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Gastroprotective and Anti-secretory Effect of *Nigella sativa* Seed and its Extracts in Indomethacin-treated Rats

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Abstract: *Nigella sativa*, seeds has been used in traditional medicine for the treatment of a variety of diseases including gastrointestinal disorders. *N. sativa* seed powder (NS), its extracts and purified fractions were studied for their possible gastroprotective effects in the indomethacin-treated rats (adult albino male rats weighing 180-220 g) to rationalize the folkloric uses. NS protected the rats against indomethacin-induced gastric effects in a dose-dependent manner. The ethanolic extract of the powder (NS-E) also attenuated the indomethacin-induced changes in gastric juice volume, pH, acid-output and ulcer index. Partition of ethanol soluble ingredients in organic solvents yielded semi-purified fractions whose anti-ulcer activity guided further chemical purification. The active fraction w as chromatographically characterized and the most purified fraction (NS-EA 51) obtained, presented anti-ulcerogenic activities in indomethacin-induced gastric ulcers in the rats. Various parameters including gastric juice volume, pH, acid-output, ulcer index and peptic activity showed that it was the most potent anti-ulcerogenic fraction which gave results comparable to Famotidine. The gastroprotective or anti-secretary, anti-peptic and anti-ulcerogenic actions of NS-EA 51 might be related to its anti-inflammatory and/or antioxidant activity.

Key words: Antiulcer, ulcer index, anti-secretary, indomethacin, *Nigella sativa*

INTRODUCTION

Several plants and herbs have been used to treat gastrointestinal disorders, including peptic ulcer, in the traditional medicines, since ancient time[1]. *Nigella sativa,* Linn. has also been used since antiquity for this purpose by Asian herbalists and pharmacists. It has been widely cultivated throughout South Europe, Syria, Egypt, Saudi Arabia, Turkey, Iran, Pakistan and India[2]. The plant was used for culinary and medical purposes by the Romans. It was widely used in the entire Old World: Europe, Asia, Africa, the Middle East and the Far East[3]. Its ethanol-water (1:1) extract possesses anti-inflammatory, anti-amoebic, anti-spasmodic, anti-tumor, CNS-depressant and plaque formation suppressant activities[4,5]. Ethanol and methanol extracts exerted smooth muscle relaxant, hypotensive, anti-bacterial against Staphylococci, anti-toxic, anti-cestodal, carcinogenesis inhibitory and anti-implantation activities[6]. The acid-ethanol extract possesses strong anti-bacterial, anti-yeast and anti-spasmodic effects. El-Dakhakhny *et al.*[7] reported on the pharmacological properties of the active principal, thymoquinone and its polymer. Houghton *et al.*[8] found that the oil possessed an anti-inflammatory action with the inhibition of eicosanoid generation and membrane lipid peroxidation. The seeds contain tannins, saponins, quinones, glucosinolates, sterols and/or triterpenes[9,10]. Arachidonic acid, eicosadienoic acid, linoleic acid and palmitic acid have been found in the seed oil while nigellicine in the roots[11,12].

Among others indications, NS has been considered anti-ulcer remedy in the traditional medicine. The present study was, therefore, carried out to validate its folkloric use. Moreover, gastric ulcers have been found common disease world over which have been caused major health problems in Pakistan. Hence, the present study was also planned to search for the effective cure of gastric ulcers, to serve the ailing humanity and to make use of the natural herbal wealth (plant source of drugs) of the country. So, to contribute to the improvement of economy of the country by involving the industrial use of natural drugs and saving the foreign exchange presently being spent on the import of different synthetic drugs.

MATERIALS AND METHODS

Plant drug: *N. sativa* seeds were purchased locally from herbal dealer in Gujranwala, Pakistan. The plant material

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was authenticated and compared with its standard in the herbarium maintained by Department of Botany, University of Agriculture, Faisalabad. Its sample was preserved in the Pharmacognosy laboratory, Department of Pharmacy, University of Sargodha. The plant material to be used was further dried under-the-shade, finely powdered and stored in well-closed cellophane bags at 4°C[13].

Chemicals: All chemicals used were of analytical grade and obtained from E. Merck (Darmstadt, FRG), BDH Poole (England) and Sigma Chemical Co. (USA). The reference antiulcer drug was famotidine (Ferozsons Laboratories Limited, Rawalpindi, Pakistan).

Test animals: Adult healthy Sprague-Dawley albino male rats weighing 180-220 g each were used. The animals were housed under standard conditions of temperature (23±2°C), humidity (55±15%) and 12 h light (7-19)[14]. These rats were provided with a free access to a standard feed made by M/S Lever Brothers, Rahim Yar Khan-Pakistan and water *ad libitum*. The rats were fasted for 24 h prior to their use in the experiments[13,14]. The animal experiments performed in this study complied with the guidelines of National Policy and with the principles and guidelines of the Canadian Council on Animal Care.

Preparation of plant extracts: Dried, ground NS (1.0 kg) was macerated with distilled water and ethanol (2.0 L each) at room temperature for 24 h separately. The dried extracts were obtained and stored in the sealed containers at 4°C. Ethanolic extract (500 g) was partitioned in succession with butanol (120.30 g), chloroform (91.50 g) and ethyl acetate (95.80 g) and residue fraction (192.40 g)[15]. The ethyl acetate fraction was chromatographed on silica gel column (6.0x100 cm, 1.0 kg) using an ethyl acetate/ethanol gradient system (1:0→0:1). The purified entities (180 mg) were obtained by 51% mixture of ethyl acetate in ethanol[16]. All the solvents were evaporated to obtain the dried materials.

For dosing all the test substances were suspended in aqueous 2.5% gum tragacanth solution or dissolved in the normal saline solutions[13].

Induction of gastric ulcer: A modified Yoshikawa *et al.*[17] method was used for production of experimental gastric ulcer in rats. The pylorus of 24 h fasted rats was ligated under light ether anaesthesia. The test substances were given orally, immediately after pylorus-ligation following the injection of indomethacin (25 mg kg^{-1} s.c.)[18] in the treated groups of rats. All the test drugs were given to the animals by oral route t.d.s for 7 consecutive days while pylorus was ligated on the 8th day[13].

The animals were divided randomly into eight groups of 6-8 rats each. The treated control, reference control and treated animals were given indomethacin (25 mg kg^{-1} s.c.)[18]. Additionally the reference control group of rats were given Famotidine 100 mg kg^{-1}[19] orally each. Animals of different treated groups received NS powder in the dose of 1.0, 1.5 and 2.0 g kg^{-1} while NS-Aq, NS-E extracts and NS-EA, NS-EA 51 fractions (equivalent to 2.0 g kg^{-1} of powder) orally. The untreated and treated control groups received 3 ml kg^{-1} of 2.5% tragacanth vehicle instead of test drug, orally[13].

Shay *et al.*[20] operative procedure was adopted. The rats were anesthetized with ether and their abdomens were opened through a midline incision. The pylorus were secured and ligated with silk suture, the abdominal wounds were closed and the animals were allowed to recover from anaesthesia. Following the pylorus ligation, drinking water was withheld and gastric juices were allowed to collect for a period of 4 h. The rats were then killed by an overdose of chloroform vapors and their stomachs were removed after clamping the oesophagus. The gastric contents were collected through the oesophagus. The gastric mucosae were washed with 3 ml of lukewarm distilled water. The gastric contents and washings were homogenized and centrifuged at 4000 rpm for 10 min. The stomachs were inflated with 10 ml of 1% formalin and the oesophagus ligated. The stomachs were then immersed in 10% formalin for 30 min to fix the inner and outer walls. The stomachs were then examined for gastric ulcers following the incisions along the grater curvatures[17,19].

Determination of gastric juice volumes, pH and acid-outputs: Volumes and pH of centrifuged gastric juice were measured by pipette and pH meter, respectively while acidities were determined by titration to pH 7 with 0.1 N sodium hydroxide solutions[13]. The acid outputs were calculated by using the following equation according to the method of Ishizuka *et al.*[21].

EqH^+/100 g 4 h = 1/antilog pH x 1000 x Volume of gastric juice (ml) x 100/body weight of animal (g).

Calculation of gastric ulcer index: All the stomachs were examined under a simple microscope. The gastric damages in the glandular regions were located in the gastric mucosae as elongated black-red lines parallel to the long axis of stomachs. The length (mm) of each lesion was measured and lesion index was calculated by adding the length of all the lesions in the stomachs[22].

Determination of drug effects on gastric pepsin activity: Pepsin activities were determined by the method of

Hirohashi *et al.*[23] from the centrifuged gastric juices using bovine serum albumin as a substrate. The test tubes containing 1.0 ml of gastric juice buffered with 5.0 ml of 0.2 N HCl and 0.2 N sodium citrate solutions (4:1) were incubated at 37° C for 30 min. The pepsin was allowed to react with bovine serum albumin 2 ml (5 mg ml^{-1}) and test tubes re-incubated at 37°C for 30 min. The unreacted protein of bovine serum albumin was then detected with the addition of 1.0 ml of Biuret reagent and read their absorbencies at 546 nm exactly after 30 min. against reagent blank. The pepsin activities were determined from the standard turbidity curve.

Statistical analysis: The data was expressed as mean±S.E.M (standard error of means) and analyzed statistically by the application of SPSS for Windows version 7.5. The Student's "t" test was applied and "P" values were determined. Differences were considered significant at $P<0.05$ and highly significant at $P<0.001$[24].

RESULTS

***N. sativa* seeds powder:** Figure 1 shows that oral administration of NS powder 1.0-2.0 g kg^{-1} doses prevented highly significantly ($P<0.001$) the indomethacin-induced increases in gastric juice volume, acid-output and ulcer index in rats. The powders also inhibited high significant decreases in gastric pH induced by indomethacin.

Ethanolic extract of *N. sativa* seeds: As shown in Table 1, oral administrations of NS-E equivalent to 2.0 g kg^{-1} of the powder decreased highly significantly ($P<0.001$) the gastric juice volume and acid output while increased gastric pH in indomethacin-treated rats (Table 1). The extract also highly significantly blocked the formation of gastric lesions in the treated animals. However, NS-Aq (Eq. to 2.0 g kg^{-1}) could not change the gastric juice volume, pH and ulcer index in the treated rats.

Purified fraction of *N. sativa* seeds: Table 2 shows that NS-EA 51 fraction inhibited highly significantly ($P<0.001$) increases in gastric juice volume, acid-output, lesion formation and pepsin activity induced by indomethacin in rats. NS-EA 51 also antagonized high significant decreases in gastric secretion pH in the treated rats. The reference drug famotidine caused the similar preventive effects on gastric pH, acid-output, ulcer index and pepsin activity. It also significantly ($P<0.05$) decreased the gastric juice volume in the treated rats.

DISCUSSION

Uncontrolled acid secretion and ulceration of gastric mucosa due to several reasons have posed serious problems to the human health all over the globe[25]. 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 antiulcer drugs has remained a field of active research[26].

In an effort to further search curative and safe agents for the treatment of peptic ulcers in our indigenous medicinal plants, the present study was undertaken. The gastroprotective efficacy of NS its extracts and purified fractions was determined in rats having indomethacin-induced ulcers. The indomethacin model has already been utilized for screening the new compounds for their antiulcer effects. Use of this model for the same purpose has been employed by several workers including Reeves *et al.*[27].

Indomethacin is a selective inhibitor of cyclo-oxygenase and it does not inhibit the lipoxygenase pathway of arachidonic acid while it strongly inhibits the cyclo-oxygenase pathway[28]. The increased level of LTs in gastric mucosa has been found to cause inflammation and pain[29]. Indomethacin potentates the secretary response elicited by histamine due to the inhibition of PGE_2 synthesis. Therefore, induction of gastric ulcers and erosions is accompanied by progressive decrease in the level of PGE_2[27,30]. So, the raised gastric secretion volume, acid out-put and pepsin activity were observed in the present study while decreased pH was found high significant in the treated control group of rats in comparison to control group animals. Similarly gastric ulcer index was also induced by indomethacin highly significantly.

In the rats having indomethacin-induced ulcerations, NS in 1.0-2.0 g kg^{-1} doses inhibited the gastric effects induced by indomethacin in a dose-dependent manner and 2.0 g kg^{-1} dose exhibited maximum preventive effects. However, the aqueous extract did not altered the gastric effects and only gastric acid out-put was significantly decreased while no change was observed in gastric secretion volume, pH and ulcer index (Table 1). On the other hand the ethanolic extract equivalent to 2.0 g kg^{-1} of powder attenuated highly significantly the indomethacin-induced effects on gastric secretion volume, pH, acid-output and ulcer index. Additionally, NS-EA and NS-EA 51 fractions were also inhibited the effects on pepsin activities induced by indomethacin (Table 2). Famotidine was used as a reference antiulcer drug which altered gastric pH, acid-output, pepsin

Table 1: Effects of *N. sativa* seed powder its aqueous and ethanolic extracts on gastric secretion volume, pH, acid-output and ulcer index in rats

Group No.	Treatments	Gastric secretion's			
		Volume (ml)	pH	Acid out-put (μEq/100 g/ 4 h)	Ulcer index (mm)
01	Normal (Untreated control)	2.20±0.06	2.82±0.01	2.82±0.15	2.62±0.26
02	Indomethacin (25 mg kg^{-1} s.c.) (Treated control)	8.60±0.45**	1.08±0.20**	340.23±14.21**	49.83±2.47**
03	*N. sativa* powder 2.0 g kg^{-1}p.o.+ Indomethacin (25 mg kg^{-1} s.c.)	2.37±0.07**	4.12±0.07**	0.17±0.03**	3.90±0.29**
04	Aqueous extract of *N. sativa* powder (Eq. to 2.0 g kg^{-1} p.o.) + Indomethacin (25 mg kg^{-1} s.c.)	6.80±0.81	1.37±0.03	207.20±5.09**	42.52±2.94
05	Ethanol extract of *N. sativa* powder (Eq. to 2.0 g kg^{-1} p.o.) + Indomethacin (25 mg kg^{-1} s.c.)	2.42±0.09**	4.06±0.06**	0.19±0.02**	4.46±0.34**

Indomethacin: Significant from untreated control (Normal) * P < 0.05; ** P < 0.001
Test drugs: Significant from treated control (Indomethacin) * P < 0.05; ** P < 0.001
All the other values are N.S. from treated control (Indomethacin)
Mean±S.E.M = Mean values±Standard error of means of six experiments

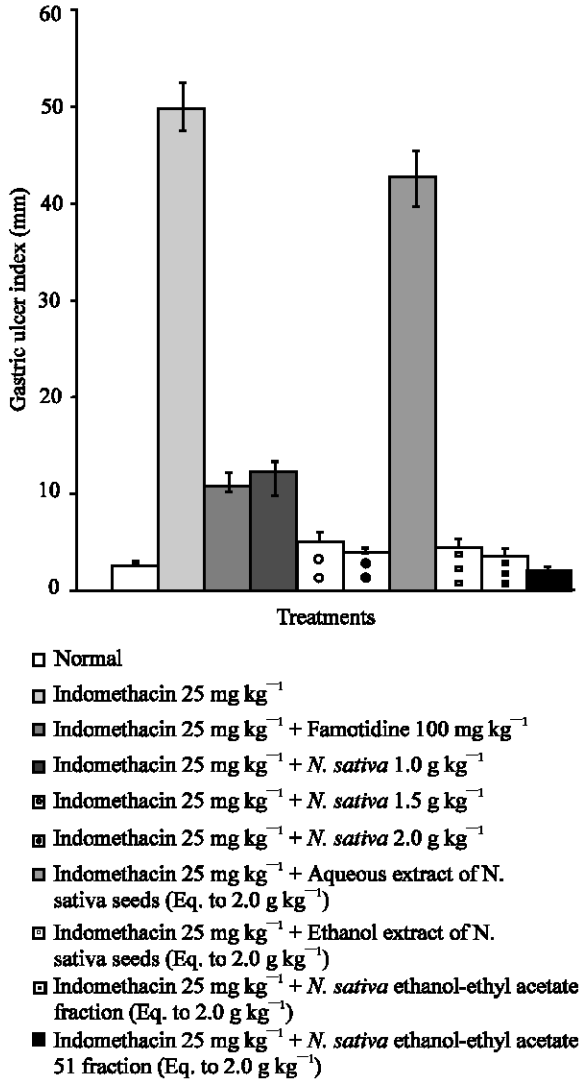

Fig 1: Comparative effects of *N. sativa* seed powder its extracts and fractions on gastric ulcer index in indomethacin-treated rats

activity, ulcer index highly significantly and gastric juice volume significantly in treated animals. But the gastric effects of NS and its fractions were greater than the reference drug. The data further suggested that NS-EA 51 exerted greater effect than all other test drugs. Therefore, it probably extracted the most of antiulcer constituents of the plant (Table 1 and 2).

From these results, it may further be speculated that the antiulcer action is exerted by purified fraction, viz., inhibition of gastric aggressive factors i.e. acid and pepsin because the test agent seems to possess the ability to interfere with the indomethacin induced-inflammatory effects which is in accordance with Al-Ghamdi[31] and El-Dakhakhny *et al.*[32]. El-Abhar *et al.*[33] also found NS oil and its constituent, thymoquinone, to be gastroprotective. Antioxidant/free radical scavenging activity of thymoquinone has been reported by Mansour *et al.*[34], Swamy and Huat[35]. Ali and Blunden[36] observed the similar activity. The oxygen free radicals serve as second messengers in pro-inflammatory signal transduction pathways[37]. Oxygen active species, such as O_2^{-1}, H_2O_2, HO^{-1} and lipid radicals, such as ROO^{-1}, RO^{-1} and hydroperoxides, generate during lipid peroxidation and metabolism[38]. The antioxidants have proved useful in those disorders in which up-regulation of inflammatory response are implicated[39]. Therefore, oxidative damage to endothelial cells, which results in nitric oxide (NO) shortage reported by Tsuchiya *et al.*[40] might be inhibited due to possible free-radical scavenging activity present in NS and its fractions.

In conclusion, the reported results have validated the folkloric use of the drug tested for use in the therapy of gastric ulcer disease. In particular, the present studies have pointed out possible gastroprotective effects of the NS-EA 51 fraction isolated from NS. Nevertheless, detailed chemical studies followed by pharmacological investigations and toxicity evaluations are still required to

Table 2: Comparative effects of purified fractions of *N. sativa* seed powder on gastric secretion volume, pH, acid-output, pepsin activity and ulcer index in indomethacin-treated rats

Group No.	Treatments	Gastric secretion's				
		Volume (ml)	pH	Acid out-put (μEq/100 g/ 4 h)	Pepsin activity (mg h^{-1})	Ulcer index (mm)
02	Indomethacin (25 mg kg^{-1} s.c.) (Treated control)	8.60±0.45	1.08±0.20	340.23± 14.21	21.53±0.91	49.83±2.47
06	Famotidine (100 mg kg^{-1} p.o.)+Indomethacin (25 mg kg^{-1} s.c.)	7.27±0.24*	2.89±0.30**	9.18±1.23**	16.11±0.02**	10.83±1.20**
07	*N. sativa* ethanol-ethyl acetate fraction (Eq. to 2.0 g kg^{-1} p.o.)+Indomethacin (25 mg kg^{-1} s.c.)	2.28±0.34**	4.61±0.24**	0.057±0.004**	15.16±0.16**	3.33±0.88**
08	*N. sativa* ethanol-ethyl acetate 51 fraction (Eq. to 2.0 g kg^{-1} p.o.) + Indomethacin (25 mg kg^{-1} s.c.)	1.96±0.89**	4.68±0.22**	0.04±0.01**	1.45±0.16**	2.00±0.40**

Test drugs: Significant from treated control * $P < 0.05$; ** $P < 0.001$ Mean±S.E.M = Mean values±Standard error of means of six experiments

isolate the pure active principle (s) of the NS and to elucidate their mode(s) of anti-ulcer actions studies are also needed.

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