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Research Article

Study the Anti-Anxiety Effect of Acute Injection of Lavandula Angustifolia Extract in Elevated Plus-Maze Test in Male Rat

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ABSTRACT

With respect to anxiety prevalence and the fact some available anxiolytic drug remain side effect, design of suitable drugs, without unwanted side effects is necessary. Lavandula angustifolia (LA) is used in herbal medicine for the treatment of anxiety. Present study investigated the acute effects of hydro-alcoholic extracts of LA on anxiety-like behavior in Elevated plus-maze (EPM) test.

The EPM is one of the most widely used animal models of anxiety. Four different groups of male Wistar rats intraperitoneally received LA extract (25, 50, 100 mg/kg), or saline (as control group) 30 min before submitting into EPM test**.** The statistical analysis of data was performed by ANOVA followed by Tukey post hoc analysis.

The results showed that compared to the control group, LA (50, 100 mg/kg) increased the percentage of entries in open arms ($P<0.01$) and time spent in open arms ($P<0.01$, $P<0.05$; respectively). LA extract has no effects on the total distance covered by animals and number of closed arm entries. The locomotor activity was not significantly changed by LA.

The LA appears to be an anxiolytic influence effect. Future investigations are essential for pharmacological providing of LA and better understanding of anxiolytic properties and neurobiological mechanisms of LA extract.

Keywords: *Anxiety, Elevated plus maze, Lavandula angustifolia, Rat*

INTRODUCTION

Anxiety is among the most common and mental disorders [1]. Many of people have been sustained anxiety in worldwide [2]. Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety [3]. However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many research to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects [4,5]. There is a need to find effective compound with fewer side effects in the treatment of patient.

Lavandula angustifolia (LA**)** is part of the Labiatae family and belongs to the Lavender genus which is natural growth in the Mediterranean region [6]. Labiates families of plants are generally known for their multiple pharmacological effects [4]. Silexan is an orally active essential oil provision from the flowering tops of LA [7-9]. Lavender species and their essential oils have been native in herbal medicine for the treatment of pain, infection, relaxation, anxiety, or improvement of sleep quality by oral administration, inhalation or topical application [10-14]. Lavender oil inhalation is used in folk medicine as anxiolytic compound [9]. Lavender is reported to be an effective medical plant treating inflammation, depression, stress, seizure and of migraine headache [12, 14, 15]. Lavender is also reported to be an effective medical plant in treatment of restlessness in case of anxious mood. Intake administration of LA has been shown with anxiolytic effect in clinical studies [7, 9, 16]. While, Kumar reported chronic IP administration of Silexan (essential oil of Lavandula angustifolia) has anxiolytic in both of rat and mice [8], the effect of acute administration of Silexan is not fully understood. Note to, anxiolytic effect of inhalation of Silexan is compared with the synthetic drug [8, 17, 18].

Pharmacological study reported anxiolytic properties of the oral application of lavender oil [19]. We examined the acute effects of hydro-alcoholic extracts of LA flower on anxiety-like behavior in male rats using the elevated plus-maze (EPM) test.

MATERIALS AND METHODS

Animal: Male Wistar rats weighing approximately 240-300g were used in this experiment. These animals were transported to a room adjacent to the test laboratory 72 h before the test. They were housed in groups of four per cage under a 12-h light-dark cycle (lights on at 07:00 h) at 23±2 °C and given free access to food and water. Groups of 10 rats were randomly assigned to different treatment groups tested in a varying order. Animals were tested repeatedly under the same experimental conditions. All experiments were carried out in a quiet room under

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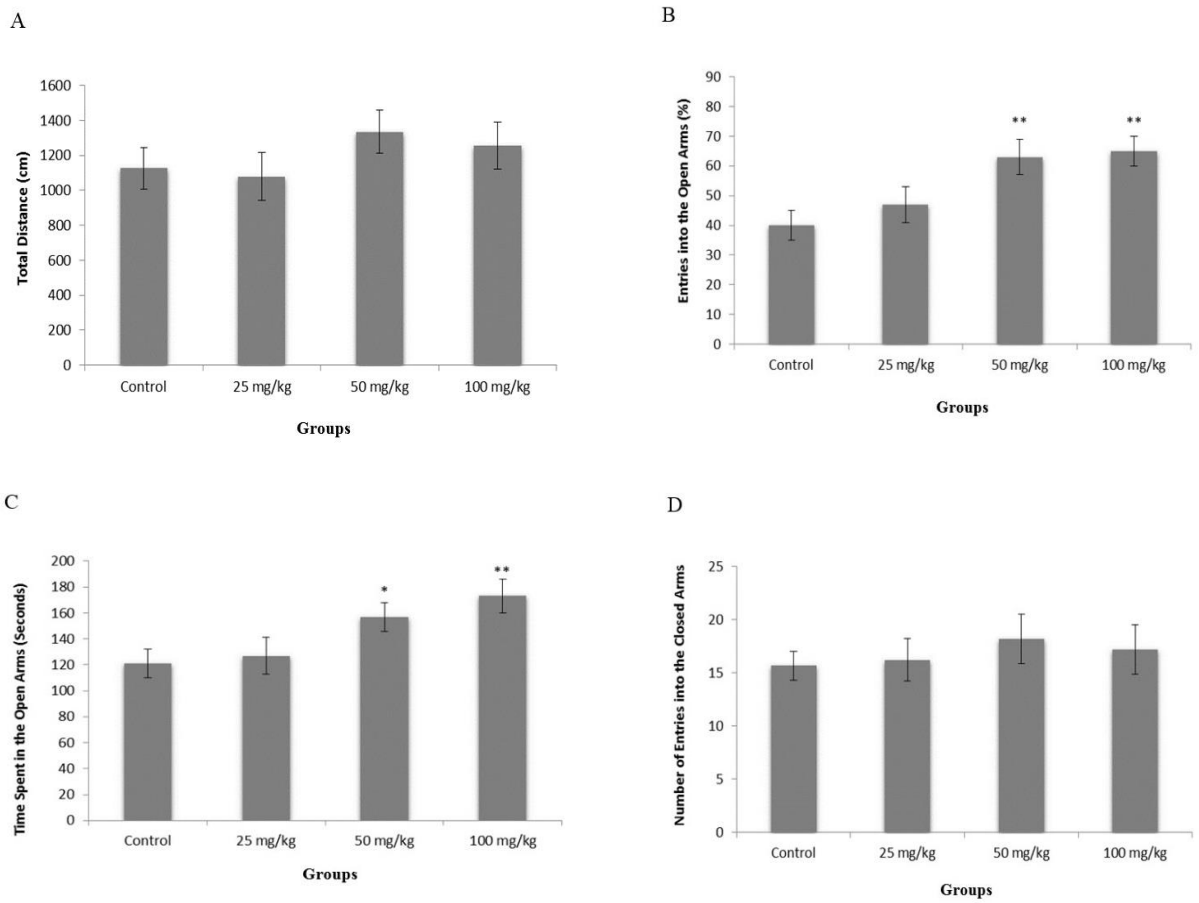

*Fig. 1: The effects of Lavende angustifolia extract (25, 50, 100 mg/kg i.p.) on total distance covered by rats (A), on the percentage of entries in open arms (B), time spent in open arms (C) and number of closed arms entry (D) during the 10 min test session in EPM. Data represent means±SEM. Animals were 10 in the treated groups. Comparisons were made by using a one-way ANOVA followed by post hoc Tukey test. *: P<0.05, **: P<0.01 in contrast with control group.*

controlled light conditions between 11:00 a.m. and 3:00 p.m. Behavioral observations took place in soundproof rooms at the same period of the day to reduce the confounding influence of diurnal variation in spontaneous behavior. Each animal was tested only once. All research and animal care procedures were approved by the Veterinary Ethics Committee of this University. All experiments were conducted in accordance with international standards of animal welfare recommended by the society for Neuroscience (Handbook for the Use of Animals in Neuroscience Research, 1997). The minimum number of animals and duration of observation required to obtain consistent data were employed.

Drugs and Preparation of the Extract: The extract of LA collected in this University. The doses are expressed as mg of dried extract/kg. Three different concentrations (25, 50, 100 mg/kg) of the LA extract were prepared by dissolving the extracts in 10 ml deionized water with 0.5% propylene glycol to form a homogenous suspension. All compound intraperitoneal (IP) administration 30 min before place in EPM session.

Elevated plus maze: Anxiolytic activity was measured using the EPM test. This test has been widely validated to measure anxiety in rodents [20-22]. Briefly, for rats, the apparatus consisted of two open arms (50 × 10 cm each), two enclosed arms (50 × 10 × 50 cm each) and a central platform (10× 10 cm), arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 100 cm above floor level. Thirty minutes after the i.p. injection of the extract (25, 50, 100 mg/kg) or diazepam (0.3, 0.6 and 1.2 mg/kg) or the solvent, each animal was placed individually in the center of the maze, facing one of the open arms. During the 10 min test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms and the distance travelled as measures of locomotion activity, was recorded [22, 23]. Entry into an arm was defined as the point when the animal places all four paws onto the arm. All tests were conducted between 08:00 and 14:00.

Each mouse was used only once. Animal behavior was taped by using a video camera located above the maze. Two 100 W lamps brightly illuminated the arena. The experimental sessions were recorded by video camera interfaced with a monitor and a VCR in an adjacent room. This apparatus allowed the measurement of activity (i.e. movement time spent) or time and distances covered in each part of the maze during a 10 min period of time. In order to record displacements and other behaviors, the

image of the EPM was divided into 10-cm squares in a transparent mask placed on the TV screen. This allowed recording of the behavioral category end-arm exploration when the animal reaches the extremity of the open arms [21, 22]. After the test, the maze was carefully cleaned with a wet tissue paper (10% ethanol solution).

Statistical analysis: Calculation of the percentage time and number of entries on the open arms with 95% confidence limits and comparisons of the results were performed using computerized analysis. The statistical analysis of data was performed by one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis. In all cases differences were considered significant if $p< 0.05$.

RESULTS

ANOVA showed that there was a significant difference in rat behavior between LA extract treated groups and control group in EPM model. The total distance covered by the LA extract treated rats during the 10 min test was not significantly ($P>0.05$) different from controls (Fig. 1A). Tukey test analysis showed that a significant increase in percentage of open arms entries in concentrations of 50 mg/kg and 100 mg/kg ($P < 0.01$), but not at 25 mg/kg in compared to the control group (Fig. 1B). In addition, the extract-treated group spent more time in the open arms in concentrations of 50 ($P < 0.05$) and 100 mg/kg ($P < 0.01$), but not at 25 mg/kg (Fig. 1C). The number of entries into the closed arms was not significantly different between the treated groups versus control group (Fig. 1D).

DISCUSSION

An increase of the time and the proportion of the entrances into the open arms without a changed locomotor activity is regarded as a powerful marker for an anxiolytic substance effect [23]. The present study showed the acute IP administration of LA extract in rats induced anxiolytic behavior and did not any significant effect on locomotor activity in EPM test.

The EPM is commonly one of the most widely used models of animal anxiety [21, 23]. Its validity in our study was supported by the observation that diazepam, a typical anxiolytic, significantly increased the time spent in the open arms. The behavior observed using the EPM in the present study confirmed the anxiolytic activity of diazepam as reported previously [4, 9, 17, 22]. In this method high dose of diazepam could not induce anxiolytic effect because it reduces animal locomotion activity in result of severe sedative effect. Therefore, EPM is not suitable to assess anxiolytic activity of the high dose of diazepam, and other anxiety survey methods, like Shuttle Box should be used.

Using this test the LA increased the percentage of time spent in the open arms. The anxiolytic result of LA extract is similar to anxiolytic drugs such as diazepam in acute or chronic without sedative effect. These results confirm LA has an anxiolytic-like and safe effect [7, 9].

LA has been affect autonomic neurotransmission [17, 24, 25]. Therapeutic anxiolytic property of lavandula oil capsule (Silexan) over 10 weeks has reported in patients with subsyndromal anxiety disorder [7]. Silexan capsule formulation induces anxiolytic effect compare to lorazepam in patients with generalized anxiety the Hamilton Anxiety Rating Scale [26]. A decreasing of state anxiety was found in subject administrated to Silexan while watching the anxiety- provoking films [16]. Other clinical studies have shown anxiolytic effects of Silexan inhalation in humans [1, 27].

Lavender oil exerts an anti-anxiety effect in open field test [8], Vogel test [28] and elevated plus-maze [8, 17, 29]. Shaw et al suggest that lavender oil does have anxiolytic effects. Rats were exposed to lavender oil for 30 or 60 min prior to open-field test were compared with the effects of chlordiazepoxide injected [18]. The IP injection of extract prior to EPM increase time spent in open arms similar to lorazepam injection in 1 week [8].

The active components of LA are thought to be linalool, linalyl acetate, cineole, terpinen-4-ol and camphor [13, 19, 25, 30]. The presence of linalool, linalyl acetate in the plant extract supports the claim that the extract has sedative effect [10]. Some studies reported the parable mechanisms. Chronic Injection of Lavender oil altered dopamine D3 receptor subtype homeostasis in the olfactory bulb and induced behavioral change [24]**.** Also, Lavender oil potent anxiolytic properties via modulating voltage dependent calcium channels [19]. Linalool, a monoterpene compound prevalent in essential oil of Lavender, interferes with glutamatergic transmission [25]. Also, cineole and terpinen have been found in the essential oil of the LA [13, 30]. It is possible these components play essential role in anxiolyic properties.

CONCLUSION

In conclusion, the results of the present study suggest that acute IP injection of LA extract produced the anxiolytic properties due to their active components and did not diminish the locomotion activities in rat. Further investigations concerning efficacy and safety of LA and its active constituent's formulation in both of rodent and humans seem to be warranted.

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