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Original Article

Evaluation of the anxiolytic effect of *Nepeta persica* Boiss. in mice

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The aim of the present study was to evaluate the anxiolytic effects of hydroalcoholic extract (HE) of *Nepeta persica* Boiss. (Lamiaceae) on the elevated plus-maze (EPM) model of anxiety. The extract of arial parts of the plant was administered intraperitoneally to male NMRI mice, at various doses, 30 min before behavioural evaluation. The HE extract of *N. persica* at the dose of 50 mg kg^{-1} significantly increased the percentage of time spent and percentage of arm entries in the open arms of the EPM. This dose of plant extract affected neither animal's locomotor activity nor ketamine-induced sleeping time. The 50 mg kg^{-1} dose of the plant extract seemed to be the optimal dose in producing the anxiolytic effects, lower or higher doses of the plant produce either sedative or stimulant effects. At 100 mg kg^{-1}, the plant extract increased the locomotor activity. These results suggested that the extract of *N. persica* at dose of 50 mg kg^{-1} possess anxiolytic effect with less sedative and hypnotic effects than that of diazepam and causes a non-specific stimulation at 100 mg kg^{-1}.

Keywords: anxiety – elevated plus-maze – *Nepeta persica* – sedative

Introduction

Anxiety disorders are the most common mental illness in the world and became a very important area of research interest in psychopharmacology. Benzodiazepines are among the first line of drugs that have been extensively used for the last 45 years to treat several forms of anxiety (1). Although benzodiazepines have well-known benefits, their side effects are prominent, including sedation, muscle relaxation, anterograde amnesia and physical dependence (2). It is because of these adverse effects that many pharmaceutical companies are conducting studies to find an alternative medicine or plant-derived medications with more specific anxiolytic effects. Some of these plants that have been tested and shown to 'calm down', tranquilize and raise mood include *Valeriana officinalis* (3–5), *Matricaria recutita* (6), *Passiflora caerulea* (7), *Salvia guaranitica* (8), *Tilia tomentosa* (9), *Tilia europaea* (10), *Stachys lavandulifolia* (11), *Echium amoenum* (12) and *Salvia reuterana* (13). *Nepeta* (Lamiaceae) is a genus of perennial or annual herbs found in Asia, Europe and North Africa. About 250 species of *Nepeta* are reported (14) of which, 67 species are available in Iran (15). *Nepeta* species are widely used in folk medicine because of their antispasmodic, expectorant, diurectic, antiseptic, antitussive, antiasthmatic and febrifuge activities (16–18). *Nepeta cataria* (Catnip) is the most famous *Nepeta* species, which has a long history of use as a tea in Europe before real tea was imported from the orient. The flowering tops of the plant have also been used as sedative drug (19). Many reports on *Nepeta* species show that the main constituents of the oil are diastereomeric nepetalactones. Nepetalactones are reported to have considerable sedative activity (20). These compounds are also responsible for their feline attractant or insect repellant properties (21). *Nepeta persica* which is native to Iran, has been proven to have similar compounds (22).

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Despite the use of *N. persica* as an anxiolytic, there is no pharmacological data to support such effects. The aim of the present study was to investigate the anxiolytic effect of ethanolic extract of *N. persica* in the elevated plus-maze (EPM), a behavioral test for anxiolytic drugs (23). In addition to EPM, we also analyzed the effects of *N. persica* on spontaneous activity and its interaction with a CNS depressant.

Methods

Preparation of the Plant Material

Arial parts of *N. persica* were collected from hights of Kolahghazi (1850 m above sea level) in Isfahan province in Iran. For preparation of hydroalcoholic extract, air-dried and powdered sample of the plant (120 g) was macerated with 360 ml of ethanol and water (8:2) for 48 h. The extract was then shaken, filtered and evaporated in a rotating evaporator under reduced pressure to give a residue (20 g). The residue was dissolved in normal saline for final suitable concentrations.

Mice

Male NMRI mice (Pasteur, Tehran) weighing 25–30 g were housed in cage (six in each cage) and kept in a room with controlled temperature (22–25°C). Mice were maintained on a 12:12 light-dark cycle and had access to food and water *ad libitum*. Tests were performed only after the mice had acclimated to the above environment for at least 7 days. All experiments were carried out between 09:00 and 13:00 h. Each mouse received a single intraperitoneal (ip) injection of drugs (diazepam, $1.5\,mg\,kg^{-1}$; ketamine, $100\,mg\,kg^{-1}$), plant extract (different doses) or vehicle (normal saline) and was tested once in the EPM. Minimum of six mice were used for each treatment group. All procedures were approved by the Ethical Committee of the Isfahan University of Medical Sciences, and conducted in accordance with the internationally accepted principles for laboratory animal use and care. Drugs Diazepam and ketamine were purchased from Sobhan Pharmaceutical Co. Iran, and Parke-Davis, France.

Elevated Plus-Maze

The EPM test is described in details elsewhere (24,25). Briefly, the apparatus comprised two open arms (35×5 cm) and two closed arms ($30 \times 5 \times 15$ cm) that extended from a common central platform (5×5 cm). The floor and the walls of each arm were wooden and painted black. The entire maze was elevated to a height of 60 cm above floor level as validated and described by Lister (23). Testing was conducted in a quiet room that was illuminated only by a dim light.

Mice were given a single ip dose of the plant extract 30 min before their placement on the EPM. To begin a test session, mice were placed on the open arm facing the center of the maze. An entry into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5-min test period. The percentage of open arm entries ($100 \times$ open/total entries) was calculated for each animal. Between each trial, the maze was wiped clean with a damp sponge and dried with paper towels.

Locomotor Activity

The actions of plant extract on spontaneous locomotor activity were measured automatically by breaking of infrared beams as described in details elsewhere (26). The units of the activity counts were arbitrary and based on the beam breaks by movement of mice. Each mouse was injected with the plant extract ($25–400\,mg\,kg^{-1}$), diazepam ($1.5\,mg\,kg^{-1}$) or vehicle (normal saline) and then, after 30-min interval, placed in a novel cage in the infrared apparatus. The locomotor activity was measured at 5-min interval for the next 15 min. Six mice were used for each treatment group. The treatments were randomized throughout the day, between 08:00 and 13:00 h, to control for diurnal variations in activity.

Ketamine-Induced Sleeping Time

The effect of plant extracts on ketamine-induced sleeping time was measured as described by Mimura *et al.* (27). After 30 min pre-treatment with the plant extracts ($25–400\,mg\,kg^{-1}$), diazepam ($1.5\,mg\,kg^{-1}$) or vehicle, mice were injected with ketamine ($100\,mg\,kg^{-1}$, ip). In the case of the control, mice were pre-treated with saline and after 30 min received only ketamine. The interval between the administrations of ketamine until the loss of the righting reflex was recorded as onset of sleep, while the time from the loss to regaining of the righting reflex as the duration of sleep (28). Diazepam ($1.5\,mg\,kg^{-1}$) was used as standard drug. Minimum of six mice were used for each treatment group.

Statistics

Statistical analysis was performed using one-way analysis of variance (ANOVA) with *post hoc* Tukey test. $P < 0.05$ was considered significant. All data are expressed as mean ± SEM.

Results

Elevated Plus-Maze Studies

In EPM, the behavior confirmed the anxiolytic activity of diazepam as reported previously (29). In order to

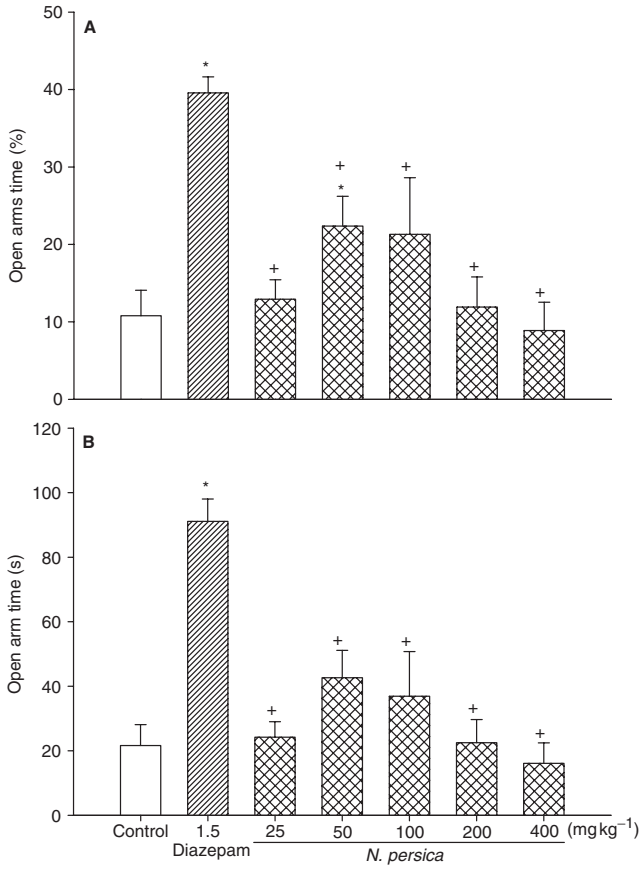

Figure 1. Diazepam, saline and different doses of *N. persica* extract on (**A**) the percentage of time spent in the open arms and on (**B**) time spent in the open arms during a 5 min test in mice. Various doses of the plant, diazepam or vehicle, were injected 30 min prior to test. Data are presented as mean ± SEM for each group of nine mice. $^{*}P<0.05$ compared with vehicle-treated control. $^{+}P<0.05$ compared with diazepam-treated group.

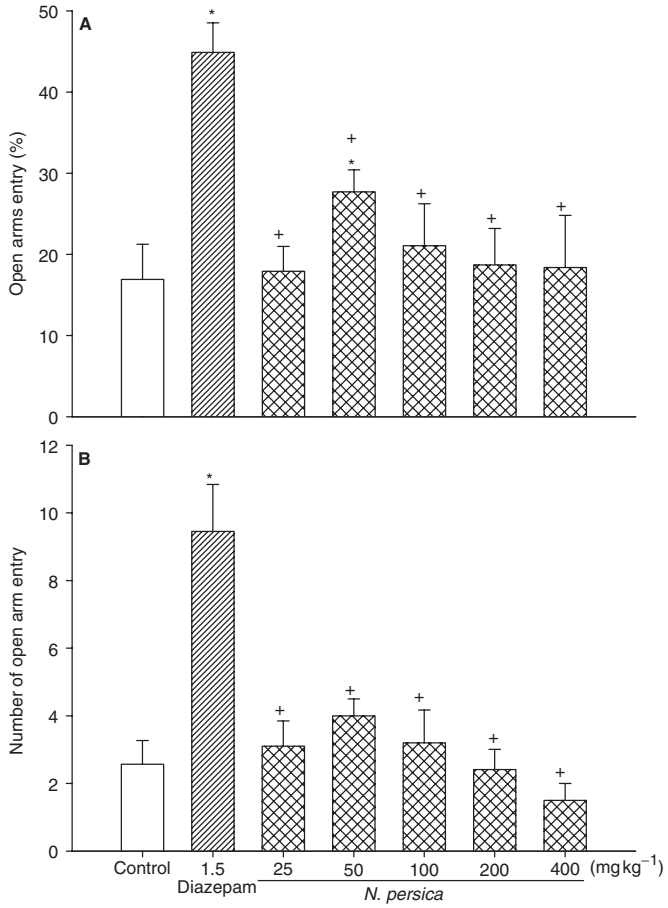

Figure 2. Diazepam, saline and different doses of *N. persica* extract on (**A**) the percentage of entries in the open arms and on (**B**) entries in the open arms during a 5 min test in mice. Various doses of the plant, diazepam or vehicle, were injected 30 min prior to test. Data are presented as mean values ± SEM for each group of nine mice. $^{*}P<0.05$ compared with vehicle-treated control. $^{+}P<0.05$ compared with diazepam-treated group.

determine the effective dose on the EPM, various doses (25–400 mg kg^{-1}) of plant extracts were tested. The hydroalcoholic extract of *N. persica* only at a dose of 50 mg kg^{-1} significantly increased the percentage of time spent [$F(2,36)=17.99$, 99%] and percentage of entries in the open arms [$F(2,36)=14.01$, 64%, $P<0.05$, Figs 1A and 2A]. Other doses of the plant extract had no significant effects on the measured EPM parameters (Figs 1 and 2). Diazepam at 1.5 mg kg^{-1} significantly increased the total arm entry (Fig. 3), while total entry was not significantly altered by the plant extract at doses of 50 and 100 mg kg^{-1} (Fig. 3).

Analyses of Locomotor Activity

Two doses (50 and 100 mg kg^{-1}) of plant extract were tested for their effects on locomotor activity. Figure 4A shows the locomotor activity counts measured at three time intervals of 5, 10 and 15 min. The dose of 100 mg kg^{-1} of the plant extract, significantly increased the locomotor activity at time intervals of 5 min [$F(1,18)=64.88$] and 10 min [$F(2,27)=29.29$, $P<0.05$, Fig. 4A]. The increase in locomotor activity was not observed with the dose of the plant extract at 50 mg kg^{-1} (Fig. 4A). Diazepam on the other hand significantly decreased the locomotor activity at three times intervals.

As shown in Fig. 4B, the total locomotor activity count measured in 15 min of the test was significantly decreased in mice pre-treated with diazepam [$F(1,18)=30$, $P<0.05$], however, this effect was not significantly changed with the plant extract at 50 mg kg^{-1}. The plant extract at 100 mg kg^{-1}, however, significantly increased the activity counts (Fig. 4B).

Time to Loss of Righting Reflex

In saline treated control mice the righting reflex was lost after 107 ± 11 s of ketamin injection. Injection of plant extract (30 min prior to ketamin) at doses of 50 and 100 mg kg^{-1} did not significantly change the latency to sleep (Fig. 5A). In mice pretreated by saline control,

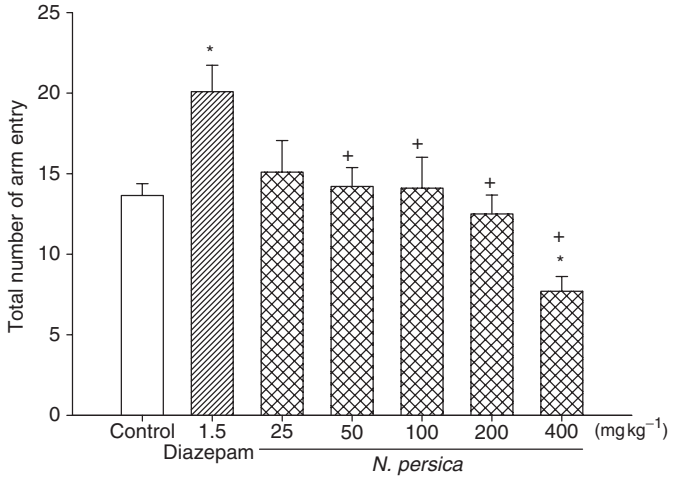

Figure 3. Diazepam, saline and different doses of *N. persica* extract on total number of entries in open and close arms. Various doses of the plant, diazepam or vehicle, were injected 30 min prior to test. Data are presented as mean ± SEM for each group of nine mice. $^{*}P<0.05$ compared with vehicle-treated control. $^{+}P<0.05$ compared with diazepam-treated group.

total sleep time was 1170 ± 259 s (Fig. 5B). Treating the mice with diazepam significantly increased the total sleeping time by 148% [$F(1,16)=10.82$, $P<0.05$, while the plant extract at dose of 50 and 100 mg/kg did not significantly alter this parameter (Fig. 5B).

Discussion

In the present study, we used the EPM model of anxiety to evaluate the anxiolytic effects of the hydroalcoholic extract of *N. persica*. This is a model which uses the natural fear of rodents to avoid open and elevated places. The ratio of open:closed area entries reflects a specific effect on anxiety, provided there is no concomitant change in the total number of entries (open + closed), however, this is not totally true for diazepam which increases preference for the open areas i.e. total entries (30). As expected, diazepam produced significant increases in open arm time and in number of entries into the open arms. Diazepam also increased the total number of entries. These data are in agreement with the results of other studies, where diazepam and other benzodiazepines have been shown to produce robust anxiolytic effects in a variety of anxiolytic screening procedures, including conflict model (31), EPM procedures (32) and other non-punishment procedures (33).

The behavioral alterations induced by the *N. persica* plant extract in the EPM provided anxiolytic effect at 50 mg kg^{-1}. However, unlike many other plant extracts (11–13) where anxiolytic effects was accompanied by sedative action, increase in the dose of *N. persica* exerted stimulation rather than sedation. An inverted U shaped dose-response curve that was seen with *N. persica* extract,

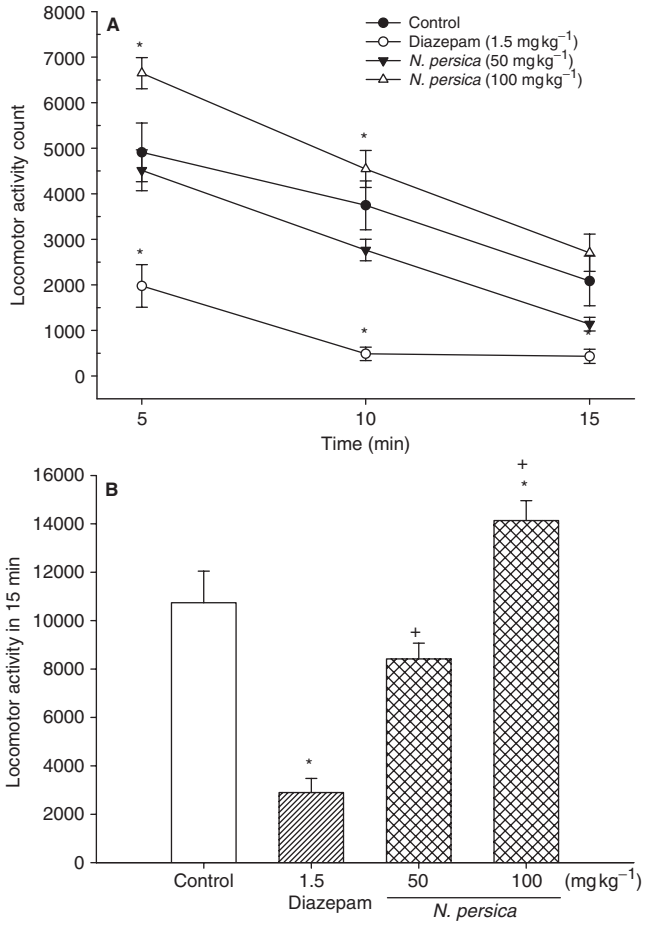

Figure 4. Diazepam, saline and different doses of *N. persica* extract on (**A**) spontaneous locomotor activity during three 5-min intervals and on (**B**) spontaneous locomotor activity during total 15 min. The locomotor activity counts (mean ± SEM) were measured over a 15-min period, beginning 30 min after the administration of saline, diazepam or different doses of *N. persica*. Data are presented as mean ± SEM for each group of six mice. $^{*}P<0.05$ compared with vehicle-treated control. $^{+}P<0.05$ compared with diazepam-treated group.

was also evident in other studies (34). Plant extract at doses higher than 400 mg kg^{-1} caused stretching postures indicative of colic pain in the mice.

The results also show that the extract of *N. persica* at both 50 and 100 mg kg^{-1} did not statistically change the latency to sleep and sleeping time induced by ketamin. This effect further confirms the absence of sedative properties of the extract. Previous studies show that *Nepeta persica* contains a group of lactones called nepetalactones (19) which is thought to be responsible for the sedative action of the plant (20). *Nepeta cataria* which is used as sedative in European folk medicine also contains monoterpenoids, a group which is thought to be composed of anxiolytic components such as linalool (19,35). The volatile oil of *N. persica* has been shown to contain 2.8% and 30.5% linalool and nepetalactones (22). Although it seems that linalool has sedative effect but the main observed effects in this case could be

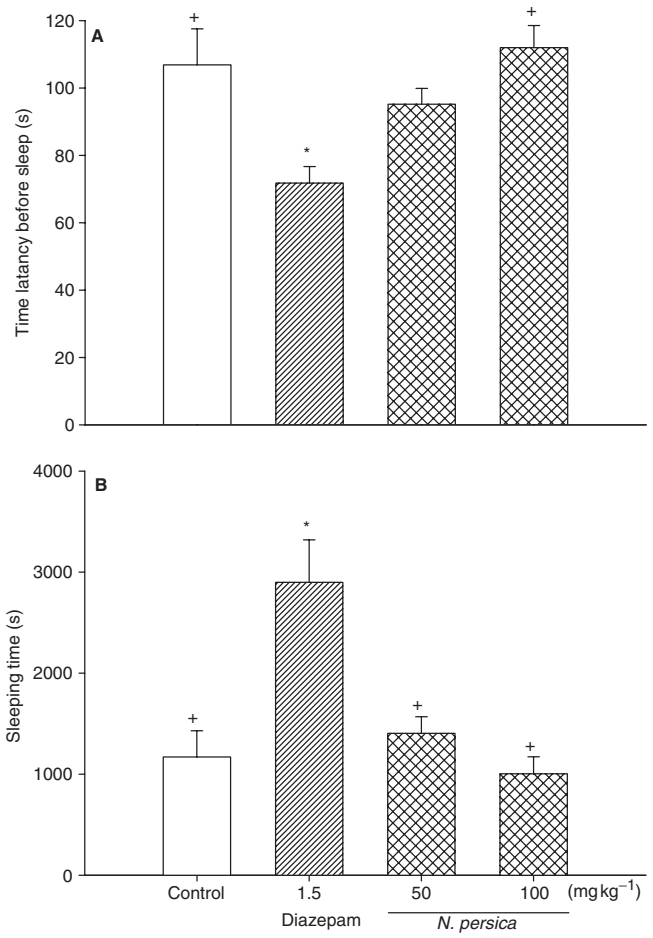

Figure 5. Diazepam and *N. persica* extract on (**A**) the latency to loss of righting reflex and (**B**) total sleep time. The interval between the administration of ketamin until the loss of the righting reflex was recorded as onset of sleep. The time from loss of righting reflex to regaining of the righting reflex was recorded as duration of sleep. Results represent mean ± SEM from six mice. $^{*}P < 0.05$ compared with vehicle-treated control. $^{+}P < 0.05$ compared with diazepam-treated group.

due to the presence of nepetalactones. Alkaloids are the most important secondary metabolites in many plants that are held responsible for their sedative and anxiolytic actions (36). However, as the major constituent of the *N. persica* is the observed anxiolytic action is more likely to be due to nepetalactones rather than alkaloids. Further investigation is required to fractionate the extract of the plant and study each fraction separately for their anxiolytic effects. Isolation of active constituent(s) of the plant extract could pave the way for the standardization of biologically active compound as in the case of Turnera aphrodisiac Ward (37).

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