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## ANTISECRETORY AND ANTIULCERATIVE EFFECTS OF ETHYL ACETATE FRACTION OF *Nigella sativa* (L.) SEED EXTRACT IN RATS

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### ABSTRACT

The present work was carried out to investigate the possible effects of ethyl acetate seed fraction of *Nigella sativa* on gastric ulcers and basal gastric secretions using the Non-Steroidal Anti-inflammatory Drug-induced (NSAID) model. Phytochemical screening according to Trease and Evans, 2002 and acute toxicity tests using the Lorke's Method, 1983 were conducted. For the mucosal integrity study, ulcer and preventive indices were analysed, while volume of gastric juice, titratable acidity, acid output and pepsin concentration were assessed for basal gastric secretion parameters. Phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, glucocinolates amongst others, while the acute toxicity studies revealed a median lethal dose above 5000mg/kg. The rats were grouped into 9 ( $n = 5$ ), with the extract fraction administered at 50, 100 and 200mg/kg subcutaneously, followed by pyloric ligation with cimetidine used as the standard drug. Five rats received normal saline 1ml/kg/rat subcutaneously (S.C) as Negative Control, Five rats received indomethacin (20 mg/kg S.C), Ten rats for the study of the effect of two different doses of cimetidine 50 and 100 mg/kg S.C (5 rats for each dose). Ten rats for the study of effect of two different doses of cimetidine (50 mg and 100 mg/kg) S.C, given 30 minutes prior to indomethacin administration (5 rats for each dose). The three experimental doses of the extract at 50,100 and 200mg/kg showed a dose-dependent decrease in both ulcer and preventive indices with the 200mg/kg dose at 0.6mm and 94% respectively. It also showed a significant ( $p < 0.05$ ) decrease in volume of gastric juice, titratable acidity, acid output and pepsin concentration in dose-dependent manner with the three experimental doses administered with the highest reduction at the 200mg/kg dose. The results obtained suggest that this fraction down-regulated all those parameters which might be attributed to the presence of the phytoconstituents present in this fraction, particularly the flavonoids. Therefore, the extract fraction of this plant possesses gastroprotective activity further explaining the folkloric use of this plant in the therapy of peptic ulcer disease.

**Keywords:** Antiulcerative, Antisecretory, *Nigella sativa*, Phytochemicals, Rats, Seed Extracts.

### INTRODUCTION

In recent years, the clinical importance of herbal drugs has received considerable attention that led to an ever increasing interest in research on different plant species to find out their therapeutic values and applications. The plant *Nigella sativa* (L.) Family Ranunculaceae has been used for medicinal purposes for centuries as a herb when pressed into oil in Asia, Middle East and Africa. It has been traditionally used for a variety of conditions and treatments related to respiratory health, stomach, intestinal, kidney, liver, circulatory and immune system support, and for general well-being. The seeds are used as carminative, aromatic, stimulant, diuretic, antihelminthic, galactagogue and to achieve diaphoresis. They are used as a condiment in curries. A tincture prepared from the seeds is useful in indigestion, loss of appetite, diarrhoea, dropsy, amenorrhoea, dysmenorrhoea and in the treatment of worms and skin eruptions. Externally, the oil is used as an antiseptic. To arrest vomiting, the seeds are roasted and given internally

(Gupta *et al.*, 2009). Gastroduodenal lesions due to hyperacidity leads to peptic ulcerations which are erosions of the mucosal epithelial lining of the gastrointestinal tract (Oliveira *et al.*, 2011).

Despite the pathophysiology of peptic ulceration not been completely elucidated, it is known that an imbalance between aggressive factors (acid and pepsin secretion) and cytoprotective factors of the gastric mucus membrane (mucus and bicarbonate secretion) result in gastric ulceration (Ramakrishnan and Salinas, 2007). It is also known that several endogenous factors are involved in the pathophysiology of the gastroprotection, which include prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), somatostatin, nitric oxide (NO) and sulfhydryl compounds (Tsukimi *et al.*, 2011).

The aetiology of gastric ulcer involves environmental factors such as alcoholic beverages and non-steroidal anti-inflammatory drugs (NSAIDs) usage, *Helicobacter pylori*, genetic factors amongst others (Konturek *et al.*, 2005).

Initially, infected individuals develop antrum-predominant gastritis accompanied by gastric acid hypersecretion (Levis *et al.*, 2002). Untreated gastric ulcer is capable of inducing upper gastrointestinal bleeding (Tortora and Grabowski, 2003). Approximately 50% of individuals who use Non-steroidal anti-inflammatory drugs (NSAIDs) develop gastric erosions, while an estimated 2 to 4% of these individuals develop clinically significant GI ulcers and bleeding sometimes leading to death (Lowe and Wolfe, 2007). The present study aimed to investigate the gastroprotective potentials of this plant via the mucosal integrity and basal gastric secretion studies.

## **MATERIALS AND METHODS**

### **Plant Material**

*Nigella sativa* air dried seeds were obtained during the month of July, 2013 from Sabon-Gari market, Zaria. Botanical identification and authentication was done at the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher herbarium specimen No: 101201 was deposited at the herbarium for future references.

### **Extraction of the Plant Material**

*Nigella sativa* dried seeds weighing about 2kg were crushed and pounded with pestle and mortar. The powder was extracted with aqueous ethanol (70%) in a Soxhlet Extractor, concentrated using rotary evaporator at reduced pressure, suspended in methanol and partitioned with ethyl acetate to obtain the ethyl acetate (ETAC) fraction. The fraction was further concentrated *in-vacuo* and the residue obtained. The extract yielded about 80% of the residue.

### **Phytochemical Screening of the Fraction:**

The preliminary analysis for the extract was conducted for the presence of flavonoids, alkaloids, saponins, steroids, glycosides, anthraquinones, resins, reducing sugars and other phytochemicals using standard procedures for analysis (Evans, 2002 and Harborne, 2007).

### **Acute Toxicity Studies:**

Lethal Dose (LD<sub>50</sub>) determination was conducted using the method of Lorke (1983). In the initial phase, male rats were divided into three groups of 3 rats each, making a total of 9 rats. The rats were treated with the ETAC fraction of the extract at doses of 10, 100 and 1000 mg/kg subcutaneously. Animals were observed for 24 hours and the number of death(s) or those that showed neurological signs were recorded. In the second phase, the animals were grouped into 4 groups of one rat each and treated with the fraction at appropriate doses subcutaneously. The rats were observed for 4 h for deaths or neurological signs, and the final LD<sub>50</sub> was calculated as the square root of the highest non-lethal dose in which the animal survived multiplied by the lowest lethal dose in which the animal died.

### **Drugs and Chemicals/Reagents:**

Cimetidine (Lek Pharma, Slovenia), Indomethacin (Liomethacin<sup>(R)</sup>) (Cheisi, Egypt), Thiopental Sodium (Abbott Laboratories, UK), Phenol Red (BDH Poole, England), Sodium Hydroxide (NaOH) (BDH Poole, England) for the preparation of 0.01N NaOH solution, Phosphate Buffered Saline (PBS), Casein Substrate Solution 1% (w/v) (Sigma-Aldrich, USA),

Hydrochloric Acid (HCl) 0.1N (Sigma-Aldrich, USA), Trichloroacetic Acid Solution 6% w/v (Sigma-Aldrich, USA). All other chemicals and reagents were analytical grade.

### **Experimental Animals and Design:**

A total of ninety adult male albino Wistar rats were used in this study. The animals were obtained from the Animal House, Faculty of Medicine, El-Kasr el-Ain, Cairo University, Egypt. Their weights ranged from 180 – 240g. They were maintained under a similar conditions of humidity, temperature and light/dark cycle respectively and each of the animals was kept in a single individual cage, with wide-meshed galvanized wire bottoms to decrease coprophagy as much as possible. The rats were given access to food and water *ad libitum* for two weeks to acclimatize, prior to the commencement of the experiment. The rats were treated in accordance to the internationally accepted principles of laboratory animal use and care. At the time of the experiment, all treatments were conducted between 9:00-10:00am to minimize variations in animal response due to circadian rhythm. The animals were divided into the following groups and subgroups for gastric mucosal damage and gastric secretion studies respectively.

### **Group I: Study of Gastric Mucosal Damage**

**Group IA;** Normal saline. Five rats received normal saline 1ml/kg/rat subcutaneously (SC).

**Group IB;** Indomethacin-treated. Five rats received indomethacin (20mg/kg S.C) for the study of gastric mucosal damage.

**Group IC;** Cimetidine-treated Ten rats for the study of the effect of two different doses of cimetidine 50 and 100mg/kg S.C on gastric mucosal damage (5 rats for each dose)

Ten rats for the study of effect of two different doses of cimetidine (50mg and 100mg/kg) S.C, given 30 minutes prior to indomethacin administration on gastric mucosal damage (5 rats for each dose).

**Group ID;** *Nigella sativa* extract treated Fifteen rats for the study of the effect of ethyl acetate (ETAC) fraction, each at three different doses (50, 100 and 200mg/kg S.C), when given 30 minutes prior to indomethacin on gastric mucosal damage (5 rats for each dose).

### **Group II: Study of Basal Gastric Secretion**

**Group IIA;** Normal saline. Five rats received normal saline (1ml/kg/rat S.C).

**Group IIB;** Indomethacin-treated. Five rats received indomethacin (20 mg/kg S.C), followed by pyloric ligation for the study of basal gastric secretion.

**Group IIC;** Cimetidine-treated. Ten rats for the study of the effect of two different doses of cimetidine, 50 and 100mg/kg S.C on basal gastric secretion (5 rats for each dose).

Ten rats for the study of the effect of cimetidine, 50 and 100mg/kg S.C, given 30 mins prior to indomethacin administration on basal gastric secretion (5 rats for each dose).

**Group IID;** *Nigella sativa*-treated. Fifteen rats for the study of the effect of (ETAC) fraction, each at three different doses (50, 100 and 200mg/kg S.C), when given 30 minutes prior to indomethacin on basal gastric secretion (5 rats for each dose).

### Induction of Gastric Ulceration

After 48 hours of starvation, the animals were weighed and maintained in their individual cages. Then, indomethacin 20mg/kg was injected subcutaneously and the animals were then deprived of both food and water for 7 h (Urushidani *et al.*, 1979). The animals were later sacrificed by decapitation following chloroform overdose (Satoh *et al.*, 1983). Their stomachs were opened along the greater curvature, rinsed slowly with running water, then stretched out as much as possible by the use of pins on No.1 Whatman's filter paper on a ceiling board.

### Quantitative Assessment of Mucosal Damage

The ulcerated areas in each stomach were measured with a transparent (mm) ruler scale, the result of each group were expressed as ulcer index (U.I) in mm of mean ulcer  $\pm$  standard error of mean (Sceповic and Radmanovic, 1984). The degree of ulceration was expressed as ulcer index (U.I). It was calculated by multiplying ulcer score by 100 (Robert *et al.*, 1968). Ulcer score for each group was calculated by dividing the total number of ulcers in each group by the total number of rats in that group (Robert *et al.*, 1968). The percentage preventive index was calculated according to the method of Hano *et al.* (1976), which is expressed as:

$$\text{Preventive Index (\%)} = \frac{\text{U.I. Indomethacin} - \text{U.I. Extract/drug plus indomethacin}}{\text{U.I. Indomethacin}} \times 100$$

U.I. Indomethacin

### Collection of Gastric Secretion

The gastric juice was collected according to the technique of Shay *et al.* (1954) as modified by Levine (1965), where oesophageal ligation was avoided. The animals were fasted for 48 hrs to ensure complete emptying of the stomach, but allowed water *ad libitum*. Each animal was weighed at the end of the fasting period. Light anaesthesia was induced using sodium thiopental 10 mg/kg intraperitoneal (Juliane *et al.*, 2009), abdomen of each rat was opened via a midline incision and the stomach exteriorated. A pyloric ligature was made using a thread with care to avoid damage to the blood vessels or traction to the stomach. The abdomen was then closed by suture, cleaned thoroughly with distilled water or saline, and the animal was allowed to recover.

After 3 hours, the rats were sacrificed by decapitation, abdomen of each of the animals were opened. The oesophagus was ligated, and the stomach removed and washed with distilled water or saline. An opening along the greater curvature was made (Nwafor *et al.*, 2000) and the gastric content drained into a graduated centrifuge tube, the volume noted, then centrifuged at 1,006  $\times g$  for 15 minutes.

### Analysis of the Gastric Juice

#### Determination of each sample volume after centrifugation

The volume of 3 hours gastric secretion was measured after being subjected to centrifugation at 1,006  $\times g$  for 15 mins.

**Determination of titratable acidity:** A given volume of the gastric juice (1ml) was titrated against 0.01N NaOH. An end point of pH 7.0 as determined colorimetrically at 280nm by phenol red was used (Grossman, 1973; Davenport, 1977). The values were calculated as micro-equivalents per litre (Meq/L), which is equal to the number of millilitres (ml) of 0.01N NaOH required to neutralize 1ml of gastric juice.

$$\text{Titratable Acidity} = \frac{\text{Volume of 0.01 N NaOH (mol) which neutralized 1ml of gastric juice}}{10}$$

**Determination of acid output:** This was calculated by multiplying the volume (ml) of the gastric juice of each animal by the titratable acidity in that animal.

**Determination of pepsin concentration:** Pepsin concentration which is the major factor involved in the proteolytic activity of gastric secretion was determined in terms of the amount of protease enzymes produced after incubation of the substrate for 30 minutes with pepsin. It was determined by the spectrophotometric method devised by Jongensen (1954) and Hawk *et al.* (1960).

### Statistical analysis

All data were expressed as Mean  $\pm$  S.E.M (standard error of the mean) using SPSS Version 20. Statistical evaluation was done by analysis of variance (ANOVA) followed by post-hoc analysis by Duncan and Scheffe. Values of  $p < 0.05$  were considered significant.

## RESULTS

### Acute Toxicity Studies

The toxicity studies of the ETAC fraction seed extract of *Nigella sativa* in the first phase after being observed for 24hr, the rats did not show any signs and symptoms of toxicity or death. In the second phase, none of the rats produced any toxic symptoms or mortality up to the dose level of 5000mg/kg body weight, hence, they were considered safe for further pharmacological screening.

As shown in Table 2, pre-treatment with 1ml/kg/rat normal saline produced an ulcer index of 0.45mm, with a preventive index of 97%. Administration of indomethacin 20mg/kg significantly elevated ( $P > 0.05$ ) the ulcer index at  $10.10 \pm 0.35$ mm. It has the highest ulcer index compared to all the groups with a zero preventive index. The two doses of cimetidine 50 and 100mg/kg alone compared to the normal saline group significantly ( $P < 0.05$ ) reduced the ulcer and preventive indices, with higher reduction at the 100mg/kg dose. Similar pattern was also obtained at the 50 and 100mg/kg plus indomethacin groups, where it showed significant reduction in both the ulcer and preventive indices more pronounced at the higher dose of 100mg/kg cimetidine plus 20mg/kg indomethacin treated group. The ethyl acetate fractions at doses of 50, 100 and 200mg/kg produced a significant decrease in the ulcer index compared to control and the standard drug cimetidine. The preventive indices also showed a dose-dependent increase from 76% at the 50mg/kg extract dose to 94% protection as obtained at the 200mg/kg extract dose.

Table 1: Phytochemical Analysis of Ethyl Acetate Fraction of *Nigella sativa* L. seed extracts.

Phytochemical Tests	Ethyl Acetate
Test for flavonoids	+++
Test for alkaloids	++
Test for tannins	+
Test for saponins	-
Test for steroids	Trace
Test for glycosides	-
Test for anthraquinones	+
Test for reducing sugars	+
Test for resins	-

+ : Presence of the constituents

- : Absence of the constituents

**Effect of Ethyl acetate fraction on ulcer and preventive indices**

Table 2: The Ulcer Index in Millimetre (mm) and Preventive Index in Percent (%) for Normal Saline, Indomethacin, Cimetidine and Ethyl Acetate-Treated Fraction of *Nigella sativa*.

Groups	Ulcer Index (mm)	Preventive Index (%)
	Mean ± SEM	Mean ± SEM
Normal Saline (1ml/kg/rat)	0.30 ± 0.45	97.03 ± 0.01 (97%)
Indomethacin (20mg/kg)	10.10 ± 0.35*	0.00 ± 0.00* (0%)
ETAC (50mg/kg) + Indo (20mg/kg)	2.40 ± 0.14*	76.24 ± 0.95 <sup>†</sup> (76%)
ETAC (100mg/kg) + Indo (20mg/kg)	1.10 ± 0.07 <sup>†</sup>	89.11 ± 0.63 <sup>†</sup> (89%)
ETAC (200mg/kg) + Indo (20mg/kg)	0.60 ± 0.11 <sup>†</sup>	94.06 ± 0.63 <sup>†</sup> (94%)
Cimetidine (50mg/kg)	2.70 ± 0.37* <sup>†</sup>	73.27 ± 0.09 <sup>†</sup> (73%)
Cimetidine (100mg/kg)	2.10 ± 0.35* <sup>†</sup>	79.21 ± 0.32 <sup>†</sup> (79%)
Cimetidine (50mg/kg) + Indo (20mg/kg)	3.30 ± 0.10* <sup>†</sup>	67.33 ± 0.32 <sup>†</sup> (67%)
Cimetidine (100mg/kg) + Indo (20mg/kg)	2.20 ± 0.35* <sup>†</sup>	78.22 ± 0.74 <sup>†</sup> (78%)

\* P<0.05 compared to Normal Saline

<sup>†</sup> P<0.05 compared to Indomethacin

Indo - Indomethacin

ETAC - Ethyl Acetate

Table 3: The Volume of Gastric Juice (ml/3hr), Titratable Acidity (mEq/L), Acid Output (μEq/hr) and Pepsin Concentration (mg/ml) for Normal Saline, Indomethacin, Cimetidine and Ethyl Acetate-Treated Fraction of *Nigella sativa* \* P<0.05 compared to Normal Saline † P<0.05 compared to Indomethacin Indo-Indomethacin, ETAC-Ethyl Acetate

Groups (n=5)	Volume of Gastric Juice (ml/3hr) Mean ± SEM	Titratable Acidity (mEq/L) Mean ± SEM	Acid Output (μEq/hr) Mean ± SEM	Pepsin Concentration (mg/ml) Mean ± SEM
Normal Saline (1ml/kg/rat)	3.68 ± 0.19	42.40 ± 1.78	52.34 ± 4.49	9.23 ± 0.23
Indomethacin (20mg/kg)	2.38 ± 0.18*	27.40 ± 4.56	22.18 ± 4.43*	6.86 ± 0.52*
ETAC (50mg/kg) + Indo (20mg/kg)	1.58 ± 0.29*	47.00 ± 3.88	25.08 ± 8.20	4.74 ± 0.46*†
ETAC (100mg/kg) + Indo (20mg/kg)	1.42 ± 0.22*	45.80 ± 2.58	18.04 ± 3.58	4.51 ± 0.27*
ETAC (200mg/kg) + Indo (20mg/kg)	1.06 ± 0.07*†	42.20 ± 4.01	13.94 ± 3.00	4.43 ± 0.28*†
Cimetidine (50mg/kg)	1.72 ± 0.15*	38.40 ± 4.71	21.62 ± 2.56*	5.12 ± 0.26*
Cimetidine (100mg/kg)	2.00 ± 0.13*	35.60 ± 4.84	19.12 ± 2.69*	5.03 ± 0.16*
Cimetidine (50mg/kg) + Indo (20mg/kg)	1.20 ± 0.13*†	34.00 ± 3.45	13.54 ± 1.81*	4.44 ± 0.31*†
Cimetidine (100mg/kg) + Indo (20mg/kg)	1.28 ± 0.12*†	31.40 ± 2.49	11.20 ± 2.38*	4.13 ± 0.27*†

**Effect of Ethyl Acetate Fraction on Basal Gastric Secretions**

Table 3 indicate the effects of the ETAC fractions of *Nigella sativa* on the gastric juice analysis, mainly on the major parameters of gastric secretion viz; volume of gastric juice, titratable acidity, acid output and pepsin concentration. It shows that indomethacin administration at 20mg/kg caused a significant (P<0.05) decrease in the volume of gastric juice compared to the normal saline control from 3.68 ± 0.19mls/3hr to 2.38 ± 0.48mls/3hr. Acid output and pepsin concentration were significantly reduced.

The parameters of basal gastric secretion for the ethylacetate fraction at 50mg/kg showed volume of gastric juice decreases significantly compared to the normal saline and indomethacin 20mg/kg control groups, while the pepsin concentration also decreases significantly (P<0.05) compared to the control with a mean value of 4.74 ± 0.45mg/ml. The ethyl acetate 100 and 200mg/kg with indomethacin 20mg/kg produced mean values of volume of gastric juice 1.42 ± 0.22 and 1.06 ± 0.07ml/3hr respectively, which were significantly (P<0.05) lower than the control groups. The titratable acidities were 45.80 ± 2.58mEq/L and 42.20 ± 4.01mEq/L, with the ethyl acetate 200mg/kg being significantly lower than the control group.

For cimetidine 50mg/kg on basal secretions, the volume of gastric juice significantly (P<0.05) decreases compared to the normal saline control, and were found to be 1.72ml/3hr. Acid output was 21.62 ± 2.56 mEq/hr which equally showed a reduction compared to control. Titratable acidity was insignificant. The 100mg/kg cimetidine, the volume of gastric juice showed a mean value 2.00 ± 0.13ml/3hr, which was less than those of normal saline and indomethacin controls, but slightly above that of cimetidine 50mg/kg. The titratable acidity was 35.60 ± 4.84 mEq/L. It decreases below that of cimetidine 50mg/kg, the

standard drug, but above that of indomethacin control. The acid output showed 19.12 ± 2.69mEq/hr, which was significant compared to the control. Pepsin concentration has been shown to be lower than that of normal saline and indomethacin control groups.

**DISCUSSION**

To evaluate the gastroprotective effect of the ethylacetate fraction of *Nigella sativa*, the model of acute ulcer induced by a non-steroidal anti-inflammatory compound indomethacin was performed. In this model, it was found that treatment with normal saline (1ml/kg/rat) which was isotonic with the plasma as a control, showed no significant (P<0.05) ulcer or lesion index with a preventive index of 97%. The group that received indomethacin 20mg/kg alone produced the highest lesion index when compared with all the treatment groups. There were gastric mucosal congestion, oedema, haemorrhage, lamina epithelial necrosis, leucocytic infiltrations, blood vessels congestion with foci of necrotic tissues in the lesions. These results are consistent with previous studies that reported similar histopathological derangement and mucosal oxidative stress effects that involves weakening of gastric mucous, leading to formation of lesions in the gastric epithelium (Valcheva-Kuzmanova *et al.*, 2007; El-Moselhy *et al.*, 2009).

As shown in Table 2 it was observed that the group that received 50 and 100mg/kg of the standard drug cimetidine alone, showed a significant decrease in gastric lesions (p<0.05) compared to the indomethacin 20mg/kg, cimetidine plus indomethacin group in the ulcer index with preventive indices of 73 and 79%, respectively. This corroborated the studies of Marivane *et al.* (2011) who reported a decrease in lesion index on the gastric mucosa of rats treated with the H<sub>2</sub>-receptor antagonist cimetidine compared to indomethacin

With regard to the effects of three different graded doses of the ETAC fraction of *Nigella sativa* (Table 2), it was found that treatment with the 50, 100 and 200mg/kg significantly reduced the lesion index compared with the control group ( $P < 0.05$ ) in a dose-dependent manner in the indomethacin-induced model. The highest dose of 200mg/kg ETAC fraction produced the highest preventive index of 96% above that of the standard drug 50 and 100mg/kg cimetidine. A similar finding was reported by Marivane *et al.* (2011) that reported a significant reduction in lesion index, total injured area and the percentage injured area when extract of *Brassica oleracea* was used on indomethacin ulcer model.

Both *Brassica oleracea* and *Nigella sativa* are found to contain some flavonoids, especially quercetin and kaempferol mainly in glycosidic form and these are secondary metabolites that are widely distributed in nature with several biological activities including gastroprotective potentials (Martin *et al.*, 1998). *Croton urucurana* with high flavonoid content also exhibited same mucosal cytoprotective potentials (Esmeraldino *et al.*, 2005; Alves *et al.*, 2008). The flavonoid content of this fraction had prevented and exerted a protective effect possibly by its inherent ability to scavenge free radicals, inhibit lipid peroxidation, increase mucous and prostaglandin contents of the gastric mucosa (Alanko *et al.*, 1999).

Through phytochemical analysis of ETAC fraction, apart from flavonoids, the presence of terpenoids, tannins, cardiac glycosides, steroids, saponins were detected amongst others. To further support and corroborate the possible fractions for the mucosal cytoprotection exhibited by this fraction of *N. sativa* containing flavonoids are the studies by Alcaraz and Hoult, 1985 that flavonoids increase mucosal prostaglandin content, inhibit histidine decarboxylase thereby decreasing histamine secretion.

The presence of saponins in the fractions of *N. sativa* to improve on mucosal integrity has been reported in several other studies where plants containing saponins have been shown to possess antiulcer activity in several experimental ulcer models. Among these, saponins isolated from the rhizome of *Panax japonicus* and the fruit of *Kochia scoparia* (which contain approximately 20% of saponins) have been demonstrated to possess gastroprotective properties (Matsuda *et al.*, 2003) in conformity with this study. The protective activities of all these saponins are not due to inhibition of gastric acid secretion, but probably due to activation of mucous membrane protective factors (Borrelli and Izzo, 2000). Moreover, several plants containing high amount of saponins have been shown to possess antiulcer activity in several experimental bioassays, probably acting as an activator of mucus membrane stabilizing factors (Morikawa *et al.*, 2006). Similarly, presence of tannins, terpenoids in *N. sativa* fractions further validate the cytoprotective property in the gastric mucosa observed in our study as reported by Al-Rehaily *et al.* (2002), where several saponins, tannins, terpenoids were found to possess gastroprotective properties. Additionally, Terpenoids are a widespread class of secondary compounds with several pharmacological activities, including anti-inflammatory and antiulcer activities (Arrietta *et al.*, 2003).

The interference of the ethyl acetate fraction of the extract using same model was also evaluated on parameters of basal gastric secretion. This method is an important procedure that reveals the possible changes of gastric secretory physiological parameters relating to volume of gastric secretion, titratable acidity, acid output relating to pH and the most important proteolytic enzyme in the stomach pepsin. The findings suggest that the fraction interfered with these major basal secretory indices of gastric juice.

Considering that the volume of gastric juice which is mainly acidic encompasses mucus, hydrochloric acid, pepsinogen, bicarbonates, intrinsic factor and protein plays a vital role in the aetiopathogenesis of gastric mucosal integrity, it is plausible to consider the two fractions as putative cytoprotective agents. This assumption was made based on the observation that the different quantities or volume of gastric juice obtained in this study showed a general inhibitory pattern with regard to its production in the stomach.

These results are in agreement with the studies of Marivane *et al.*, (2011), who reported a significant decrease in volume of gastric juice on a similar ulcer model after using *Green propolis*.

With regard to the titratable acidity, the ethyl acetate fractions at 50, 100 and 200mg/kg administered showed a reduction that was significant ( $P < 0.05$ ). This decrease in stomach acidity facilitated the healing of gastric ulcers, because exposing the mucosa to high concentrations of acid favours mucosal epithelial damage (Laine *et al.*, 2008). Contact between stomach acid and the mast cells of the submucosa and lamina propria causes mast cell degranulation and the release of histamine, which stimulates hydrochloric acid secretion by parietal cells and produces inflammation and acute oedema at the site of contact (Rodrigues *et al.*, 2008). Overall, concentration of hydrogen ions in the gastric juice decreases reflective of high pH, further aggravating the aggressive factors (Lullmann *et al.*, 2000). Consistently, hyperacidity is known to result due to uncontrolled hypersecretion of hydrochloric acid from parietal cells of gastric mucosa through the proton pump  $H^+ - K^+ ATPase$  (Kishor *et al.*, 2007).

All the ethyl acetate fractions of 50, 100 and 200mg/kg administered showed a reduction that was significant in terms of volume of gastric juice, with the 200mg/kg significantly decreasing compared to both indomethacin and control. A similar finding was reported by Halter *et al.* (1988) and Hatazawa *et al.* (2006). The titratable acidity, acid output were also decreased though insignificantly.

#### **CONCLUSION**

The reported results have validated the folkloric use of *N. sativa* in the therapy of peptic ulcer disease. *N. sativa* offers protection against NSAIDs-induced gastric ulceration and down-regulate basal acid secretions to promote mucosal cytoprotection. The presence of phytoconstituents in this medicinal plant might be responsible for those pharmacological actions.

#### **FUTURE PERSPECTIVES**

In this context extracts and active principles from plants could serve as leads for the development of new drugs. Therefore, this plant specie(s) have a great potential to be used as a gastroprotective agent either alone or in combination with others.

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