

# Gastroprotective Effect of an Aqueous Suspension of Black Cumin *Nigella sativa* on Necrotizing Agents-Induced Gastric Injury in Experimental Animals

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## ABSTRACT

**Background/Aim:** Previous studies on "Black seed" or "Black Cumin" *Nigella sativa* (NS) have reported a large number of pharmacological activities including its anti-ulcer potential. These studies employed either fixed oil, volatile oil components or different solvent extracts. In folkloric practices, NS seeds are taken as such, in the form of coarse dry powder or the powdered seeds are mixed with water. This study examines the effect of NS aqueous suspension on experimentally induced gastric ulcers and basal gastric secretion in rats to rationalize its use by herbal and Unani medicine practitioners. **Materials and Methods:** The study was conducted at the Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Acute gastric ulceration was produced by various noxious chemicals (80% ethanol, 0.2 M NaOH, 25% NaCl and indomethacin) in Wistar albino rats. Anti-secretory studies were undertaken in a separate group of rats. Gastric wall mucus contents and non-protein sulfhydryl concentration were estimated, and gastric tissue was examined histopathologically. **Results:** An aqueous suspension of Black seed significantly prevented gastric ulcer formation induced by necrotizing agents. It also significantly ameliorated the ulcer severity and basal gastric acid secretion in pylorus-ligated Shay rats. Moreover, the suspension significantly replenished the ethanol-induced depleted gastric wall mucus content levels and gastric mucosal non-protein sulfhydryl concentration. The anti-ulcer effect was further confirmed histopathologically. **Conclusion:** These findings validate the use of Black seed in gastropathies induced by necrotizing agents. The anti-ulcer effect of NS is possibly prostaglandin-mediated and/or through its antioxidant and anti-secretory activities.

**Key Words:** Habbatul-Barakah, *Nigella sativa*, stomach, ulcer, gastric acid

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Gastric ulcers, one of the most widespread diseases, is believed to be due to an imbalance between aggressive and protective factors.<sup>[1]</sup> The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs.<sup>[2]</sup> These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility.<sup>[3]</sup> Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF," leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defenses (mucus, bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide).<sup>[4]</sup> The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently, there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources. A large

percentage of world population relies on natural remedies to treat a variety of diseases. Due to socio-economic factors, faith and ancestral experience, medicinal herbs and spices are considered as an indispensable part of traditional medicine.<sup>[5]</sup> Besides this, a large number of spices and herbs have been evaluated by various researchers for their anti-ulcer effects to achieve a favorable outcome.<sup>[6-12]</sup> The seeds of *Nigella sativa* L. (Ranunculaceae) (NS) known as Black seed or Black cumin ("Al Habbah Al Sawda" or "Habbatul-Barakah") have long been used in folk medicine in the Middle East, Far East and in the Indian subcontinent as a traditional medicine. The Ayurvedic, Unani and herbal medicine practitioners extensively used this age old spice for a wide range of illnesses, including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems as well as a diuretic, and to promote menstruation and increase lactation.<sup>[13-15]</sup> Its use in skin conditions as eczema has also been recognized worldwide.<sup>[16]</sup> NS contains more than 30% of a fixed oil and 0.40-0.45 w/w of a volatile oil. The volatile oil has been shown to contain

18-24% thymoquinone (TQ) and 46% monoterpenes.<sup>[16]</sup> Recent clinical and experimental studies have shown several therapeutic effects of NS extracts including its antioxidant hepatoprotective,<sup>[17]</sup> immunomodulatory,<sup>[18]</sup> anti-inflammatory<sup>[19]</sup> and anti-tumor<sup>[20]</sup> activities. However, recently the active principle of NS oil TQ has shown to possess a gastroprotective activity in rats.<sup>[16]</sup> In other studies, TQ has demonstrated an antimicrobial activity<sup>[21]</sup> and was beneficial for treating doxorubicin-induced nephropathy in rats.<sup>[22]</sup> Although much work has been done on crude extract of NS and its constituents, to our knowledge, there is no report available on its aqueous suspension dosage form. NS seeds are customarily consumed either in their intact or powdered forms. Therefore, the present study was undertaken to determine the effect of an aqueous suspension of NS seeds against various necrotizing agents and indomethacin-induced gastric ulcer in rats. Anti-secretory assessment was performed on pylorus-ligated Shay rats. Gastric wall mucus and non-protein sulphhydryl contents were estimated and gastric tissue was histopathologically examined.

## MATERIALS AND METHODS

The present study was carried out at the Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

### Plant material and preparation of aqueous suspension

NS seeds were purchased from a local herb dealer in Riyadh, were identified under expert guidance and preserved for future reference. The seeds were ground to a very fine powder and used as an aqueous suspension for treatment in different experiments. Wistar albino rats of either sex, approximately of same age, weighing 150-200 g were obtained from the Animal Care Center, College of Pharmacy, King Saud University. They were maintained under standard conditions of temperature, humidity and light (12 h dark, 12 h light) and provided with Purina chow and free access to water. Before testing, the animals were fasted for 36 h with access to water *ad libitum*. All experimental procedures were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

### Gastric lesions induced by necrotizing agents

Each rat in the test group was given 1 ml of different necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl) that are known to produce gastric lesions.<sup>[23]</sup> NS suspension was given orally 30 min before administering the necrotizing agents. Animals were killed under ether anesthesia 1 h after treatment with the ulcerogenic agents. The stomach was excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using a binocular magnifier and ulcers were scored.<sup>[24]</sup> Lesions were also assessed by two observers

blinded to experimental protocols.

### Histopathological evaluation

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/or anti-ulcerogenic activity of NS. The tissues were fixed in 10% buffered formalin and were processed using a VIP tissue processor. The processed tissues were embedded in paraffin blocks and about 5- $\mu$ m thick sections were cut using an American optical rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures.<sup>[25]</sup> The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema and erosions using an arbitrary scale for the assessment of severity of these changes.<sup>[26]</sup>

### Determination of anti-secretory activity

To determine anti-secretory activity, the 36-h fasted rats were anesthetized under light ether and the pylorus was ligated. Care was taken to avoid bleeding or occlusion of the blood vessels. Aqueous suspension of NS was administered intraperitoneally, immediately after pyloric ligation.<sup>[27]</sup> The animals were sacrificed 6 h after the pyloric ligation. The stomachs were removed, contents collected, measured, centrifuged and subjected to analysis for titratable acidity against 0.01 N NaOH at pH 7 and the total acid output was calculated.

### Gastric wall mucus determination

The glandular segments of the stomach were removed and weighed, to determine gastric wall mucus in rats after receiving 80% ethanol only or ethanol plus NS suspension.<sup>[28]</sup> Each segment was transferred immediately to 1% Alcian blue solution (in sucrose solution, buffered with sodium acetate at pH 5), and the excess dye was removed by rinsing with sucrose solution. The dye complexed with gastric wall mucus was extracted with magnesium chloride solution. A 4-ml aliquot of blue extract was then shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of glandular tissue (net) was then calculated.

### Indomethacin-induced gastric mucosal ulceration

Indomethacin was suspended in 1.0% carboxymethyl cellulose in water (6 mg/ml) and administered orally to the fasted rats at 30 mg/kg body weight (0.5 ml/100 g). Control rats were treated similarly with an equivalent amount of the vehicle.<sup>[29]</sup> The animals were sacrificed 6 h after the treatment. The stomachs were excised, rinsed with normal saline and ulcers were scored.

### Estimation of non-protein sulphhydryl (NP-SH) groups

Gastric mucosal NP-SH was measured to analyze the oxidant/antioxidant balance. The glandular stomach was removed

**Table 1: Effect of *Nigella sativa* on the various necrotizing agents in rats**

Ulcer Index (Mean ± S.D.)	Treatment (Dose)			F-value	P-value
	Control	<i>Nigella sativa</i> (250)	<i>Nigella sativa</i> (500)		
80% EtOH	8.0 ± 0.0*	4.0 ± 1.8*	2.17 ± 16*	27.77	<0.0001
0.2 M NaOH	7.83 ± 0.41*	6.0 ± 1.67*	1.83 ± 1.72*	26.68	<0.0001
25% NaCl	7.0 ± 1.67**	3.83 ± 1.33	3.83 ± 1.94	7.22	0.006

\*Statistically significantly different from each other (ANOVA followed by Duncan's multiple range test), \*\*Statistically significantly higher than other two groups (ANOVA followed by Duncan's multiple range test)

**Table 2: Effect of *N. sativa* on ethanol-induced histopathological lesions in gastric mucosa of rats**

Treatment and dose (mg/kg., body weight)	Histopathological Lesions							
	Congestion	Haemorrhage	Edema	Necrosis	Inflammatory changes	Dysplastic changes	Erosions	Ulceration
Control (distilled water)	-	-	-	-	-	-	-	-
Ethanol, 80%	+++	++	+	+	+	+	++	++
<i>N. sativa</i> (250) + ethanol, 80%	-	-	-	-	-	-	-	-
<i>N. sativa</i> (500) + ethanol, 80%	-	-	-	-	-	-	-	-

- = Normal, + = Moderate effect, ++ = severe effect, +++ = Intensely severe effect

and homogenized in ice-cold 0.02 M ethylene-diamine-tetraacetic acid. The homogenate was mixed with distilled water and 50% (w/v) aqueous tri-chloroacetic acid and centrifuged; the supernatants were mixed with phosphate buffer (pH 8), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) was added and the sample was shaken. The absorbance was read within 5 min of addition of DTNB at 1/12 nm, against a reagent blank with no homogenate.<sup>[50]</sup>

### Statistical analysis

The readings shown are mean ± standard error of means. The mean determination of treatment groups was compared statistically with that of control by using ANOVA followed by Duncan's multiple range test.

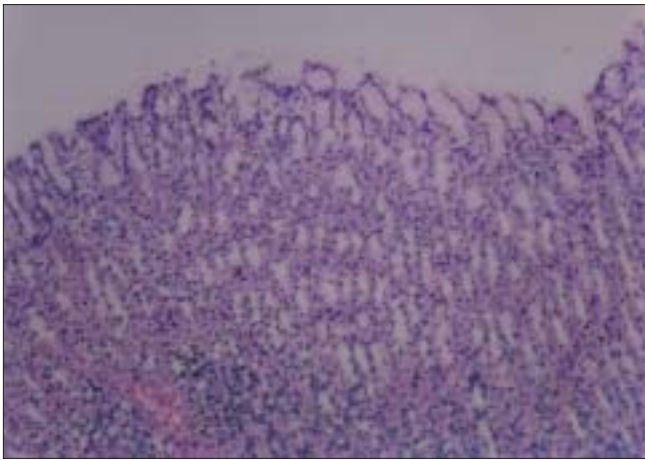
## RESULTS

Aqueous suspension of NS significantly reduced gastric ulcers induced by ethanol ( $P < 0.0001$ ), NaOH ( $P < 0.0001$ ) and NaCl ( $P < 0.006$ ) at 250 and 500 mg/kg body weight compared to the values obtained in the control [Table 1]. Pretreatment with NS suspension (250 and 500 mg/kg body weight) completely protected the gastric mucosa against different histopathological changes (congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes, erosions and ulceration) caused in ethanol-treated rats [Table 2 and Figures 1-4]. In addition, treatment with NS aqueous suspension resulted in a significant decrease in the volume of basal gastric secretion and titratable acidity after pyloric ligation for 6 h ( $P < 0.0001$  and  $P < 0.005$ , respectively, at 250 and 500 mg/kg body weight), while NS treatment did not influence the ulcer index at high dose ( $P 0.57$ ) [Table 3]. Treatment with ethanol (80%) caused a significant ( $P < 0.0001$ ) decrease in the mucus content of the gastric wall in untreated animals [Table 4]. The depleted gastric mucus

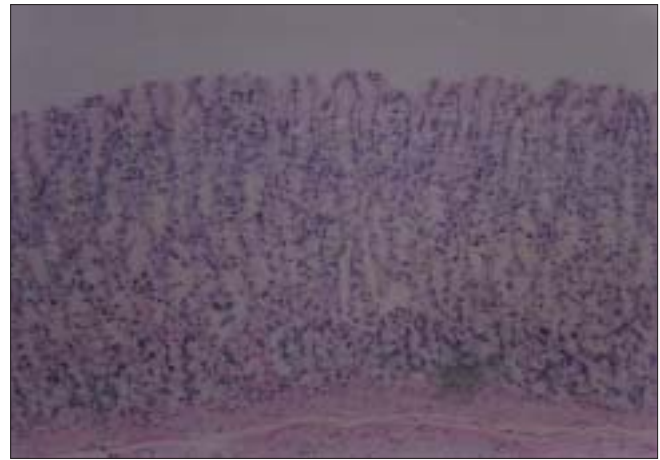
was significantly replenished ( $P < 0.0001$ ) after pretreatment with NS suspension at both doses. Table 5 shows a significant dose-dependent reduction ( $P < 0.002$ ) of stomach ulceration produced by indomethacin after pretreatment with NS suspension at both doses. In addition, ethanol (80%) induced a significant decrease ( $P < 0.0001$ ) in NP-SH concentrations in the gastric tissue as compared to the control and NS-treated (normal) animals [Table 4]. An increased level of NP-SH concentration was observed after pretreatment with NS aqueous suspension at 250 and 500 mg/kg body weight.

## DISCUSSION

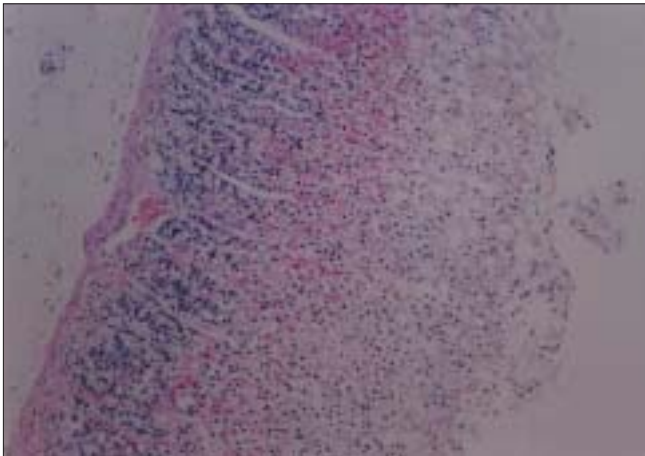
The results of this study showed that oral administration of an aqueous suspension of NS prevents gastric mucosal injuries caused by ethanol and strong alkalies, the most commonly employed tests in the evaluation of anti-ulcer and cytoprotective activities.<sup>[31-34]</sup> It is suggested that oxygen radicals may contribute to the formation of ethanol-induced gastric mucosal lesions and antioxidants are protective against the damage caused by these oxidants.<sup>[35]</sup> The cytoprotective effect of black cumin observed in the present study, which could be attributed to the endogenous generation of PG, was responsible for maintaining the cellular integrity of the gastric epithelium; therefore, such endogenous release of PG would play a physiological role in protecting the gastric mucosa.<sup>[23]</sup> On the other hand, it has been reported that plants and/or spices sometimes exhibit their cytoprotective action through their mild irritant property.<sup>[6,9,10]</sup> Furthermore, Robert *et al.*<sup>[23]</sup> have also described the ability of a mild irritant in protecting gastric mucosa against strong irritants. This protection is called "adaptive cytoprotection." These results were further supported by histopathological findings. Pretreatment with NS suspension prevented histopathological changes in the form of congestion, hemorrhage, edema, necrosis,



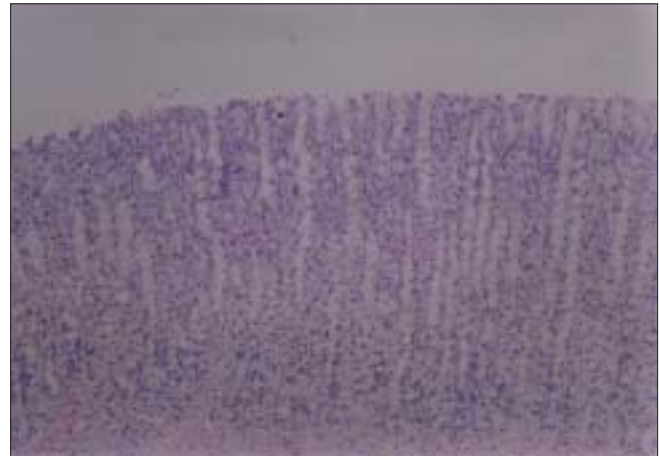
**Figure 1:** Section through gastric mucosa of control rat showing normal appearance (H&E, x100)



**Figure 3:** Section through gastric mucosa of rat treated with *Nigella sativa* (250mg/kg) and ethanol (80%, 1 ml). There are no significant histopathological changes (H&E, x100)



**Figure 2:** Section through gastric mucosa of rat treated with ethanol (80%, 1 ml) showing mucosal ulceration, inflammation and haemorrhage (H&E, x100)



**Figure 4:** Section through gastric mucosa of rat treated with *Nigella sativa* (500 mg/kg) and ethanol (80%, 1 ml). No significant histopathological changes are seen in the mucosal layer (H&E, x100)

**Table 3: Effect of *Nigella sativa* on gastric secretion, titratable acidity and ulceration in 6-hr pylorus ligated (Shay) rats**

Outcome variables (Mean $\pm$ S.D.)	Treatment (Dose)			F-value	P-value
	Control	<i>Nigella sativa</i> (250)	<i>Nigella sativa</i> (500)		
Volume of gastric content	8.0 $\pm$ 1.3*	1.8 $\pm$ 0.8	2.0 $\pm$ 2.9	18.41	<0.0001
Titratable acid	135.0 $\pm$ 4.0*	58.9 $\pm$ 64.9	32.2 $\pm$ 49.9	7.7	0.005
Ulcer Index	0.33 $\pm$ 0.5	0	0.33 $\pm$ 0.8	0.59	0.57

\*Statistically significantly higher than the two treatment groups (ANOVA followed by Duncan's multiple range test)

**Table 4: Effect of *Nigella sativa* on gastric wall mucus and non-protein sulphhydryl concentrations in rats**

Outcome variables (Mean $\pm$ S.D.)	Treatment (Dose)				F-value	P-value
	Control	80% Ethanol only	<i>Nigella sativa</i> (250)	<i>Nigella sativa</i> (500)		
GWM concentration ( $\mu$ g/g)	450.8 $\pm$ 47.5*	291.9 $\pm$ 8.2	311.6 $\pm$ 16.0	321.9 $\pm$ 24.2	35.71	<0.0001
Alician blue g net tissue NP-SH ( $\mu$ mol/100mg concentration)	8.5 $\pm$ 0.80*	5.89 $\pm$ 0.39**	7.47 $\pm$ 0.44	7.97 $\pm$ 0.88	53.22	<0.0001

\*Statistically significantly higher than the 3 treatment groups (ANOVA followed by Duncan's multiple range test), \*\*Statistically significantly lower than the other 2 treatment groups and control group (ANOVA followed by Duncan's multiple range test), GWM = Gastric wall mucus, NP-SH = Non-protein sulphhydryl

**Table 5: Effect of *Nigella sativa* on the gastric damage induced by indomethacin in rats**

Treatment	Dose (mg/kg, p.o.)	Ulcer index (Mean ± S.D.)	F-value	P-value
Control	-	30.50 ± 5.9*		
<i>Nigella sativa</i>	250	14.00 ± 12.7	9.56	0.002
<i>Nigella sativa</i>	500	4.60 ± 14.4		

\*Statistically significantly higher than the 2 treatment groups (ANOVA followed by Duncan's multiple range test)

inflammatory and dysplastic changes, erosions and ulceration caused by the destructive stimuli in the gastric tissue. This confirms the cytoprotective ability of the NS suspension, which may be attributed to chemical components such as TQ, a major and active constituent of NS oil.<sup>[36]</sup> In some earlier studies, TQ has been reported to exhibit a significant protective action on gastric mucosal lesions induced by different necrotic agents.<sup>[37,38]</sup> A significant reduction in basal gastric secretion and inhibition of gastric ulcers by NS suspension may involve direct reduction of gastric secretion through one or more of possible mechanisms.<sup>[39-41]</sup> Moreover, gastric acid is an important factor for the genesis of pyloric ligation ulcer in rats.<sup>[27]</sup> However, gastric hypersecretion is unlikely to be the sole major mechanism that contributes to the development of gastric mucosal lesions.<sup>[42,43]</sup>

Our findings are in agreement with previous data reporting that an aqueous extract of Black cumin did not only inhibit gastric secretion but also reduced the ulcer index in rats.<sup>[44,45]</sup> The current data clearly demonstrated that, at the very least, NS suspension inhibited gastric acid secretion. The gastroprotective effect observed in the present study might be due to a possible relationship between mucosal injury, inhibition of acid secretion and the antioxidant nature of NS.<sup>[46]</sup>

It has been reported that certain anti-ulcer drugs increase the amount of gastric mucus secretion in gastric mucosa.<sup>[47,48]</sup> This mucus consists of mucin-type glycoproteins, which can be detected by amounts of Alcian blue.<sup>[49]</sup> The gastric wall mucus depletion induced by 80% ethanol is also one of the pathogenic mechanisms responsible for gastric lesions.<sup>[50]</sup> Alcian blue dye binds with negatively charged materials. The increase in bound Alcian blue indicates the protective effect of orally administered NS suspension, which may occur via the formation of protecting complexes between the aqueous suspension and mucus that act as a barrier against several necrotizing agents introduced in the stomach.<sup>[51]</sup> In the present study, NS suspension replenished the ethanol-induced decreased concentration of gastric wall mucus. Thus, the possible mechanism of gastric mucosal protection by NS suspension is due to the reinforcement of resistance of the mucosal barrier by a protective coating. Administration of indomethacin caused gastric side effects ranging from endothelial microvascular damage to development of macroscopic gastric mucosal lesions, which

is attributed mainly to the inhibition of biosynthesis of cytoprotective PG, resulting in overproduction of leukotrienes and other products of the 5-lipoxygenase pathway.<sup>[52]</sup> These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H<sup>+</sup> and Na<sup>+</sup> ions reducing the transmucosal potential difference and induce formation of erosions and ulcers.<sup>[53-54]</sup> In this assay, NS suspension was able to produce a significant reduction of the gastric mucosal damage induced by indomethacin, indicating a probable local increase in PG synthesis. Additionally, NP-SH is thought to be involved in protecting gastric mucosa against various chemical agents.<sup>[55]</sup> Our observations showed a significant reduction in the NP-SH content of gastric mucosa after 80% ethanol administration. However, pretreatment with NS aqueous suspension prevented this depletion. An elevated NP-SH level is reported to protect gastric damage against various noxious chemicals.<sup>[56]</sup> These findings clearly showed that the possible involvement of NP-SH in the ulcer protective potential is through the antioxidant activities of NS suspension. Thus, NS treatment appears to strengthen the mucosal barrier, which is the first line of defense against endogenous and exogenous ulcerogenic agents.

The results of the present study establish the anti-gastric ulcer and anti-secretory properties of NS aqueous suspension, substantiates its use against gastric disorders in Unani and Arab traditional medicine. The effects of NS suspension is possibly PG-mediated and/or through its free radical scavenging and anti-secretory properties.

Earlier reports have indicated that 88 mg/kg/day administration of NS oil for two weeks significantly increased glutathione levels in gastric mucosa but failed to suppress acidity.<sup>[37]</sup> However, in the current study a single dose of aqueous suspension induced a significant elevation in NP-SH levels and suppressed basal gastric secretion and titratable acidity. It appears therefore, that the aqueous suspension of NS is more effective than oil suspensions. Similarly, in another study, administration of high doses of NS oil and its major compound TQ at 50 and 100 mg/kg body weight demonstrated a gastroprotective effect, which was attributed to its free radical scavenging effect.<sup>[57]</sup> To our knowledge, the present investigation is the first reporting that NS aqueous suspension possesses cytoprotective, anti-ulcer and anti-secretory capacity. In comparison with different dosage forms, the dynamics of the whole seed aqueous suspension with all chemical constituents and their synergistic effect was both advantageous and convenient to consume.

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