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Amelioration of Non-steroidal Anti-inflammatory Drug Induced Gastropathy by *Nigella sativa* Oil- An Experimental Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MNH and RAK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MN and AAK managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the protective effect of *Nigella sativa* oil against Non-steroidal anti-inflammatory drug induced gastropathy.
Methodology: Thirty wistar albino rats (15-200 gm) of either sex were divided into five groups each containing six animals. Group I & II were administered distilled water 0.5 ml daily p.o for 5 days, Group III was administered distilled water 0.5 ml daily p.o for 5 days and ranitidine 30 mg/kg p.o on 5th day and group IV & V were test groups, administered with *Nigella sativa* oil (NSO) at a dose of 1 & 2 ml/kg/day 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 400 mg/kg p.o was administered in fasted rats. 5 hrs after aspirin administration the rats were sacrificed and gastric contents were analysed for pH and acid output while the stomach was taken out for ulcer index

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calculation and histological examination.
Results: Aspirin caused marked gastric damage evidenced in group II which was prevented by ranitidine as well as *Nigella sativa* oil (NSO). Among the test groups the protection was in a dose dependent manner.
Conclusion: *Nigella sativa* oil (NSO) showed a dose dependent protective effect against aspirin induced gastropathy.

Keywords: Nigella sativa oil; aspirin; gastropathy; gastroprotection.

ABBREVIATIONS

NSO- Nigella sativa oil; NSAIDs- Non-steroidal anti-inflammatory drugs; C.I- Confidence interval.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs. They are prescribed commonly as an analgesic, antipyretic & anti-inflammatory agent and the low dose aspirin used as an antithrombotic agent also [1]. The Kolbe Company in Germany started producing salicylic acid in 1860. Acetylsalicylic acid (aspirin) which is more palatable form of salicylic acid was introduced into the market by Bayer in 1899 [2]. One of the study showed that the attributed risk for hospitalization with NSAIDs associated gastrointestinal problems is 1.3-1.6% annually while the risk of death was 0.15% [3]. In another study it was found that in women of regular aspirin user (≥2 standard [325 mg] tablets/week) the multivariate RR of gastrointestinal bleeding was 1.43 (95% C.I, 1.29-1.59) compared to non-regular users [4].

Among the NSAIDs, aspirin still the prototype drug used as an antiplatelet agent, anti-inflammatory agent and antipyretic agent. The different doses of aspirin used for different purposes like low dose as an antiplatelet agent while the high doses used as an anti-inflammatory & antipyretic agent. Both the lower and higher doses have significant risk of gastrointestinal damage which may be manifest as dyspepsia, gastritis, ulceration, haemorrhage. In a cohort study it was found that the incidence rate of upper GI bleeding was 37.9/10000-years [5]. The incidence of upper GI bleeding depends upon both duration and dose of aspirin use but the dose has more pronounced effect, higher the dose more will be the chances of bleeding [4]. In a meta-analysis it was found that the risk of major GI bleeding increased with low doses of aspirin (75-325 mg/day) alone (OR, 1.55; 95% CI, 1.27-1.90), compared with inert control reagents. This risk is increased with accompanying use of other antithrombotic or anticoagulants [6].

Nigella sativa which is commonly known as Black cumin or Kaloonji belongs to family *Ranunculaceae*. Seeds of *Nigella sativa* are small dicotyledonous, trigonus, angular, black in colour with aromatic odour and bitter taste [7]. Seeds are the source of oil. It is reported of various pharmacological activities such as antidiabetic, antioxidant, anti-tumour, antibacterial immunomodulatory and wound healing [8-14].

Although, many drugs are still available such as Proton pump inhibitors, H_2-blockers but are not free from their own adverse effects. So, there is a continuous search for new drugs or their source which minimise the NSAIDs (like aspirin) induced adverse effects. In this line of search we have planned to evaluate the protective activity of Nigella sativa oil against aspirin induced gastropathy on the basis of above background.

2. METHODOLGY

2.1 Institutional Animal Ethical Committee (IAEC) Approval

The study protocol was approved by the IAEC, Jawaharlal Nehru Medical College, A.M.U; Aligarh (U.P), India [Registration no. 401/CPCSEA dated 08.05.2012]. All the animal experimental procedures were carried out as per the rules and regulations of IAEC and CPCSEA under the “Guidelines for Care and Use of Animals in Scientific Research” (INSA 1992 and 2000).

2.2 Chemical and Drugs

All chemicals and drugs used were of analytical grade.

Test drug *Nigella sativa* oil (Kalonji oil, Mohammedia products, Aamir nagar, Shah Sahab Mohalla, Karimnagar– 505001, A.P., India) was procured from local market at Aligarh. As per manufacturer's information, it was prepared by steam distillation. Packing size was 200 ml, batch no. 020 and date of manufacture was Feb., 2011.

Drugs- Aspirin (Reckitt Benckiser, India) and Tablet Ranitidine (Unique Biotec Ltd.)

Chemicals- 0.1 N Sodium hydroxide [NaOH] (Central Drug House Pvt. Ltd.), Phenophthalein indicator (Orchid chemicals) and Formaldehyde (Central Drug House Pvt. Ltd.)

2.3 Experimental Animals

A total 30 healthy wistar albino rats weighing 150-200 g of either sex procured from Central Animal House, Jawaharlal Nehru Medical College, A.M.U, Aligarh were housed under standard condition (temperature 27±2°C, Humidity 30-70% & 12 hour light/dark cycles), and fed with standard pellet diet and water ad libitum. All the animals were acclimatized to laboratory conditions for one week prior to experimental procedure.

2.4 Experimental Design

All the rats were randomized and grouped in 5, each group contained six animals. Gastric damage was induced by aspirin 400 mg/kg p.o single dose in 36 hours fasted rats [15]. Different groups were treated as below…….

- Group I (Normal control): Distilled water (DW) 0.5 ml/day p.o × 5 days
- Group II (Negative control): DW (0.5 ml/day p.o x 5 days) + (Aspirin 400 mg/kg p.o on 5^{th} day)
- Group III (Standard control): DW (0.5 ml/day p.o x 5 days) + (Ranitidine 30mg/kg p.o on 5^{th} day) + (Aspirin 400 mg/kg p.o on 5^{th} day 1 hour after Ranitidine administration)
- Group IV (NSO 1): *Nigella sativa* oil (1ml/kg p.o x 5days) + (Aspirin 400 mg/kg p.o on 5^{th} day 1hour after NSO administration)
- Group V (NSO 2): *Nigella sativa* oil (2 ml/kg p.o x 5 days) + (Aspirin 400 mg/kg p.o on 5^{th} day 1hour after NSO administration)

After 5 hours of aspirin administration the animals were sacrificed under Sodium pentobarbitone (50 mg/kg i.p) and dissection was done.

2.5 Collection of Gastric Content

After dissection, stomach was exposed the pyloric end was ligated with a thread while the cardiac end held firmly with forcep. Then pyloric end (distal to the ligature) and cardiac end were cut and the gastric content was poured into a centrifuge tube. The content was centrifuged at 4000 rpm for 10 minutes. Then the supernatant was collected with help of pipette and its volume measured and titration was done with 0.1 N NaOH & phenolphthalein as an indicator. pH and acid output was measured [16].

$$pH = -\log [H^+]$$

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

2.6 Collection of Tissue Sample

Stomach was dissected along the greater curvature with taking precaution not to damage the tissue. The cut-opened stomach 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 ulcer index with the help of Image J software (32-bit Java) available at NIH (*http://rsbweb.nih.gov/ij/download.html*) [17].

$$\text{Ulcer index (U.I)} = 10/X$$

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 negative test group

2.7 Histological Examination

After scanning the tissues, they were preserved in 10% formalin for histological analysis. The tissues were processed and made cut sections

with the help of microtome. Slides were made and stained with haematoxylin and eosin and then the slides were observed under photomicroscope.

2.8 Statistical Analysis

All the data were presented as Mean ± Standard Error of Mean (SEM) and were analysed by SPSS-20 software package. The groups were evaluated 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.

3. RESULTS AND DISCUSSION

3.1 Results

Different parameters were measured which are shown in Table 1 and Figs. 1, 1 & 3. After analysing the different parameters and the histological examination [Figs. 4 & 5] it was found that aspirin caused massive damage to the gastric mucosa evidenced by highly significant decrease in gastric pH, increase in acid output and ulcer index in Group II (negative control group) as compared to Group I (normal control) (p<0.001) [Table 1 and Figs. 1, 2 & 3]. The histological findings showed distorted micro-architecture & disorganised glandular pattern with loss of surface epithelium. It was also associated with marked congestion of mucosa and haemorrhage in the interstitium [Fig. 4]. While the Group III (standard control group) which was treated with ranitidine and aspirin the damages are significantly less as compared to Group II (p<0.001) [Table 1 and Fig. 1, 2 & 3]. And the histological micro-architecture was very akin to normal control [Fig. 4]. Test groups (Group IV & V) showed improvement in the deranged parameters and histological architecture. They showed a dose dependent improvement against aspirin induced gastropathy evidenced by increase in pH, decrease in acid output and ulcer index as compared to Group II (p<0.001-0.05). They also showed improvement in the distorted micro-architecture in dose dependent fashion [Fig. 5] as compared Group II.

3.2 Discussion

Gastric mucosal epithelium is in constant assault by many offending agents in the form of exogenous or endogenous substances. So, nature has provided a very effective barrier mechanism which provides basically a defence against the noxious agents. This barrier mainly occur at three levels- Pre-epithelial, Epithelial and Post-epithelial. Pre-epithelial level mainly composed of mucus layer, bicarbonate & surface active phospholipids; epithelial level composed of epithelial lining which secretes mucus & bicarbonate; while the post-epithelial layer composed of mucosal/sub mucosal blood flow [18]. Any breech in these defense mechanism leads to damage to the gastric mucosa.

Prostaglandins (PGs) especially PGE_2 & PGI_2 which constitute the first barrier are very important for maintaining the mucosal integrity. These PGs directly stimulate mucous & bicarbonate secretions by stimulating epithelial cells and inhibit gastric acid secretion from parietal cells by inhibiting cAMP dependent pathway which directly stimulate H^+ K^+ ATPase pump through EP_3 receptor [19].

In our study we used aspirin which caused severe damage to the gastric mucosa as found in aspirin only treated group i.e Group II as compared to normal control as evidenced by the different parameters we used and the histological findings. Aspirin damages the mucosa in two different manners: PG-dependent &

Table 1. Effect of *Nigella sativa* oil (NSO) on gastric damages induced by aspirin

Group no.	Group name	pH	Acid output (µEq/100 g body weight)	Ulcer index	% Protection
I.	Normal control	1.89±0.09	11.42±1.29	----	----
II.	Negative control	0.90±0.03###	119.68±9.38###	3.48 ±0.32###	----
III.	Standard control	1.61±0.03***	11.90±0.64***	0.91±0.05***	73.85%
IV.	NSO 1	1.04±0.102*	86.54±11.03*	2.90±0.11*	16.67%
V.	NSO 2	1.31±0.04***	60.78±3.34***	2.11±0.14***	39.36%

*[NSO (1 & 2) - Nigella sativa oil (1 & 2 ml/kg). # (p<0.05), ## (p<0.01) and ### (p<0.001) when compared with Normal control; *(p<0.05), ** (p<0.01) and *** (p<0.001) when compared with Negative control.]*

PG-independent manner. PG-dependent manner due to irreversible inhibition of Cyclooxygenase (COX) leads to decreased levels of PGs which are protective to the gastric mucosa. PG-independent mechanism is due to the direct effect of aspirin. Aspirin is an acidic drug because of its acidic character it remain in non-ionised form in the stomach (due to acidic pH of gastric content) and hence easily diffuse inside the mucosal epithelial cells where they get ionised because of neutral cytoplasmic pH and then remain trapped inside the cells (called as "Ion trapping mechanism") causing cell organelle damage. The main target inside the cell is mitochondria where aspirin inhibits oxidative phosphorylation by changing the mitochondrial transmembrane potential (MTP), leading to the liberation of cytochrome-C from mitochondrial inter-membranous 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 [20].

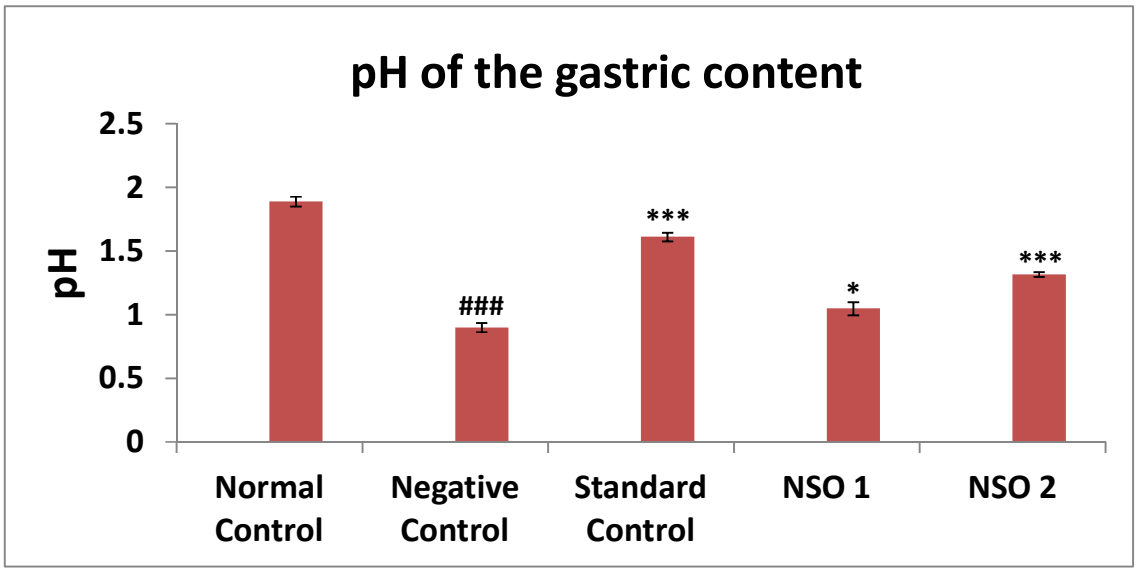

Fig. 1. Showing pH of the gastric content in different experimental groups

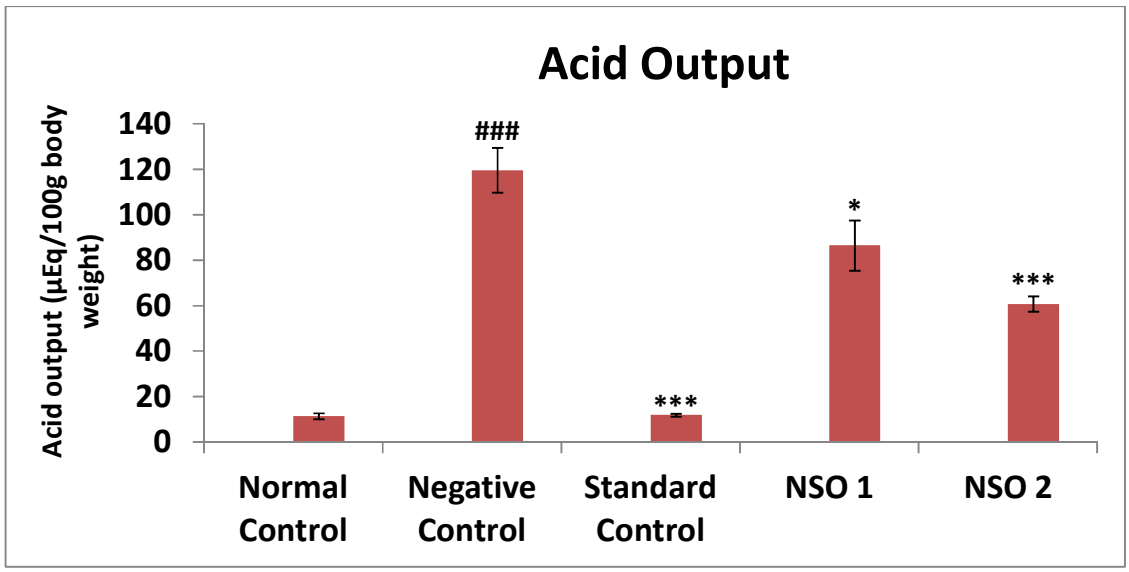

Fig. 2. Showing gastric acid output in different experimental groups

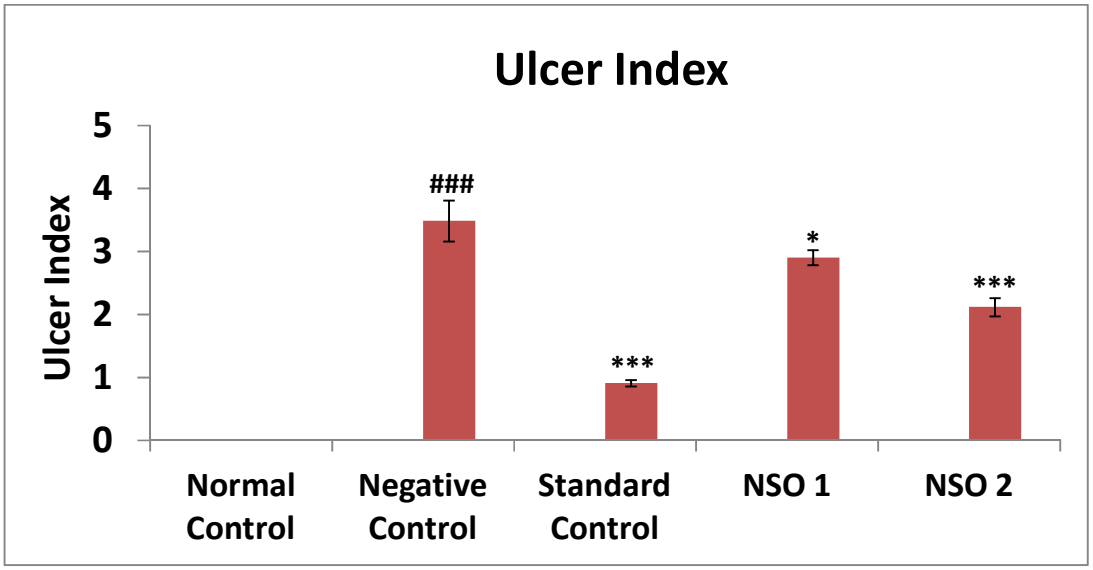

Fig. 3. Showing ulcer index in different experimental groups

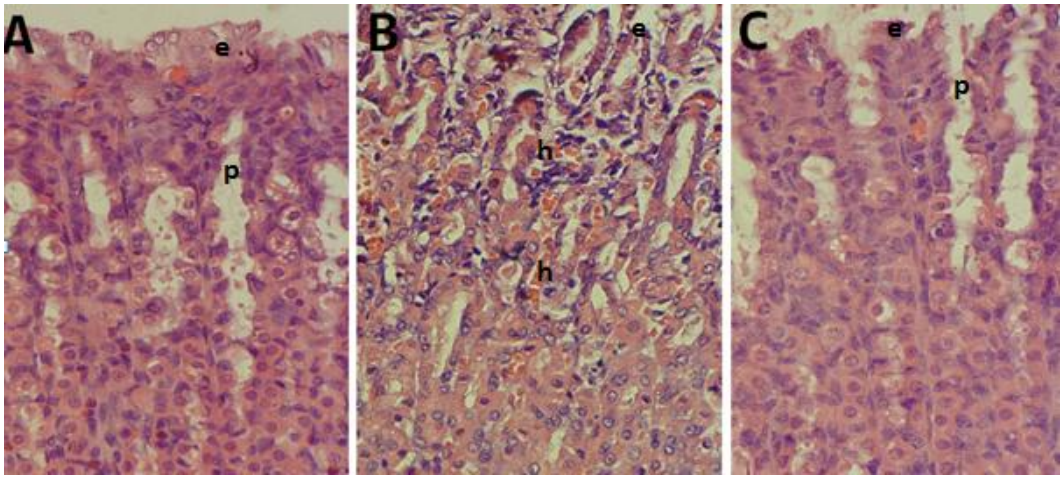

Fig. 4. Microscopic photograph of rat stomach stained with H&E on 400X

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.

In our study we found that the test drug (i.e *Nigella sativa* oil, NSO) showed dose dependent protection against aspirin induced gastric damages. NSO at a dose 2 ml/kg showed maximum protection among test group rats as compared to aspirin only treated rats ($p<0.001$), while NSO at a dose 1 ml/kg showed relatively less protection but has significant protective effect as compared to aspirin only treated rats ($p<0.05$).

The protective effect of *Nigella sativa* oil may be due to its constituents like thymoquinone, nigellone. Thymoquinone is the most important constituent. In previous studies it was found that thymoquinone has propensity to increase mucus production & also has free radical scavenging action [21]. Beside that this thymoquinone has somehow inhibitory effect on H^+-K^+ ATPase pump, this inhibition results in decrease gastric acid production [22]. The constituents of *Nigella sativa* also have property to nitric oxide production which results in local vasodilatation. This local vasodilatation leads to increase in submucosal blood flow. Increase in submucosal blood flow results in early clearance of offending

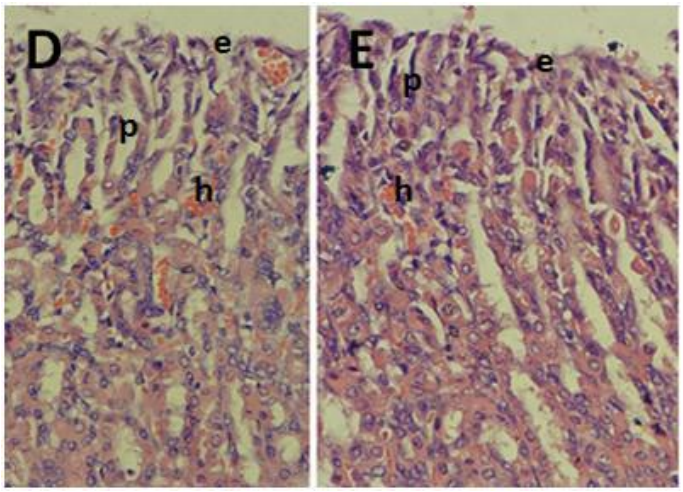

Fig. 5. Microscopic photograph of rat stomach stained with H&E on 400X

D. Nigella sativa oil (1 ml/kg) group showing relatively improved distorted architecture although, the gastric pits (p) are not clearly well defined and some areas of haemorrhage (h). E. Nigella sativa oil (2 ml/kg) group showing relatively few areas of haemorrhage (h), epithelial lining (e) is relatively defined with defined gastric pits

agents from the site of inflammation [23]. So, the probable mechanism of gastroprotection by *Nigella sativa* occurs at various steps. Like it inhibits the H^+-K^+ ATPase pump activity increases the mucous & prostaglandins production and increases the submucosal blood flow. Hence, the gastroprotective effect may be due to the composite effect of all the mechanisms. For complete elucidation of the mechanism further study needs.

4. CONCLUSION

So, on the basis of above findings it may be concluded that *Nigella sativa* oil has protective effect against aspirin induced gastropathy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was taken from Institutional Animal Ethical Committee (IAEC) before starting the study [Registration no. 401/CPCSEA dated 08.05.2012]

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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