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Acute Toxicity Studies of Petroleum Ether, Methanol and Aqueous Extracts of *Nigella sativa*

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ABSTRACT

The purpose of the study was to test the acute oral toxicity of the different extracts of the plant *Nigella sativa*. Acute toxicity of petroleum ether, methanol and aqueous extracts of *Nigella sativa* was evaluated in Swiss mice. The acute toxicity studies were carried out based on OECD guidelines 423. The animals were administered orally with a single dose of 100, 250, 500, 750, 1000, 2000 mg/kg body weight of each extract. Signs of toxicity and mortality were noted after 1, 4 and 24 h of administration of the extract for about 14 days. The highest dose administered (2000 mg/kg body weight) do not produce mortality or changes in general behaviour of the test animals. These results indicate the safety of the oral administration of petroleum ether, methanol and aqueous extracts of *Nigella sativa*.

Keywords: Acute toxicity studies, Anaphylaxis, *Nigella sativa*, Methanolic extract.

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INTRODUCTION

Nigella sativa belongs to the family Ranunculaceae is commonly known as Black seed, black caraway, fennel flower, roman coriander, Kalonji and black cumin. [1] It is an annual flowering plant, and is native to south west asia. The plant grows up to a length of 25 to 35 cm. The leaves are linear and the flowers are bluish and white colour. Fruit of this plant is like a capsule and it consists of 3 to 7 follicles. The *Nigella sativa* seed extracts is used to suppress the cough. [2] It is also has anticarcinogenic activity. [3-4]

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The extracts of the seeds are very well used to treat abdominal pain, flatulence, diarrhea and polio. [5] The entire plant is having uricosuric activity [6], antioxidant activity [7] and anti-inflammatory activity. [8-9] The oil from the seeds of *Nigella sativa* was used in cooking also. It is also having antihelminthic activity [10], antiviral activity [11] and also has antimicrobial activity. [12] The seeds are having properties like carminative, diaphoretic and stimulatory activity. It is also used to treat eczema and in asthma. [13-14] Previously reported chemical constituents of *Nigella sativa* were linoleic acid, thymoquinone, nigellone, damascenine, melanthin, nigilline, anethole and dithymoquinone. [15-16] Plants or drugs must be ensured to be safe before they could be used as medicines. By conducting toxicity tests in appropriate animal models, acute toxicity studies, we ensuring the safety of drugs. So, the objective of the present study includes the analysis of the acute toxicity

profile of the petroleum ether, methanol and aqueous extracts of *Nigella sativa* with reference to behavioural aspects, in Swiss Albino mice. The limit test dose of 2000 mg/kg body weight was used following OECD guidelines. [17-18]

MATERIALS AND METHODS

Plant material collection

The plant material of *Nigella sativa* (Retz.) was collected from market in tirupati. They were identified and authenticated in Department of Botany, S. V. University, Tirupathi. The plant materials were coarsely powdered with the help of rotary grinder and stored in airtight plastic containers. The prepared powder was used for preparation of extracts.

Preparation of extracts

The collected plant material was washed, dried at room temperature for about 15-20 days under shade and was treated with a rotary grinder for size reduction. The fine powder which was prepared is used for preparation of extracts. Dried plant material (100 g) was extracted with Soxhlet apparatus using 400 mL petroleum ether for 48 h. After defatting, the marc was dried with the help of hot air oven at 50°C, packed in soxhlet apparatus and further extracted with 400 mL of 95% Methanol until it does not show the presence of any residue on evaporation. The aqueous extract was prepared by using cold maceration with 3% methanol-water for 7 days with occasional shaking. The solvents were removed from the extracts under reduced pressure with the help of rotary vacuum evaporator.

Experimental animals

Acute oral toxicity test was performed as per the Organization for Economic Co-operation and Development (OECD) guidelines 423. [19] Experiments were performed using healthy young Swiss albino mice weighing 25-35 g. [20]

Housing and Diet

The animals were housed in polypropylene cages (55 × 32.7 × 19 cm) at a standard condition of temperature (22 ± 2°C) relative humidity (60 ± 5%). Lighting was controlled to supply 12 h of light and 12 h of dark cycle for each 24-h period. The animals were fed with standard laboratory animal food pellets along with water *ad libitum*.

Grouping of animals

The animals were randomly divided into 3 batches. Each batch contains 7 groups and each group containing 4 mice. Group 1 (Control Group), Group 2 : Receives 100 mg/kg, Group 3 : Receives 250 mg/kg, Group 4 : Receives 500 mg/kg, Group 5 : Receives 750 mg/kg, Group 6 : Receives 1000 mg/kg, Group 7 : Receives 2000 mg/kg of a specific extract of *Nigella sativa*.

Mode of administration

The test substance was administered orally at a single dose using specially designed mice oral needle. Animals were fasted 3 h, before the administration of

drug (only food was withheld for about 3 h but not water).

Administration Dose

After the period of fasting, animals were weighed and test substance was administered orally at a dose of 100, 250, 500, 750, 1000 and 2000 mg/kg. After the administration of extract, food for the mice was withheld for 2 h.

Test substance administration volume

Based on the body weight of the animal, on the treatment day, the quantity of the test substance was calculated. The administration volume of the extract was 1ml/kg body weight of the animal.

Observation period

Animals were observed individually at least once during the first 30 min, after administration of extract, and also observed periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. All the mice were observed at least twice daily with the purpose of recording the symptoms of any ill-health or behavioural changes and for the mortality if any.

Acute toxicity studies

Direct observation parameters include alertness, writhing, gripping strength, pinna reflex, corneal reflex, tremors, righting reflex, skin colour, urination, pupils diameter, subcutaneous swellings, and abdominal distensions. The time of death, if any, was also recorded. After administration of the extract, food was withheld for further 1-2 h. The number of survivors was noted after 24 h and then they were observed for further 14 days and calculate the percentage of mortality.

Statistical Analysis

Data are presented as a mean ± SEM (Standard Error of the Mean). Comparisons were made between the treated groups by the use of single way analysis of variance (ANOVA). P< 0.05 was considered as the level statistical significance.

RESULTS AND DISCUSSION

The present study conducted as per the OECD guidelines 423 revealed that the above extracts did not produce any mortality throughout the study period of about 14 days even when the limit dose was maintained at 2000 mg/kg body weight. The oral LD₅₀ was indeterminable being in excess of 2000 mg/kg body weight. So, testing the extracts at a higher dose may not be necessary and the extracts were non-toxic. Tables 1, 2, 3 indicates the parameters observed before and after the administration of the Petroleum ether, Methanolic and Aqueous extracts of *Nigella sativa* respectively. The parameters observed were normal even at the highest dosage of 2000 mg/kg body weight of the animal. This clearly indicated that the above extracts of *Nigella sativa* do not produce any oral toxicity.

Table 1: Effect of petroleum ether extract of *Nigella sativa* on acute oral toxicity test in mice

S. No.	Response	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Torch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal
4	Corneal reflex	Present	Present	Present	Present	Present	Present	Present
5	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Righting reflex	Present	Present	Present	Present	Present	Present	Present
7	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Pinna reflex	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Pupils diameter	Normal	Normal	Normal	Normal	Normal	Normal	Normal
12	Subcutaneous swelling	Absent	Absent	Absent	Absent	Absent	Absent	Absent
13	Abdominal distensions	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 2: Effect of methanolic extract of *Nigella sativa* on acute oral toxicity test in mice

S. No.	Response	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Torch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal
4	Corneal reflex	Present	Present	Present	Present	Present	Present	Present
5	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Righting reflex	Present	Present	Present	Present	Present	Present	Present
7	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Pinna reflex	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Pupils diameter	Normal	Normal	Normal	Normal	Normal	Normal	Normal
12	Subcutaneous swelling	Absent	Absent	Absent	Absent	Absent	Absent	Absent
13	Abdominal distensions	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 3: Effect of aqueous extract of *Nigella sativa* on acute oral toxicity test in mice

S. No.	Response	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Torch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal
4	Corneal reflex	Present	Present	Present	Present	Present	Present	Present
5	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Righting reflex	Present	Present	Present	Present	Present	Present	Present
7	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Pinna reflex	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Pupils diameter	Normal	Normal	Normal	Normal	Normal	Normal	Normal
12	Subcutaneous swelling	Absent	Absent	Absent	Absent	Absent	Absent	Absent
13	Abdominal distensions	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 4: % of mortality of mice after 14 days of treatment with different extracts of *Nigella sativa*

Groups	No. of mice	Dose administered (mg/kg)	Petroleum ether extract		Methanol extract		Aqueous extract	
			No. of mice died	% of mice died	No. of mice died	% of mice died	No. of mice died	% of mice died
1	6	Control(Water)	0	0	0	0	0	0
2	6	100	0	0	0	0	0	0
3	6	250	0	0	0	0	0	0
4	6	500	0	0	0	0	0	0
5	6	750	0	0	0	0	0	0
6	6	1000	0	0	0	0	0	0
7	6	2000	1	16	1	16	0	0

Values are mean $\pm$ S.E.M., n=6, * P <0.05 as compared with the control group

The medium lethal dose (LD₅₀) of the extracts is higher than 2000 mg/kg body weight and hence, in a single dose administration, the plant extracts does not have any adverse effect. Table 4 indicates the percentage of Mortality after 14 days of treatment with Petroleum ether, Methanolic and Aqueous extracts of *Nigella sativa*.

The non-toxic nature of petroleum ether, methanol and aqueous extracts of *Nigella sativa* is evident by the

absence of mortality of the test animals at oral treatment of 2000 mg/ kg body weight. The normal behaviour of the test animals during a period of 14 days suggests the non-toxic nature of the above extracts. Hence *Nigella sativa* could be safe up to the dose of 2000 mg/kg body weight of the animal. Further studies are warranted for determining chronic toxicity of the extracts.

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