

Research article

Effectiveness of black cumin seed oil (*Nigella sativa* L.) as a gastroprotective in white rats induced by aspirin and gastric histopathology

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Abstract

Objective: Gastric ulcer is damage in mucosal tissue, sub mucosa until the lining of the gastric muscle; the causes of gastric ulcer include gastric acid hypersecretion. Black cumin (*Nigella sativa* L.) seed oil has a gastroprotective effect because it contains *timoquinone* (TQ) compounds. This study aims to determine the gastroprotective activity of black cumin seed oil in rats induced by aspirin. **Method:** Black cumin (*Nigella sativa* L.) seed oil was given in 3 concentration variations, namely 0.025; 0.05; and 0.075 ml/kg. Phytochemical screening tests in black cumin seed oil were carried out which included tests of flavonoids, saponins, tannins and terpenoids. Observations included macroscopic (number of ulcers, pH of ulcer and index of ulcer) microscopically (histopathological test). **Result:** The results of the phytochemical screening test obtained positive results on the test of saponin and terpenoid compounds. While the results of macroscopic observations showed that giving of black cumin seed oil with a concentration of 0.075 ml/kg in rats induced by aspirin showed a more optimal gastroprotective outcome. **Conclusions:** Histopathological tests showed that doses that have a gastroprotective effect can restore mucosal cell cohesion that has been damaged by erosion.

Introduction

Gastric ulcer is caused by an imbalance between aggressive factors that can damage the mucosa (gastric acid, non-steroidal anti-inflammatory drugs or NSAIDs, alcohol, and *Helicobacter Pylori* bacteria) and defensive factors that maintain the integrity of the gastric mucosa for example, mucin, bicarbonate, and prostaglandin [1]. The high mortality rate caused by gastric ulcer disease is perforation and bleeding, which is caused by frequent over consumption of NSAIDs [2].

Black cumin (*Nigella sativa* L.) is an amazing herb with a religious background and that is grown in Middle East, Mediterranean regions, Southern Europe, India, Pakistan, Syria, Saudi Arabia, and Turkey. Black cumin (*Nigella sativa* L.) has been used as a preventive substance against many disease in Asian Countries [3]. According to the study of Ahmad *et al.*, (2013) states that the potential of black cumin are as antidiabetic, anticancer, immunomodulator, anti-inflammatory, antibacterial, antioxidant, neuroprotective and bronchodilator [4]. The main ingredients of black cumin (*Nigella sativa* L.) are thymoquinone, dithymoquinone, thymohydroquinone, nigellidine and nigellimine, and thymol which act as antioxidants [5].

Materials and methods

Tools

The tools used in this study were: laboratory glassware tool, surgical scissor, caliper, cover glass, digital camera (Sony), electric microscope, electric balance (Libra Mas), oral sonde, spatula, syringe 3 ml (Onemed), pH meter and animal scale (Presica Geniweigher).

Materials

The materials used in this study were: HabbaSyifa black cumin seed oil, BPOM TR 11332346, acetosal, omeprazole (Ferron), Na-CMC, chloroform, Formalin Neutral Buffer 10% solution for fixation, Haematoxylline-eosin.

Experimental animals

The experimental animals used in this study was wistar strain male white rats (*Rattus Norvegicus*), aged 2-3 months, weighing 200-250 grams. The sample was obtained by take offhand the subject of the research that was found from the existing population. Rats were divided into six groups comprising of 4 animals each. Group I: Administered only aspirin (300 mg/bw) orally without treatment.

Group II: Given omeprazole (3.6 mg/kg bw) orally before 1 h were induced by aspirin at a dose 300 mg/kg bw orally (positive control).

Group III: Given carboxymethylcellulose (CMC) 0.5% before 1 h were induced by aspirin at a dose 300 mg/kg bw orally (Negative control).

Group IV: Given black cumin seed oil 0.025 ml/bw before 1 h were induced by aspirin at a dose 300 mg/kg bw orally.

Group V: Given black cumin seed oil 0.05 ml/bw before 1 h were induced by aspirin at a dose 300 mg/kg bw orally.

Group VI: Given black cumin seed oil 0.075 ml/bw before 1 h were induced by aspirin at a dose 300 mg/kg bw orally.

Methods

Phytochemicals test of black cumin seed oil

a. Flavonoid test

A total of 4 ml of the sample was put in a test tube, added 1.5 ml of methanol 50% and heated. And then, added Mg powder and 5-6 drops of HCl. The formation of red or orange color indicates the presence of flavonoid compounds.

b. Saponin test

A total of 2 ml of the sample was dissolved in 2 ml of water and shaken vigorously until the foam formed for less than 10 minutes as high as 1-10 cm. When adding 1 drop of 2 N HCl the foam did not disappear.

c. Tannin test

A total of 1 ml sample was added with 3-4 drops of FeCl₃ 10% solution. Formation of blue or green color indicates the presence of flavonoid compounds.

d. Terpenoid test

A total of 2 ml of sample was added to 2 ml of chloroform, then added with 3 ml of H₂SO₄. Formation of a reddish brown color indicates the presence of terpenoids [6].

Measurement of gastric fluid pH

Measurement of gastric fluid pH were carried out by means of gastric fluid accommodate and then, used of pH meter to determine the pH of gastric fluid [7].

Assessment of the number of ulcers

Rats gastric that have been dissected, stretched and observed the number of ulcers. Assessment of the number of ulcers can be seen in table 1.

Table 1. Assessment of the number of ulcers [7].

Value	Explanation
1	Normal gastric
2	Bleeding spots or number of ulcers 1
3	Number of ulcers 2-4
4	Number of ulcers 5-7
5	Number of ulcer 8-10
6	Number of ulcers was more than 10 or perforation

Assessment of ulcer severity

Assessment of ulcer severity by means of a rat gastric that has been dissected, then the diameter of the gastric ulcer was measured using a caliper. According to Vogel [8] the assessment of the severity of ulcer formed in the gastric mucosa was observed according to the criteria in table 2. Based on the scores obtained, then the ulcer index was calculated by adding up the scores obtained by using the formula:

$$U = U_N + U_S + U_P \times 10^{-1}$$

Where,

U = ulcer Index

U_N = average number of ulcers for each animal

U_S = average severity of ulcers.

U_P = percentage of animals with ulcers

Table 2. Assessment of ulcer severity.

Value	Explanation
1	Normal gastric
2	Bleeding spots or ulcers with a diameter of 0.5mm
3	Ulcers with a diameter of 0.5-1.0 mm
4	Ulcers with a diameter of 1.0-1.5 mm
5	Ulcers with a diameter of 1.5-2.0 mm
6	Ulcer with a diameter more than 2.0 mm or perforation

Statistical analysis

The data were analyzed by using SPSS 23.0 software. Comparisons of the measured parameters among all groups were made using ANOVA test with significance level was accepted $p < 0.05$.

Results and discussion

Phytochemical test of black cumin seed oil

In this study, phytochemical tests were conducted on the sample, which aimed to find out secondary metabolites what that contained. Phytochemical tests conducted in this study include tests of flavonoid, saponin, tannin and terpenoid. Phytochemical test results from black cumin seed oil can be seen in table 3.

Table 3. Phytochemical test results of black cumin seed oil.

No.	Compound Test	Result
1.	Flavonoid	-
2.	Saponin	+
3.	Tanin	-
4.	Terpenoid	+

(+) There were secondary metabolites present, (-) there were no secondary metabolites present.

Phytochemical test results of black cumin seed oil showed positive results on the compounds test of saponin and terpenoid.

Test of gastroprotective effect of black cumin seed oil (*Nigella sativa* Linn)

Observation of gastroprotective effect of black cumin seed oil was done by calculating the number of ulcers and gastric pH from each treatment group. The results of the observation of average number of ulcers and gastric pH for each group are shown in table 4.

Based on table 4 above obtained results, namely on the giving of black cumin seed oil at a concentration of 0.025 ml/kg bw shows the formation of the most ulcer, namely (1 ulcer). The decrease in the number of ulcers was in tandem with the increase in concentration, and at a concentration of 0.05 ml/kg bw the average of ulcer formed was (0.3), while at concentration of 0.075 ml/kg bw ulcers was not formed.

Table 4. Average results of the number of gastric ulcers.

Giving	Average of gastric ulcer (mm)	Average of gastric pH
Aspirin	5 ± 1.00	2.3±0.20
Positive control (Omeprazole dose 3.6 mg/kg bw)	0	4.1±0.15
Negative control (CMC)	4±1.00	2.4±0.20
Black cumin seed oil 0.025 ml/kg bw	1± 0.00	2.4±0.25
Black cumin seed oil 0.05 ml/kg bw	0.3±0.57	3.6±0.89
Black cumin seed oil 0.075 ml/kg bw	0	4.1±0.15

The results of the average number obtained were lower than those given only aspirin for 7 days, which were formed (5 ulcers). The measurement of gastric pH value showed that the lowest concentration of black cumin seed oil was 0.025 ml/kg bw obtained pH value 2.4, while the concentration of 0.05 ml/kg bw obtained pH value 3.6. The highest pH value was obtained by giving 0.075 ml/kg bw of black cumin seed oil and omeprazole. The pH value obtained by giving aspirin was the lowest pH value. The results of the calculation of gastric pH of rats given black cumin seed oil showed that the pH value increased with the increase in the concentration of black cumin oil given. The average graph of gastric ulcer and pH value can be seen in figure 1.

The effect of gastric protection can also be observed by observing the gastric ulcer index, which was characterized by a decrease in value. The results of calculating the index gastric ulcer average are shown in table 5.

Table 5. Index of gastric ulcer.

Giving	Ulcer Index
Aspirin	0.86
Positive control (Omeprazole 3.6 mg/kg bw)	0.2
Negative control (CMC)	0.83
Black cumin seed oil 0.025 ml/kg bw	0.53
Black cumin seed oil 0.05 ml/kg bw	0.43
Black cumin seed oil 0.075 ml/kg bw	0.2

Based on table 5 that on giving black cumin seed oil, the lowest ulcer index value was at the highest concentration, at a concentration of 0.075 ml/kg bw the ulcer index value (0.2) was the same as positive control (Omeprazole). The lowest concentration of 0.025 ml/kg bw has the highest ulcer index value (0.53).

Black cumin seed oil has a gastroprotective effect on rat gastric, this was appropriate with the observation of the amount of ulcer and the pH value produced. According to Magdy, *et al.*, (2012) the gastroprotective effect produced by black cumin seed oil was due to the presence of important constituent compounds, namely timoquinone (TQ) which was included in terpenoids [9]. Timoquinone reduces the secretion of gastric acid by inhibiting the K⁺/H⁺ ATP-ase pump. Inhibition of this enzyme cause in inhibition of neutrophil infiltration and increases nitric oxide production, thereby increasing blood flow to the submucosa [10]. Based on research conducted by Al-Douri, *et al.*, (2010) the content of timoquinone in black cumin seed oil can provide anti-inflammatory effects [11]. Timoquinone activates the effect of leukotrienes which causes controlled inflammatory processes, thus minimizing tissue damage [12].

Histopathological results of rats gastric

The results of microscopic observations on the normal gastric showed the results of cohesion between mucosal cells looks good and there was no erosion or ulcer in the gastric mucosal tissue. Gastric histopathology can be seen in figure 2.

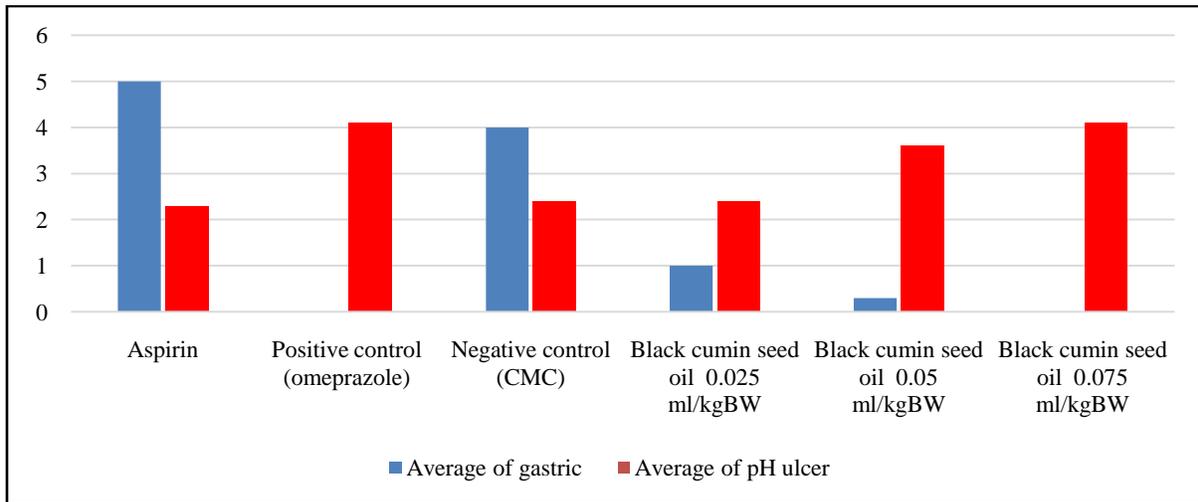


Figure 1. The average graph of gastric ulcer and pH value.

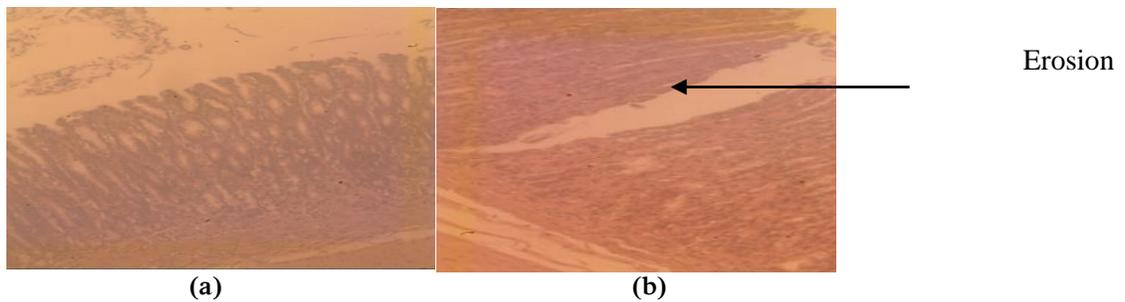
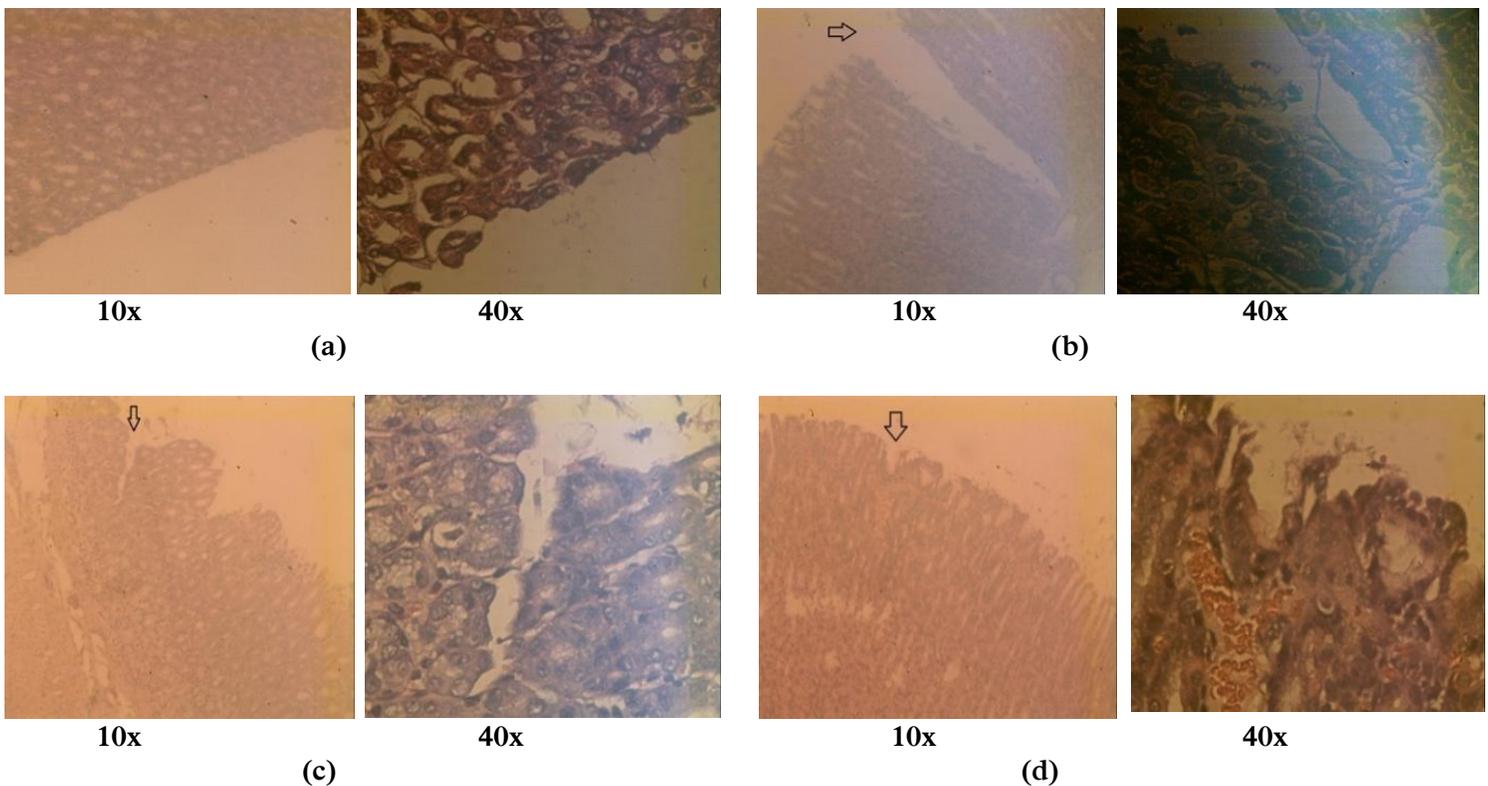


Figure 2. Gastric histopathology. (a) Cohesion between mucosal cells looks good, (b) there was erosion in epithelial cells in the gastric mucosa.

Based on study by Frezza *et al.* (2001) aspirin enter in the gastrointestinal tract (gastric) causing exfoliation of epithelial cells and can reduce mucus secretion which was

a protective barrier against acid attacks [13]. Figure 3 shows histopathological results.



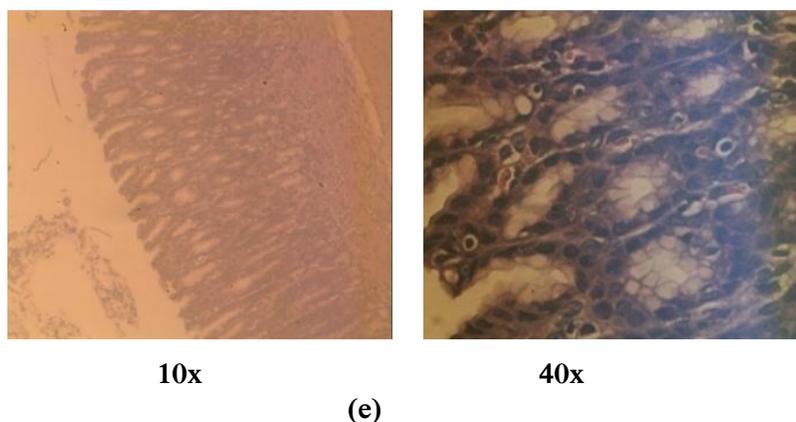


Figure 3. Histopathological results. (a) Positive control of omeprazole 3.6 mg/kg bw; (b) Negative control of CMC; (c) Black cumin seed oil 0.025 ml/kg bw; (d) Black cumin seed oil 0.05 ml/kg bw; (e) Black cumin seed oil 0.075 ml/kg bw.

Conclusion

Based on the results of the study it can be concluded that black cumin seed oil has a gastroprotective effect in rats induced by aspirin, at a concentration of 0.075 ml/kg bw.

References

1. Amandeep K, Singh R, Sharma R, and Kumar S. Peptic Ulcer: a review on etiology and pathogenesis. *International Research Journal of Pharmacy* 2012; 3(1): 86-90.
2. Hill, AG. Management of perforated duodenal ulcer. *East African Medical Journal* 2001; 78(7): 346.
3. Ermumcu, MSK, Nevin, S. Black Cumin (*Nigella Sativa*) and its active component of thymoquinone: Effects on health. *Journal of Food and Health Sciences* 2017; 3(4): 170-183.
4. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, And Siddique N. A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine* 2013; 3(5): 337-352.
5. Khan Z, Noorul H, Nesar A, Vartika S, Khalid M, Prashant S, Zeeshan A, Zohrameena S. Pharmacological activity of *Nigella Sativa*: A Review. *World J Pharm Sci.* 2016; 4(5): 234-241.
6. Tiwari P, Bimlesh K, Mandeep K, Gurpreet K, Harleen K. Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Scientia.* 2012; 1(1): 98-106.
7. Thabrew MI, Arawwawala LDAM. An overview of in vivo and in vivo models that can be used for evaluating anti-gastric ulcer potential of medical plants. *Ausyin boil* 2016; 1(2):1007.
8. Vogel, H.G. *Drug discovery and evaluation.* Springer-Verlag, Germany 2002; 867-873.
9. Magdy MA, El-Abhar H and Al-Maraghy N. Thymoquinone: Novel gastroprotective mechanisms. *European Journal of Pharmacology* 2012; 693(1-3): 126-131.
10. Abdelwahab SI, Sheikh BY, Taha MME, How CW, Abdullah R, Yagoub U, El-Sunousi R and Eid EM, Eltayeb. Thymoquinone loaded nanostructured lipid carriers: preparation, gastroprotection, in vitro toxicity and pharmacokinetic properties after extravascular administration. *International Journal of Nanomedicine* 2013; 8: 2163-2172.
11. Al-Douri, Asmaa S, and Al-Kazaz, SGA. The effect of *Nigella sativa* oil (black seed) on the healing of chemically induced oral ulcer in rabbit (experimental study). *J. Al-Ra dain Dent J.* 2010; 10: 151-157.
12. Al-Douri AS, Sahar GA. The effect of nigella sativa oil (Black Seed) on the healing of chemically induced oral ulcer in rabbit (Experimental Study). *Al – Rafidain Dent J.* 2010; 10(1): 151-157.
13. Frezza M, N Gorji, M Melato. The histopathology of non-steroidal anti-inflammatory drug induce gastroduodenal damage: Correlation with helicobacter pylori, ulcers, and haemorrhagic event. *J Clin Pathol.* 2001; (54): 521-525.