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Original Article

Anti-ulcer activity of NS-EA 51 – A fraction of *Nigella sativa* seed, in histamine plus pylorus-ligated and hypothermia plus restrain stressed rat models

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ABSTRACT

Background: In spite of all recent advancements, the complete cure of gastric ulcer disease has awaited, to be explored. Earlier we reported the potent anti-secretary and anti-ulcer activities of ethanol extract and a fraction of *Nigella sativa*, seed (NS) in indomethacin-induced gastric ulcers-models. Therefore, anti-ulcer activity of purified fraction of NS (NS-EA 51) in different experimental gastric ulcer-models was evaluated in the present study.

Methods: Anti-ulcer activity of NS-EA 51 was evaluated in histamine plus pylorus ligation (PL) and hypothermia-restrained stress, rats (adult male albino; weighing 180–220 g). Changes in the gastric secretion volume, pH, acid-output, pepsin activity, mucus secretion and ulcer indices were observed in histamine plus PL rats. Effects on gastric mucus secretion and ulcer indices were also evaluated in the hypothermic-restrain stressed models.

Results: NS-EA 51 antagonized histaminic effects on gastric juice volume, pH, acid-output, lesion formation and pepsin activities. Fraction also inhibited gastric ulcer formation induced by hypothermic-restrained stress in this study. But it did not show any significant change in the gastric mucus secretion in above models. The anti-ulcer effects of NS-EA 51 found comparable to the Famotidine, a reference anti-ulcer agent.

Conclusion: NS-EA 51 fraction isolated from NS may prove an effective anti-ulcer tool.

1. Introduction

Uncontrolled acid secretion and ulceration of gastric mucosa due to several reasons have posed serious problems to the human health all over the globe.[1] Many natural products and modern synthetic drugs have been used to treat the gastric ulcer disease but so far a complete cure has not been discovered and exploration of new anti-ulcer drugs has remained a field of active research.[1] Since centuries a number of medicinal plants have been used in the treatment of gastric

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ulcer.[2] The modern drugs have also been used to treat the disease in different combinations as double, triple and quadruple therapy regimens.[3–5] In spite of all these developments, side/adverse effects and recurrence of gastric ulcer disease occurs even after long-term therapies.[6–8] Therefore, the treatment of this disease has continued to be the big therapeutic challenge to the pharmacologists.

In an effort to further search curative and safe agents for the treatment of gastric ulcer in the indigenous medicinal plants, present study was undertaken. For this purpose, a highly reputed and quite frequently used medicinal plant in the traditional medicine, *Nigella sativa* (Kalonji) seed was selected. In our previous study, we reported that the ethanol extract, ethyl acetate fraction (NS-EA) and purified fraction (NS-EA 51) of *N. sativa* seed protected the rats against gastric ulcers, induced by indomethacin.[9] Therefore, it was planned to test the purified fraction of *N. sativa* seed (NS-EA 51) for its anti-ulcer effects in the histamine plus PL and hypothermia-restrain stressed models.

2. Methods

2.1. Plant drug and extracts

N. sativa seeds were purchased locally from herbal dealer in Gujranwala, Pakistan. The plant material was authenticated and compared with its standard in the herbarium maintained by Department of Botany, University of Agriculture, Faisalabad, Pakistan. A specimen (NS. Ph. 102) of this drug was preserved in the Pharmacognosy Laboratory, Department of Pharmacy, the Islamia University of Bahawalpur, Pakistan.[2]

Dried, ground NS (1.0 kg) was macerated with ethanol (2.0 lit) at room temperature for 24 h. Dried extract was obtained and stored in the sealed containers at 4 °C. Extract (500 g) was partitioned in succession with butanol (120.30 g), chloroform (91.50 g) and ethyl acetate (95.80 g) and residue fraction (192.40 g). The ethyl acetate fraction was chromatographed on silica gel column (6.0 × 100 cm, 1.0 kg) using an ethyl acetate/ethanol gradient system (1:0 → 0:1). The purified entities (NS-EA 51; 180 mg) were obtained by 51% mixture of ethyl acetate in ethanol.[2,9]

2.2. Animals

Adult healthy Sprague–Dawley albino male rats weighing about 180–220 g were used in this experiment. The rats were obtained from University of Agriculture, Faisalabad and National Institute of Health (NIH), Islamabad (Pakistan). The animals were housed under the standard conditions of temperature (23 ± 12 °C), humidity (55 ± 15%) and 12 h light (7.00–19.00).[9] Animals were provided with a free access to a standard feed (M/S Lever Brothers, Rahim Yar Khan, Pakistan) and water *ad libitum*. The rats were fasted for 12 h prior to their use in the experiments. They were fed according to a strict schedule (6.00, 14.00 and 20.00 h).[9] The animals were divided randomly into different groups, 6–8 animals each that were used in accordance with the principles and guidelines of the Gandhara University Council on Animal Care in this study.

2.3. Chemicals

All chemicals used *i.e.* histamine, alcian blue, bovine serum albumin, ether, gum tragacanth, hydrochloric acid, sodium citrate, Biuret reagent, sodium hydroxide, sodium-potassium tartrate, potassium iodide, cupric sulfate, sucrose, magnesium chloride and diethyl ether were of analytical grade that were obtained from E. Merck (Darmstadt, FRG), BDH Poole (England) and Sigma Chemical Co. (USA). The reference anti-ulcer drug, famotidine was taken from Ferozsons Laboratories Limited, Rawalpindi, Pakistan.

2.4. Gastric ulcer induction

2.4.1. Histamine plus pylorus ligation

The method of Tanaka et al.[10] was used to produce the experimental gastric ulcer in the rats. The test drugs were suspended in 2.5% gum tragacanth solution before their administration (intra-gastric gavages, *ig*), followed by histamine 25 mg kg^{-1} of body weight injection (*sc*) in pylorus-ligation (PL) treated groups of rats.

5 ml kg^{-1} of body weight, 2.5% gum tragacanth vehicle was given orally (*ig*) to each animal in the untreated and treated control groups.[2] The treated control, reference control and treated groups of animals were administered histamine 25 mg kg^{-1}. Additionally the reference control group of rats were given a single dose of Famotidine 100 mg kg^{-1} orally and animals of different treated groups received a single dose of NS-EA 51 (equivalent to 2.0 g kg^{-1} of body weight, NS powder) orally (*ig*).[11,12]

Starodub et al.[13] operative procedure was adopted. The rats were anaesthetized with ether and their abdomens were opened through a midline incision. The pylorus of rats under trial were secured and ligated with silk suture, the abdominal wounds were stitched in separate layers, which were then allowed to recover from anesthesia. Following PL, drinking water was withheld and gastric juices were allowed to collect for a period of 4 h.[12]

2.4.2. Hypothermic-restraint stress

The method of Ichikawa et al.[14] was used to produce the experimental gastric ulcer in the rats. The animals of different groups were placed in restraint cages and immersed to the level of the xiphoid in the ice-cold water at 4 °C for 2 h.[11] The same animals were then used for experiments. The different groups of rats were treated by above-mentioned protocol without PL separately. All the rats were killed by an overdose of ether and their stomachs were removed to visualize the gastric ulcers following the incisions along the greater curvatures.[13,14]

2.5. Gastric secretion volume, pH and acid-output determinations

The volumes and pH of all supernatants of centrifuged gastric juices were measured separately.[15] Acid outputs were calculated by following equation according to the method of Ishizuka et al.[11]

$EqH^{+}/100\ g/4\ h = 1/\text{antilog pH} \times 1000 \times \text{Volume of gastric juice (ml)} \times 100/\text{body weight of animal (g)}$.