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Comparative effects of balm hydro alcoholic extract and diazepam on reducing anxiety in mice

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ABSTRACT

Anxiety is a normal feeling which is experienced in threatening situations and can affect neural system. Balm (*Melissa officinalis*) is proposed in traditional medicine for adjusting the anxiety. This study was carried out to compare the effects of balm extract and diazepam on anxiety adjustment. 50 female were studied in five treatments groups including control, diazepam, 50, 100, and 200 mg/kg of balm's hydro-alcoholic extract. Drug and extract doses were injected IP. After receiving the last dose, anxiety was induced using dark box and was evaluated by an elevated plus-maze. Frequency of entrance to open and close arms and also the spent time in those arms was measured as anxiety index. Hydro-alcoholic extract in 200 mg/kg dose increased the time of presence in open arms significantly which shows anxiety reduction. Also, movement activities of mice were significantly higher in this dose in proportion to diazepam. According to results, hydro-alcoholic extract of balm in 200 mg/kg dose can be good replacement for diazepam in reducing anxiety reflexes.

Keywords: Balm, Anxiety, Diazepam, Elevated plus-maze, Mice

INTRODUCTION

Almost all human individuals have experienced stress, discomfort or expectation of unpleasant events. Anxiety plays important role in psychology studies of abnormal people. Few people experience a week without anxiety. Shorter periods of anxiety will harass an ordinary person [1].

Anxiety is a natural feeling which is experienced in threatening situations. Mental state, concerns and anxiety are associated with physiological changes (increase in heart beat, blood pressure respiration rate, and muscles tonus) and prepare the person to defend or escape. In other words, anxiety is a wide unpleasant concern which is accompanied with stimulation signs of the autonomic nervous system [2]. Nowadays, a lot of people experience anxiety because of numerous problems. The continuation of this state can cause turbulence in normal life. Wide spread of anxiety forces many people to permanent use of anti-stress drugs especially benzodiazepines. One of these drugs is diazepam (Valium) as an analgesics and hypnotics drug which is classified as long-acting benzodiazepines [3]. Although these drugs have high security policies and new types of them have more specific effects, but they have side effects like drug resistance and withdrawal symptoms when the drug is stopped abruptly. Therefore, searching for effective drugs with fewer side effects is continued [4].

Since effective matters of herbal drugs are in balance with other matters biologically, they will not be accumulated in body and don't have side effects or have low side effects and are substantially superior to synthetic drugs [5].

Balm is one of the medicinal plants which are mentioned in both traditional and modern medicine for anxiety reduction. This plant (*Melissa officinalis*) is one of the oldest pharmaceutical plants from Lamiaceae family. Various

properties have been ascribed to this plant. Sedative, antioxidant, anti-spasm, carminative, anti-bacteria, antiviral and anti-inflammation effects are some properties of this plant [3].

Bisto Vichtel showed that balm reduced the signs of neurological disorders like stress, anxiety and irritability [6]. This plant protects neural cells and destroys free radicals. Methanolic and aquatic extracts of this plant are both controllers of mono aminoxidase (MAO) which methanolic extract is stronger. Therefore, balm has been reported as antidepressant also [7, 8].

The goal of this study was investigating the effects of balms hydro-alcoholic extract on reducing anxiety of mice in proportion to diazepam.

MATERIALS AND METHODS

Fifty female mice weighing approximately 25-30g were used in this experiment. Mice were kept in Standard polycarbonate cages with stainless steel perforated ceiling. Samples had free access to food and water. Cages floors were covered by sawdust which was replaced every two days. Mice were divided into five groups with ten members in each group: control group did not receive any drug, Diazepam group which received 1.2 mg/kg of diazepam and three experimental groups which received 50, 100, and 200 mg/kg doses of extract in peritoneum. Treatment groups received drug 30 minutes before the test.

- Anxiety evaluation:

Elevated plus-maze was used to evaluate the anxiety [9]. The machine has two open arms (50×10 cm) and two closed arms (40×50×10 cm). Open and closed arms were opposite and were 50 cm higher than the room floor (Figure 1).

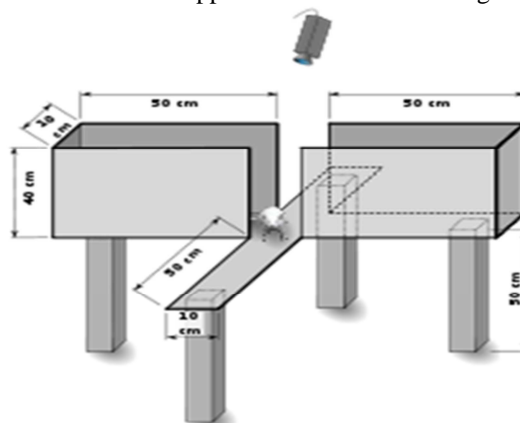


Figure 1. plus-maze was used to evaluate the anxiety

This model of anxiety evaluation is experimental and doesn't need to train the animal [10]. This evaluation is based on two instincts: curiosity of rodents and avoiding from open environments so that animal wants to stay in closed arms. Four parameters were measured: the number of times the animal enters or stays in open or closed arms [10]. Being of all four legs in arms is evaluated as 'Staying'. Percent of entrance to open arm (OAE %) and percent of staying in open arms (OAT %) and movement activity were calculated as follows:

$OAE\% = \frac{\text{the number of entrance to open arms}}{\text{total number of entrances to open and close arms}} \times 100$

$OAT\% = \frac{\text{Openarm time}}{\text{total time in both arms}} \times 100$

Movement activity = the number of entrance to closed arm + the number of entrance to open arm

Significant increase in percentage of entrance to open arms and percentage of staying in open arms plus no movement activity shows anxiety reduction in this test. However, OAE% in proportion to OAT % has less sensitivity in recording Anxiety and anxiolytic actions of animal.

- Statically Analysis:

The experimental design was a completely randomized design. Data were analyzed by SPSS (18) software.

RESULTS AND DISCUSSION

Figure 2 shows that the injection of balm's hydro-alcoholic extract in dose of 200mg/kg led to an increase in the time of animal presence in open arms or OAT% ($P < 0.05$).

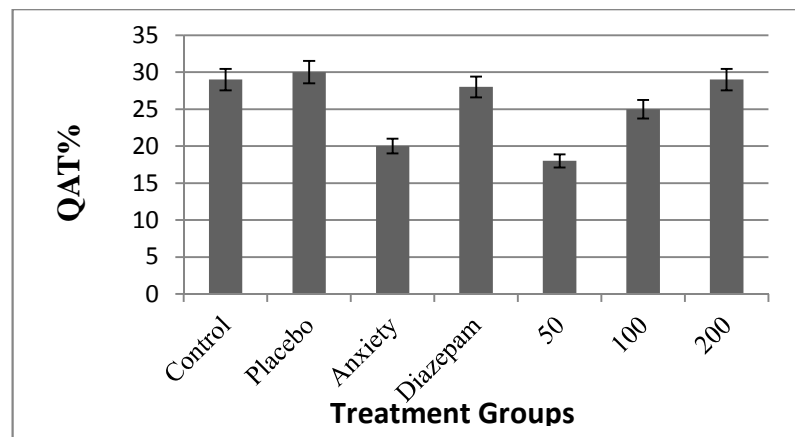


Figure2. Time of animal presence in open arms percentage (QAT %) in All Groups

Figure 3 shows that dose of 200mg/kg extract and diazepam led to increase in movement activity in maze ($P < 0.05$).

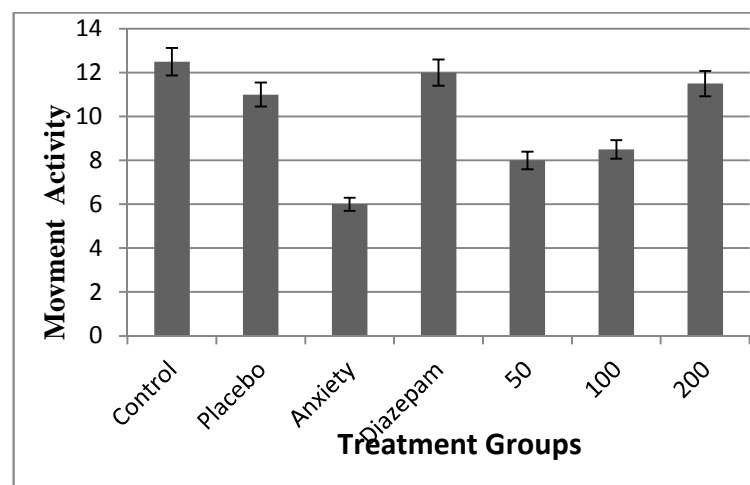


Figure 3. Movement activity in all Groups

Figure 4 shows no significant difference between treatment groups and control group in percentage of open arm entries or OAE% ($P < 0.05$).

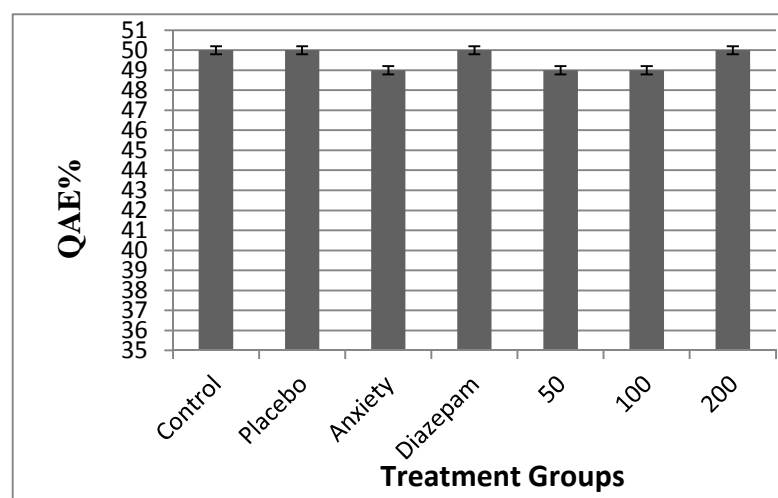


Figure 4. Percentage of open arm entries (OAE %) in all Groups

Anxiety is one of the most studied excitements and many theories have been proposed about it. Among these theories, those which are on the basis of clinical cases are really important. Some researchers believe that anxiety is an excitement including insolency that there is no source for it[4].

Various problems of individual, social, and global life need permanent adaptation of people. A little anxiety is essential to move human[11]. But anxiety more than human tolerance is known as problem.

Several methods are used to treat anxiety, but most of them are in drug therapy and psychotherapy.

The importance of traditional medicine and herbal drugs is obvious [12]. Results of this study showed that 100 mg/kg dose of balm hydro-alcoholic extract increased the time of animal presence in open arms of maze which is assessed as an indicator of reduced anxiety [13]. Also this dose increased movement activity.

Since increase in open arms entrance and presence time were known as indicators of reduced anxiety, if both are increased or decreased or at least one of them have significant difference with control group, it is known as significant difference of anxiety level. Therefore, considering that none of groups had significant difference in percentage of open arm entrance, we can conclude that balm has anxiolytic effects and reduces anxiety reflexes [14].

In spite of many researches about pharmaceutical plants in traditional medicine, there is no scientific research about comparing a synthetic drugs and balm. But modern medicine researches show many benefits of this plant including sedative, anti-oxidant, anti-spasm, carminative, antiviral and anti-inflammation properties [15].

In a research about the role of opioid receptors on the anxiolytic effect of aquatic extract (sodden) of balm, 5 mg/kg dose had anxiolytic effect whereas higher doses showed hypnotic effect on mice; therefore the effect was dose dependent and probably has appeared via opioid receptors [16,17].

In another research, about anticonvulsant and sedative effects of hydro-alcoholic extract of balm on seizures of rats induced by pentylenetetrazole, potential effect of this plant were confirmed and announced that it is enforced via GABA-ergic system probably [18].

Results of this study showed that Balm plant has compound which reduce anxiety but more researches are necessary to prove this and identify the effective compounds.

CONCLUSION

According to results, hydro-alcoholic extract of balm in 200 mg/kg dose can be good replacement for diazepam in reducing anxiety reflexes.

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