ANTIDIARRHEAL ACTIVITY OF METHANOLIC EXTRACT OF NIGELLA SATIVA SEEDS IN RODENTS

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ABSTRACT

Background: Nigella sativa (N. sativa) has a special place in herbal medicines that are used in plant – based treatment of different ailments.

Aim: The aim of this study was to evaluate the antidiarrheal activity of methanolic extract of seeds of N. sativa.

Methodology: The seeds of N. sativa were collected, identified, and authenticated with the help of a plant taxonomist. The seeds were dried at room temperature, grounded, passed through a 60-mesh sieves, and extracted with hydroalcoholic solvent. Adult albino rats weigh between 150 and 200 gm of both sexes were used for experimental procedure. At the time of experiment, rats were randomly divided into five groups with six rats per group. Group 1 (negative control) received 10ml/kg in distilled water. Group 2 (positive control) received 1mg/kg Loperamide. Group 3, Group 4, Group 5 (test groups) received four different doses respectively such as (100, 200, 300 and 400 mg/kg) of the crude extract and solvent fractions.

Results: N. sativa extract significantly reduced GIT motility, number of defecations and water content in the feces when compared with the standard drug Loperamide (1mg/kg) in a dose dependent manner. N. sativa extract at 300mg/kg extract showed significant results as compared to 100, 200 and 400 mg/kg.

Conclusion: The study concludes with the validation of anti-diarrheal effects of N. sativa seeds provided that the GIT motility decreases in a dose dependent manner. The results also pave way for more study on the chemical constituents (flavonoids, alkaloids, and tannins) present in N. sativa seeds as a possible reason for this medicinal property.

Keywords: Nigella sativa, Loperamide, GIT motility, tannins, flavonoids, anti-diarrheal.

INTRODUCTION

Diarrhea is the second leading causes of death especially in children [1]. To treat involuntary muscles spasm, synthetic antispasmodic drugs, antidiarrheal, anticholinergics, and antibiotics are used. Due to severe side effects, the shift to natural and herbal medicines is paramount [2]. In recent decades, the concepts of nutraceuticals have become popular among health-conscious individuals. These concepts have embraced the attention of dietitians, nutritionists, food scientists, physicians, as well as food and pharmaceutical industries [3]. GIT disorders can lead to reduced life eminence and increased risk of anxiety and depression [4].

Nigella sativa (N. sativa) is also called ***Black seed*** **or** ***Black cumin*** (Kalonji). It belongs to the family Ranunculaceae [5]. N. sativa is a small herb, mostly cultivated in India, Southern Europe, Pakistan, Syria, Turkey, Saudi Arabia, North Africa, and Southwest Asia. In India, it is found in Punjab, Himachal Pradesh, Gangetic plains, Bihar, Bengal, Assam, and Maharashtra 8]. It is a small shrub with tapering green leaves and rosaceous white and purplish flowers [6]. Its ripe fruit contains tiny seeds, dark black in color, known as "Habba Al-Sauda" [7] [8]. The black seeds contain protein, fat, carbohydrates, alkaloids that isoquinoline alkaloids, terpenes, saponins, thymoquinine, Thymohydroquinine, dithymoquinone, p-cymene carvacrol, 4-terpineol, t-anethol, sesquiterpene, α-pinene [9].

N. sativa, used in the form of essential oil, paste, powder, and extract, has been indicated in traditional medicine for many diseases, such as asthma, bronchitis, rheumatism, headache, back pain, anorexia, amenorrhea, paralysis, inflammation, cancer, eczema, and diarrhea [10, 11]. N. sativa is effective in GIT disorders such as abdominal pain, irritable bowel syndrome (IBS), spasm, ulcerative colitis, and diarrhea. The seed of this plant is commonly known as black seed and is referred to by the Prophet Muhammad (PBUH) as having healing powers [12].

The description of the phytochemical composition, antimicrobial activity of N. sativa seeds was approved using different extracts. N. sativa seed oil has thymoquinone, avoids liposomes from suffering non-enzymatic lipid peroxidation. [13] N. sativa seeds are often used to treat a wide range of (illnesses, including bronchitis, diarrhea, rheumatism, asthma, and skin conditions) [14]. The seeds are used to stimulate the immune system, fight parasite infections, performance as a digestive aid and anti-diarrheal [15].

MATERIAL AND METHODOLOGY

Chemicals/reagents: Loperamide hydrochloride, castor oil, charcoal, distilled water, methanol, and chloroform.

Equipment: Weighing balance, freezer, bloating paper.

Plant Collection: The seed of N. sativa were purchased from an eco-geographical area of Pakistan, Rahim yar khan. The crushed seed powder was dried at room temperature, ground, and passed through a 60 mesh seize. Plant material was recognized

and authentic by a plant taxonomist at the GCU Lahore (voucher number).

Preparation of extract

The N. sativa seed was shade dried then crushed and extracted with the ratio of 100g (1kg) by soaking the powder in 80 % hydroalcoholic solvent using (80% methanol: 20%distilled water) with daily shaking at room temperature for 12 days. The extract will then filter using Whatman filter paper. The filtrate was then dried at room temperature. The crude extract was suspended in distilled water before administration to the animals. Stock solution of N. sativa extract by using distilled water and given to the animals according to the body weight in concentration of 100mg/kg, 200mg/kg, 300mg/kg. N. sativa extract was stored in an airtight flask in a refrigerator till further solvent fractionation and experimental procedure.

Animals

Adult rats of both sexes, weighing between 150 and 200g were used. Animals remained provided with animal feed and tap water. Before the experimental procedure, food was reserved, and water was allowed. In this study all animals were cared for in accordance with international guidelines.

Experimental Design

Anti-diarrheal activity

Rats were kept in six cages containing 6 rats each. At the time of the experiment, the rats were randomly divided into five groups with six rats in each group. G1 (negative control) receiving 10ml/kg distilled water, G2 (positive control) getting 1mg/kg Loperamide and G3, G4, G5 (test groups) receiving different doses (100, 200, 300, and 400 mg/kg) of the N. sativa methanolic extract and solvent portions. All rats remained given their respective treatment orally using oral gavage. Throughout the experiment, the rats were handled as per the global standard rules set for the Consideration and Utilization of Research Center Creatures. All administrated doses were intraperitoneal. Rats were placed in cages linked with white blotting paper. 1 ml of castor oil was given orally to the rats 1 hour after the above treatment. Paper was altered every hour after recording its weight. In the laboratory, the filter paper was dried for the last 14 hours, and it was reweighted. Fecal water content was calculated. Rats were observed up to 5hr after administration of CO for the presence of diarrhea. Diarrhea was considered as watery (moist) unformed stool. The quantity of wet stool was counted consistently for 5 hours. In the control group the total number of wet stools was 100%.

Gastrointestinal motility

The gathering of rodents was abstained for 18 hours yet could unreservedly admittance to water. Six groups of six animals, groups A, B, C and D were administered charcoal suspension, 10% charcoal suspension 5% gum acacia were given orally 1 hour after castor oil treatment, 60 minutes later the animals were given intra gastric. N. sativa and group E treated with 2mg/kg Loperamide. Group F (control group) were treated with typical saline prior to getting the charcoal for 40 minutes of observation. period. Each rat was sacrificed and dissected. The small digestive tract was taken out, and all-out length (cm) was estimated. The

movement of charcoal from the pylorus was equally measured. The intestinal charcoal transit was expressed as a percentage of the distance moved by charcoal to the total length between the pylorus and the caecum.

RESULTS

Preliminary phytochemical analysis

Crude extract of N. sativa was evaluated for the presence or absence of auxiliary metabolites like alkaloids, flavonoids, tannins, terpenoids, and steroidal compound, glycosides, phenols and saponins utilizing the methodology using the procedure described by Software [16].

Castor oil induced diarrhea

The animals of control group appeared diarrhea in 30 minutes later administration of castor oil for the 4 hours. Loperamide 2mg/kg was largely eliminated by intraperitoneal injection (50.13%). Impact of the Nigella sativa separate was not generally as powerful as Loperamide at 100mg/kg, however in portion 200, 300, 400 mg/kg. The concentrate of N. sativa created a dose dependent reduce in the no. of defecation over 4 hours, ($p<0.001$). This is discussed and presented in Table 1 and Graph 1 and 2.

GIT motility test

GIT motility was measured by calculating the distance (cm) travelled by charcoal. N. sativa reduced the gastrointestinal motility in a dose dependent manner. At dose of 400mg/kg it significantly reduced the GIT motility compared control group. Loperamide 1mg/kg caused a significant reduction in the propulsive movement and length of the intestine insulated by charcoal, shown in Table 2 and Graph 3 and Graph 4.

$T\bigcirc - T1/T\bigcirc \times 100$

$T\bigcirc$=length of intestine

T1=distance travelled by charcoal in intestine

Effect of oral dose of methanolic extract administer to mice.

The acute toxicity study, all rats given the methanol extract stay alive. They seemed dynamic and healthy, without any indication of any signs of unusual behavior.

DISCUSSION AND CONCLUSION

Individuals ordinarily utilize various part of plant for the treat sicknesses remembering diarrheal illness with no logical reason for their well-being and viability [17]. Moreover, the study conducted on the methanolic extract of the seed of N. sativa seeds resulted in antidiarrheal effects against CO induce diarrhea in decreasing defecation frequency in rats. Consequently, this study was intended to assess the antidiarrheal impact of the 80ME seed extract of N. sativa, albino rats' model beside CO induce GIT fluid accumulate as compared with the earlier study [18] .

Castor oil consist of 90% ricinoleate, the active ingredient i.e., ricinoleic acid, which has an inflammatory, irritated action on the mucosa layer of intestine therefore it can reduce prostaglandin, increment the penetrability of the mucosal cells and cause changes in the transport of electrolyte

causing loose bowels. PG secretion N. sativa extract [19]. The standard drug i.e. Loperamide used for the positive control is a synthetic opiate agonist as a result in the myenteric plexus of massive gut activated μ opioid receptors [20]. Receptors occur presynaptic at the end parasympathetic cholinergic innervation of gastrointestinal smooth muscle which facilitatory affects smooth muscle contractility [21]. As a result, Loperamide (standard drug) decrease individually delay fecal volume , delay fluid and reduce less electrolyte, causes increases fecal volume & majority viscosity discomfort [22].

Anti – diarrheal activity of the N. sativa extract followed mechanism:

a) N. sativa extract can proliferation the reabsorption of NaCl & water by reducing motility of intestine by charcoal food [23].
b) Alkaloids and flavonoids are inhibit prostaglandin, secretion induced by castor oil [24].
c) The chemical constituents of methanolic extract N. sativa in which the presence of flavonoids, alkaloids, and tannins. The methanolic extract of N. sativa reduce the diarrhea by increasing reabsorption of electrolyte and water on the standard drug like Loperamide [25].

N. sativa has a diarrheal effect due to found tannins, alkaloids, saponins, flavonoids, sterols, sesquiterpene, diterpenes, terpenes, flavonoids, terpenoids, these components are responsible from the biological activities of N. sativa extract. The extract of nigella sativa encounter the standards for the drugs acceptability as an antidiarrheal, these criteria include the formation of moist or unformed feces in animals and inhibition of GIT propulsive action of the GIT [26].

The successive excretion of stools of low consistency, because of the disturbance in water transport and electrolyte in the intestine known as diarrheal disease, different causes lead to diarrhea the mechanism involves in diarrhea. Osmotic diarrhea, in which increase intra – luminal osmotic solutions and decrease in absorption of water. Inflammatory diarrhea and infectious diarrhea, the viral, bacterial and pathogens disrupts of epithelium of the intestine, immune response to inflammatory condition in bowel [27]. During infectious diarrhea, the extract may contribute as an antidiarrheal activity. The N. sativa extract restrained unconstrained, and agonist prompted compression of rodent ileum and decreased digestive motility. These effect contributed to the observed antidiarrheal activity [28].

In the diarrhea, abdominal pain is associated with changes in bowel habits and disrupts intestinal smooth muscle contraction these are common symptoms to produced diarrhea, to treat the diarrhea by selectively blocking voltage dependent Ca+ channels [29]. The contraction of smooth muscle is dependent on the concentration of intracellular Ca+2. The 80ME of N. sativa inhibits the contraction of the rat ileum by blocking voltage dependent Ca+ channels, the extract caused a dose dependent (100, 200, 300, 400mg/kg) relaxation of spontaneous contraction, due to Ca+ blockade. The crude extract of N. sativa seeds exhibits spasmolytic and antidiarrheal activity,

inhibits the smooth muscle contraction, GIT motility to treat diarrhea [30].

The effect of N. sativa extract was like that of Loperamide. Therapeutic effect (standard drug) thought to remain antimotility and anti-secretory properties [30]. 80ME N. sativa extract, percentage defecation inhibition , wet fecal output weight, total stool output weight were detected in a dose dependent manner [31].

Many countries, pharmacopoeias define a wide variety of plant species follows as remedies for abdominal cramps & diarrhea, Therapeutic studies of plants showed that they perform a combination of mechanisms more than one, Calcium channels block the opening K+ channels [32].

To conclude, the present study supports claims by traditional medical practitioners about the use of methanolic extract of N. sativa in the treatment of diarrhea. These results also in line with the finding of the previous study conducted on seeds of N. sativa in terms of percentage protection of defecation to inhibit the SM contraction. Oral administration of 80 M.E of the seed extract of N. sativa provided protection against CO induced diarrhea in albino rats, showed a significant delay in the onset of diarrhea, and decreased the frequency of wet feces. The N. sativa seed extract indicated the antimotility effect at its higher doses, thus the study also elevated the acute toxicity the plant extract in which the plant is found to be nontoxic, which ensures the safe use of the plant extract in folk medicine.

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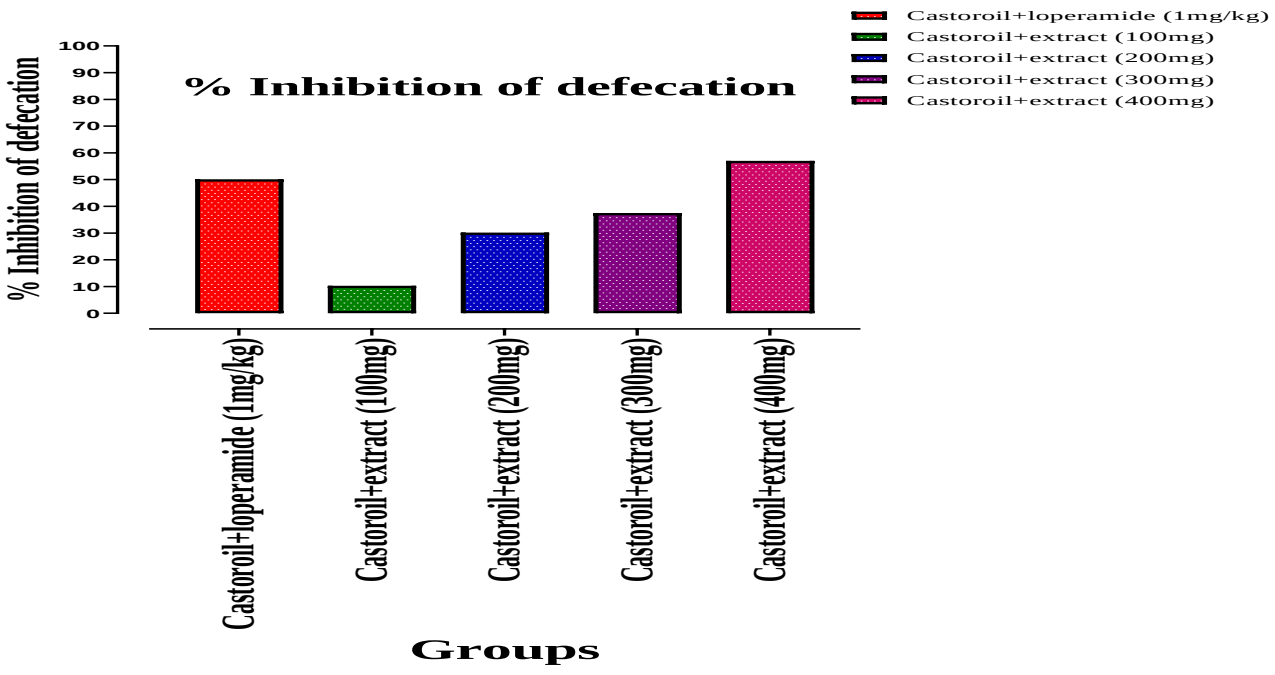
Table 1: Effect of methanolic extract of seeds of N. sativa on castor oil induced diarrhea in rats.

Treatment	Mean defecation in 4hr (g/kg body wt.)	% Inhibition of defecation
CO +N/S (2ml/kg)	24.62±0.36	0
Castor oil + Loperamide (1mg/kg)	13.30±1.8**	50.13%
Castor oil + extract (100mg)	22.1±0.61*	10.3%
Castor oil + extract (200mg)	17.62±0.21**	30.18%
Castor oil + extract(300mg)	15.2±0.22**	37.5%
Castor oil + extract (400mg)	11.05±0.29**	56.99%

The methanolic extract was administered intraperitoneally one hour before CO administered, value is communicated as mean SEM from the research when contrasted with castor oil + saline treatment. SEM, standard error of mean, **P<0.01, * P<0.001.

Graph 1: Graphical representation seeds of N. sativa on castor oil induced diarrhea in rats.



Graph 2: Graphical representation of defecation inhibition through dose of seeds of N. sativa and castor oil induced diarrhea in rats.

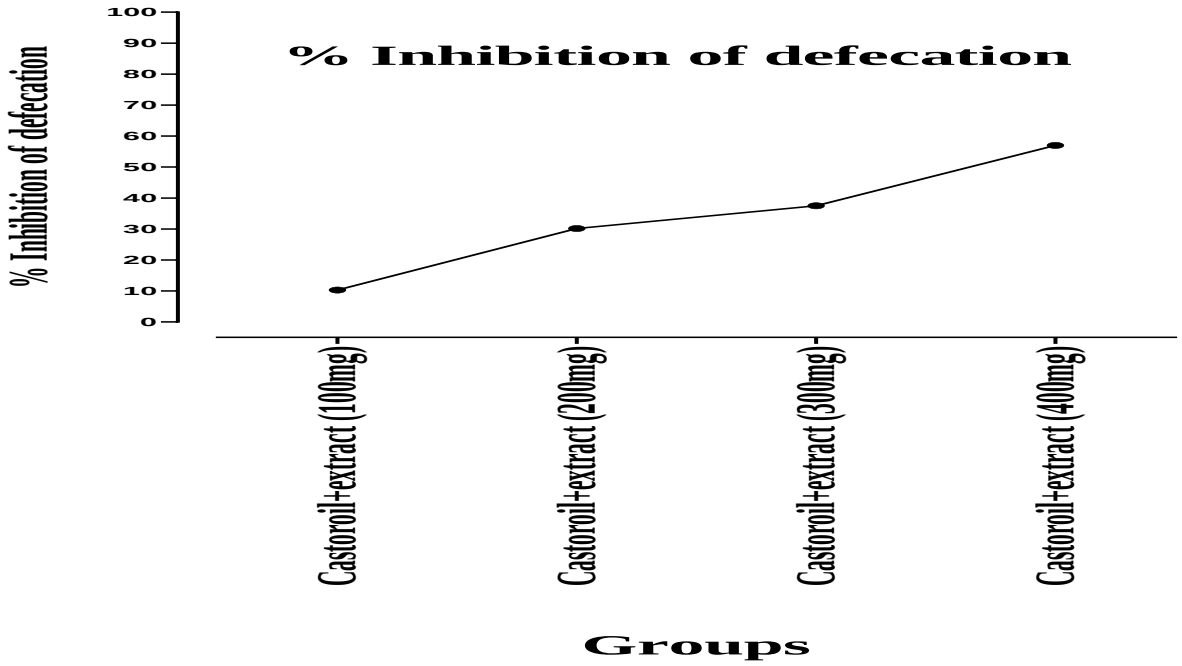
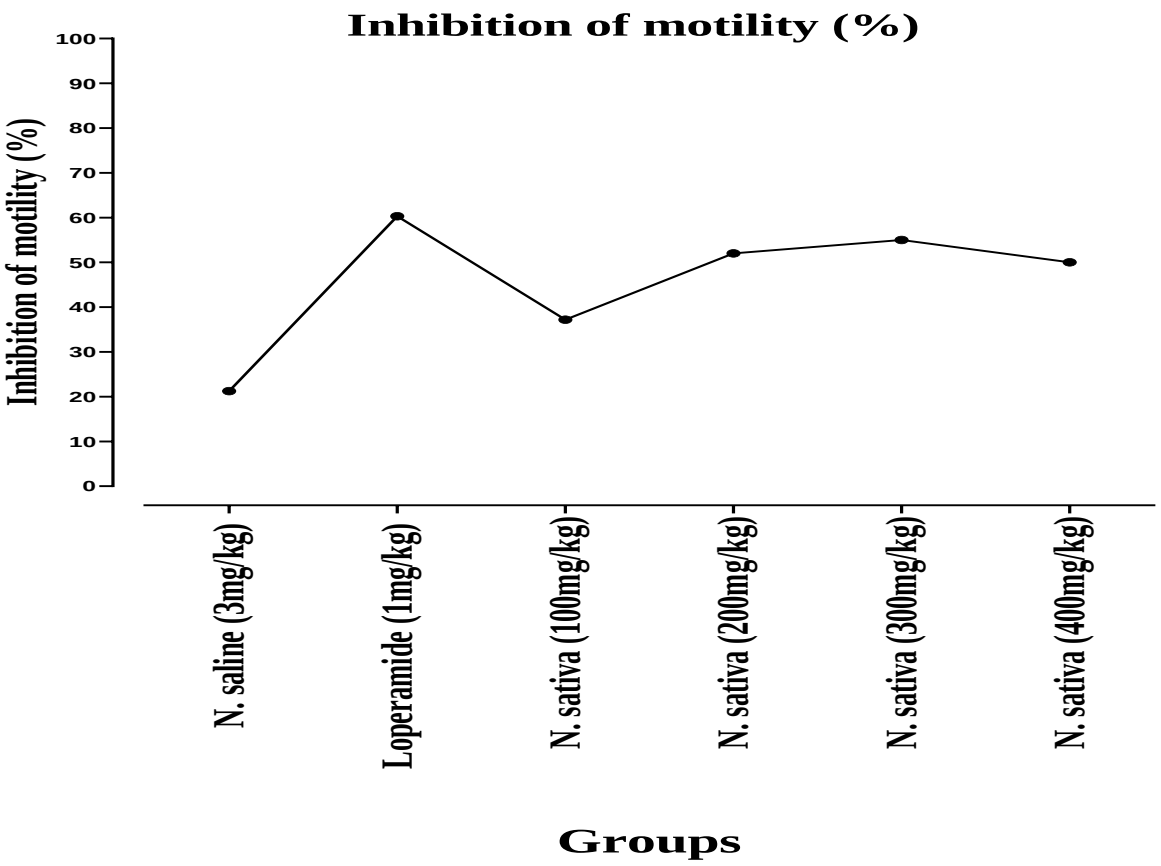


Table 2. Effect of methanolic extract of N. sativa extract on gastrointestinal motility communicated as distance travelled by the charcoal suspension as percent of the absolute intestine length.

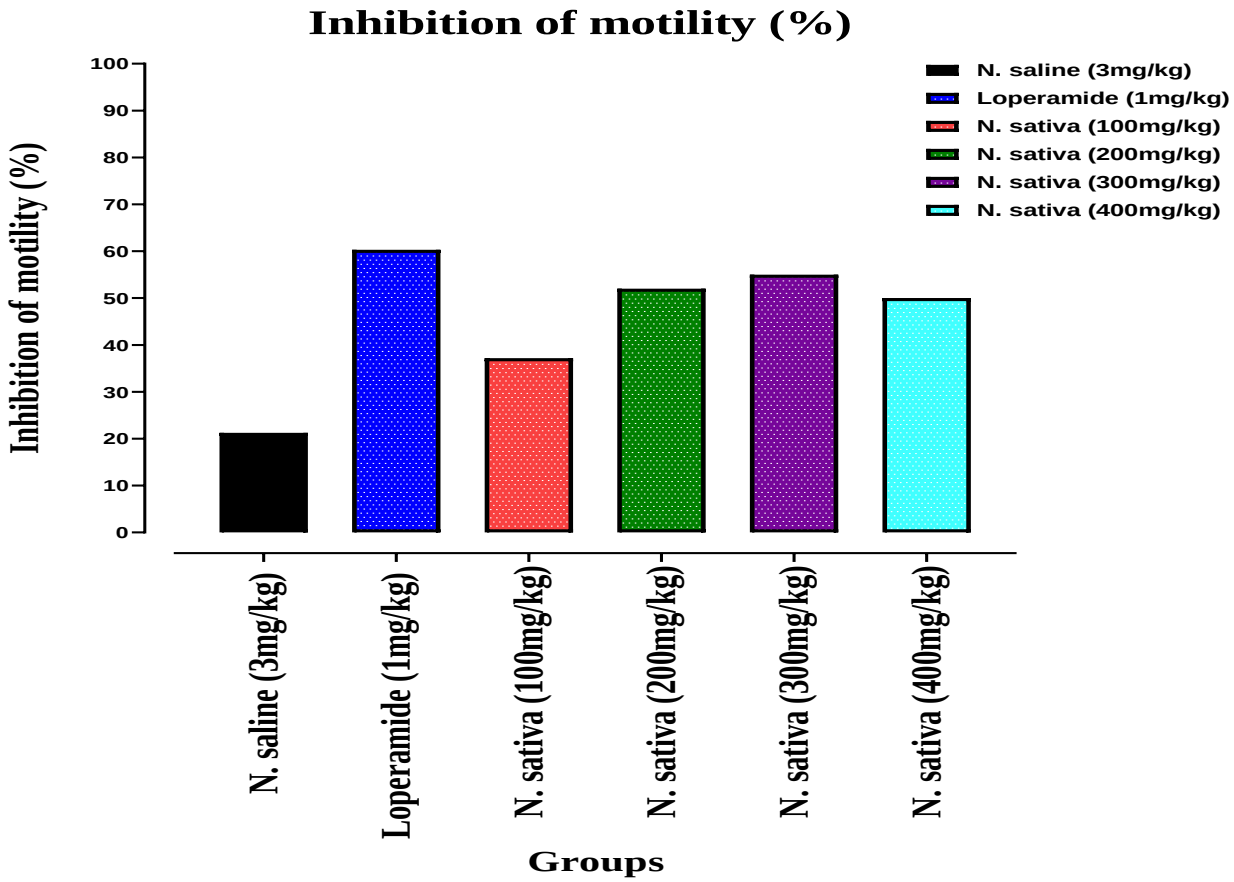
Treatment	Dose	Total length of intestine(cm)	Distance travelled by charcoal(cm)	Inhibition of motility (%)
Normal Saline	3ml/kg	100.93±1.08	80.7±0.50	21.24
Loperamide	1ml/kg	107.58±2.8	41.928±3.10	60.3
N. sativa	100mg/kg	103±1.95	66.1±2.39	37.2
N. sativa	200mg/kg	104.3±3.50	52.3±5.34	52
N. sativa	300mg/kg	106.2±3.70	50.1±3.11	55
N. sativa	400mg/kg	108.72±4	44.08±3.5	50

Results are conveyed in mean ±SEM. Distance travelled by unpaired test followed by one way ANOVA. At dose of 400mg/kg it significantly reduced the GIT motility when it was compared with control group.

Graph 3: Graphical representation of N. sativa extract on inhibition of motility of GIT in rats.



Graph 4: Graphical representation of effect of N. sativa extract, Loperamide, and Normal Saline on inhibition of GIT motility in rats.



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