

Original Article

PROTECTIVE EFFECT OF NIGELLA SATIVA AGAINST ASPIRIN INDUCED GASTRIC DAMAGE IN RATS

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ABSTRACT

Objective: To evaluate the gastroprotective effect of *Nigella sativa*.

Methods: Ethanolic and aqueous extracts of *Nigella sativa* were prepared with the help of Soxhlet's apparatus. Forty two wistar albino rats (150-200g) of either sex were divided into seven groups of 6 each. Rats of group I and II were administered distilled water 0.5ml daily p.o for 5days, Group III were administered distilled water 0.5ml daily p.o for 5days and ranitidine 30mg/kg p.o on 5th day and group IV to VII administered with *Nigella sativa* aqueous & ethanolic extracts at a dose of 200mg/kg & 400mg/kg p.o respectively for 5 days. Except group I all groups were fasted for 36 hrs and on 5th day 1 hour after the last dose of ranitidine or test drug administration, aspirin 400mg/kg p.o was administered. After 5hrs of aspirin administration rats were sacrificed and gastric contents were analysed for pH and acid output while the stomach was taken out for ulcer index calculation and histological examination.

Results: Aspirin administration caused marked gastric damage in negative control group which was prevented in ranitidine and *Nigella sativa* extracts treated groups significantly. The protective effect was maximum with ranitidine followed by ethanolic extract and then aqueous extract in dose dependent manner.

Conclusion: Ethanolic and aqueous extracts showed significant protection against aspirin induced gastric damages in rats.

Keywords: *Nigella sativa*, Aspirin, Gastroprotection.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs worldwide. In the US alone, more than 111 million prescriptions are issued annually, at an approximate cost of \$4.8 billion [1]. NSAIDs are used very commonly in long term basis like in rheumatoid arthritis, post myocardial infarction etc. In spite of this they have significant adverse effects related burden to the community. For the average patient with rheumatoid arthritis taking NSAIDs, the attributed risk of hospitalisation with NSAIDs associated gastrointestinal problems is 1.3 to 1.6% annually and risk of death is 0.15% [2]. The estimated direct costs of hospitalization range from \$1,800 to \$8,500 per patient per hospitalization, based on different studies [3]. Among the NSAIDs the prototype drug aspirin has still significant role as an Antiplatelet, anti-inflammatory, analgesic and antipyretic agent. Its higher doses have anti-inflammatory, analgesic and antipyretic effect while the lower doses for antiplatelet activity in post myocardial infarction. Both higher and lower doses have significant risk to develop gastric damages which may be in the form of dyspepsia, gastritis, haemorrhage, ulceration or even sometimes perforation. In a case-control study it was found that the relative risk for aspirin users to develop symptomatic ulceration is 2.9 as compared to non-users [4]. Another study revealed that the odds ratio for peptic ulcer bleeding in a dose dependent manner as for 75mg/day, 2.3; 150mg/day, 3.2 and 300mg/day, 3.9 [5].

Nigella sativa commonly known as "Kalonji/Black cumin" belongs to family *Ranunculaceae* is an annual herb. Seeds are small dicotyledonous, trigonous, angular, rugulose-trabecular, and black externally with white inside. Odour of seed is aromatic with bitter taste [6]. It is reported to have various pharmacological activities like antidiabetic, analgesic & anti-inflammatory, antioxidant, antitumor, wound healing and very few reports on gastroprotective activity against NSAIDs induced damage [7-12].

Although, to prevent gastric damage many of the drugs are available like proton pump inhibitors, H₂ blockers but they also have adverse effects. Newer drugs which have minimal adverse effects with maximum benefit are continuously searched worldwide to prevent

the gastric damages induced by NSAIDs. So, in this regard study was planned to evaluate protective effect of *Nigella sativa* against aspirin induced gastric damages.

MATERIALS AND METHODS

Institutional Animal Ethical Committee (IAEC) approval

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Jawaharlal Nehru Medical College, A.M.U; Aligarh on 13.04.2012 (Registration no. 401/CPCSEA dated 08.05.2012). All animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA under the "Guidelines for Care and Use of Animals in Scientific Research" (INSA 1992 & 2000).

Plant material & extraction

The seeds of *Nigella sativa* were purchased from Pharmacy of Ajmal Khan Tibbiya College, Aligarh Muslim University; Aligarh (U.P) and authenticated by Dr. (Mrs) Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum (RHMD) of National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. The sample specimen was deposited at RHMD, NISCAIR; New Delhi bearing ref. no. NISCAIR/RHMD/Consult/ 2013/2316/96. Seeds were powdered with the help of a grinder. For aqueous extract 100 gm of seed powder was extracted separately with 300ml of distilled water for 72 hours by using Soxhlet's apparatus. The extract so obtained was filtered. Then the filtrate was collected in Petri dish and evaporated till dryness in an incubator. Similarly for ethanolic extract finely powdered, 100 gram of powdered seeds extracted separately in 300 ml of absolute ethanol for 72 hours using Soxhlet's apparatus. The extract so obtained was collected in Petri dish and evaporated till dryness at 35-40°C in an incubator. The yields of aqueous and ethanolic extracts were found to be 12.62% and 48.50% respectively.

Chemicals and drugs

All chemicals and drugs used were of analytical grade.

Chemicals

0.1N Sodium hydroxide {NaOH} (Central Drug House Pvt. Ltd.)

Phenolphthalein indicator (Orchid chemicals, China)

Formaldehyde (Central Drug House Pvt. Ltd.)

Drugs

Tablet Aspirin (Reckitt Benckiser, India)

Tablet Ranitidine (Unique Biotec Ltd.)

Animals

Forty two wistar albino rats (150-200g) of either sex procured from Central Animal House, Jawaharlal Nehru Medical College; A.M.U; Aligarh and were housed under standard condition (temperature $27 \pm 2^\circ$ C, Humidity 30-70% & 12 hour light/dark cycles), and fed with standard pellet diet and water *ad libitum*. They were acclimatized to the laboratory condition for 1 week prior to experimental study.

Experimental design

All the rats were randomly divided into seven groups each containing six rats. Gastric damage was induced by aspirin 400mg/kg orally single dose in 36 hours fasted rats with water *ad libitum* [13]. Different groups were treated as below

- Group I (Normal control): Distilled water 0.5ml daily p.o for 5days.
- Group II (Negative control): Distilled water 0.5ml daily p.o for 5days.
- Group III (Standard control): Distilled water 0.5ml daily p.o for 5days + ranitidine 30mg/kg p.o on 5th day.
- Group IV (NSAE 200): *Nigella sativa* aqueous extract 200mg/kg p.o for 5 days.
- Group V: (NSAE 400): *Nigella sativa* aqueous extract 400mg/kg p.o for 5 days.
- Group VI (NSEE 200): *Nigella sativa* ethanolic extract 200mg/kg p.o for 5 days.
- Group VII (NSEE 400): *Nigella sativa* ethanolic extract 400mg/kg p.o for 5 days.

All the groups (except group I) were given Aspirin 400mg/kg p.o on 5th day 1hour after standard drug (i.e Ranitidine) or test drugs (i.e *Nigella sativa* extracts) administration and after 5 hours of Aspirin administration rats were sacrificed under sodium pentobarbitone (50mg/kg i.p) anaesthesia and dissection was done.

Collection of gastric content

Stomach was exposed, pyloric end of stomach was ligated with a Thread and dissected out, the gastric contents were poured from the opened cardiac end, collected in a tube. The contents were centrifuged at 4000 rpm for 10 minutes. Then the supernatant was collected, their volume measured and titration done with 0.1 N NaOH & phenolphthalein as an indicator. pH and acid output (in $\mu\text{eq}/100\text{g}$ body weight) were measured as [14].

$$\text{pH} = -\log [\text{H}^+]$$

$$\text{Acid output} = \frac{[\text{Normality} \times \text{Volume (ml)}]}{\text{Body weight (g)}} \text{ of gastric content} \times 10^5$$

Collection of tissue sample

Stomach was cut along the greater curvature and was kept in a petridish containing normal saline then it was dried with a blotting paper. Then it was sandwiched between the two transparency sheets and scanned in a scanner. Scanned images were saved and evaluated for ulcer index with the help of Image J software (<http://rsbweb.nih.gov/ij/download.html>).

$$\text{Ulcer index} = 10/X$$

Where X= Total mucosal area/Total ulcerated area

$$\% \text{ Protection} = \frac{(U_c - U_t) \times 100}{U_c}$$

Where U_c - Ulcer index of negative control group

U_t - Ulcer index of test group

Finally, the tissue was preserved in 10% Formalin for histological examination.

Histological examination

The tissue was processed and sections were cut. The slides were prepared and stained with haematoxyline and eosin stain and the histological changes were observed under photomicroscope of 400X magnification.

Statistical analysis

All the values were presented as Mean \pm Standard Error of Mean (SEM). The groups were compared by one way analysis of variance (ANOVA) followed by post hoc "Dunnett's Multiple comparison test" to analyse statistical significance. $P < 0.05$ was considered to be significant.

RESULTS

Different parameters were measured for the gastroprotective effect of *Nigella sativa* which are shown in **Table 1**. Administration of aspirin showed massive gastric damages evidenced by highly significant decrease in gastric pH, increase in acid output and ulcer index in group II as compared to group I [$p < 0.001$]. Among the test groups ethanolic extract groups showed better protection as compared to aqueous extract groups. And the effect was found to be in dose dependent manner. Group VII showed maximum protective effect evidenced by significant improvement in all parameters as compared to group II [$p < 0.001$], this was followed by group VI, group V [$p < 0.05-0.001$]. Although the group IV (NSAE 200) showed preventive effect on the damages caused by aspirin but it was not found to be statistically significant as compared to group II [$p > 0.05$].

Table 1: Effect of *Nigella sativa* on gastric damages induced by aspirin

Group No.	Group Name	pH	Acid Output ($\mu\text{Eq}/100\text{g}$ body weight)	Ulcer Index	% Protection
I.	Normal Control	1.89 ± 0.09	11.42 ± 1.29	----	----
II.	Negative Control	$0.90 \pm 0.03^\#$	$119.68 \pm 9.38^\#$	$3.48 \pm 0.32^\#$	----
III.	Standard Control	$1.61 \pm 0.03^{***}$	$11.90 \pm 0.64^{***}$	$0.91 \pm 0.05^{***}$	73.85%
IV.	NSAE 200	0.98 ± 0.10	102.40 ± 10.46	3.11 ± 0.12	10.63%
V.	NSAE 400	$1.08 \pm 0.08^{**}$	$85.38 \pm 9.58^*$	$2.88 \pm 0.11^*$	17.24%
VI.	NSEE 200	$1.09 \pm 0.07^{***}$	$78.68 \pm 7.09^{***}$	$2.75 \pm 0.10^{**}$	20.98%
VII.	NSEE 400	$1.36 \pm 0.08^{***}$	$50.33 \pm 3.94^{***}$	$1.82 \pm 0.08^{***}$	47.76%

[NSAE (200 & 400) - *Nigella sativa* aqueous extract (200 & 400mg/kg), NSEE (200 & 400) - *Nigella sativa* ethanolic extract (200 & 400mg/kg). # ($p < 0.001$) when compared with Normal control; * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) when compared with Negative control.]

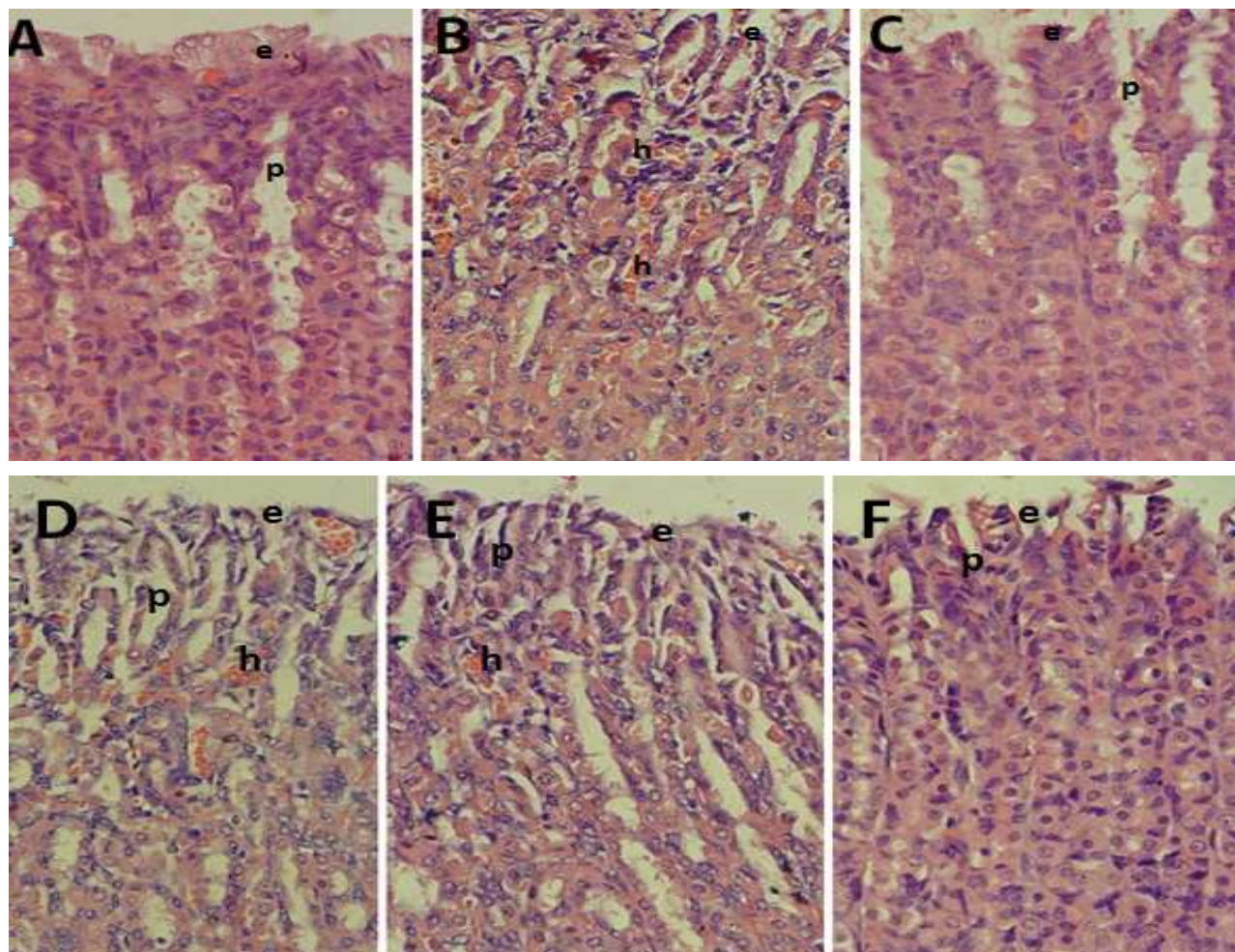


Fig. 1: Photomicrograph of rat stomach stained with H&E; original image X 400 magnification.

A. Normal control group showing normal architecture with intact epithelial lining (e), gastric pits (p) and cells with centrally placed nuclei. **B.** Negative control group showing complete distortion of the micro architecture with haemorrhagic (h) areas, loss of surface epithelium (e). **C.** Standard control group showing almost normal micro architecture with intact tubular glands and centrally placed nuclei. **D.** *Nigella sativa* aqueous extract (400mg/kg) group {NSAE 400} showing relatively distorted architecture, gastric pits (p) are not clearly well defined and some areas of haemorrhage (h). **E.** *Nigella sativa* ethanolic extract (200mg/kg) group {NSEE 200} showing relatively few areas of haemorrhage (h), epithelial lining (e) is relatively defined. **F.** *Nigella sativa* ethanolic extract (400mg/kg) group {NSEE 400} showing well defined epithelial lining (e) and gastric pits (p), cells having centrally placed nuclei, no area of haemorrhage and the micro architecture is almost normal.

Histological examinations

Normal Control Group: Histology of stomach on staining with haematoxylin and eosin showed normal architecture. The glandular part of the stomach had normal gastric pits with intact tubular epithelium [Fig 1A].

Negative Control Group: The stomach of aspirin-only treated rat on staining with haematoxylin and eosin showed a distorted micro architecture. There was disorganised glandular pattern with loss of surface epithelium. It was also associated with marked congestion of mucosa and haemorrhage in the interstitium [Fig 1B].

Test Groups: Among the test group minimal distortion in the histological architecture was observed in positive control group which was treated with ranitidine to the extent that it leads very

akin to normal control [Fig 1C]. This was followed by NSEE 400mg/kg and NSEE 200mg/kg treated groups [Fig 1E & 1F]; while the aqueous extract treated group showed only minimal improvement in the histo-architecture very close to negative control group [Fig 1D].

DISCUSSION

The gastric epithelium is under constant assault by a series of endogenous noxious factors, including HCl, pepsin and bile salts as well as exogenous substances such as medications, alcohol and microorganisms. A highly intricate biologic system is in place to provide defence from mucosal injury and to repair any injury which consists of barrier at three levels: Pre-epithelial, Epithelial and Post-epithelial [15]. Pre-epithelial barrier consists mainly of a mucous layer that contains mucous & bicarbonate are secreted by gastric epithelial cells, and surface active phospholipids, which prevents epithelial cells from the contact with luminal noxious agents such as gastric acid. Epithelial barrier consists of surface epithelial cells their tight junctions and restoration of damaged region whenever there is breach in the pre-epithelial barrier (restitution). And the mucosal blood flow within the gastric sub mucosal layer comprises the post-epithelial defense barrier which provides an adequate supply of micronutrients and oxygen in order for epithelial cells to secrete mucous and bicarbonate. It also removes acid and other toxic metabolic by-products [15-16]. Prostaglandins (PGs) play a key role in gastric epithelial defense by enhancing the pre-epithelial, epithelial, post-epithelial defense mechanisms: PGs (PGE₂ & PGI₂) stimulate the secretion of bicarbonate and mucous, inhibit gastric acid secretion from parietal cells by inhibiting cAMP dependent pathway which directly stimulate H⁺ K⁺ ATPase pump and are also

important in maintaining epithelial cell restitution and mucosal blood flow [17].

The present study showed that aspirin at a dose of 400mg/kg p.o single dose in 36hrs fasted rats caused significant damage to the gastric mucosa. This was evidenced by significant decrease in gastric pH, increase in acid output and ulcer index as compared to normal control ($p < 0.05$) [Table 1]. Histological findings also supported the gastric mucosal damage [Fig 1B].

Aspirin breaches gastric defense mechanism in two different manners: PG-dependent and PG-independent manner. Since PGs play a critical role in maintaining gastric mucosal defense system, the irreversible inhibition of COX by aspirin leading to decreased mucosal PGs synthesis. Aspirin damages the mucosa in PG-independent manner by "ion trapping mechanism". Since aspirin is a weak acid, at gastric pH it remains unionised hence get easily diffused across gastric mucosal epithelial cells and ionised at neutral pH of cytoplasm. Within the cell it is trapped and accumulated causing cellular injury. Among the cell organelles mitochondria are the ultimate target for aspirin. It inhibits oxidative phosphorylation by changing the mitochondrial transmembrane potential (MTP), leading to the liberation of cytochrome C from mitochondrial intermembranous space into cytosol and to the release of ROS such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), thereby causing caspase 9 and caspase 3 activation and cellular lipid peroxidation, all resulting in cellular apoptosis [16].

Among the test groups (treated with *Nigella sativa*) there was dose dependent protection. The maximum protection was shown by *Nigella sativa* ethanolic extract (400mg/kg) and *Nigella sativa* ethanolic extract (200mg/kg) which were statistically significant as compared to negative control group ($p < 0.001$). In aqueous extract groups only the 400mg/kg dose showed significant protection as compared to negative control group ($p < 0.01$). The histological results also revealed that the damages were less in ethanolic extract followed by aqueous extract groups in a dose dependent manner [Fig 1D, Fig 1E, Fig 1F].

Aspirin is non-steroidal anti-inflammatory drug (NSAID) which inhibits cyclooxygenase (COX) enzyme irreversibly. COX enzyme converts arachidonic acid to unstable intermediate PGG_2 and PGH_2 . These intermediates further leads to production of other prostaglandins (PGs). PGE_2 and PGI_2 have direct inhibitory action on gastric acid secretion, stimulate mucus production through EP_3 receptor and also increase the submucosal blood flow [17]. The gastroprotective effect of *Nigella sativa* may be due to its important constituent thymoquinone (TQ) [18-19]. In previous studies it was found that TQ has property to increase mucus production. Beside this it decreases acid secretion by inhibiting H^+K^+ ATPase pump. It also inhibits neutrophil infiltration and increase nitric oxide production hence increase submucosal blood flow [19-20]. So, the gastroprotective effect of *Nigella sativa* is due to the collaborative effect of all the described mechanism.

CONCLUSION

So, in view of the above findings it may be concluded that *Nigella sativa* ethanolic and aqueous seed extracts has significant gastroprotective activity against aspirin induced gastric damage.

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