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Evaluation of Gastro-Protective Activity of Nigella Sativa Seeds Extract in Wistar Rat

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Abstract: *Nigella sativa is commonly known as black seed traditionally used in various diseases. In this investigation to find out gastroprotective activity in rats. This paper reports on the investigation of the acute toxicity and gastroprotective effect of the Nigella sativa seed extract on Ibuprofen-induced ulcers. The extract did not show acute toxicity in mice treated with 2 g/kg p.o., but exhibited significant antiulcer activity in rats at doses of 300, and 400mg/kg p.o., with the reference drug omeprazole. The results provide evidence for the usage of Nigella sativa as angastroprotective agent, which has been based previously only on ethnopharmacological claims.*

Keywords: Nigella sativa, gastroprotective activity, acute toxicity, herbal plants

I. INTRODUCTION

Peptic ulcer is a disease characterized by the rupture of the protective barrier of the epithelial mucosa of the esophagus, stomach, or duodenum [1]. These lesions can affect the mucosa and other deeper layers of the gastrointestinal wall and damage the muscle tissue, leading to complications such as hemorrhage and perforation [2, 3]. The etiology of gastric ulcers is complex and multifactorial, mainly attributed to an imbalance between protective factors (mucus barrier, cytoprotective prostaglandins, antioxidants, bicarbonate secretion, and appropriate microcirculation) and harmful factors (highly acidic environment in the gastric lumen and pepsin activity) [4].

Furthermore, gastric ulcers may involve exogenous agents such as stress, *Helicobacter pylori* infection, smoking, alcoholism, and prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs). These aggressive factors can increase the production of reactive oxygen species (ROS) that can promote a strong inflammatory response [5]. Despite the protective barrier provided by the epithelial layer, several agents and pathogens can cause inflammation by activating the epithelium, polymorphonuclear neutrophils, and macrophages to produce inflammatory cytokines and other mediators that contribute further to oxidative stress [6]. In addition, the current antiulcer therapy does not focus on these pathophysiological events. Thus, to investigate new, effective, and safe alternatives, extensive pharmacological studies based on natural products have been conducted [7].

Nigella sativa (N. sativa) (Family Ranunculaceae) commonly known as black seed, have been used for thousands of years as a spice and food preservative, as well as a protective and curative remedy for several disorders. Traditionally, there is a common Islamic belief that blackseed is a universal remedy for all ailments, but cannot prevent aging or death. Blackseed is also known as the curative black cumin in the Holy Bible and is described as Melanthion by Hippocrates and Dioscorides and as Gith by Pliny. During the last two decades, many studies have been conducted, on the effect of N. sativa seed extracts on various body systems in vitro or in vivo. Seed extracts reveal a broad spectrum of pharmacological activities including immunopotentiation and antihistaminic, antidiabetic, anti-hypertensive, anti-inflammatory, and antimicrobial activities. Many of these activities have been due to the quinone constituents of the seed.[8]

II. MATERIALS AND METHOD

2.1 Animals

Swiss albino mice weighing between 20-25 g, were obtained from Wockhardt Ltd, Aurangabad. Animals were housed under standard laboratory conditions of temperature 25 ± 1°C with free access to food (Amrut rat and mice feed, Sangli, India.) and water. The experiments were performed during the light cycle (12 12 h). The experiments were carried out

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according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee.

2.2 Drugs and Chemicals

Iboprofen was purchased from Merck, Mumbai. All the solvents, chemicals used were of analytical grade and chemicals required for sensitive biochemical assays were purchased from Merck. All drug solutions were freshly prepared in saline before each experiment.

2.3 Collection and Extraction of Plant Material

Nigella sativa seeds were purchased from a local herb dealer, identified under expert guidance and preserved for future reference. The seeds were dried and ground to a very fine powder and subjected to successive extraction by using different solvent in increasing the order of polarity (pet.ether, chloroform and methanol) in soxhlet apparatus until the eluent became colourless. The prepared extract will be concentrated under reduced pressure and stored in air tight container away from direct sunlight.

2.4 Extractionand Isolation of Flavonoids

Nigella sativa seeds were collected and dried under the shade condition, crushed with thehelp of grinder and stored in the airtight container. The dried crushed Nigella sativa seeds were weighed anddefatted with petroleum ether (60-80 °C) in Soxhlet's extractor. The marc was dried and againextracted with methanol for 72hrs in Soxhlet's extractor. The ethanolic extract was evaporatedusing rotary evaporator. [9]

2.5 Preliminary Phytochemical Evaluation of Nigellasativa Seeds Extract

Testforsaponins

- Foamtest
- Haemolytictest

Testforalkaloids

- Dragendorff'stest
- Mayer's test
- Wagner's test
- Hager's test

Testforflavonoids

- Shinoda test
- Alkaline reagent test
- Zinchydrochloridetest

Test for phenols

Test for Triterpenoids

Libermann-Burchard Test

Sulfur powder test Test for lignins

Thionine test for lignin

2.6 Experimental Protocol

The animals were divided in following experimental groups, each group comprising of six animals...

Group I: Animals served as Control Ibuprofen (200mg/kg, p.o)

Group II: - Animals served as Standard (Omeprazole Tablet 3.6 mg/kg bw)

Group III: Animals served as Test 1 (Low dose)

Group IV: Animals served as Group IV: Test 2 (High dose)

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In-Vitro anti-oxidant activity

1. Determination of DPPH Scavenging Assay

DPPH radical scavenging activity of Nigella Sativa was determine according to the method reported by Blois [19]. An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mm methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula. [10]

% of inhibition = absorbance of control – absorbance of sample / absorbance of control ×100

2. Determination of Fecl3 Scavenging Antioxidant Assay (FSAA):

The ferric chloride scavenging assay was performed according to Benzie and Strain (20) with some modifications. The stock solutions included 300 mm acetate buffer (3.1 g CH3COONa.3H2O and 16 ml CH3COOH), pH 3.6, 10 mM TPTZ (2, 4, 6-Tripyridyl-striazine) solution in 40 mm HCl, and 20 mM FeCl3.6H2O solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml FeCl3.6H2O solution and then warmed at 37°C before using. The solutions of plant samples and trolox were formed in methanol (250 mg/mL). 10 mL of each of sample solution and BHT solution were taken in separate test tubes and 2990 mL of FSAA solution was added in each to make total volume up to 3 mL. The plant samples were allowed to react with FSAA solution in the dark for 30 min. Readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 595 nm. The FRAA values were determined as micromoles of trolox equivalents per mL of sample by computing with standard calibration curve constructed for different concentrations of trolox. Results were expressed in TE µg/ml.[10]

3. Determination of Phosphor- Molybdenum Scavenging Assay:

The antioxidant activity of the nigella sativa seed extract was determined by the phosphormolybdenum Method as described by Prieto et al. 0.3 ml of extract was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 m Mammoniummolybdate). The reaction mixture was incubated at 95ċ for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam) against blank. Methanol (0.3 ml) in place of extract was used as the blank. The total antioxidant capacity was expressed as the number of equivalents of Ascorbic acid (AAE).[10]

4. Toxicity Studies

The acute oral toxicity test was performed following the internationally accepted OECD Test Guideline 420. Ten adult female Sprague–Dawley rats weighing between 180 and 200 g were randomly divided into 2 groups. Animals in the control group received 10 mg/kgOmeprazole via oral gavage, while animals in the test group received a high dose of NSSE (2000 mg/kg). General appearances and behavioral signs were closely monitored. Changes in skin, fur, eyes, and mucous membrane were recorded at 1, 2, 4, and 6 h and every 24 h daily for 14 days. Sacrifices and necropsies were made on the 15th day to examine the gross pathological changes in the internal organs.[11]

5. Pharmacological Studies:

1. Fixed dose method (OECD guideline No. 420) of CPCSEA will be followed to carry out LD50 of the extracts. For this purpose wistar rats of either sex will be used.
2. Evaluation of the extracts for gastroprotective effect by different experimentally induced ulcer models. The experimental models (screening models) will be used in the study are outlined as below:

III. EVALUATION OF GASTROPROTECTIVE ACTIVITY

3.1 Ibuprofen-Induced Gastric

Ibuprofen induced Gastric Ulcer some adaptations were applied to the method described by Nwafor et al., with the exception of the substance used to induce gastric ulcer; 200 mg/kg of Iboprofen in 0.5% carboxymethyl cellulose was administered via intragastric gavage. After 5 h, the rats in all groups were euthanized and measured for gastric ulcer.[12]

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IV. INVESTIGATION OF GASTROPROTECTIVE MECHANISMS

4.1 Investigation of Gastroprotective Mechanisms

A. Pylorus Ligation

After 48 h of fasting, the rats were randomly allocated into 3 groups (n = 6 each). The control group received omeprazole 10 mg/kg via intragastric gavage. The test group received NSSE at a dose of 400 mg/kg. One hour later, pyloric ligation was performed . The gastric content was collected from all 18 rats and centrifuged to measure the volume, pH, and total acidity. Titration with 0.1 N sodium hydroxide was performed using phenolphthalein.[12]

B. Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by the post hoc least-significant difference (LSD) test was used to determine the statistical significance ($p < 0.05$) of the differences among groups. Student's t-test was used to determine the statistical significance ($p < 0.05$) from the control group.[13]

V. RESULTS AND DISCUSSION

Phytochemical Investigation

Table 1: The phytochemical investigation for various chemical constituents in Nigella sativa seeds extract is given below.

Chemical constituents	Name of the test	Procedure	Observation	Inference
Flavonoids	Shinoda test	2-3 ml of Extract + few drops of cone. HCI+0.5 gm magnesium turnings	Slight pink color	Flavonoids present
	Lead acetate test	2-3 ml of Extract lead acetate	Yellow color ppt	Flavonoids present
	NaOH test	2-3 ml of Extract + increasing amt. of NaOH	Yellow color which decolorized on addition of acid	Flavonoids present
	$FeCl_3$ test	-3 ml of extract+5% Fe Chution	Deep blue color2	Tannins & phenolic comp. present
	HNO_3TEST	2-3 ml of Extract +Dil HNO_3	Reddish yellow color	Tannins & phenolic comp. present
	Acetic acid test	2-3 ml of Extract+ Acetic acid solution	Red color	Tannins & phenolic comp. present
Tannins & phenolic comp.	$KMnO_4$	2-3 ml of Extract+ $KMnO_4$ solution	Decolorisation $KMnO_4$	Tannins & phenolic comp. present
Alkaloids	Mayer test	2-3 ml of Extract+ mayer's solution	Cream color ppt	Alkaloids present
	Dragondroffs test	2-3 ml of Extract+ Dragondroffs reagent	Orange color ppt	Alkaloids present
	Wagner test	2-3 ml of Extract+ Wagner reagent	Raddish brown ppt	Alkaloids present
	Hagers test	2-3 ml of Extract+ Hagers reagent	Yellow color ppt	Alkaloids present

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Table 2: Experimental group of animals and corresponding treatment given

GROUP	TREATMENT
1	Control
2	Standard(Omeprazole)
3	NSSE 300 mg/kg
4	NSSE 400 mg/kg

Antioxidant Activity

1. DPPH (2,2-diphenyl-1-picrylhydrazyl)radical scavenging activity:

Free radical scavenging activities of the extract of Nigella sativa seed was assessed by the DPPH assay. It was significant decrease in the concentration of DPPH radical due to scavenging potential of the extract. The results show that extract of Nigella sativaseed had the highest DPPH scavenging activity with an IC50 value 27.62±0.26 µg/ml., and percentage of DPPH free radical inhibition. (fig. 1)

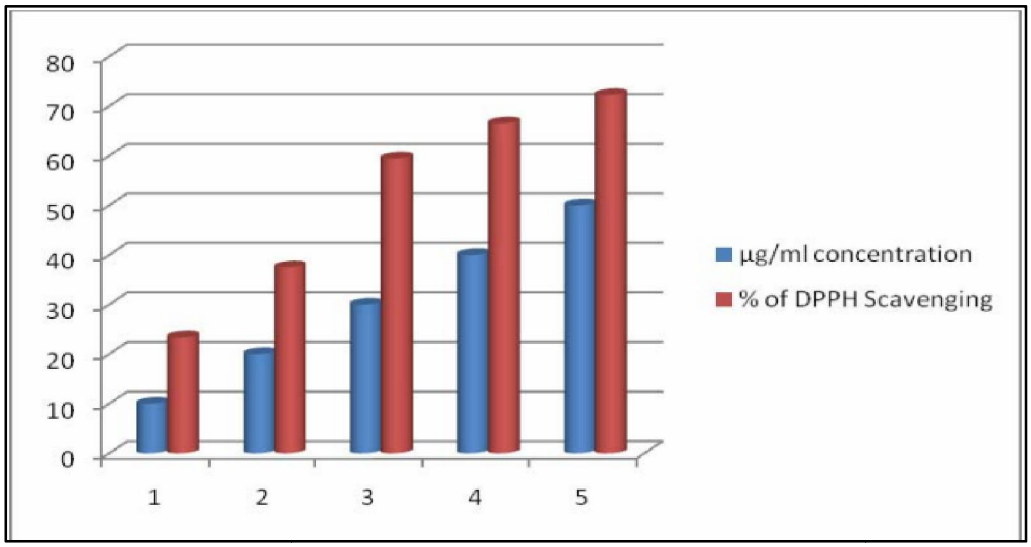

Figure 1: %DDPH radical scavenging activity of methonolic extract of Nigella sativa seed

2. Fecl3 Radical Scavenging Activity

Free radical scavenging activities of the extract of Nigella sativa seed was assessed by the Fecl3 assay. It was significant decrease in the concentration of Fecl3 radical due to scavenging potential of the extract. The results show that extract of Nigella sativaseed had the highest Fecl3 scavenging activity with an IC50 value 48.39±1.20 µg/ml., and percentage of Fecl3 free radical inhibition (fig. 2)

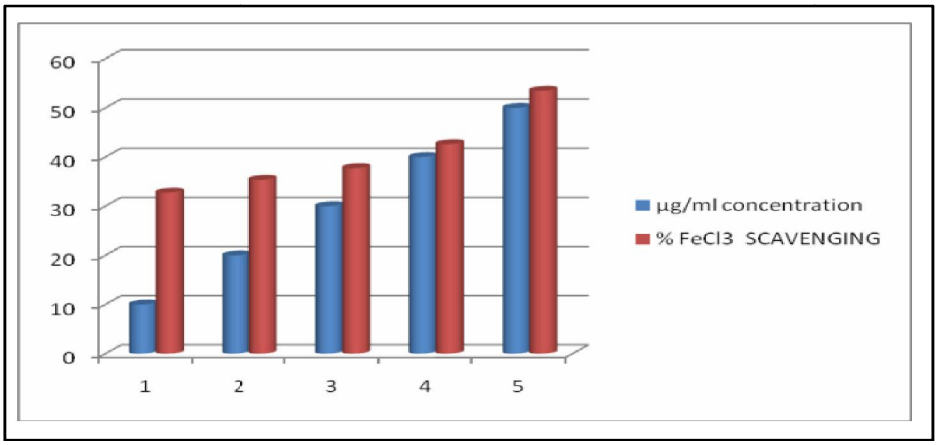

Figure 2: $FeCl_3$ radical scavenging of methonolicextract of Nigella sativa seed

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3. Phosphor-Molybdenum Radical Scavenging Activity

Free radical scavenging activities of the extract of Nigella sativaseed was assessed by the phosphor-molybdenum assay. It was significant decrease in the concentration of phospho-molybdenum radical due to scavenging potential of the extract. The results show that extract of Nigella sativa seed had the highest phosphor-molybdenum scavenging activity with an IC50 value 32.36±0.22 µg/ml., and percentage of phosphor-molybdenum free radical inhibition (fig.3)

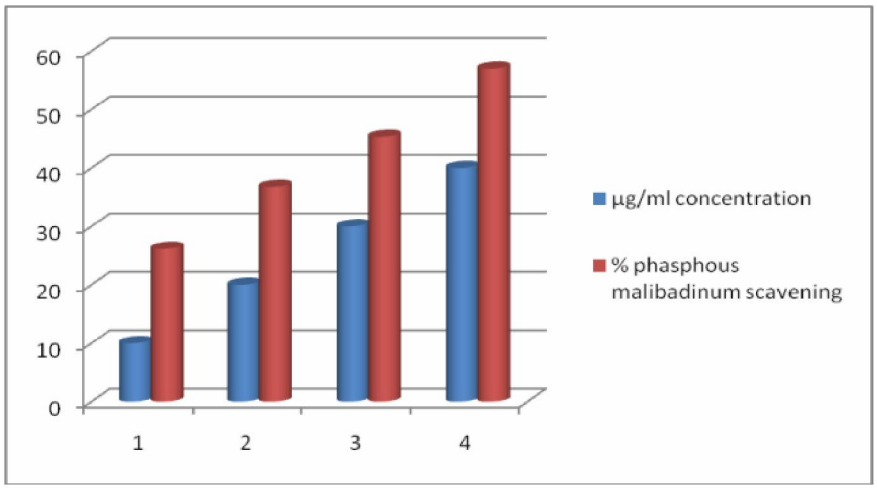

Figure 3: %phosphor-molybdenum radical scavenging activity of methonolic extract of Nigella sativa seed

Gastroprotective Activities of NSSE in Rats

Effect of NSSE on Ibuprofen-Induced Gastric Ulcer

Damage of gastric glandular mucosa caused by Ibuprofen was visible in the control group with an ulcer index of 9.70 ±2.75 mm. On the contrary, the rats that received pre-treatment with either omeprazole or NSSE at all doses displayed significantly fewer gastric lesions. The maximum ulcer inhibition of 9.70 ±2.75 (78.48%) was seen at the dose of 400 mg/kg, while the inhibition rate of omeprazole was 85.34% (Table 3).

Table 3: Effect of NSSE on gastric mucosa in the Ibuprofen-induced gastric ulcer.

Sr no	Group	Dose (mg/kg)	Ulcer index(mm)	Inhibition
1	Control	-	9.70 ±2.75	-
2	Standard(omeprazole	10	1.55±0.55	85.34 %
3	NSSE Dose 1	300	4.38±0.22	54.77%
4	NSSE Dose 2	400	2.57±1.30	78.48%

Data are represented as mean ±SEM(n=5).One way analysis of variance (ANOVA)followed by the post hoc least significant difference (LSD) test was used to determine the significant difference from the control group *p<0.05
NSSE=*NIGELLA SATIVA* Seed extract

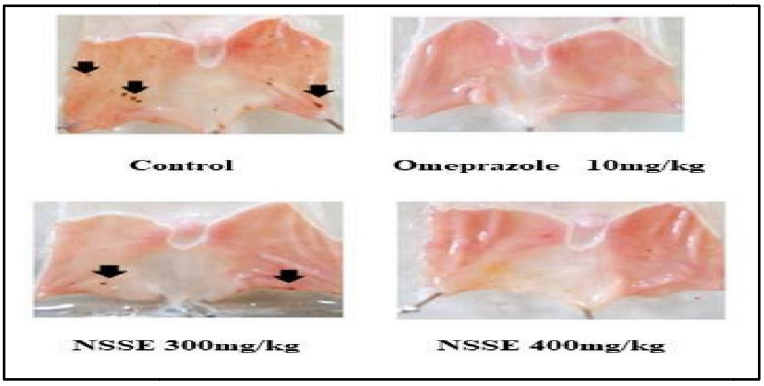

Figure 4: Comparison of the effects of pre-treated omeprazole at 10 mg/kg and NSSE at 300, and 400 mg/kg on gastric surface from 3 different gastric ulcer models in rats versus control: acidified ethanol-induced gastric ulcer; Black

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arrows indicate the characteristic necrotic bands forming gastric ulcers or the spots and small erosions forming gastric ulcers.

Mechanisms of Gastro-protection of NSSE

Effect of NSSE on Gastric Secretion Following Pyloric Ligation in Rats

After ligation of pylorus, the gastric volume and total acidity of the NSSE group were slightly decreased, whereas the gastric pH was slightly increased. However, these findings are insignificant when compared to those of the control group (Table 4). Only the omeprazole-pre-treated group showed significant elevation of gastric pH and reduction in gastric volume and total acidity.

Table4: Effect of NSSE on Gastric Secretion Following Pyloric Ligation in Rats

Sr no	Group	Gastric volume	Gastric pH	Totlly acidity(mEq/100 g/h)
1	Control	4.38±0.26	2.47±0.35	3.69±1.37
2	Standard(omeprazole 10 mg/kg)	2.65±0.26*	5.70±1.35*	0.54±0.01*
3	NSSE Dose 1(300 mg/kg)	2.52±0.22	1.78±0.61	1.45±1.81
4	NSSE Dose 2(400 mg/kg)	3.94±0.12	2.45±0.51	2.94±1.35

Data are represented as mean ±SEM(n=5).One way analysis of variance (ANOVA)followed by the post hoc least significant difference (LSD) test was used to determine the significant difference from the control group *p<0.05
NSSE=*NIGELLA SATIVA* Seed extract

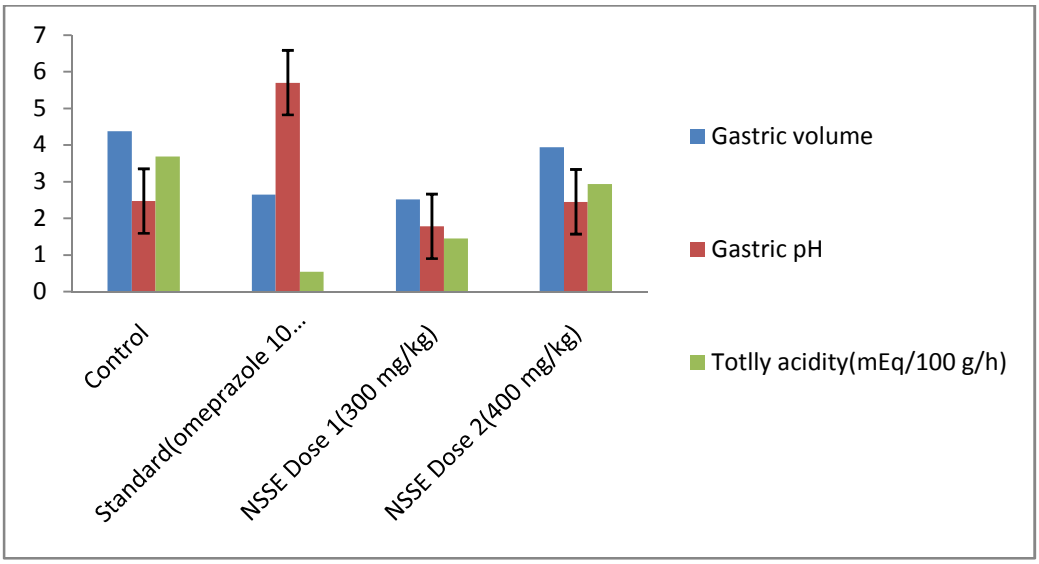

Fig 5: Effect of NSSE on Gastric Secretion Following Pyloric Ligation in Rats

Data are represented as mean ±SEM(n=5).One way analysis of variance (ANOVA)followed by the post hoc least significant difference (LSD) test was used to determine the significant difference from the control group *p<0.05
NSSE=*NIGELLA SATIVA* Seed extract

VI. CONCLUSION

The results of the present study showed that the Nigella sativa seed extract shows gastroprotective activity in ratsin comparision of omeprazole. These results support the traditional belief about the beneficial effect. Further studies are required for determining (confirming) the protective effect of *NS.*

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