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An investigation on LD50 and subacute hepatic toxicity of *Nigella sativa* seed extracts in mice

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Nigella sativa seeds (blackseed) have been used in traditional medicine for the treatment of a variety of diseases including diarrhea and asthma, and have been shown to have various useful pharmacological effects. In this study, acute and subacute toxicity of the aqueous, methanol and chloroform extracts of the seeds have been investigated. To determine their LD50, the aqueous, methanol and chloroform extracts were administered orally, in 4 different doses, 6, 9, 14 and 21 g/kg. Mortality rate and weight changes have also been measured in all groups for 3 and 7 days, respectively. No mortality has been observed in all groups and with all doses. Methanol extracts in all doses and chloroform extract in the dose of 21 g/kg significantly decreased animals weight. Hepatic toxicity of the extracts was also investigated in the dose of 6 g/kg/day orally for 14 consecutive days by measuring ALP, SGOT and SGPT activity in blood and hepatic histological study. Degenerative changes in hepatic cells have been observed only with aqueous extract of the seeds. In conclusion, *Nigella sativa* extracts are relatively nontoxic in the acute toxicity test, but the possibility of hepatic damage with its aqueous extract should be considered.

1. Introduction

Nigella sativa (black cumin) is an annual Ranunculaceae herbaceous plant growing to 30 cm and has an upright branching stem, fine deeply cut leaves, gray-blue flowers and toothed seedpots. The plant is native to Western Asia and the Mediterranean region for its seeds. The seeds contain 40% fixed oil, a saponin (melantin) and up to 1.4% volatile oil (Chevallier 1996).

The seeds of *Nigella sativa* have been used traditionally for centuries in the Middle East, Northern Africa and India for the treatment of various diseases (Brutis and Bucar 2000; Gilani et al. 2004).

The plant extracts and essential oil showed a broad range of pharmacological effects such as antidiabetic (Farah et al. 2002), spasmolytic and bronchodilatory (Gilani et al. 2001), antioxidant (Burits and Bucar 2000), hepatoprotective (Nagi et al. 1999), nephroprotective (Ali 2004), antitumor (Worthen et al. 1998; Khan et al. 2003) and antiulcer (El-Dakhakhny et al. 2000; El-Abhar et al. 2003) effects in various studies. The extracts also showed antimicrobial activity (Hanafy and Hatem 1991). It is used traditionally in Iran as laxative, carminative and intestinal antiprotozoal drug (Amin 1990). As the seeds are used widely for nutritional and medical purposes, this study was carried out to clarify some aspects of possible acute and subacute toxic effects of the seeds extracts.

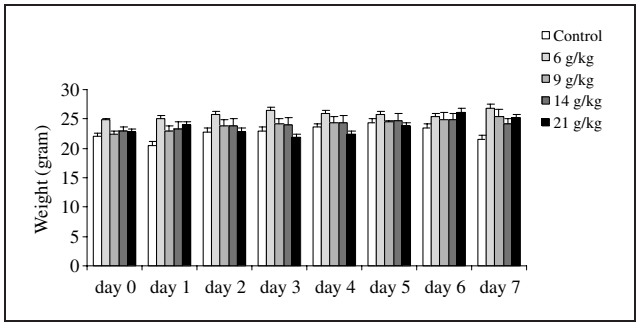

Fig. 1: Effects of acute administration of aqueous extract of *Nigella sativa* on animals weight

2. Investigations and results

2.1. Acute toxicity

Oral administration of 6, 9, 14 and 21 g/kg of aqueous, methanol and chloroform extracts of *Nigella sativa* seeds did not result in animals mortality or significant morbidity.

One animal receiving an aqueous extract in a dose of 21 g/kg and 2 animals from each group receiving doses of 14 and 21 g/kg methanol extract and 2 animals receiving 21 g/kg chloroform extract had hypoactivity and malaise at the first day after dosing but the others were normal. Malaise and hypoactivity were overcome at day 2 or 3 in the affected mice.

Animals weight was not significantly affected by single dose administration of the aqueous extract in 7-day observation (Fig. 1). Methanol and chloroform extracts in 21 g/kg

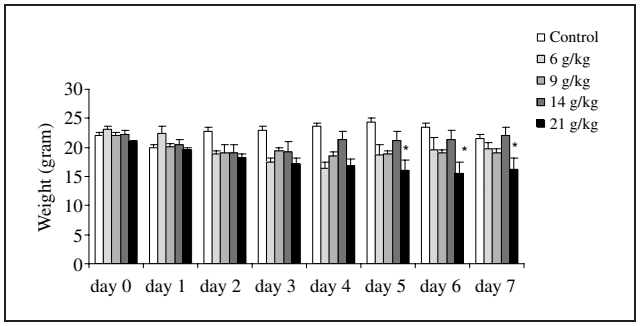

Fig. 2: Effects of acute administration of methanol extract of *Nigella sativa* on animals weight
* $P < 0.05$

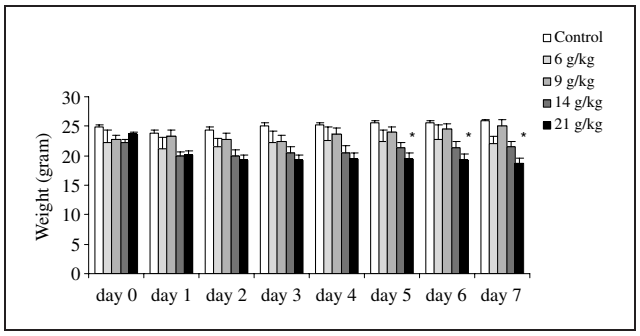

Fig. 3: Effects of acute administration of chloroform extract of *Nigella sativa* on animals weight
* $P < 0.05$

groups, caused significant reduction in the weight of animals as compared with control groups ($p > 0.05$) at days 5, 6 and 7 after dosing (Figs. 2 and 3).

2.2. Subacute toxicity

2.2.1. Weight monitoring

Administration of a single daily dose of 6 g/kg of aqueous, methanol or chloroform extracts of *Nigella sativa* seeds for 14 consecutive days did not cause any significant reduction in animals weight (data not shown).

2.2.2. Effect of extracts on hepatic enzymes

As shown in Fig. 4, administration of an aqueous extract of the seeds resulted in decreased serum levels of alkaline phosphatase (ALP) ($P < 0.01$), as compared with control group. This extract did not change significantly the levels of SGPT or SGOT (Figs. 5 and 6).

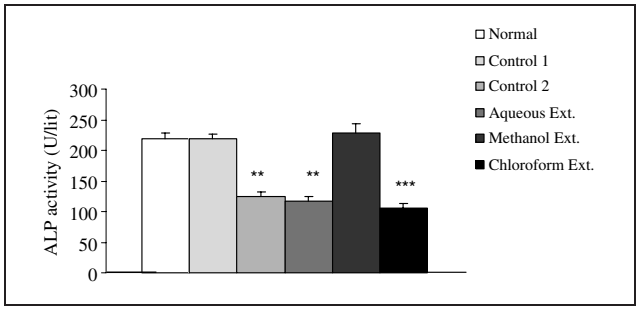

Fig. 4: Effects of aqueous, methanol and chloroform extracts of *Nigella sativa* on mouse serum Alkaline Phosphatase (ALP) activity in subacute (14 days) experiment
** $P < 0.01$, *** $P < 0.001$

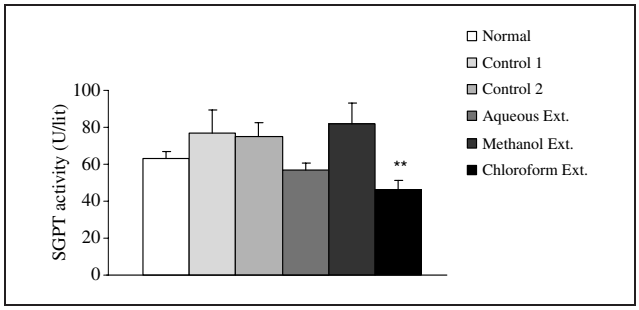

Fig. 5: Effects of aqueous, methanol and chloroform extracts of *Nigella sativa* on mouse Serum Alanine Aminotransferase (SGPT) activity in subacute (14 days) experiment
** $P < 0.01$

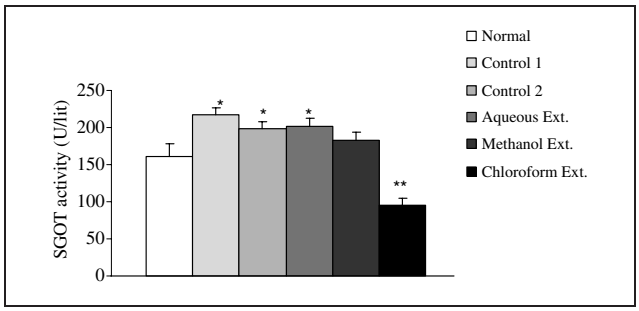

Fig. 6: Effects of aqueous, methanol and chloroform extracts of *Nigella sativa* on mouse serum Aspartate Aminotransferase (SGOT) activity in subacute (14 days) experiment
* $P < 0.05$, ** $P < 0.01$

Methanol extract did not cause any significant change in the levels of these hepatic enzymes (Figs. 4, 5 and 6).
Both control group 2 ($P < 0.01$) and the chloroform extract treated group ($P < 0.001$) showed a significant decrease in ALP activity (Fig. 4). There was not any significant difference in ALP activity between control group 2 and chloroform extract. Chloroform extract, on the other hand, caused a significant decrease in serum levels of SGPT ($P < 0.001$) (Fig. 5) and SGOT ($P < 0.01$) (Fig. 6).

2.2.3. Histopathologic examination

Hepatic samples of aqueous extract treated animals showed pathological changes including diffuse infilterative centers around the portal and central veins, granulation of hepatocyte cytoplasms and dilation of portal spaces. Samples of methanol extract treated animals showed diffuse infilterative centers and two cases of cloudy inflammation and fatty changes. Samples of chloroform extract treated animals showed variable inflammatory cells (less than methanol samples) and dilation of central veins. No cloudy inflammation or fatty changes were observed in this group of samples.
Variable degrees of cellular inflammation and dilation of central and peripheral veins have also been observed in normal and control groups 1 and 2.

3. Discussion

Results of acute toxicity tests with aqueous, methanol and chloroform extracts of *Nigella sativa* seeds showed a wide margin of safety for these extracts. These results are in agreement with other studies that showed a high degree of safety in the acute systemic administration of seed extracts (El-Daly 1998) and essential oil (Khanna et al. 1993; Zaoui et al. 2002).

Some mice in test groups showed significant hypoactivity and malaise after administration of high doses of methanol and chloroform extracts. As this effect has been observed previously with the administration of thymoquinone, one of the main ingredients of the seeds (Badary et al. 1998), these effects are probably due to the presence of thymoquinone in the extract.

Animals weight was reduced in test groups receiving high doses (21 g/kg) of methanol and chloroform extracts in days 5 to 7 (Figs. 2 and 3). Hypoactivity and malaise in test groups and the resultant anorexia might partly contribute to this effect. Some experiments showed that hypoglycemic effect of seeds oil is time-dependent and increases with increasing in the administration period (Al-Hader et al. 1993; Farah et al. 2002). This effect may also contribute to weight reduction and may explain why weight reduction was seen in days 5 to 7 of methanol and chloroform extracts administration. The effects of the acute administration of the extracts may be extended to 7 days. More experiments are needed to clearly define the importance of this effect.

Animals in subacute toxicity experiments were all well. There was not any significant effect of the extracts on their weight or overall health in 14 days of administration.

Aqueous, methanol and chloroform extracts of the seeds did not cause any pathological changes in plasma concentrations of ALP, SGPT or SGOT (Figs. 4, 5 and 6). There was no increase in these enzymes levels. Chloroform extract, on the other hand, caused significant decrease in the levels of SGPT and SGOT. Aqueous extract, also, decreased the level of ALP. These effects suggest a protective action of the extracts on hepatic function. Tennekoon et al. (1991) observed similar results with hepatic enzymes of rats treated with boiling water extract of the seeds. They did not observe a significant decrease in ALP levels in treated animals. They used ether anesthesia for the administration of extract and this might affect the results. Others showed significant decrease in elevated levels of liver enzymes due to hepatotoxic substances and suggested a protective effect of *Nigella sativa* seeds (Al-Ghamdi 2003) and oil (Kanter et al. 2003) against drug-induced hepatic damage. The seeds oil also had protective effects against gentamicin-induced nephrotoxicity (Ali 2004). The most known suspected substance in oil and extracts is thymoquinone and it exerts this effect via its antioxidant action.

Histopathological examination of mice livers in all test groups showed variable degrees of minor pathological changes. These changes were also observed in control and normal groups. Similar histopathological changes were also observed by Tennekoon et al. (1991).

Overall, subacute experiments show that *Nigella sativa* seeds extracts have wide margin of safety in 14 days administration. The promising results of this study emphasize a great margin of safety for *Nigella sativa* seeds.

4. Experimental

4.1. Materials

Nigella sativa seeds were collected from local herbal drugs shops. Methanol and chloroform were purchased from Merck Company.

4.2. Extraction

4.2.1. Reflux extraction

Aqueous and methanol extractions were performed by this method. 150 g of blackseed powder were extracted with 500 ml of water or methanol for 12 h at 95 °C and 70 °C, respectively. The extracts were filtered using filter paper. In order to obtain a completely dry extract, the resultant extracts were transferred to glass dishes and were left at 50 °C in an oven for 24 h. Then, they were left at 4 °C until their use in toxicity assessments.

4.2.2. Wetting procedure

1500 g of ground blackseed in 500 ml chloroform were incubated at 25 °C for 4 days, during which vibration was carried out twice a day. The resultant solution was filtered and dried up as previously described in reflux extraction.

4.3. Toxicological assessment

4.3.1. Acute toxicity

The aqueous and methanol extracts were dissolved in distilled water and the chloroform extract in sesame oil and administered by gavage. Animals of each sex were isolated in separate cages and all animals were kept under fasting conditions for 6 h before dosing. Doses of 6, 9, 14 and 21 g/kg of the extracts were administered to 6 young virgin mice (5–6 weeks age) of both sexes with the weight of 20–25 g. The volume of administration was 10 ml/kg. Animals were under close observation for 72 h after dosing. The effect of the single dose of the extracts on animals weight was also recorded for 7 days.

4.3.2. Subacute hepatic toxicity

Male mice weighing 25–30 g were separated randomly to different groups of normal, control group 1, control group 2 and test groups. Each group consisted of at least 8 animals. Normal group received nothing, control group 1 received distilled water, control group 2 received sesame oil and the test groups received 6 g/kg/day of aqueous, methanol and chloroform extracts (as solutions of 10 ml/kg) for 14 consecutive days. The extracts were dissolved in distilled water and sesame oil as described above and administered orally. Animals were anesthetized by ketamine and xylazine (80 mg/kg and 16 mg/kg ip, respectively) and their blood were collected for the assessment of enzyme activity and their livers were taken for histopathological studies. Alkaline phosphatase (ALP), alanine aminotransferase (SGPT) and aspartate aminotransferase (SGOT) activity were determined for the evaluation of hepatic cell damage. The effect of extracts administration on animals weight was also investigated.

4.4. Statistical analysis

The data were analyzed using ANOVA and Tukey-Kramer multiple comparisons tests.

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