^[3] hepatoprotective,^[4] and gastroprotective effects.^[5] *Nigella sativa* is increasingly used in the folk medicine for alleviating various gastric symptoms either alone or in combination with principal anti-ulcer drugs, thus a possible herb-drug interaction may be seen when the drugs and herbs are taken together.

This study, therefore, was designed to investigate a possible interaction between the oil of *Nigella sativa* and ranitidine on gastric mucosal lesion induced by ethanol and stress in rabbits.

MATERIALS AND METHODS

The study was approved by the institutional Ethical Committee and carried out between August 2006 and April 2007.

Plant materials and procedure of extraction

The seeds of *Nigella sativa* were purchased from a local market in Basrah, south of Iraq. The seeds were identified and authenticated by an expert local pharmacist. Voucher specimens were kept in the Department of Pharmacology. The seeds of *Nigella sativa* were powdered mechanically using an ordinary blender for 6 minutes. Eighty grams of the powder were

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dissolved in petroleum ether, and then the fixed oil of *Nigella sativa* was extracted by soxhlet at 60-90°C for 18 hours. The extract was then concentrated using rotary evaporator for 30 minutes.

Treatment of rabbits

The experiments were carried out on 30 locally bred sexually mature male rabbits. They were maintained on free excess to food and drinking water. Prior to the day of the experiment each rabbit was kept in a restrain cage for 48 hours. During restrain the rabbits had free access to drinking water only. On the day of the

experiment the rabbits were allocated randomly to receive either *Nigella sativa* oil orally in a dose of 10ml/kg or ranitidine 10mg/kg I.M.; (ranitidine, Glaxo-Smith-Kleine, UK) physiological saline administered orally or the combination as shown in (Table-1). A child stomach tube was used for administration of oral treatments. It was advanced into the stomach through a wooden tongue depressor with a hole in the center to prevent the animal chewing the tube. The rabbits were divided into five groups, six each. Treatments were given as presented in (Table-1).

Table 1. Study design

Treatments (N=6 in each group)	Time	
	0 time	1 hour after treatment
Control (Group 1)	10ml/kg saline (oral)	10ml/kg saline (oral)
Absolute ethanol (Group 2)	10ml/kg saline (oral) + 1ml/kg saline (I.M)	5ml/kg ethanol (oral)
Ranitidine (R) (Group 3)	10mg/kg ranitidine (I.M)+ 10ml/kg saline (oral)	5ml/kg ethanol (oral)
oil of <i>Nigella sativa</i> (NS) (Group 4)	10ml/kg nigella oil (oral)+ 1 ml/kg saline (I.M)	5ml/kg ethanol (oral)
(NS + R) (Group 5)	10ml/kg nigella oil (oral)+ 10mg/kg ranitidine (I.M)	5ml/kg ethanol (oral)

Four hours after starting the experiment and before sacrificing, the rabbits were exposed to a short chloroform anesthesia. Two milliliters of blood were taken directly from the heart by an ordinary syringe for the measurement of serum MDA. The rabbits were then sacrificed by cervical dislocation. The stomach was exposed, the lower esophagus and the first part of the duodenum were ligated, and the stomach contents were collected and stored in a deep freezer (-10 °C). The stomach was then opened along the greater curvature, gently washed in a

tap water and then fixed on a piece of cork for macroscopical examination. Ulcers were scored using ordinary ruler with the help of magnifying lens; ulcer index which is the sum of length of all ulcers was calculated for each animal. A piece of stomach tissue was taken from the largest ulcerated area for the measurement of tissue MDA. The stomach then fixed with 10% formalin for histopathological examination. Gastric acidity was measured by a digital pH meter. Serum and stomach tissue MDA level were measured spectrophotometrically by

thiobarbituric acid assay method.^[6] Statistical analysis was carried out using SPSS (version 11), analysis of variance (ANOVA) was used to compare means of groups followed by unpaired t-test to compare different groups means. P-value less than 0.05 is considered significant.

RESULTS

Effect on ulcer index

Ethanol administration induced gastric ulceration in all treated rabbits, with a mean ulcer index of 91.7±19.4mm. The administration of the oil of *Nigella sativa* in a dose of 10ml/kg orally one hour before ethanol

administration reduced the mean ulcer index to 43.3±8.7mm as compared to the ethanol treated value, p<0.01. Ranitidine 10mg/kg administered intramuscularly one hour before ethanol administration had reduced ulcer index to 22.5±9.4 mm which was significantly lower than that of the ethanol treated group (p<0.01). The mean ulcer index of the combination of *Nigella sativa* oil (10ml/kg orally) and ranitidine (10mg/kg I.M) given one hour before ethanol administration was 95±13.7, which was not statistically different from the ulcer index of the ethanol treated group (91.7±19.4 mm) (Table-2).

Table 2. Effect of *Nigella sativa*, ranitidine and their combination on ulcer index, serum and stomach tissue MDA in rabbits.

Treatments Groups	Ulcer index (mm) Mean ±SD	Serum MDA µmol/l	Stomach tissue MDA nmol/mg
Group 1(control)	0.0	0.63±0.08	0.33±0.08
Group 2 absolute ethanol	91±19.4	1.18±0.1	1.12±0.3
Group 3 oil of <i>Nigella sativa</i> (NS)	43.3±8.7**	0.26±0.0*	0.50±0.06*
Group 4 ranitidine (R)	22.5±9.4**	0.41±0.0*	0.63±0.1*
Group 5 (NS + R)	95.0±13.7	0.44±0.1*	0.98±0.2

Significantly different from the corresponding value of the ethanol treated group, *p<0.05, ** p<0.01

Effect on serum and stomach tissue MDA levels

The administration of ethanol had significantly increased the mean MDA value in the serum to 1.18±0.1 µmol/liter compared to 0.63±0.08µmol/liter in the control on normal saline instead of ethanol (p<0.05).

The administration of the oil of *Nigella sativa* one hour before ethanol administration significantly reduced the mean serum MDA levels from 1.18±0.1 µmol/literto 0.26 ± 0.04 µmol/liter (p<0.05)in the rabbits treated with ethanol. The intramuscular administration of ranitidine produced an effect on MDA level similar to that of *Nigella sativa*. Ranitidine reduced the mean serum level of MDA to 0.41±0.07µmol/liter compared to 1.18±0.1 µmol/liter in the ethanol treated group (p<0.05).

The combination of *Nigella sativa* and ranitidine reduced the mean serum MDA level to 0.44±0.1 µmol/liter which was slightly higher than that of *Nigella sativa* or ranitidine given separately, but significantly lower than the MDA level in the ethanol treated group (1.18±0.1 µmol/liter) (Table-2).

The mean MDA value in stomach tissue homogenate of the control group was 0.33±0.08 nmol/mg, this was significantly increased to 1.12±0.3 nmol/mg in stomach tissue taken from the ulcerated area of rabbits in the ethanol treated group, P<0.05.

The administration of the oil of *Nigella sativa* or ranitidine significantly reduced the mean values of stomach tissue MDA from 1.12±0.3

nmol/mg in the ethanol treated group to 0.50 ± 0.06 nmol/mg in the *Nigella sativa* treated rabbits and to 0.63 ± 0.1 nmol/mg in the group which received ranitidine, $P < 0.05$. The mean stomach tissue MDA of the combination of *Nigella sativa* and ranitidine was 0.98 ± 0.2 nmol/mg which was insignificantly different from the mean stomach tissue MDA of the ethanol treated group (1.12 ± 0.3 nmol/mg) P -value > 0.05 (Table-2).

Table 3. Effect of *Nigella sativa*, ranitidine and their combination on gastric pH in rabbits.

Treatments	pH
Absolute ethanol	1.36 ± 0.4
<i>Nigella sativa</i> (NS)	$3.5 \pm 2.1^*$
Ranitidine (R)	Complete inhibition of gastric secretion
Combination (NS+R)	1.97 ± 0.86

Significantly different from the ethanol treated group, $p < 0.01$

Effect on gastric pH

The administration of *Nigella sativa* oil had resulted in an elevation of gastric pH from 1.36 ± 0.4 in the ethanol treated group to 3.5 ± 2.1 in the *Nigella sativa* treated group ($p < 0.01$).

In the ranitidine group there was complete inhibition of gastric acid secretion, and therefore, it was not possible to measure the pH.

The combination of *Nigella sativa* extract with ranitidine had resulted in a pH value of 1.97 ± 0.86 which was slightly higher than 1.36 ± 0.4 in the ethanol treated group but the difference was statistically not significant.

Histopathological examination

Histopathological examination of gastric sections revealed that absolute ethanol elicited gastric ulceration, necrotic mucosal lesions with wide areas of polymorph nuclear cell infiltration in all rabbits treated with ethanol. One hour before ethanol, the administration of *Nigella sativa* extractor ranitidine demonstrated a clear reduction of mucosal damage and reversal of histological changes, while the combination of *Nigella sativa* and ranitidine had failed to reduce ethanol induced gastric damages. These changes are presented in (Figure-1).

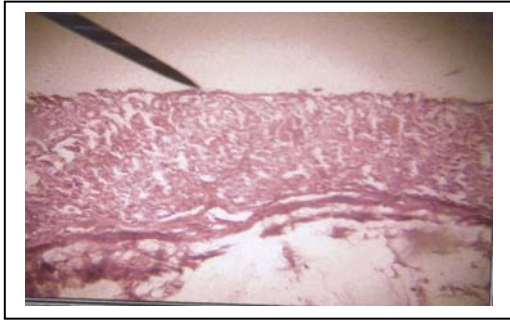


Figure 1A



Figure 1B

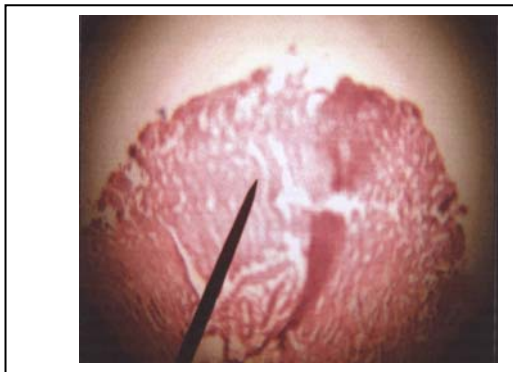


Figure 1C

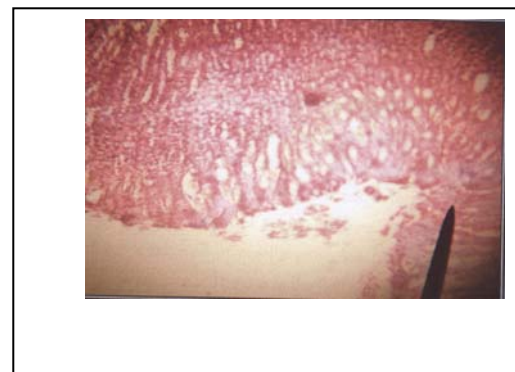


Figure 1D

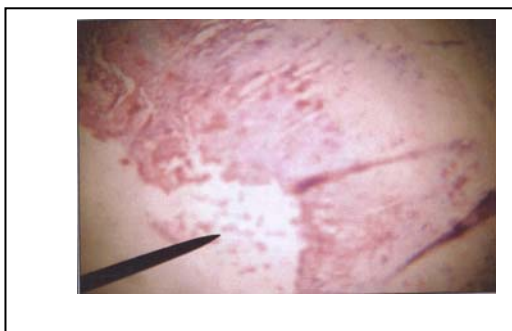


Figure 1E

Figure 1. Histopathological examination of gastric mucosa of the rabbits; figure 1A. Normal gastric mucosa in a rabbit treated with normal saline (control) (X 100); figure 1B. Gastric mucosa from a rabbit treated with absolute ethanol administered orally. Large gastric ulceration and necrotic mucosal lesions with wide areas of polymorphonuclear cells infiltration are seen; figure 1C. Small superficial gastric ulceration in a rabbit treated with I.M. ranitidine one hour before ethanol; figure 1D. Normal gastric mucosa in a rabbit treated with the oil of *Nigella sativa* one hour before absolute ethanol treatment; figure 1E. Large gastric mucosal ulceration with inflammatory cell infiltration in a rabbit treated with combination (*Nigella sativa* orally and ranitidine I.M.) one hour before absolute ethanol treatment.

DISCUSSION

The model of gastric ulceration induced by orally administered ethanol is well established; however, the mechanism behind the ulcerogenic effect of ethanol is not well understood. Several factors could contribute to that effect such as stimulation of gastric acid secretion,^[7] direct mucosal damage,^[8,9] decreased synthesis of prostaglandins, release of leukotriens,^[10] and free radicals generation.^[11, 12] The oil of *Nigella sativa* reduced the ulcer index in all treated animals, which was associated with reduction in both serum and stomach tissue MDA. These results are in agreement with other studies which demonstrated antiulcer effect of *Nigella sativa* against ethanol induced gastric ulceration in experimental animals.^[13,14] Important factor suggested to contribute to the antiulcer effect of *Nigella sativa* is probably its antioxidant activity.^[13] *Nigella sativa*, in addition, was shown to decrease the release of leukotriens which causes mucosal tissue injury and hypoxia.^[15,16] The antiulcer effect of *Nigella sativa* in this study is comparable to that of ranitidine. It was found in animals that an oral dose of 150 mg/kg of the oil of *Nigella sativa* produced comparable antiulcer effects to ranitidine in a dose of 20mg/kg given orally in ethanol induced gastric ulceration.^[16] Despite the established antiulcer effect of ranitidine and the observed antiulcer effect of *Nigella sativa*, unexpectedly their combination had a negative effect on gastric mucosal protection. Thus the gastroprotective effect observed with either treatment given separately was significantly reduced when the two treatments were given in combination. The loss of gastro protective effect of the combination was confirmed by histopathological examination and was also associated with elevation of serum and stomach tissue MDA levels and reduction of pH towards ethanol alone treated values. The mechanism of such interaction is not clear, however our results can at least exclude interaction between

ranitidine and *Nigella sativa* at the level of absorption from the gastrointestinal tract since *Nigella sativa* was given by oral route and ranitidine by intramuscular route. *Nigella sativa* was found to significantly decrease gastric mucosal histamine contents;^[14] a possible site for interaction. Many drugs in which drug interaction with herbs are reported are substrates of cytochrome P450s (CYPs) and/or P-glycoprotein (P-gp),^[17] in which the plasma concentrations is reduced with or without increased clearance of the concomitantly prescribed drugs.^[18] Recently it was reported that *Nigella sativa* exerted an effect on CYP2D6 and CYP3A4 mediated metabolism of dextromethorphan in human liver microsomes in vitro and in human volunteers.^[19] As Cytochrome P450 pathway is involved in ranitidine metabolism,^[20] an interaction between ranitidine and *Nigella sativa* with reduction in ranitidine plasma concentration cannot be ruled out. In conclusion our findings revealed that although *Nigella sativa* has gastro-protective potentials against ethanol-induced gastric ulcer, it has a significant interaction with ranitidine as manifested by disappearance of the anti-ulcer effect of both when the two were given together. Until further wider studies to explore such interaction, the simultaneous use of *Nigella sativa* and ranitidine should be discouraged.

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