https://doi.org/10.48047/AFJBS.6.14.2024.6354-6368

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

ULCER-PROTECTIVE EFFECT OF *NIGELLA SATIVA* OIL IN INDOMETHACIN PLUS PYLORUS LIGATION INDUCED GASTRIC ULCERS IN RATS

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Volume 6, Issue 14, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.6354-6368

ABSTRACT

Aim:- The ulcer protective potential of *Nigella sativa* oil (NSO) was evaluated in acute gastric ulcer model in albino wistar rats induced by Indomethacin and pylorus ligation (PL).

Experimental Procedure:- CMC(1%) [1ml/kg/day] was used as a vehicle as well as in normal control group. Three toxic groups i.e. pylorus ligated, Indomethacin (20mg/kg/p.o.) treated and Indomethacin plus PL rats, were developed. Omeprazole (10mg/kg/p.o.), a proton-pump inhibitor (PPI) anti-ulcer drug, was used as a standard drug in standard group. The effect of test drug NSO (2.5ml/kg/p.o.) and the Omeprazole(standard) (10mg/kg/p.o.) were compared with toxic groups using evaluation parameters like Ulcer index (UI), gastric wall mucus determination, total acidity, gastric juice volume and biochemical estimation of reduced glutathione (GSH) in blood as parameters.

Results:- The Indomethacin (20mg/kg/day, p.o.) treated PL rats, produced substantial ($p<0.01$) increase in levels of blood GSH , mean ulcer index and mean total acidity and substantial decrease ($p<0.01$) decrease in the mean gastric wall mucus thickness but less substantial ($p<0.05$) increase in mean gastric juice volume was seen. NSO treatment (2.5 ml/kg/day, p.o.) showed moderate ulcer protective effects as evident by substantial ($p<0.01$) reduced in blood GSH levels, mean total acidity, gastric juice volume and UI. The gastric mucus thickness was increased substantially ($p<0.01$) with NSO treatment.

Conclusion:- The results exhibits that oral treatment with *Nigella sativa* oil exhibits ulcer protective characteristics by increasing the gastric mucosa and decreasing the acidity of gastric juice.

Keywords: - *Nigella sativa* oil (NSO), Pylorus ligation (PL), Reduced Glutathione level (GSH), Ulcer index (UI), Omeprazole

INTRODUCTION

Peptic ulcer disorder has become a substantial cause of morbidity and mortality for more than a century. The pathophysiology of peptic ulcer disease is based on an imbalance of violent and protective stomach factors[1].

Peptic ulcer, which is the beginning of the small intestine, is a sore on the lining of the stomach or duodenum. In less general terms, peptic ulcers can form in the esophagus just above the stomach, a tube that connects the mouth to the stomach. Peptic ulcers are considered gastric ulcers in the stomach. A duodenal ulcer is the one which arises in the duodenum. At the same time, people may have both gastric and duodenal ulcers[2]. Symptoms of peptic ulcers cause intestinal irritation and inflammation 2-3 hours after following a meal or on an empty stomach. Such signs include weight loss, reduced appetite, swelling, fatigue, and vomiting. Some can also experience blood in stools and vomiting, and black stools that suggest gastrointestinal bleeding.

The underlying causes of peptic ulcers are complex and multifaceted. The disorder's development is influenced by a combination of factors including excessive acid secretion and a compromised mucosal membrane. The etiological factors range from H. pylori infection to an imbalance in aggressive agents like pepsin, hydrochloric acid, and bile reflux, as well as the generation of free radicals, stress, consumption of spicy foods, lifestyle habits, alcohol intake, 5-hydroxy tryptamine, platelet-stimulating elements, medications, and cytoprotective elements. These protective factors encompass activities like maintaining the mucous-bicarbonate barrier, promoting prostaglandin production, ensuring surface-active phospholipids, sustaining mucous blood flow, facilitating cell renewal and migration, fostering enzymatic and non-enzymatic antioxidant defenses, and facilitating growth factor interactions.[3-5].

The lifetime chance of developing peptic ulcer is roughly 10%. It can occur more than once in a man’s lifetime. About half a million individuals develop in the United States per year and 10% of the adult population is projected to develop in western countries during their lifespan [6]. Gastric ulcer is a global issue affecting 4-10% of the world's population with a fairly high prevalence in India. Peptic ulcer originates because of unknown reason [7]. The highest prevalence (56.5%) of peptic ulcer in India was found to be among the semi-skilled employees and the lowest (2.5%) among management and technical classes [6].

Various drugs have been employed in the treatment of ulcers, although only a few were initially utilized. Over time, these treatments have stood the test of time. The most effective medications are those that target the reduction of gastric acid release. H_2 receptor antagonists have significantly transformed the management of peptic ulcers, facilitating ulcer healing and sustaining remission through maintenance therapy [8][9]. Subsequently, proton-pump inhibitors (PPIs), introduced in 1989, emerged as more potent options, gradually replacing antacid medications [10]. PPIs selectively inhibit the H^+K^+ ATPase in parietal cells, forming a cornerstone of ulcer therapy by correlating ulcer healing rates with the degree of acid suppression. While ulcers often resurface post-healing, the groundbreaking introduction of Helicobacter pylori (H. pylori) eradication therapy has extended acid suppression maintenance [10].

The second category of drugs is aimed at strengthening the mucosal barrier and has been established as a defense against NSAIDs in its main application. The most commonly used agent was Misoprostol, a prostaglandin analogue, but it is limited to abdominal side effects (like diarrhea), especially at higher doses. Salts of sucralfate and bismuth also facilitate the recovery of ulcers by enhancing mucosal repair.[11]. In light of other increasingly successful treatments, cytoprotective therapies have been

obsolete. Present ulcer therapy consists of *H pylori* eradication of H pylori-positive peptic ulcer and PPIs for recovery and avoidance of drug induced gastro-toxic effect, but due to the use of this drug there is also a recurrence problem. Therefore, modern and powerful medications are required for very successful care. [12].

Despite recent interest in the discovery of drugs through molecular simulation, combination chemistry and other synthetic chemistry approaches, plant medicines are still proving to be an invaluable source of human medicines. At present, at least 120 distinct chemical compounds are obtained from plants that are known to be essential herbal drugs. According to the methodological evidence available in the USA, 41% of pharmaceuticals derived from herbal medicines. Since the last two decades in the United States, growing consumer frustration with the expensive prescription drugs and their side effects, along with a sudden interest to reforming and reuse of natural remedies, has resulted in increased demand for herbal medicines [13].

Many herbal medicines have been proved experimentally to be equally effective in the treatment of peptic ulcer disease (PUD), like Aloe vera (*Aloe barbadensis*) [14], Ginger (*Zingiber officinale*) [15], black cumin seeds (*Nigella sativa*) [16], Banana (*Musa paradisciaca*) [17], Fennel (*Foeniculum vulgare*) [18], Turmeric (*Curcumin longa*) [19], Holy basil or tulsi(*Ocimum tenuiflorum*) [20] and other spices.Various preparation in Ayurvedic and Unani medicines containing the above mentioned plants have been proved to be effective and is widely used in the treatment of gastric ailment.

Drug consists of dried *Nigella sativa* beans. The herb is spread and often grown in the tropical subtropical regions of India [21]. In the Middle Eastern countries, Nigella sativa is traditionally known as 'Habib al Barakah'' the Blessed Seed' and the Prophet's medicine because of its good healing properties for many diseases. It has been used in the Middle East as well as in ports in Asia and Africa for thousands of years, and is now well known in the USA and Europe. Widely referred to as Kalaunji, Mangaraila, Black seed. Black Crop, Upakuncika (Sanskrit) and Habat Albaraka (Arab). According to the Arab proverb, 'Black seed is a cure for any disease except death.'[22]

Nigella Sativa belongs to the Ranunculaceae family. Its seeds are characterized by their flattened, oblong, funnel-shaped structure, measuring about 0.2 cm in length and 0.1 cm in width. They are black in color, possess a distinct odor, a mildly aromatic quality, and a bitter taste [23]. Within literature, Nigella Sativa is attributed with various properties including being a carminative, diaphoretic, digestive aid, diuretic, emmenagogue, excitant, lactagogue, laxative, resolvent, stimulant, stomachic, tonic, and vermifuge. In Ayurveda and Unani medicinal systems, it is additionally recognized as an abortifacient and diuretic. Moreover, it is employed for the treatment of ailments such as ascites, coughs, eye sores, hydrophobia, jaundice, paralysis, piles, and tertian fever[23].

Seeds of Nigella Sativa contain diverse esters of structurally distinct unsaturated fatty acids along with terpene alcohols (approximately 7%). Additionally, trace amounts of alkaloids are present, falling into two distinct categories: isochinoline alkaloids, specifically nigellimin and nigellimin-N-oxide, and pyrazole alkaloids, which encompass nigellidin and nigellicin. In the essential oil fraction (averaging around 0.5% and peaking at 1.5%), thymoquinone has been identified as the primary component, constituting up to 50% of the oil composition. Additionally, p-cymene accounts for 40%, pinene makes up to 15%, and minor components such as di-thymoquinone and thymohydroquinone are also detected. A few terpene derivatives are present in trace quantities, including carvacroi, carvone, limonene, 4-terpineol, and citronellol [24].

Nigella sativa oil have been traditionally found useful in a number of diseases like asthma, cough, lethargy, nervous tension, backache, rheumatoid arthritis, high blood pressure, stomach complaint, diarrhea, ear-ache, hair loss, sinusitis and many more[25]. Experimentally *Nigella sativa* oil have been

found very effective in the treatment of certain ailments like diabetes [26], hypertension [27], rheumatoid arthritis [28], osteoporosis [29], neurological and mental illness [30], cardiovascular diseases [31]. But it was studied only in ethanol-induced ulcer [32].

Preclinically, *Nigella Sativa* oil (NSO) has been studied for its gastroprotective effect in ethanol induced and ischemia reperfusion injury [33], but surprisingly it has not been studied in Indomethacin induced gastric ulcer. After *H. pylori* positive gastric ulcer, NSAIDS induced ulcer is the second most common type of disorder[32]. Many experiments proved that free radicals and lipid peroxidation contributes mainly in the formation of gastric ulcer. Further *Nigella sativa* oil (NSO) has been proven for free radical scavenging, lipid peroxidation inhibitory and cytoprotective effect [34]. So it will be fruitful to assess the gastroprotective effect of *Nigella sativa* oil (NSO) in Indomethacin-induced gastric ulcer.

Owing to the wide-spread use of aspirin and other NSAIDs, the prevalence of peptic ulcer disease and its associated complications remains a significant concern. In cases of ethanol-induced ulcers, the underlying mechanism primarily involves ischemia reperfusion and the generation of free radicals, without necessitating the inhibition of prostaglandins. On the contrary, the development of gastric ulcers due to Indomethacin occurs through various pathways, which encompass the inhibition of prostaglandin synthesis in conjunction with the formation of free radicals. Notably, a study by Martin et al. in 1994 highlighted that Naringin exhibited more pronounced antiulcer activity against ethanol-induced gastric ulcers, yet its effectiveness was comparatively limited against ulcers induced by Indomethacin. Consequently, in the current analysis, the model of Indomethacin-induced gastric ulcers was selected, given the widespread usage of NSAIDs, with the aim of exploring whether Nigella sativa oil (NSO) could exert any impact on such ulcers. It's important to acknowledge that demonstrating the potency and therapeutic attributes of herbal medicines often requires additional time for empirical validation.

Indomethacin, classified as an NSAID, is commonly prescribed to address conditions like arthritis, gout, and myocardial infarction. However, its usage among patients suffering from these ailments can lead to the development of peptic ulcer disease (PUD). Therefore, the primary objective of the current study was to assess the potential gastroprotective impact of Nigella sativa oil (NSO) in experimental rats afflicted with Indomethacin-induced gastric ulcers [32].

MATERIALS AND METHODS

Animals

Albino Wistar rats, irrespective of gender, with a weight ranging between 150-200g, were utilized for the study. The rats were housed in cages, ensuring their well-being, and the ambient temperature was maintained at an optimal 25±5 degrees Celsius. A standard light-dark cycle of 12 hours was upheld. The rats were provided with a pellet diet and unrestricted access to water. Prior to commencing the experiments, the rats were given ample time to acclimate to the laboratory conditions. Ethical clearance from the Institutional Animal Ethics Committee (IAEC), specifically from the Faculty of Pharmacy at Integral University (IU/M.Pharm./CPCSEA/10/23/JULY 15, 2011), was acquired before initiating the study.

Chemicals and Drugs Required

Alcian blue, Carboxy methyl Cellulose, diethyl ether, disodium EDTA, disodium o-phosphate, hydrochloric acid, magnesium chloride, m-phosphoric acid, phenolphthalein indicator, potassium chloride, sodium acetate, sodium chloride, sodium citrate, sodium hydroxide, and sucrose. All the

chemicals used in the present study were of analytical grade. The drugs used are Indomethacin, *Nigella sativa* oil, Omeprazole.

The *Nigella sativa* Oil (NSO) is procured from the local market and stored in refrigerator at 4℃. A specimen sample of the same was preserved in the section of the Faculty of Pharmacy, Integral University, Lucknow, for future reference. The drug was administered according to protocol.

Dosage

Acute toxicity of *Nigella sativa* fixed oil (NSO) has been studied in mice. The LD50 values obtained when administered orally and intraperitonally were 28.8 ml / kg body wt. p.o (26.2-31.6) and 2.06ml / kg body weight (1.86-2.26) respectively. Chronic toxicity was examined in rats treated daily with an oral dosage of 2 ml / kg body wt. for twelve weeks. No substantial changes were found in the liver, kidney, heart and other organs after 12 weeks of treatment with NSO. The low toxicity of NSOs with a high range of LD50 values and organ stability mark the safety margin of its use as a therapeutic drug [35].

Experimental Procedure

For the evaluation of the antiulcerogenic activity of Nigella sativa oil (NSO), 36 albino wistar rats were taken and divided into six groups, each consisting for six animals. All rats were deprived of food but not water for 36 hours before sacrifice. Group I, II, III & IV received 1% CMC(1ml/kg, p.o) for 10 days whereas Group V & VI received pre-treatment with Omeprazole(10mg/kg, p.o) and *Nigella sativa* oil (NSO) (2.5ml/kg, p.o) respectively. Indomethacin was administered in all the groups, except I & II before 6 hours of sacrifice. Group II received 1% CMC (1ml/kg, p.o) while Group IV & V received Indomethacin 2 hours before pyloric ligation. Pyloric ligation was performed on the 11^{th} day in groups II, IV V & VI under ketamine anaesthesia [36] and water was withheld. Gastric juice was allowed to accumulate for the next 4 hours. Group II, IV, V & VI were sacrificed after 4 hours of pyloric ligation by cervical dislocation method. The stomach was then removed after clamping the esophagus and gastric juice was collected in centrifuge tube for the measurement of total acidity. Group I & III were sacrificed after 6 hours of the prescribed treatment by the same cervical dislocation method. After this the stomachs were weighed and immediately immersed in alcian blue solution, for the determination of gastric wall thickness. After that, a small portion of the stomach was taken and kept in 10% formalin solution for histopathological study. Rest of the portion of the stomach was homogenized in EDTA for the assessment of other parameters like ulcer index and tissue glutathione.

Reduced Glutathione Estimation

Briefly, 100 µl of freshly collected blood was mixed with 0.9 ml of distilled water, and a precipitating solution consisting of Glacial Metaphosphoric acid (1.67 g), Na_2EDTA (0.2 g), and NaCl (30 g) in 100 ml of distilled water. This mixture was promptly blended. After allowing a 5-minute incubation at room temperature, the resulting mixture was subjected to centrifugation (3000g at 4℃ for 15 minutes). To 500 µl of the clear supernatant, 2 ml of a 0.3 mol/l phosphate solution and 250 µl of DTNB solution (200 mg in 100 ml of 1% sodium citrate solution) were added and thoroughly mixed. A blank solution was prepared using 1 ml of 0.3 mol/l phosphate solution, 1 ml of distilled water, 0.5 ml of the precipitating solution, and 250 µl of DTNB solution. The spectrophotometric readings for both the blank and sample reaction mixtures were taken against water at 412 nm using a Shimadzu UV-1700 spectrophotometer.

Statistical Analysis

Data analysis was conducted using Microsoft Excel 2007 (Windows XP) to calculate the mean and standard deviation. Statistical evaluations involved ANOVA followed by Dunnett's t-test. The presented values are represented as Mean ± SEM. A significance level of $p < 0.01$ was deemed noteworthy.

RESULTS

Several experiments have been conducted in all classes of experimentally induced gastric ulcers in rats to determine the efficacy of Nigella sativa oil on indomethacin-treated pylorus-ligated induced gastric ulcers in rats to determine alleviate blood glutathione (GSH) levels, gastric wall mucus (barrier mucus), ulcer index, overall acidity and gastric juice volume.

The following pharmacological effects were observed:

1. Effect on ulcer index

A significant rise ($p < 0.01$) in the mean ulcer index was observed in rats treated with CMC + pylorus ligation (Group II), Indomethacin (Group III), and the combination of Indomethacin and pylorus ligation (Group IV), in comparison to the mean ulcer index of the healthy rats in the normal control group (Group I).

Furthermore, there was a noteworthy increase ($p < 0.01$) in the mean ulcer index in the Indomethacin-treated pylorus ligated rats (Group IV) and the rats treated only with Indomethacin (Group II) when compared to the rats treated with CMC + pylorus ligation (Group II). Conversely, a substantial decrease ($p < 0.01$) was observed in the blood GSH levels of Group V rats (treated with Omeprazole, 10mg/kg, p.o.) and Group VI rats (treated with NSO, 2.5 ml/kg, p.o.) compared to the blood GSH levels in the Indomethacin-treated pylorus ligated rats (Group IV).

2. Gastric wall mucus

A significant reduction ($p < 0.01$) in the mean thickness of the gastric wall mucus was observed in rats subjected to CMC + pylorus ligation (Group II), Indomethacin treatment (Group III), and the combined Indomethacin and pylorus ligation treatment (Group IV), compared to the mean gastric wall mucus thickness of the healthy rats in the normal control group (Group I).

In rats treated with Indomethacin (Group III), there was no substantial alleviation ($p > 0.05$) in the mean gastric wall mucus thickness. However, in Indomethacin-treated pylorus ligated rats (Group IV), the alleviation was slightly significant ($p < 0.05$) compared to the rats treated with CMC + pylorus ligation (Group II). Conversely, there was a noteworthy increase ($p < 0.01$) in the mean gastric wall mucus thickness in Group V rats (treated with Omeprazole, 10mg/kg, p.o.) and Group VI rats (treated with NSO, 2.5 ml/kg, p.o.) in comparison to the blood GSH levels in the Indomethacin-treated pylorus ligated rats (Group IV).

3. Effect on total acidity of gastric juice

A substantial increase ($p < 0.01$) in the mean total acidity of gastric juice was observed in pylorus ligated rats that were administered Indomethacin (Group IV rats), in comparison to the mean total

acidity of gastric juice in rats treated with CMC + pylorus ligation (Group II). Conversely, there was a significant reduction ($p < 0.01$) in the mean total acidity of gastric juice in Group V rats (treated with Omeprazole, 10mg/kg, p.o.) and Group VI rats (treated with NSO, 2.5 ml/kg, p.o.) compared to the mean total acidity of gastric juice in pylorus ligated rats treated with Indomethacin (Group IV).

4. Effect on volume of gastric juice

A relatively modest increase ($p < 0.05$) in the mean gastric juice volume was observed in pylorus ligated rats treated with Indomethacin (Group IV), compared to the mean gastric juice volume in rats treated with CMC + pylorus ligation (Group II). Conversely, there was a significant reduction ($p < 0.01$) in the mean gastric juice volume in Group V rats (treated with Omeprazole, 10mg/kg, p.o.) and Group VI rats (treated with NSO, 2.5 ml/kg, p.o.) in comparison to the mean gastric juice volume in pylorus ligated rats treated with Indomethacin (Group IV).

Table 1: Effect of Nigella sativa oil on blood GSH, gastric wall mucus, ulcer index, total acidity and vol. of gastric juice

Groups	Ulcer Index score	GSH (mg/100 ml blood)	Total acidity (meq/lit)	Vol. of gastric juice(ml/100g)	Gastric wall Mucus (µg/g of stomach wt.)
I Vehicle control CMC [1ml (1%)/kg/day, p.o]	0.00	14.97 ± 0.10	_	_	21.62 ± 0.42
II CMC [1ml (1%)/kg/day, p.o.]+ pylorus ligation	10.53 ± 0.13$^{\$\$}$	25.17 ± 2.35$^{\$\$}$	33.09 ± 0.09	1.17 ± 0.10	16.25 ± 0.36$^{\$\$}$
III Indomethacin (20mg/kg/day, p.o.)	20 ± 0.50$^{\$\$,\#\#}$	30.98 ± 4.56$^{\$\$,\#}$	_	_	16.36 ± 0.23$^{\$\$,n.s.}$
IV Indomethacin (20mg/kg/day, p.o.)+ pylorus ligation	25.17 ± 0.16$^{\$\$,\#\#}$	35.57 ± 7.81$^{\$\$,\#\#}$	45.82 ± 0.18$^{\#\#}$	1.75 ± 0.25$^{\#}$	12.54 ± 0.33$^{\$\$,\#}$
V Omeprazole(10mg/kg/day) + Indomethacin (20mg/kg/day, p.o.)+ pylorus ligation	12.13 ± 0.20**	14.09 ± 0.36**	21.1 ± 0.10**	0.65 ± 0.15**	24.75 ± 2.09**
VI Nigella sativa Oil (2.5 ml/kg/day, p.o.) + Indomethacin (20mg/kg/day,p.o)+ pylorus ligation	14.31 ± 0.31**	15.33 ± 0.72**	21.17 ± 0.17**	0.8 ± 0.20**	22.12 ± 0.52**

APPENDICES

The comparisons were done by ANOVA followed by Dunnett's t test. All values are expreseed as Mean ± SEM, n=6

$$ p< 0.01, as compared to vehicle control group (i.e. group I)

n.s. p>0.05 (non substantial) as compared to CMC + pylorus ligation treated group (i.e. group II)

 # p<0.05, as compared to CMC + pylorus ligation treated group (i.e. group II)

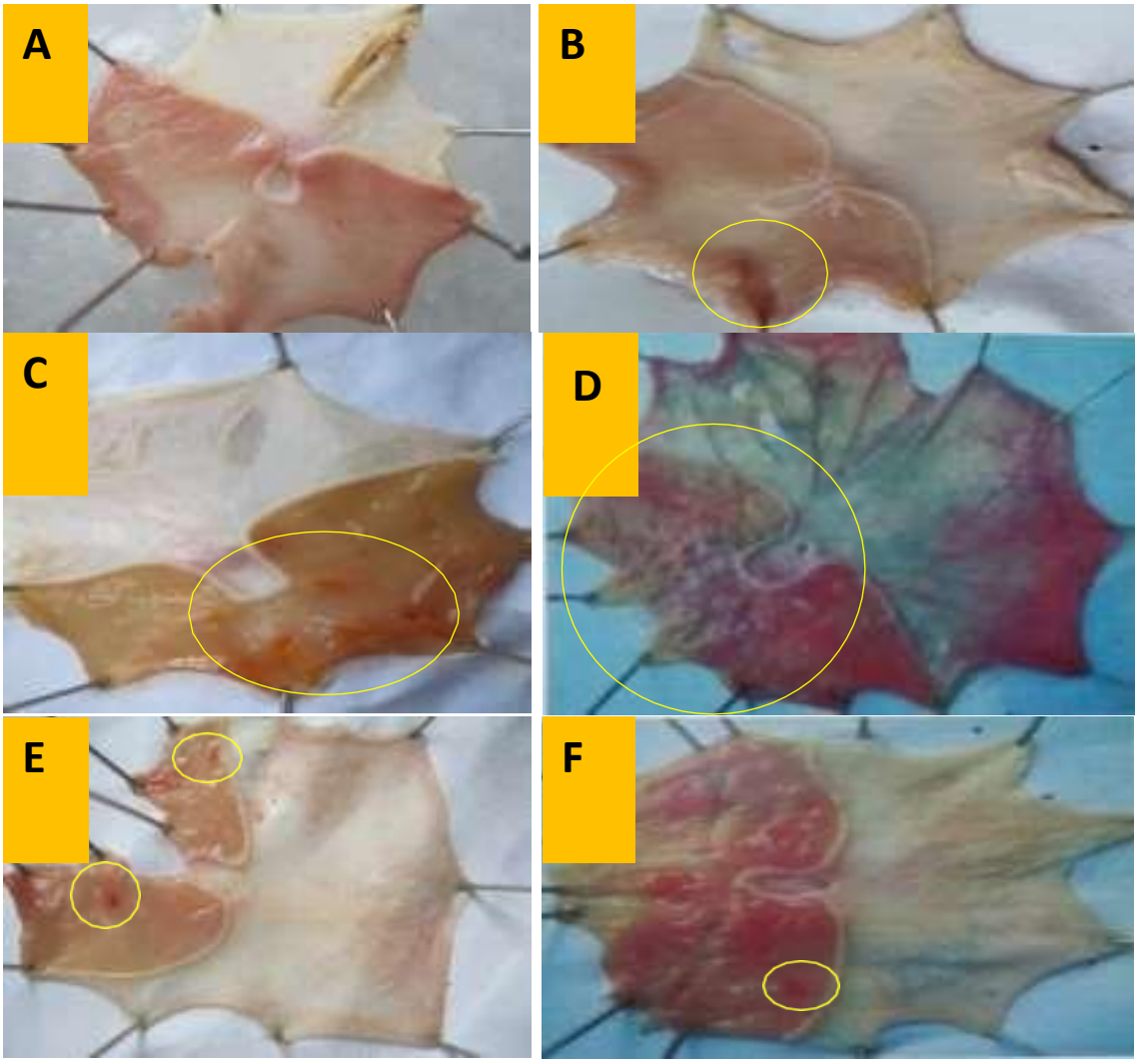
p<0.01, as compared to CMC + pylorus ligation treated group (i.e. group II)

**** p< 0.01,as compared to indomethacin +pylorus ligation treated c group(i.e. group IV)**

5. Effect on Reduced Glutathione level in blood (GSH)

A significant elevation ($p < 0.01$) in the reduced Glutathione (GSH) level in blood was evident in rats subjected to CMC + pylorus ligation (Group II), rats treated with Indomethacin (Group III), and the combination of Indomethacin and pylorus ligation (Group IV), in comparison to the GSH levels in healthy rats from the normal control group (Group I). Conversely, a marked reduction ($p < 0.01$) in GSH levels was observed in Group V rats (treated with Omeprazole, 10mg/kg, p.o.) and Group VI rats (treated with NSO, 2.5 ml/kg, p.o.) in comparison to the blood GSH levels in pylorus ligated rats treated with Indomethacin (Group IV).

Figure 1: Comparison of differently treated stomachs of albino rats



Gross structure of the stomach:- A) Vehicle control (Group I) treated with 1% CMC-No ulcers and healthy stomach wall. B) CMC+Pyloric ligation (Group II)- A few superficial ulcers with redness in stomach wall. C) Indomethacin (Group III)- Deep and superficial ulcers alongwith redness. D) Indomethacin+pyloric ligation (Group IV)-More deep ulcers/perforations and superficial ulcers along with redness and weaker stomach as compared with Group II. E) Omepraole+Indomethacin (Group V)- No deep ulcers and redness but a few superficial ulcers only and comparatively healthy stomach wall to Group IV. F) NSO+Indomethacin (Group VI)- No deep ulcers only superficial ulcers and a little redness comparatively healthy stomach wall to Group IV.

DISCUSSION

India is a vast nation with diverse cultural and dietary patterns, which may produce regional variations in the frequency and natural course of peptic ulcers [37, 38]. The comparatively high prevalence of peptic ulcer in South India was due to the sloppy diet, which involved little chewing. It has been shown that saliva has a buffering ability and a preventive effect on the development of peptic ulcer [39].

Peptic ulcer is a prevalent disorder; 5 to 10% of all people experience peptic ulcers in their lifetime. While ulcer disease is a prevalent cause of morbidity, it is a comparatively uncommon cause of death [40]. In general, if all patients with duodenal ulcer are included, the ulcer hemorrhage mortality rate is approximately 5% [41]. Peptic ulcer appears to be caused by an imbalance between the protective mechanisms of the gastro-duodenal mucosa and adverse forces. For all peptic ulcerations, gastric acid and pepsin are needed. *H.pylori's* primary role cannot be overemphasized. H. Pylori is present in virtually all duodenal ulcer cases and approximately in 70 percent of the gastric ulcer patients.

In the treatment of hyperacidity, gastric and duodenal ulcers, herbal medicines have been shown to be very effective.To supplement or intensify the function of synthetic drugs, they may be used as combination therapy. Furthermore, in hyperacidity, gastric and duodenal ulcer prophylaxis, herbal medicines have been used successfully. Infusions of spices, herbal mixtures, tinctures, herbal preparations are offered as herbal medicinal preparations[42].

Gastrointestinal damage associated with NSAIDs is believed to stem from both their local and systemic impacts. The utilization of Indomethacin was chosen for ulcer induction due to its ability to deplete the protective mucosal layer by inhibiting prostaglandin production [43]. It's acknowledged that non-steroidal anti-inflammatory drugs (NSAIDs) exhibit varying degrees of effectiveness in inhibiting the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes. Indomethacin notably disrupts prostaglandin (PG) synthesis by targeting both COX-1 and COX-2 enzymes. Both COX-1 and COX-2 enzyme inhibition is necessary to induce gastric damage. While COX-2 enzyme inhibition is associated with Indomethacin's anti-inflammatory function, COX-1 inhibition is linked to gastrointestinal side effects (GIS).

The initiation of ulceration in pylorus ligation is attributed to the digestive impact of concentrated juice and disruption in gastric blood supply [44]. Therefore, the combination of Indomethacin and pyloric ligation was employed in this research to induce significant ulceration. Pyloric ligation is a standard procedure utilized to assess the anti-secretory and acid-inhibitory effects of drugs.

The current investigation demonstrated that the vehicle control group (Group I), which received the standard diet supplemented with CMC, exhibited standard levels of blood GSH, gastric wall mucus thickness, ulcer index. Similarly, animals in the CMC + pylorus ligated group (Group II rats) exhibited usual levels of total acidity and gastric juice volume.

The administration of Indomethacin (20 mg/kg/day, p.o.) to pylorus ligated rats (Group IV rats) was associated with a significant increase in blood GSH levels, a notable decrease in the average thickness of gastric mucus, and an elevation in the mean ulcer index in comparison to the healthy rats in the normal control group. Furthermore, there was a significant rise in the mean total acidity of gastric juice as compared to the group of rats subjected to CMC + pyloric ligation (Group II).

The impact of the test substance, Nigella sativa oil, at a dosage of 2.5 ml/kg/p.o. (Group VI), and the standard drug Omeprazole at a dosage of 10 mg/kg/p.o. (Group V), were contrasted with the effects observed in rats treated with Indomethacin and pylorus ligation (Group IV rats). The current study examined the oral administration of Nigella sativa oil (2.5 ml/kg/p.o.) in Group VI rats and the standard drug Omeprazole (10 mg/kg/p.o.) in Group V rats, both of which led to a significant reduction in blood GSH levels compared to the pylorus-ligated rats treated with Indomethacin (Group IV rats). A similar decline in GSH levels was noted in the study by Tahir et al. in 2019 [45], where they investigated the anti-ulcer activity of the medicinal plant Hyssop (*Hyssopus officinalis*).

The significance of the mucosal component in the development of peptic ulcers has been emphasized, leading to the introduction of the term "cytoprotection," which signifies safeguarding the mucosa against damage through mechanisms other than merely inhibiting acid secretion [46]. The current understanding highlights the effectiveness of preventing peptic ulcer disease by enhancing the protective mechanisms of the gastric and duodenal mucosa rather than solely addressing aggressive factors.

Administration of Nigella sativa oil at a dosage of 2.5 ml/kg/p.o. (Group VI rats) and the standard drug Omeprazole (10 mg/kg/p.o.) in Group V rats led to a slight elevation in the average thickness of the gastric wall mucus when compared to pylorus ligated rats treated with Indomethacin (Group IV rats).

Our findings are in line with the outcomes presented by Khushtar et al. in 2018 [47], where they observed an enhancement in gastric mucus thickness (referred to as barrier mucus) in rats treated with *Polygonum bistorta* against Indomethacin-induced gastric ulcers in rats.

Various grading systems have been developed which, by measuring ulcer indices, calculate gastric ulceration. While the greater emphasis was assigned to the number of ulcers in the measurement of severity in all scoring systems, other factors should not be ignored, such as the ratio between the area of gastric mucosa and the area of ulceration and/or perforation, and the frequency distribution of the scale of the ulcer. Ulcer index extension can be a measure of the strength of stress or ulcerative ability of pharmacological agents. [48].

Significant reduction in the mean ulcer index was observed in rats treated with the test drug (Group VI) and standard drug (Group V rats) in comparison to pylorus-treated rats treated with Indomethacin. This decrease in the ulcer index aligns with the findings of Khushtar in 2016 [49], who documented a reduction in ulcer index when administering Tirphala hydroalcoholic extract for indomethacin-induced gastric ulcers [50].

The connection between acid production and peptic ulcers has been acknowledged by numerous researchers [51-53]. The adage 'no acid, no ulcer' and the medications employed to reduce acid secretion have been the prevailing pharmacological foundation of ulcer treatment for many years [54].

The mean total acidity in rats subjected to Indomethacin treatment and pylorus ligation (Group IV rats) showed a notable increase when compared to the mean total acidity in rats treated with CMC and pylorus ligation (Group II rats). Upon administration of the test drug, Nigella sativa oil, to Group IV rats at a dose of 2.5 ml/kg/p.o. over a 10-day period, a significant reduction in mean total acidity was observed in NSO-treated rats (Group VI) as compared to rats with Indomethacin-treated pylorus ligation (Group IV rats). When Group IV rats were treated with the standard drug Omeprazole (10 mg/kg/p.o.) (Group V rats), the mean total acidity was significantly lowered.

Gastric secretion inhibition has been studied in order to protect the gastro-duodenal mucosa against pylorus ligation injuries [55]. Sanmugapriya and Venkataraman [56] have shown that reducing the amount of gastric juice lowers the average acidity and hence the index of ulcers..

Less important differences occurred in the mean volume of gastric juice in Indomethacin treated pylorus ligated rats (Group IV rats) relative to the mean total volume in CMC treated pylorus ligated rats (Group II rats). Important decrease was observed in the mean volume of gastric juice when *Nigella sativa* oil was administered at a dosage of 2.5 ml / kg / p.o. (Group VI rats) and standard drug Omeprazole (10mg / kg / p.o.) (Group V rats) relative to Indomethacin-treated pylorus-treated rats (i.e. group IV rats). Our study corroborates the findings of Surender [57], Sanmugapriya and Venkataraman [56] investigating the anti-secretory potential of *Ocimum basilicum* Linn and *Strychnous potatorum* Linn seeds.

The findings of this research have shown that NSO therapy has anti-secretory efficacy, as observed by a reduction in overall acidity and volume of gastric juice. Furthermore, NSO therapy provides cytoprotection by increasing the thickness of the mucus wall (barrier mucus) and reducing blood GSH levels.

Clinically *Nigella Sativa* oil (NSO) has been found useful in Rheumatoid Arthritis [58] [59] [60] and preclinically it has been established as cardioprotective [61] [62], antiosteoporotic [63] [29] and antiosteoarthritic [64] [65]. Powdered seeds of *Nigella Sativa* has been found effective in osteoporosis in rat model [63] [66]. NSAIDs have been used in the management of certain diseases like Rheumatoid arthritis, osteoarthritis, angina, myocardial infarction, gout and migraine [12]. Many studies indicated that the use of NSAIDs in these chronic disorders have led to the development of peptic ulcer [67] [68]. Based on the above observation, *Nigella sativa* oil (NSO) might be useful in these chronic disorders, thereby providing dual benefit by treating them as well as preventing ulcer formation against NSAIDs. Furthermore, *Nigella sativa* oil (NSO) should be evaluated clinically in the treatment of gout, migraine, myocardial infarction and osteoporosis.

CONCLUSION

The present research found that Indomethacin (20 mg / kg / day, p.o) treated pylorus ligated rats provided a substantial increase in blood GSH levels, mean ulcer index and mean total acidity and a substantial decrease in mean gastric wall mucus thickness but a less substantial increase in mean gastric juice volume.

Nigella sativa oil therapy showed modest preventive activity as shown by a substantial decrease in blood GSH levels, mean overall acidity, amount of gastric juice and mean ulcer index. Gastric wall mucus thickness was slightly improved with *Nigella sativa* oil treatment.

Our findings indicated that oral therapy with *Nigella sativa* oil exhibits ulcer preventive properties by improving the gastric mucosa and reducing the acidity of the gastric juice.

CONFLICT OF INTEREST

The authors report no conflict(s) of interest.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmacy, Integral University, Lucknow for their support in work, continuous motivational support, providing the adequate research atmosphere and the necessary

facilities to conduct the experiments related to research work.

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