International Journal of Veterinary Sciences and Animal Husbandry 2018; 3(3): 33-36

ISSN: 2456-2912
VET 2018; 3(3): 33-36

www.veterinarypaper.com
Received: 22-03-2018
Accepted: 26-04-2018

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The effect of *Nigella sativa* (black seed) on ethanol induced ulcer in wistar albino rats

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Abstract
The study was carried out to determine the protective role of the aqueous extract of *Nigella sativa* on ethanol induced intestinal ulcer. A total of twenty (20) healthy Wistar albino rats were randomly divided into four groups of five rats each. The control was pre-treated with normal saline, the second group was pretreated with a standard ulcer drug (Gestid suspension), the third group was pretreated with aqueous extract of *Nigella sativa* at low dose (320mg/kg) and the fourth group was pretreated with aqueous extract of *Nigella sativa* at a high dose of 640mg/kg. The findings at the end of the experiment revealed that the control group showed distortion in the intestinal mucosa, damaged submucosa, basement membrane, blood vessel and muscular layer. The Gestid group showed an intact submucosa and mild damage to the intestinal lining. The low dose showed a distorted intestinal mucosa and the high dose group showed mild damage to the intestinal mucosa. The aqueous extract of *Nigella sativa* administered at 320 mg/kg body weight and 640 mg/kg body weight dose appeared to have protected the intestine from ethanol-induced ulcer at the end of the experimental study.

Keywords: *Nigella sativa*, ethanol, intestinal ulcers and wistar rats

1. Introduction
Peptic ulcers are pathological lesions in the gastrointestinal tract that usually occur in the stomach and duodenum. Major etiologic factors of peptic ulcer include the following: Helicobacter pylori infection, the excessive use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol consumption, cigarette smoking, psychological and physiological stress [1]. Gastric ulcer is commonly characterized by necrosis, infiltration of neutrophils, reduction in blood flow, induction of oxidative stress, and secretion of inflammatory mediators [2-3], impairment of the balance between aggressive (increased acid secretions) and protective factors, stress, trauma, sepsis, hemorrhagic shock, burns, pulmonary and liver diseases, helicobacter pylori, use of cigarettes and alcohol, and steroidal and non-steroidal anti-inflammatory drugs. These have been shown to play a role in gastric ulcerogenesis [4]

Gastric ulcers are common gastrointestinal (GI) disorders that affect approximately 10% of the world population. They are multifactorial and complicated disorders resulting from breakdown of the equilibrium between the defense mechanisms of the gastric mucosa and aggressive factors [5]. It is estimated that 14.5 million people worldwide are affected by gastric ulcers in 2007, with a mortality of 4.08 million individuals [2]

Ethanol is considered as an agent that induces extreme gastric ulcers as it stimulates severe instabilities in the gastric mucosa [5-6] Ethanol performs its lethal effect on gastric epithelium and leads to the formation of typical necrotic injuries due to a decrease in bicarbonate secretion and mucus production which provides a protective cover over the gastric mucosa in a normal condition. It also aggravates a reduction in gastric blood flow, induction of oxidative stress, increased activity of xanthine oxidase, an increase in malondialdehyde (MDA) level, solubilization of components of the mucus of the stomach and a decrease in glutathione level [6-7]. Gastric ulcers are locally treated in some communities in northern Nigeria using *Nigella sativa*.

Nigella sativa is also called *Habbatulsauda* or *Habbat al Baraka* and is known around the world by many names because of its ancient popular history and medicinal value. It is also known as black caraway, Roman Coriander, carvi (French), Schwarz kummel (German),

Kalonji (Hindi/Urdu), Kezah (Hebrew), Chernushka (Russian). It is an annual herbaceous plant that grows about 16-24 inches in height. The name *Nigella sativa* comes from the Latin word *Nigellus*, meaning black. It is translated as "seed of blessing". The seed is called black cumin or black seed in English, while in old Latin it was called Panacea meaning cure all. It comes from a Small rectangular Black seed which is known as Blessed seed (Habbat al Baraka or Habbatulsauda). *Nigella sativa* is a member of the Ranunculacea family [8]. Black cumin or Black seed has even been described as a 'miracle herb' and a cure for every disease except death" (SahihBukhari).

Black cumin seed have been used to successfully keep healthy for over 3,300 years. It is one of the early providers of life. The miracle seed has been used by millions of people to treat various ailments for century in different parts of the world especially in Mediterranean, Middle East, and Indian, Pakistan, Bangladesh and also in Europe [8].

This study was carried out to determine the protective role of an aqueous extract of black seed (*Nigella sativa*) in the treatment of duodenal or intestinal ulcer.

2. Materials and Method

2.1 Collection and identification of plant materials

The seeds of *Nigella sativa* were purchased from a traditional herbalist in Damaturu; North-Eastern Nigeria. The seed was identified and authenticated by a plant taxonomist of the Department of Biological Science, University of Maiduguri, Borno State.

2.1.2 Ethical approval of the Research Protocol

The study protocol was approved by the Ethics Committee for Experiments on Animals of University of Maiduguri (number UM/HA/UGP/16.17-010) All experiments were carried out in accordance with the constituted Ethics Committee of the institution within which the work was undertaken and it conformed to the provisions of the Declaration of Helsinki (as revised in 2000)

2.1.3 Extraction procedures

The *Nigella sativa* seeds were winnowed and crushed to smaller fragments using a pestle and a mortar. 100g of the crude extract was soaked in 1 liters of distilled water for 24 hours. The extract was then filtered. The liquid component was removed using an evaporator. The extract was then poured on a tray for complete dryness, after drying; the yield was measured to be 35.4%.

2.1.4 Animal Husbandry

Twenty (20) adult male and female Wistar albino rats weighing between 65.9g to 89.6g were obtained from Department of Biochemistry animal house, University of Maiduguri. The rats were acclimatized for two weeks to let them adapt to the animal house condition. The rats were housed in a cross ventilated room with temperature, humidity and 12 hours light/ 12 hours dark cycle maintained. The rats were fed with standard laboratory pellet and water ad-libitum.

2.1.5 Experimental design

Twenty (20) adult Wistar rats weighing between 65.9g to 88.6g were used for this study. The rats are grouped into four (I, II, III and IV) with each group containing 5 rats. The animals were fasted for 18 hours prior to the start of the experiment to make sure the stomach was empty.

Group I, received 1ml of ethanol by oral route and served as positive control group and were sacrificed 1h later.

Group II, received aqueous extract of *Nigella sativa* (320mg/kg body weight) by oral route 30 minutes prior to ulcer induction with ethanol. This group was sacrificed 1h after inducing ulcer.

Group III, received aqueous extract of *Nigella sativa* (640mg/kg body weight) by oral route 30 minutes prior to ulcer induction with ethanol. This group was sacrificed 1h after inducing ulcer

Group IV, received a standard ulcer drug (Gestid) 30 minutes prior to inducing ulcer with ethanol and were sacrificed 1h after inducing ulcer.

2.1.6 Preparation of solution

Stock solution of *Nigella sativa* was prepared by dissolving four grams (4g) in 20ml of distilled water to obtain a concentration of 0.2g/ml. Calculation of the actual concentration carried out by substituting weight of each rat.

2.1.7 Tissue processing

After the examination of the morphology of intestine, the duodenum were fixed in 10% formalin and tissue processing and staining was carried out using Hematoxylin and Eosin (H & E) and cresyl violet.

3. Results

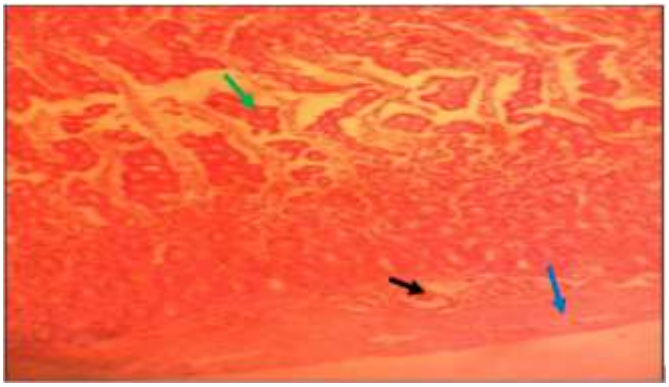

Fig 1: Photomicrograph of small intestine of rats pre-treated with normal saline showing distorted intestinal mucosa (green arrow), blood vessel (black arrow) and muscular layer (blue arrow) Haemotoxylin and Eosin (H and E) ×100.

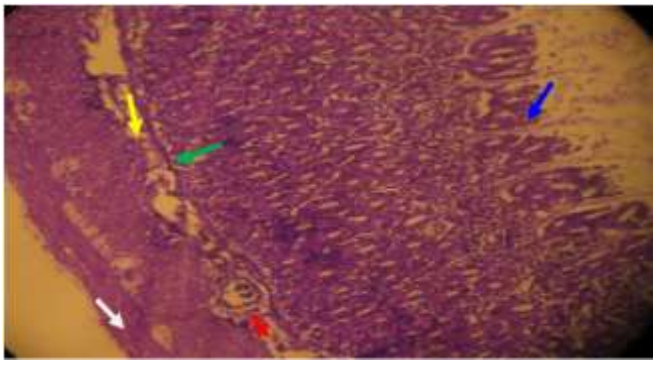

Fig 2: Photomicrograph of small intestine of rats pre-treated with alcohol showing distorted intestinal mucosa (blue arrow), blood vessel (red arrow), muscular layer (white arrow), basement membrane (green arrow) and distorted sub mucosal layer (yellow arrow), cresyl violet stain x100.

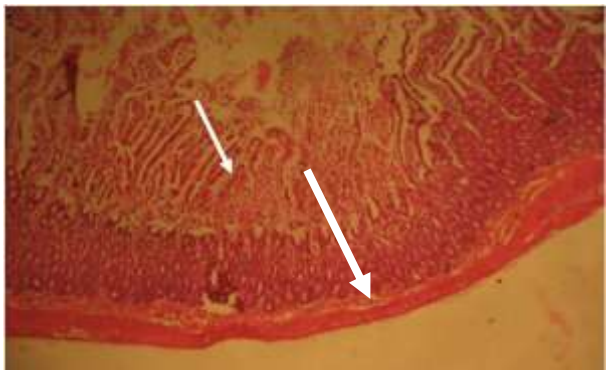

Fig 3: photomicrograph of the small intestine of rats treated with standard drug (Gestid) showing the mucosa and disruption of the epithelial lining (white arrow). H&E stain (×100)

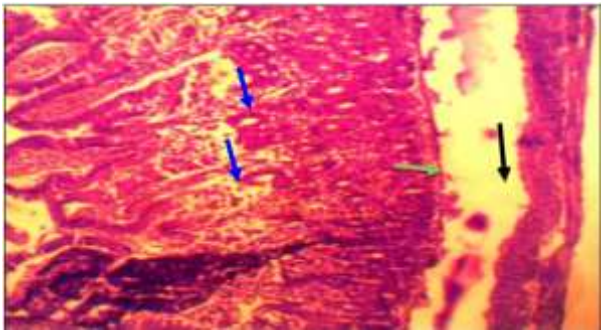

Fig 4: Photomicrograph of intestine of rats pre-treated with Gestid suspension showing mild damages to the intestinal mucosa (blue arrows), normal basement membrane (green arrow) and damaged submucosal layer (black arrow), cresyl violet stain x100.

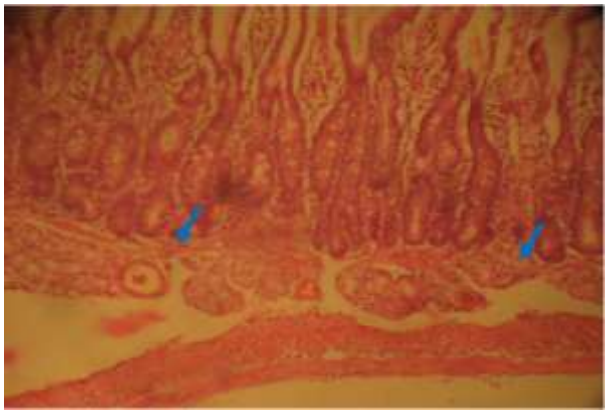

Fig 5: Photomicrograph of intestine of rats treated with aqueous extract of *Nigella sativa at* 320 mg/kg showing little disruption of basement membrane (blue arrow). H&E stain (×100)

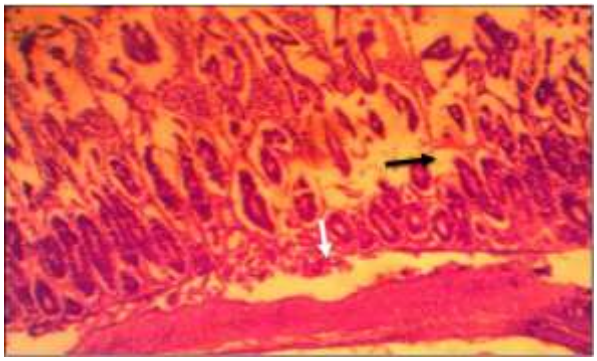

Fig 6: Photomicrograph of Intestine of rats pre-treated with aqueous extract of *Nigella sativa* at 320mg/kg showing mild damage in intestinal mucosa (black arrow) and damaged basement membrane (white arrow), cresyl violet stain x100.

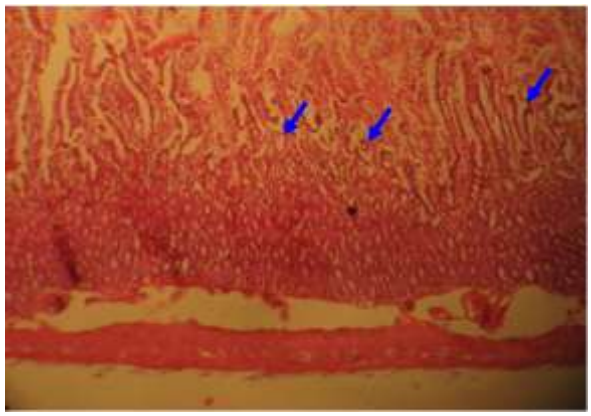

Fig 7: photomicrograph of intestine of rats treated with aqueous extract of *Nigella sativa at* 640 mg/kg showing mild distortion in the intestinal mucosa (blue arrow). H&E stain (×100).

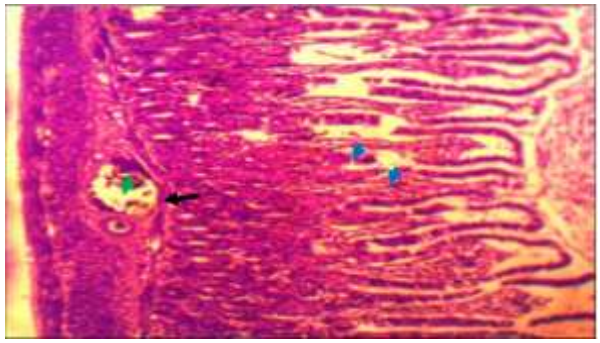

Fig 8: Photomicrograph of intestine of rats pre-treated with aqueous extract of *Nigella sativa at* 640 mg/kg showing normal basement membrane (black arrow), normal blood vessel (green arrow) and damaged intestinal mucosa (blue arrow), cresyl violet stain x100.

4. Discussion

The distortion of the mucosal layer and the basement membrane that was noticed in rats pre-treated with normal saline showed that normal saline did not protect the intestine in ethanol –induced intestinal ulcer. Alcohol consumption therefore resulted in irritation and inflammation of intestinal mucosa which eventually led to intestinal ulcer and affect mucous production as observed in figures I and II. This is also in accordance with studies conducted by [9-10]. The submucous layer in this group also showed distortion as was also found in the animals in group 2 (Fig 3-4). The glandular epithelium was observed to be thicker in the group treated with Gestid. This group also showed a mild mucosal layer disruption as well as mild damage to blood vessels and basement membrane. This drug is standardly used for the treatment of gastric ulcer, but it offered less protection on the intestine in the experimental group because the stomach digested the drug before it reached the intestine for absorption wheras ethanol passed directly into the intestine where its absorption caused the damage observed in the micrograph for this group damage. This is in agreement with [9] who experimented on the effect of ulcer on standard drug antacid in treatment of gastric ulcer. It was discovered that because the stomach acid was neutralized, it also offered mild protection to the intestines.

The experimental group that was pre-treated with aqueous extract of *Nigella sativa* in low dose (320 mg/kg) showed mild disruption of the intestinal villi but the mucosal distortion was less compared to the rat pre-treated with normal saline, this is in agreement with [12-13] who reported that when Wistar rats that were induced with ulcer were given

Nigella sativa, it reduced distortion in the mucosal layer of the intestine.

Aqueous extract that was administered in high dose (640 mg/kg) showed an high improvement in the structure of the intestinal villi, the mucosa membrane and the blood vessels irritation were less, this may had occured as a result of the thymoquinone substance that is present in the *Nigella sativa*. Reports according to [12] shows that *Nigella sativa* has an antioxidant and anti-secretory activities, which might had contributed in protecting the mucosal layer of the intestine.

4.1 Author contributions

Concept and design: Abwage F. and Ishaya H.B.
Administrative support: Dibal N. I., Ishaya H. B. and Attah M. O. O.
Provision of study materials: Dibal N. I. and Abwage F.
Data collection and assembly: Abwage F. and Dibal N. I.
Data analysis and interpretation: Attah M. O. O., Abwage F. and Dibal N.I
Manuscript writing: All Authors
Final approval of manuscript: All Authors.

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