Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(4): 725-732

Received: 01-05-2017
Accepted: 02-06-2017

Orooba MS Al-Shaha
Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad

Samir Abed Mohammed
Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad

Correspondence
Orooba MS Al-Shaha
Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad

Gastro protective effect of oil extract of *Nigella sativa* Seeds against Aspirin-Induced Gastric Ulcer in Albino Rats

Orooba MS Al-Shaha and Samir Abed Mohammed

Abstract
Seeds of *Nigella sativa* L. (Rananculacene) is widely used in the treatment of gastric ulcer as a traditional medicine. In this study, our aim is to investigate gastro-protective activity of *Nigella sativa* oil and its constituent thymoquinone in aspirin induced ulcer models in albino rats. Group A and B received tap water 0.5ml daily. Group C were administered Esomeprazole + Clarithromycine (0.5 and 7mg/kg) daily. And group D received *N. sativa* oil extracts at 50mg/kg for 5 days. To induce gastric ulcers, aspirin (400mg/kg) was given orally to all groups except group A. The gastric contents were evaluated by biochemical parameters and gastric ulceration was studied by comparing the volume of gastric juice, free acidity, total acidity, ulcer index and by histopathological study. Oral administration of extract showed significant gastric protection as the ulcerated areas were remarkably decreased. Histological observations showed less edema and leucocytes infiltration when compared with the ulcer control group which exhibited severe gastric mucosal injuries.

Keywords: *Nigella sativa,* Gastric Ulcer, Albino Rats, gastric mucosal injuries

Introduction
Gastric ulcers are a serious problem in many parts of the World. The etiology of gastric ulcers is influenced by various factors. Ulcers are worsened by inadequate dietary habits, excessive ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) [1], stress, hereditary predisposition and infection by *Helicobacter pylori* [2-4].
We focused on gastric ulcers induced by aspirin because it is commonly and continuously used to treat several conditions like arthritis and as an analgesic etc, its ulcer may be characterized through the release of various intermediates such as lipoxygenase, oxygen free radicals, and cytokines. Several studies [5-8] showed that administration of alcohol and aspirin causes deterioration of gastric mucosa by increasing neutrophils infiltration, which then delays the healing process of ulcerated gastric tissues [9, 10].
Several pharmaceutical products have been employed for the treatment of gastroduodenal ulcers and peptic diseases, resulting in decreased mortality and morbidity rates. However they are not completely effective and they produce many adverse effects [11]. Esomeprazole is a proton pump inhibitor which has been widely used as an acid inhibitor agent for the treatment of disorders related to gastric acid secretion for about 15 years [12]. Commonly used drugs have several side effects like osteoporosis, disturbance in small intestine flora, kidney stones, anemia and increased chance of occurrence of drug-induced diseases such as gastric cancer.
Therefore, due to the side effects of conventional medicine. In recent years, there is growing interest in alternative therapies and the use of natural products, especially those derived from plants [11, 13]. Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of gastric ulcer [13, 14]. *Nigella sativa* Linn (*N. sativa*) commonly known as black seed or black cumin, is an annual herb from the botanical family of *Ranunculaceae* contains more than 30% of fixed oil and 0.4-0.45% of volatile oil. The volatile oil contains 18.4-24% thymoquinone (TQ) [15]. The seeds of the plant have been used as a natural remedy to treat many diseases, including asthma, hypertension, and others for over 2000 years [16-20]. The aim of this study was to evaluate the protective role of oil extract of *N. sativa* and thymoquinone (TQ) on gastric ulceration induced by aspirin on albino rats.

Materials and Methods

Plant extraction and phytochemical investigation

100 grams of *N. sativa* dry seed were triturated by mortar, then weighted again, then macerated with 500 ml of 70% ethanol, after maceration overnight, the extract was filtered and the marc was macerated again overnight. The filtrates were combined together and concentrated under vacuum then mixed with 100 ml of distilled water and fractionated using 70 ml x 3 times of petroleum ether, chloroform and methanol. All the organic fractions were dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The remaining fraction was mixed with equal volume of methanol and evaporated to dryness under vacuum [21].

Phytochemical investigations

Preliminary investigations for the chemical constituents of the petroleum ether, chloroform and methanol extracts were done using ammonia vapor, ethanolic KOH, Meyer,s and Dragendorff,s reagents. Utilizing HPLC (Waters, USA), petroleum ether, chloroform and methanol fractions were analyzed for their thymoquinone contents using C18 (25 Cm) column, methanol, acetonitrile, and 20 Mm potassium dihydrogen phosphate buffer (KH_2PO_4) with pH adjusted to 4.5 in the ratio of (50:20:30%, *v*/*v*/*v*) as a mobile phase with a flow rate 1.5ml /min and detection at 295nm, using thymoquinone standards [22].

Experimental design

Adult male Wister albino rats weighing 200–250 g rats were obtained from the animal house of department of pharmacology and toxicology-college of veterinary medicine. 24 male rats divided into 4 groups each group with 6 rats.

_ Group A (Normal control): Distilled water 0.5ml daily p.o for 5days.

_ Group B (Negative control): Distilled water 0.5ml daily p.o for 5days.

_ Group C (Standard control): Esomeprazole + Clarithromycine (0.5mg/kg and 7mg/kg) p.o on 5th day.

_ Group D (NSAE 50): *Nigella sativa* oil extract 50mg/kg p.o for 5 days.

All the groups (except group A) were given Aspirin 400mg/kg p.o on 5th day 1hour after standard drug (i.e Esomeprazole + Clarithromycine) or test drugs (i.e *Nigella sativa* extracts) administration and after 5 hours of Aspirin administration rats were sacrificed under sodium pentobarbitone (35mg/kg i.p) anaesthesia and dissection was done. The stomachs were removed, and opened along the greater curvature. Stomach was gently rinsed with water to remove gastric contents and blood. The gastric contents were evaluated by biochemical parameters and gastric ulceration was studied by comparing the volume of gastric juice, free acidity, total acidity, ulcer index and by histopathological study.

Collection of gastric juice

After removal of the stomach, the gastric content was collected in a graduated test tube for physical examination. The contents were then centrifuged at 2000 revolutions per minute (rpm) for 10 minutes. The supernatant fluid was subjected to biochemical analysis.

Determination of anti-secretory activity

To determine anti-secretory activity, the supernatant fluid was analysed for titratable acidity against 0.01 N NaOH at pH 7 and the total acid output was calculated.

Free and total acidity: Amount of 0.01 N NaOH required to titrate to the methyl yellow end point is the measure of the free acid present. The amount of 0.01N NaOH required to titrate from the beginning to the phenolphthalein end point is a measure of the total acid present in the sample [10].

$$acidity = \frac{Volum\ of\ NaOH \times Normality \times 100}{0.1}\ mEq/l$$

Measurement of ulcer index

Following the method described by [23] a scoring system of Zero to Three, based on the number and the severity factor of the ulcer, has been employed. Accordingly, the severity factor of the ulcer was defined in relation to the length of the ulcer (lesion) as follows: Severity factor Zero = No visible lesions, Severity factor One = Lesion < 2 mm, Severity factor Two = Lesion 2 - 4 mm and Severity factor Three = Lesion > 4 mm. The ulcer index was calculated as the total number of ulcers per stomach multiplied by the severity factor. To assess the ulcer index subsequent to each experiment the abdomen of each animal was opened, the stomach was removed and cut along the greater curvature and gently rinsed with distilled water. The stomach was then pinned out on a flat surface with the mucosal surface uppermost. The levels of severity of the induced gastric ulcers were assessed by calculating the Ulcer Index as described below [24].

$$\%protection = \frac{[(Uc - Ut) \times 100]}{Uc}$$

Where: Uc Ulcer index of ulcer control group, Ut Ulcer index of test group

Measurement of Mucus Production.

Gastric mucus production was measured in the rats. The gastric mucus of each rat was obtained by gently scraping the mucosa with a glass slide and the collected mucus was weighed by using a precision electronic balance [25].

Macroscopic examination of the stomach

All animals were killed 5 hours after aspirin administration. The stomach was removed and opened along the greater curvature and gently washed with distilled water. Gross of mucosal lesions were recognized as hemorrhage or erosions with damage to the mucosal surface and the ulcers of the gastric mucosa appear as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach.

Histopathological Examination

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. The tissues were processed according to the standard procedure and sections were cut stained with haematoxylin and eosin. The slides were examined microscopically for morphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes.

Statistical analysis

For all the above methods, the results were expressed as Mean ±SEM. Statistical analysis was done using one way ANOVA test.

Results

Extraction

Extracts of Nigella sativa oil seed were isolated successfully by using different solvents. The results are presented in Table 1

Table 1: The percentage yield of different extracts of *Nigella sativa* seed.

Solvent	Color of extracts	Percentage yield (%w/w)
Petroleum ether	Light brown	37%
Chloroform	Light brown	16%
Methanol	Dark brown	27%

Nigella sativa, a fixed oil yielding medicinal plant, belonging to the family *Ranunculaceae*, were selected for present investigation. The dried seeds were subjected to traditional method for extraction of fixed oil and it was found that dried seeds with petroleum ether contain more percentage of oil content than the other chloroform and methanol (37%, 16% and 27%) respectively. The color of oil is Light to dark brown; odor is aromatic having agreeable taste.

In phytochemical screening of the present study carried out in the *Nigella sativa* revealed the presence of medicinal active constituents.

The phytochemical active compounds of *Nigella sativa* were qualitatively analyzed for seeds and the results are presented in Table 2. In these screening process alkaloids, glycosides, saponins, phenol, tannins, sterols, flavanoids, and terpenoids shows different types of results in different solvents extracts. Among these phytochemicals, Alkaloids, Flavonoids, Glycosides and Phenols were absent in all solvent extracts except methanol, whereas saponins were absent in all solvent extracts. Tannins and Terpenoids are present in all solvent except chloroform. Steroids were present in all solvent extract. So Alkaloids, Flavonoids, Phenol compounds, Glycosides and Steroids were present in petroleum ether extract.

Table 2: Results of phytochemical screening of petroleum ether, chloroform and methanol seeds oil extract of *Nigella sativa*.

No.	Name of the Phytochemical	Petroleum ether	Chloroform	Methanol
1	Alkaloids	+	-	-
2	Flavonoids	+	-	-
3	Phenol	+	-	-
4	Tannins	+	-	+
5	Glycosides	+	-	-
6	Steroids	+	+	+
7	Saponins	-	-	-
8	Terpenoids	++	-	+

+ indicates presence of the Phytoconstituents, ++ indicates present in more quantity of the Phytoconstituent, – indicates absence of the Phytoconstituents

HPLC estimation of thymoquinone

HPLC method has been used to determine the quantity of thymoquinone in three different extracts formulations. Thymoquinone has retention time of R_f 4.48 min at 295 nm. At this wave length there is no interference neither from dithymoquinone (thymoquinone dimer) nor from thymol. A typical chromatogram of thymoquinone was presented in Figure 1.

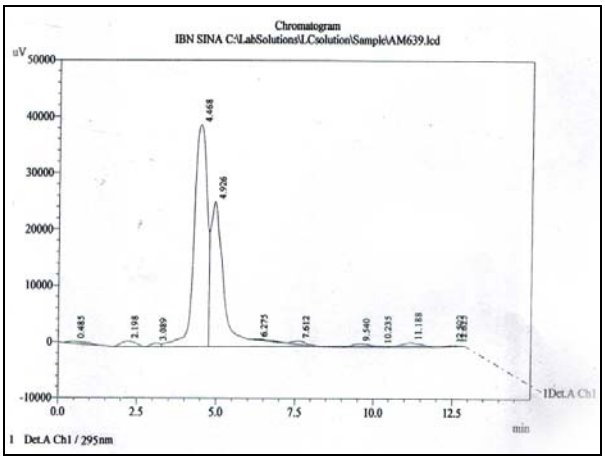

Fig 1: Chromatogram of thymoquinone standard sample

Linearity

The calibration curve of thymoquinone was constructed over the range of 50-700 ng/ml. Linear regression data for the plot confirmed the good linear relationship (Table 3). The correlation coefficient (R_2) was 0.9989 (n=7) which was highly significant (P<0.05). The linear regression equation was Y=13.092x+1212.1, where Y is response and x is amount of thymoquinone (Figure 2).

Table 3: Linear regression data for the calibration curve of thymoquinone (n=7).

Linearity range ng/ml	50-700
Regression equation	y = 13.391x + 1258.5
Correlation coefficient	0.9995
Slope ± SD	13.391 ± 0.394 8
Intercept ± SD	1258.5 ± 270.39
Standard error of slope	0.218
Standard error of intercept	136.12
95% confidence interval of slope	8.303 - 9.319
95% confidence interval of intercept	805.83 - 1501.50

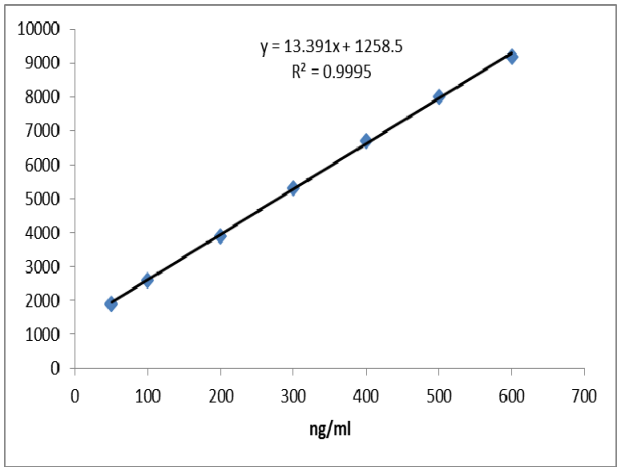

Fig 2: Regression curve of standard thymoquinone.

High and low valued quality control sample (700 and 50 ng/ml) were assayed seven times a day on the same day and several days during a one week period, to evaluate the precision of the assay. The within day variability (coefficient of variation) were 1.0 and 2.0 respectively for 700 and 50 ng. Table 4 summarizes the results for the quantitative assay of thymoquinone as the main active constituent of the volatile oil in *Nigella sativa* oil.

Table 4: Analysis of thymoquinone content in *Nigella sativa* oils of petroleum ether, chloroform and methanol seed extract of *Nigella sativa*.

Black seed oil	Thymoquinone mg/ml
Petroleum ether extract	7.58 ± 0.00 a
Chloroform extract	0.2 ± 0.00 b
Methanol extract	2.4 ± 0.00 c

± Standard Error of three replications. Different letters in the same column represent significant ($p < 0.05$) differences.

Oil sample of Petroleum ether has a higher thymoquinone content than oil sample of Methanol and Chloroform.

Protective effect of oil *extract N. sativa* against aspirin induced gastric damage in rats.

Different parameters were measured for the gastroprotective effect of *Nigella sativa* which are shown in Table (5). The results of this study show no lesion was seen in any of control animals (A) and show that oral administration of an oil extract of *NS* prevents gastric mucosal injuries caused by aspirin, the most commonly employed test in the evaluation of anti-ulcer and cytoprotective activities. Biochemical parameters like volume of gastric juice, total acidity and ulcer index were noted in all the four groups respectively.

Administration of aspirin showed massive gastric damages evidenced by highly significant decrease in gastric pH, increase in total acidity and ulcer index in ulcer control group (B) as compared to group (A) and group (D)($p<0.05$). Among the oil extract by petroleum ether group (D) showed maximum protective effect 100% evidenced by significant improvement in all parameters as compared to group B ($p<0.05$), this was followed by group C (standard control) 67.1%($p<0.05$).

Effect of *N. sativa* oil Extract on the Production of Mucus Content in Gastric Mucosa.

The mucus content of gastric mucosa in rats treated with aspirin (ulcer control group) was significantly reduced when compared to standard control or the extract groups. The pre-treatment of rats with *N. sativa* oil extracts significantly elevated the mucus content in the aspirin -induced ulcerated rats (Table 5). The increases of mucus production revealed that the oil extract had potential to induce the mucus secretion and thereby protect the stomach layer from any injury caused by noxious and other agents.

Gross Examination of Gastric Lesions

No lesions were seen in any of control animals while oral administration of aspirin produced multiple mucosal lesions in the rat stomach in group B. Severe lesions were seen with extensive visible hemorrhagic necrosis of gastric mucosa. Pretreatment with oil extract of *Nigella Sativa* inhibited the aspirin-induced gastric mucosal injury and significantly reduced erosions and ulceration in rats compared with groups B and C ($P<0.01$). Table (6) and Figure (3 A- D).

An ulcer control group showed severe damage and extensive visible hemorrhagic necrosis of gastric mucosa due to the induction of aspirin (Figure 3 b). However the standard control group showed Mild lesions of gastric mucosa when compared to the lesions in the negative control group. No hemorrhagic bands of ulcers or injuries were observed in the gastric mucosa when the rats were pretreated with the oil extract of *N. Sativa* (D) (Figures 3 (D). This oil extract manifested a significant protection (100% ulcer inhibition) against aspirin associated gastric ulcers in rats and showed complete protection and did not reveal any abnormality. Complete remission was seen in2/6 (33%) rats of group C while no remission was observed among animals of group B. The protection capacity of oil *Nigella Sativa* was almost differed from Esomeprazole and Clarithromycin and the difference was found to be significant ($P<0.01$) (Table 6).

Histological examinations of the stomach

The stomach tissue slides were examined microscopically for morphological changes like congestion, haemorrhage, oedema, necrosis, inflammatory and dysplastic changes, erosions and ulceration caused by the destructive stimuli of aspirin in the stomach tissue. Negative control group did not showed any pathological changes (Figure 4). Rats in the positive control group showed high damage of the epithelium surface and necrosis lesions penetrating into mucosa and severe edema of submucosa layer. In addition, leucocyte infiltration was present too (Figure 5). The histological result showed that the rats pretreated with Esomeprazole and Clarithromycin had less protection of stomach mucosa by mild leucocyte infiltration and edema in submucosal layer as well as less disruption to the surface epithelium and deep mucosa (Figure 6).

Pre-treatment with oil extract of *N. sativa* prevented histopathological changes and did not show any significant leucocyte infiltration, edema, and disruption of deep mucosa (Figures 7).

Table 5: Protective effect of Oil *Nigella sativa* against aspirin induced gastric damage in rats

Biochemical Parameters	Normal Control (A)	Ulcer Control (B)	standard Control (C)	Oil NS pt. ether (D)	LSD value
Vol. of gastric juice (ml)	0.60±0.16 c	2.50±0.14 a	1.82 ±0.2 b	0.66±0.13 c	0.331 *
pH	1.89 ±0.09 a	0.90 ±0.03 b	1.09 ± 0.07 b	1.61±0.03 a	0.594 *
Total Acidity (µEq /100 g BW.)	14.34 ±1.29 c	28.68 ±9.38 a	22.53±1.42 b	15.61±0.65 c	4.511 *
Mucus weight (g)	0.55 ± 0.02 a	0.20 ± 0.03 b	0.40 ± 0.04 a	0.48 ± 0.02 a	0.178 *
Ulcer Index	00.00 c	3.48 ±0.32 a	1.12 ±0.10 b	0.00±0.00 c	1.263 *
% Protection	100.00% a	00.00% c	67.81% b	100.00% a	12.272 *

* ($P<0.05$). Means having with the different letters in same row differed significantly.

Table 6: Comparison of antiulcerogenic protective effects of *Nigella sativa* with (Esomeprazole and Clarithromycin) from ulcer by Aspirin after 5 days of treatment in albino rats.

Groups (n=6)	Gross examination of stomach				Microscopic examination of stomach				
					Abnormality of mucosa				Number of animals with lesion
	Mucosal appearance	Loss of mucosal integrity		Number of animals with lesion	Surface	Glands	Lamina propria	Inflammation	
		Erosions	Ulceration						
A- (n=6)	6	0	0	0	0	0	0	0	0
B- (n=6)	1	5	5	5	5	5	5	6	6
C- (n=6)	3	4	4	4	4	3	4	4	4
D- (n=6)	5	0	0	0	0	0	0	0	0
Chi-square	8.39 **	7.96 **	7.52 **	7.52 **	7.52 **	7.04 **	7.52 **	8.20 **	8.20 **

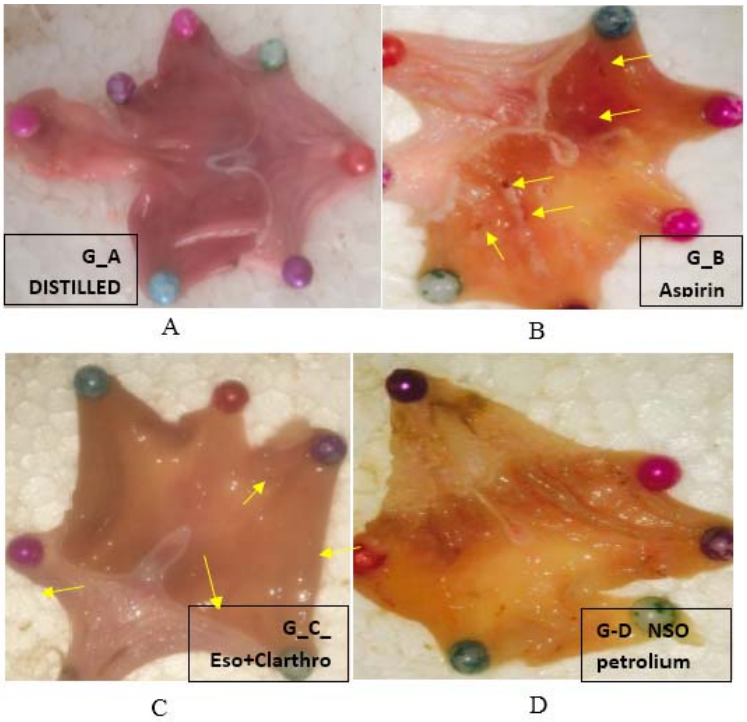

Fig 3: Gross examination of the gastric mucosa in rats. (a) Untreated rats (normal control). Intact gastric mucosa tissues are seen; (b) rats pretreated with distilled water. Severe lesions are seen with extensive visible hemorrhagic necrosis of gastric mucosa;(c) rats pretreated with Esomeprazole and Clarithromycin. Mild lesions of gastric mucosa are observed compared to the lesions in ulcer control group; (d) rats pretreated with *N. sativa* oil; No lesions are formed which indicates full protection of oil extract against gastric ulcers.

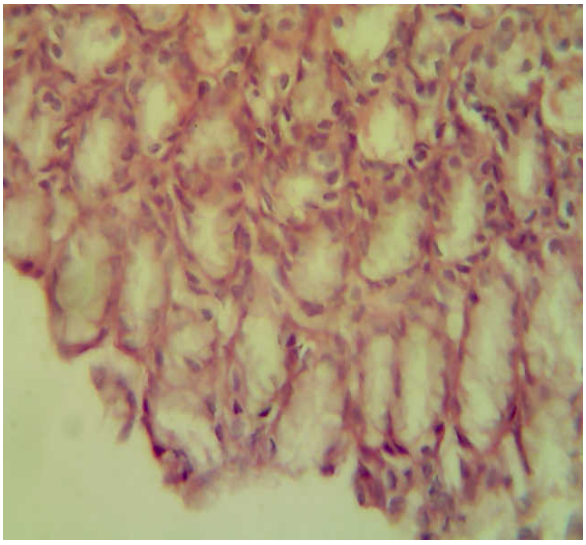

Fig 4: Section in the stomach of rats treated with Distilled water only (Negative control). Intact gastric mucosa layer are seen (H&E stain 400X).

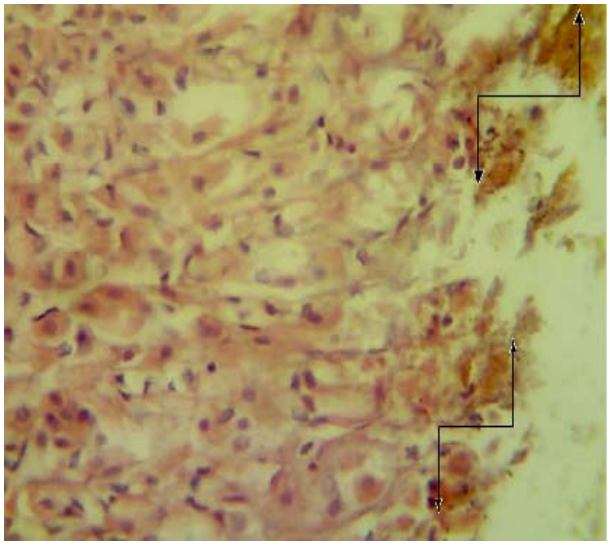

Fig 5: Section in the stomach of rat treated with Distilled water befor exposured to Asprin shows severe destruction to surface epithelium, local necrosis,hemorhage in the epithelial cells and odema (H&E stain 400X).

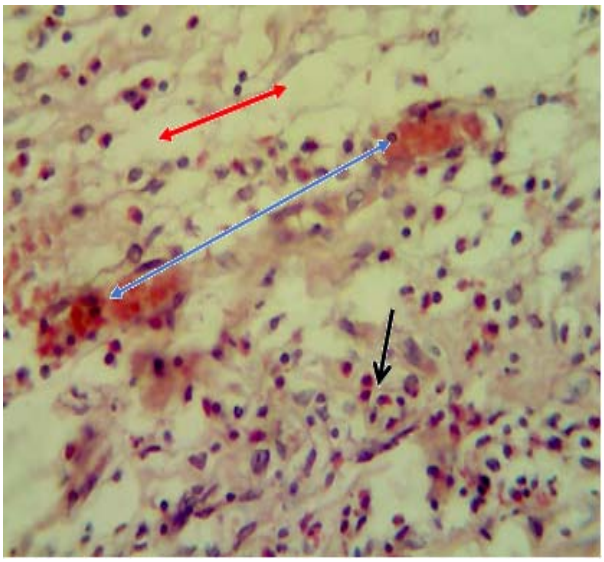

Fig 6: Section in the stomach of rat treated with Esomeprazole + clarthromycin befor exposured to Asprin shows inflmattory cells particularly neutrophils and mononuclear cells infiltration in the submucosa and mucosa ➔ in addition to congested blood vessels ↔ and odema (H&E stain400X).

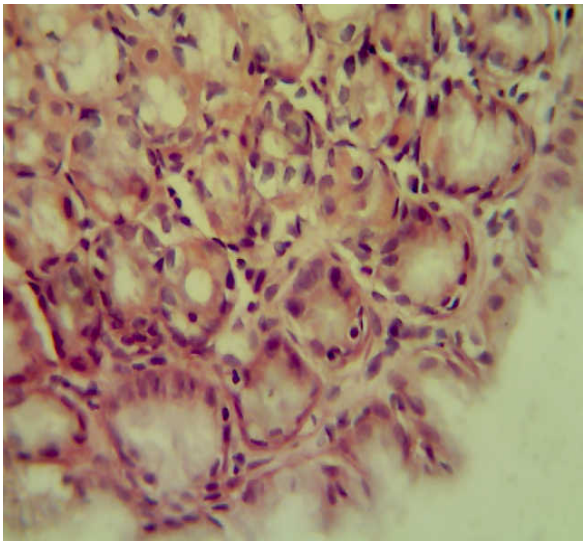

Fig 7: Section in the stomach of rat treated with *N. sativa* oil befor exposured to Asprin shows no clear lesions and No disturbance to gastric mucosa layer. (H&E stain 400X)

Discussion

Oil content in most *Nigella seeds* investigated, so far, is higher than 30% [26]. *Nigella* seeds from Iraq afforded 37%, 16% and 27% of oil after petroleum ether, chloroform and methanol extraction, respectively (Table 1). These yields are very similar to those repeatedly reported for *Nigella* seeds cultivated in countries presenting weather conditions close to those encountered in Saudi Arabia [27]. This result is similar to another research that *Nigella* seeds are composed of 28-36% fixed oils [22]. In this study, phytochemical screening for all three extracts showed significant indication of the presence of metabolites; Alkaloids, Tannins, Flavonoids Terpenoids, Phenol and steriods were found to be present in all the sequential extracts of *N. sativa seed* whereas saponins were absent in all solvents.

The detected phytochemical compounds are known to be beneficial in medicinal as well as physiological activities as they can be utilized in the formulation of new drugs to treat various diseases and disorders. So *N. sativa* seeds extract could be seen as an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit [28].

Thymoquinone has retention time of R_f 4.48 min at 295 nm. At this wave length there is no interference neither from dithymoquinone (thymoquinone dimer) nor from thymol. Dithymoquinone and thymol were detected at 260 and 275 nm respectively. These results are in agreement with [29] who showed that analysis of thymoquinone resulted in a sharp, symmetrical, and well resolved peak at R_f value of (0.48±0.04) at 295 nm.

Plant extracts are some of the most attractive sources of new drugs and have shown promising results in the treatment of gastric ulcers. Several folk medicinal plants and herbs have been used to treat gastrointestinal disorders or gastric ulcer. Uncontrolled acid secretion and ulceration of gastric mucosa due to several reasons is a serious problem to human health all over the world. The group that received aspirin 400mg/kg alone produced 100% ulcer induction with a mean ulcer score of 3.48 ±0.32 accompanied by a reduction in mucus content and increase total acidity. Moreover, there was a significant increase in gastric juice. Aspirin administration in rats, produced the highest lesion index when compared with all the pre-treatment groups. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms [30]. The excess gastric acid formation by prostaglandin (PG) includes both increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin [31]. Inhibitions of prostaglandin synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells. Prostaglandin is a major component of the protective factors that maintain gastrointestinal mucosal integrity and microcirculation[32]. Prostaglandins could reduce the damage caused by necrotizing agents (such as alcohol, aspirin, bile salts) in the gastric mucosal barriers. In addition, the prostaglandin would inhibit the movement of acid and pepsin into the mucus layer. To prevent the diffusion of hydrogen ions into the gastric mucosa, bicarbonate is required to regulate mucus pH to an optimized acidity of the stomach that served as barrier for acid component [33].

The result showed aspirin-induced gastric ulceration has been used as suitable method to study gastric ulcer this was confirmed by [34]. In gasroprotective study show that aspirin could injure the epithelium of stomach and disrupt the vascular endothelium and increase the permeability of the vessels and develop edema in submucosal layer of the stomach as well as epithelial lifting this result was agreement with gasroprotective study by [7,8]. Aspirin also caused dissolution of mucus constituents and reduced the mucus contents. These changes would elevate the flow of sodium and potassium ions into the lumen and pepsin secretion. Furthermore, aspirin was able to trigger direct toxic effect on the body and indirectly alter the mucosal flow in gastric mucosa by increasingly transcapillary fluid filtration and finally the epithelial lining was ruptured [35]. Gastric mucosal congestion, oedema, haemorrhage, lamina epithelial necrosis, leucocyte infiltrations, blood vessels congestion with foci of necrotic tissues in the lesions in positive Control group these results are consistent with previous studies that reported similar histopathological derangement and mucosal oxidative stress effects that involves weakening of gastric mucous, leading to formation of lesions in the gastric epithelium [36,37].

In the present study, the result showed that the rats pretreated with *N. sativa* oil extracts significantly reduced gastric ulcer and able to decrease acidity in aspirin induced gastric ulcer models; this can emphasize the effect of the major constituent Thymoquinone (TQ) in reducing acid secretion in the stomach and decrease in the acid level is attributed directly to its ability to normalize the proton pump activity.

This result agreement with [38, 39] reported that *Nigella sativa* oil increases mucin production, a finding that supports ours, since TQ is the oil's main constituent.

TQ also reduced peptic activity of gastric juice as shown in this study, possibly via inhibition of histamine release, and/or decreased gastric acidity, which attenuates the acid-stimulated activation of pepsinogens to the active pepsin in the gastric juice. TQ reduced neutrophils invasion as evidenced by the decreased activity of Myeloperoxidase (MPO), specific marker of acute inflammation, as well as in the histological results. This finding mimics that of [40] in an experimental colitis model, and another mechanism for TQ gastroprotective action [41]. Pretreatment with Nigella sativa and esomeprazole + clarithromycin prevented histopathological changes such as congestion, haemorrhage, oedema, inflammatory and dysplastic changes. The findings of this study were in well agreement with various data reporting that alcoholic extract of *Nigella sativa* reduced the number of lesions, lesions length, lesions breadth, lesions area and ulcer index in rats [42,43]. The current study demonstrated that *Nigella sativa* has gastro-protective activity as well as esomeprazole and clarithromycin.

Reference

1. Wynne HA, Campbell M. Pharmacoeconomics of non-steroidal anti-inflammatory drugs (NSA IDs). Pharmacoeco. 1993; 3(2):107-123.
2. Garcia Rodriguez LA, Sonia Hernandez Diaz. Risk of uncomplicated Peptic ulcer among users of Aspirin and Non-aspirin Non-steroidal anti-inflammatory drugs. Am Jo Epidemiol. 2004; 159:23-31.
3. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Brazilian Journal of Medical and Biological Research. 2002; 35:523-534.
4. Dash VB. Peptic Ulcer and Gastritis: Herbal Cure, Health Harmony, New Delhi, India, 2002.
5. Wallace JL. How do NSAIDs cause ulcer disease. Practice and Research Clinical Gastroenterology. 2000; 14(1):147-159.
6. Chari ST, Singer MV. Alcohol and gastric acid secretion in humans. *Gut*. 1993; 34(6):843-847.
7. Ismail IF, Golbabapour S, Hassandarvish. Gastroprotective activity of *Polygonum chinense* aqueous leaf extract on ethanol-induced hemorrhagic mucosal lesions in rats. Evid Based Complement Alternat Med. 2012, 9.
8. Golbabapour S, Gwaram NS, Hassandarvish P. Gastroprotection studies of Schiff base zinc (II) derivative complex against acute superficial hemorrhagic mucosal lesions in rats. PLOS ONE. 2013; 8(9):1-9.
9. Suzuki Y, Ishihara M, Ito M. Anti-Ulcer Effects of Antioxidants, Quercetin, α-Tocopherol, Nifedipine and Tetracycline in Rats. Japanese Journal of Pharmacology. 1998; 78:435-441.
10. Cheng CI, Koo M. Effect of *centella asiatica* on ethanol induced gastric mucosal lesions in rats. Life Science Journal. 2000; 67:2647- 2653.
11. Rates SM. Plants as source of drugs. Toxicol. 2001; 39(5):603-613.
12. Ekstrom P, Carling L, Wtterhus S, Wingren P, Anker-Hansen O, Lundegardh G. Prevention of peptic ulcer and dyspeptic symptoms with omeprazole in patients receiving continuous non-steroidal anti-inflammatory drug therapy. Scandinavian Journal of Gastroenterology. 2004; 31(8):753-8.
13. Schmeda-Hirschmann G, Yesilada E. Traditional Medicine and Gastroprotective Crude Drugs. Journal of Ethnopharmacology. 2005; 100(1-2):61-66.
14. Alkofahi A, Atta AH. Pharmacological screening of the anti-ulcerogenic effects of some Jordanian medicinal plants in rats. Journal of Ethnopharmacology. 1999; 67(3):341-345.
15. El-Kadi A, Kandil O. The black seed (*Nigella sativa*) and immunity: its effect on human T cell subset. Federation proceedings. 1987; 46:1222.
16. Tasar N, Sehirli O, Yiginer O, Suleymanoglu S, Yuksel M, Yegen B *et al*. Protective effects of *Nigella sativa* against hypertension-induced oxidative stress and cardiovascular dysfunction in rats. Marmara Pharmaceutical Journal. 2012; 16:141-149.
17. Najmi, A, Nasiruddin M, Khan RA, Haque SF. Indigenous herbal product *Nigella sativa* proved effective as an antihypertensive in metabolic syndrome. Asian Journal of Pharmaceutical and Clinical Research. 2013; 6:61-64.
18. Forouzanfar F, Bazzaz BS, Hosseinzadeh H. Black cumin (*Nigella sativa*) and its constituent (thymoquinone): A review on antimicrobial effects. Iranian Journal of Basic Medical Sciences. 2014; 17:929-938.
19. Kolahdooz M, Nasri S, Modarres SZ, Kianbakht S, Huseini HF. Effects of *Nigella sativa* L. seed oil on abnormal semen quality in infertile men: A randomized, double-blind, placebo-controlled clinical trial. Phytomedicine. 2014; 21:901-905.
20. Sobhi W, Stevigny C, Duez P, Calderon PP, Atmani D, Benboubetra M. Effect of lipid extracts of *Nigella sativa* L. seeds on the liver ATP reduction and alpha-glucosidase inhibition. Pakistan Journal of Pharmaceutical Sciences. 2016; 29:111-117.
21. Azra K. Phytochemical Screening of *Syzygium Cumini* Seeds. Indian Journal of Plant Sciences. 2014; 3(4):2319-3824.
22. Ahmad I, Tripathi J, Sharma M, Karchulli MS, Umer L. *Nigella sativa* a medicinal herb with immense therapeutic potential (a systematic review). International Journal of Biological and Pharmaceutical Research. 2014; 5:755-762.
23. Gretzer B, Ehrlich K, Maricic N, Lambrecht N, Respondek M, Peskar BM. Selective cyclo-oxygenase-2 inhibitors and their influence on the protective effect of a mild irritant in the rat stomach. British Journal of Pharmacology. 1998; 123:927-35.
24. Akilandeswari S, Senthamarai R, Valarmathi R, Shanti S, Prema S. Screening of gastric antiulcer activity of *Sida acuta burm*. International Journal of PharmTech Research. 2010; 2(2):1644-1648.
25. Wasman S, Mahmood A, Salehhuddin H, Zahra A, Salmah I. Cytoprotective activities of *Polygonum minus* aqueous leaf extract on ethanol-induced gastric ulcer in rats. Journal of medicinal plant research. 2010; 4(24):2658-2665.
26. Matthaus B, O¨zcan MM. Fatty acids, tocopherol, and

sterol contents of some *Nigella* species seed oil. Czech Journal of Food Sciences. 2011; 29:145-150.
27. Atta MB. Some characteristics of *Nigella* (*Nigella sativa L*.) seed cultivated in Egypt and its lipid profile. Food Chemistry. 2003; 83:63-68.
28. Abdurohaman MY. Phytochemical Extraction and Screening of Bio Active Compounds from Black Cumin (*Nigella sativa*) Seeds Extract. American Journal of Life Sciences. 2015; 3(5):358-364.
29. Prawez Alam, Hasan Yusufoglu, Aftab Alam. HPTLC densitometric method for analysis of thymoquinone in *Nigella sativa* extracts and marketed formulations. Asian Pacific Journal of Tropical Disease. 2013; 3(6):467-471.
30. Goel RK, Gupta S, Shankar R, Sanyal AK. Anti-ulcerogenic effect of banana powder (*Musa sapientum var. paradisiaca*) and its effect on mucosal resistance. Journal of Ethnopharmacology. 1986; 18:33-44.
31. Rainsford KD. Mechanisms of gastro-intestinal ulceration by non-steroidal anti-inflammatory/ analgesic drugs. Advances in inflammation research. 1984; 6:51-64.
32. Alarcon DL, Martin MJ, Lacasa C, Motivan V. Antiulcerogenic activity of flavonoids and gastric protection. Journal of Ethnopharmacology. 1994; 42:161-170.
33. Vadgama P, Alberti KG. The possible role of bicarbonate in mucosal protection and peptic ulceration. Digestion. 1983; 27(4):203-213.
34. Bae DK, Park D, Lee SH. Different antiulcer activities of pantoprazole in stress, alcohol and pylorus ligation-induced ulcer models. Laboratory animal research. 2011; 27(1):47-52.
35. Lam EKY, Tai EKK, Koo MWL. Enhancement of gastric mucosal integrity by *Lactobacillus rhamnosus* GG, Life Sciences. 2007; 80(23):2128-2136.
36. Valcheva-Kuzmanova S, Krasnaliev I, Galunska B, Belcheva A. Influence of DL-alpha tocopherol acetate on indomethacin-induced gastric mucosal injury in rats. Autonomic and Autacoid Pharmacology. 2007; 27(3):131-136.
37. El-Moselhy MA, Abdel-Hamid NM, Abdel- Raheim SR. Gastroprotective effect of nicorandil in indomethacin and alcoholinduced acute ulcers. Appl. Biochem. Biotechnology. 2009; 153(3):449-459.
38. El-Dakhakhny M, Barakat M, Abd El-Halim M, Aly SM. Effects of *Nigella sativa* oil on gastric secretion and ethanol induced ulcer in rats. Journal of Ethnopharmacology. 2000; 72:299-304.
39. Zaman R, Akhtar MS, Khan MS. Gastroprotective and Antisecretary Effect of *Nigella Sativa* Seed and Its Extracts In Indomethacin-Treated Rats. Pakistan Journal of Biological Sciences.2004; 7:995-1000.
40. Mahgoub AA. Thymoquinone protects against experimental colitis in rats. Toxicology Letters. 2003; 143:133-143.
41. Abdel-Fattah AM, Matsumoto K, Watanabe H. Antinociceptive effects of Nigella sativa oil and its major component, thymoquinone, in mice. European Journal of Pharmacology. 2000; 400:89-97.
42. Peter C, Konturek, Brozozowask T. Effect of *Helicobacter Pylori* or Delay in Ulcer Healing Induced by Aspirin in Rats. Journal of Ethnopharmacology. 2000; 70:1-17.
43. Arooba MSI, Mohammed kT. The effect of propolis on growth inhibition of *Helicobacter pylori* isolates from peptic ulcer patient. Kufa Journal For Veterinary Medical Sciences. 2011; (2)1:44-58.