SUPPLEMENTARY MATERIAL

Effect of *Nigella sativa* L. seed extract on cisplatin induced delay in gastric emptying in Sprague-Dawley rats

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**ABSTRACT**

The aim of this study was focused on investigating the possible protective effect of *Nigella Sativa* L. seed extract against cisplatin-induced delay in gastric emptying, in a rat model. Twenty-five male Sprague-Dawley rats were divided into five equal groups as follows: Group I or control group, Group II (cisplatin 10 mg/kg, i.p at day 5), Group III (*Nigella sativa* L. 250 mg/kg for 5 days + cisplatin 10 mg/kg, i.p on day 5), Group IV (*Nigella sativa* L. 500 mg/kg for 5 days + cisplatin 10 mg/kg, i.p on day 5) and Group V (ondansetron 3 mg/kg/day, per os + cisplatin 10 mg/kg, i.p on day 5). Phenol red meal was adopted to estimate gastric emptying in different groups of the rats. Gastric emptying was significantly increased (p<0.01) in *Nigella sativa* L. seed extract-pretreated rats (Group III and Group IV) when compared to cisplatin...
treatment alone (Group II). However, ondansetron produced significantly (p<0.01) better reversal than *Nigella sativa* L. seed extract.

**Keywords:** *Nigella sativa* L.; Gastric emptying; Cisplatin; Ondansetron

**Experimental**

**Animals**

25 Sprague-Dawley male rats, weighing 150g-200g, were obtained from Central Drug Research Institute (CDRI), Lucknow, India. Upon arrival to our laboratory, animals were housed in polypropylene cages (60 × 40 × 20 cm) that were furnished with wood shaving bedding, which was changed every 1–2 days. Cages were placed adjacent to each other under environmentally controlled conditions (temperature: 22°C; humidity: 50%) with a 12 h light/12 h dark cycle (lights on between 08:00 and 20:00 hours). Animals were housed in cages with a maximum of four rats per cage and given food in the form of dry chow pellets food and water *ad libitum*. The animals were deprived of food 24 h prior to the experiment but allowed free access to water until 2 h before the experiment. The experiment commenced after acclimatization for one week to the animal house conditions. During the first week, rats were habituated to the testing procedures and to daily handling by the investigator. The study received the approval of the Institutional Animal ethical committee of Integral University, Lucknow, India. Animals were cared for in accordance with the internationally accepted principles for laboratory animal use and care and the procedures followed were in accordance with the standards set forth in the Guide for the Care and Use of Laboratory Animals (Published by the National Academy of Science, National Academy Press and Washington, D.C)

**Collection and Preparation of NS seed extract**

The dried seeds of *Nigella sativa* were purchased from a local market in Lucknow, north India and identified and authenticated in the Department of Pharmacognosy, Faculty of Pharmacy, Integral University, Lucknow, India, where a voucher specimen was deposited with Ref. No.: IU/PHAR/HRB/15/02. The seeds of NS were washed, dried, and crushed to a powder with the help of mortar and pestle. The powdered seeds were extracted by repeated maceration using 70 % (v/v) ethanol for 3 days, and the mixture was then filtered using Whatmann filter paper no. 1. The extract was dried by evaporation using rotary vaporizers under reduced pressure at a
temperature of 40-45 °C. The residue filtrate obtained was then dried by steam bath at 40 °C and kept in a refrigerator at 8 °C for experimental usage. The yield of the aqueous ethanol extract was 16 % in weight by weight (w/w).

**Experimental design**

Experiments were done in the laboratory of pharmacology, Faculty of Pharmacy, Integral University, Lucknow India. Twenty five Sprague-Dawley male rats were divided into five equal groups. Group I received single intraperitoneal (i.p) injection of isotonic saline on the 5th day of experiment (control); group II were given Cisplatin (10mg kg\(^{-1}\)) intraperitoneally on the 5\(^{th}\) day. Group III and IV were provided NS seed extract at 250mg kg\(^{-1}\) (p.o) and 500mg kg\(^{-1}\) (p.o) for the first five days of experiment respectively, followed by intraperitoneal injection of cisplatin (10mg kg\(^{-1}\)) 30 min after last dose of oral administration of NS seed on 5\(^{th}\) day. Similarly, group V were given cisplatin(10mg kg\(^{-1}\)) intraperitoneally 30 min after last dose oral administration of Ondansteron (given for first five days of experiment at the dose of 3 mg kg\(^{-1}\)). The extracts and drugs(Ondansteron) were suspended in 1% sodium carboxy methyl cellulose to administer them orally in the volume of ml/kg. Cisplatin was dissolved in normal saline (2.5 mg cisplatin powder per ml of saline) at 50°C and cooled to 37°C before administration.

**Measurement of gastric emptying**

Gastric emptying of non-nutrient solution was determined according to the method described previously (Sharma and Gupta, 1997). Briefly, a test meal (0.05% phenol red in a 1.5% aqueous methyl cellulose solution) in a volume of 1.5 ml/rat was given by gastric tube after 30 minutes of administration of cisplatin. Thirty minutes after the meal was administered, the animals were anaesthetized with thiopental sodium (40mg kg\(^{-1}\), i.p) and then sacrificed by cervical dislocation. The stomach was removed and homogenised in 100 ml of 0.1 N NaOH. Proteins (in 5 ml of homogenate) were precipitated with 0.5 ml of trichloroacetic acid (20% w:v), centrifuged and separated out. The supernatant was mixed with 4 ml of 0.5 N NaOH and the absorbance of the sample was read at wavelength of 560 nm. Phenol red recovered from stomach of five rats were anesthetized (with thiopental sodium; 40mg kg\(^{-1}\), i.p) and sacrificed immediately after administration of methyl cellulose meal served as standard stomach. The percentage of gastric emptying in different groups was calculated as:
Gastric emptying (%) = 1 - (Amount of phenol red recovered from the test stomach)/(Average amount of phenol red recovered from the standard stomach) x 100

The parameters were expressed as mean ±standard error of mean (SEM). The values were analyzed by one-way analysis of variance (ANOVA) followed by the Dunnett’s t-multicomparsion post hoc test. The level of statistical significance was set at p < 0.01. Analysis was performed using SPSS software package Version 20.0.

References
Sharma SS, Gupta YK. 1997. Effect of antioxidants on cisplatin induced delay in gastric emptying in rats. Environmental Toxicology and Pharmacology. 3: 41-46.
Supplemental file (Figure) Legend

Figure S1. Effect of *Nigella sativa* seed (NS) extract on cisplatin induced delay in gastric emptying in rats. Means showing different letters (a, b, c) between columns different significantly at p <0.01. Data were analyzed by ANOVA followed by Dunnett’s test. Each bar represents mean percentage of gastric emptying and standard error of five animals in each group (n= 5)

Figure S1.