Comparison of anxiolytic effects of root extract of winter cherry (*Withania somnifera*) with the effects of diazepam in male wistar rats

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Abstract

Introduction: Anxiety is a generalized and extremely unpleasant feeling of vague dread of unknown origin which is accompanied by physical feelings like exhaustion, chest tightness, palpitations, sweating, headache, sudden desire to urinate, restlessness, and a desire to move. Anxiety is one of the most common psychiatric disorders which lead to problems in many aspects of individual, family, and social life. According to some studies, one out of every four people experiences a type of an anxiety disorder in lifetime. Medicinal plants play an important role in treating the disease and they cause milder symptoms in comparison with chemical drugs. Hence in this research, the anxiolytic effect of the extract of winter cherry (Withania somnifera) was compared with the effect of diazepam which is a chemical drug.
Materials and methods: After extraction of essence from the roots of the plant, the collected root extract was injected intraperitoneally with doses of 150, 100, 75, 50, and 25،mg/kg. Diazepam was injected with a dose of 1 mg/kg and the same volume of saline was also injected 30 min before assessing anxiolytic effects. Plus-maze apparatus was used to study animal behavior.
Results: The largest and the smallest amount of OAT were attributed to doses of150 mg/kg and 25 mg/kg, respectively. The best dose of root extract was 75 mg / kg,becausehigher amounts of the root extract did not result in a significant difference.
Conclusion: Winter cherry root extract reduces anxiety, and a dose of 75 mg/kg generated an effect similar to that of diazepam. However, it reduced locomotor activity.

Keywords: Winter cherry, diazepam, anxiolytic, rats

Introduction

Herbal medicine has been used for thousands of years as an alternative method of medicine. Supply and demand for medicinal plants is increasing annually. In the Declaration of Alma-Ata in 1978, World Health Organization (WHO) noted that a large number of people believe in herbal medicines and use them for their own health care. Accordingly, the use of herbal medicines and more generally the traditional medicines was included in public health program in 2000 (1).
Bozidan (Withania somnifera) is a plant with several important medicinal properties. This herb has anti-inflammatory, anti-anxiety effects; it facilitates the normal functioning of liver and relieves the mucus secretions. In recent years, it has been proved that the plant can be used for the treatment or cure

of asthma, ulcers, insomnia, bronchitis, pulmonary tuberculosis, rheumatism, excessive thinness, dementia in old ages, and stroke (2). The results of different researches show that this plant is helpful for relieving anxiety, cognitive disorders, and some neurological disorders (3). Bozidan, as an adaptogen, is applied for patients with fatigue (with an origin of nervous exhaustion) and disability caused by mental and physical stress; it has immune stimulating properties which is useful for patients suffering from severe reduction in white blood cell count (4). The maximum dose is 5 mg and it should be administered under medical supervision. The plant has slim and smooth cylindrical roots with a light cream color; its interior part is white and tastes bitter mucilage. The local names of the bush are "Kopinak, Boodmar, Lakri, Lalehbar, and Shokran." The scientific name of the plant is Withania somnifera and it is from dark potatoes (Solanaceae) family (2).

It is an upright shrub with radiating branches, white colored, and is all downy. Its flowers are yellow - green and it has round and yellow berry fruit which turns black after drying. In early summer, the fruit reaches its fruition. The plant has a fleshy and green leaf which protects soil from storms. Its leaves are leathery, tough, and spear-like (5). The plant is found in dried parts of India and the Himalayas at an altitude of over 1700 meters. In Iran, this plant is native to the South East parts of the country and is among the plants commonly grown in the city of Saravan, Iranshahr, Soldier, Sib, Suzan, Zabol, and the vicinity of Zahedan especially Askolabad; it generally grows in plains and mountains (hillsides) (3).

Chemical constituents: The plant contains a bitter alkaloid (Somniferin) with a sedating effect and it has resins, fats, and pigments. Glucose-lowering, plant sterol, eiporanol mixture of saturated and unsaturated fatty acids, a small amount of main the alkaloid nicotine, somniferin , withanine, withania, and a withanine -like substance have been extracted from the plant so far (2).

Simmon et al. in 1994 concluded that beta amyloid is an important pathological factor which induces Alzheimer disease and it induced the arrangements of beta sheet in brain that causes the death of nerve cells in the brain (6). Koboyama in 2002 concluded that withanolide A could be obtained from methanol extract of winter cherry and could repair the damage caused by amyloid beta in the cerebellar cortex and hippocamp. (6). The National Research Institute of the United States, in 2002, announced that 8.5% of people in the United States have at least once tried anxiolytic drugs. Benzodiazepines misuse among psychiatric patients and those who abuse substances is 23% more than normal people (7). Due to the side effects of chemical drugs, this study assessed the anxiolytic effect of winter cherry (Withania somnifera) compared with the effect of diazepam as a chemical drug.

Materials and methods

In this study we used large laboratory rats (adult male Wistar rats) with an average weight of 200-250 grams. The animals were purchased from Razi Institute, Karaj. The physical condition of the place where the animals were kept was as follows: housed with a 12-h light/dark cycle and a temperature of 22 ± 2°C without noise. The animals were fed using special rats' food (pellets) produced by Pars Poultry feed Corporation.

Extraction: An adequate amount of winter cherry (Bozidan) root was purchased from traditional pharmacies of the city of Qom. After grinding and powdering the dried root of the plant, it was soaked for 72 hours in 70% ethanol. The ethanol was separated in a rotary device. Then, the moisture content was determined and it was applied in specific doses.

Experimental groups: 42 male Wistar rats were divided into seven groups of six. One control group, five groups of root extract

with doses of 150, 100, 75, 50, and 25 mg/kg, and one group of diazepam with a dose of 1 mg/kg were injected intraperitoneally half an hour before taking the test.

Behavioral tests: Plus-maze device was used for the assessment of fear behavior. The standard Plus-maze device is designed to determine the level of fear and anxiety in rats. It is made of wood and it has two open arms and two closed arms; the closed arms have tall walls. Each open and closed arm has a dimension of 10 × 50 cm; there is a wall of 40 cm height at the two ends of closed arm and there is an edge of 1 cm at end of the open arm made of glass. Closed arm represents security and open arm indicates exploratory activity. Five minutes after injection of the extract, mice are placed in a central area of the device. During five minutes, when the animals could move freely through the maze, the following parameters were measured with a stopwatch through observations.

-Number of entry into the open arms compared with entry into the open and closed arms (OAE)

-Time spent in the open arm compared with the total time of the trial (OAT)

- The number of times animals entered the opens and closed arm (locomotor activity)

Statistical analysis

Statistical analysis was performed using SPSS software via one-way-ANOVA and post hock Tukey tests. The results of statistical analysis are shown as mean ± SD.

Results

Figures obtained from the statistical results showed significant mean differences between the experimental and control groups. As shown in Figure (1) the maximum value of OAT (time spent in open arm) was observed at a dosage of 159 mg/kg (91.7%) and then at doses of 100 and 75 mg/kg, respectively. The minimum value for this variable (16.72%) was observed in the control group. Thus, it can be concluded that the least level of anxiety was observed in rats that were in the treatment of 150 mg / kg dosage, and the maximum stress was observed in the control group.

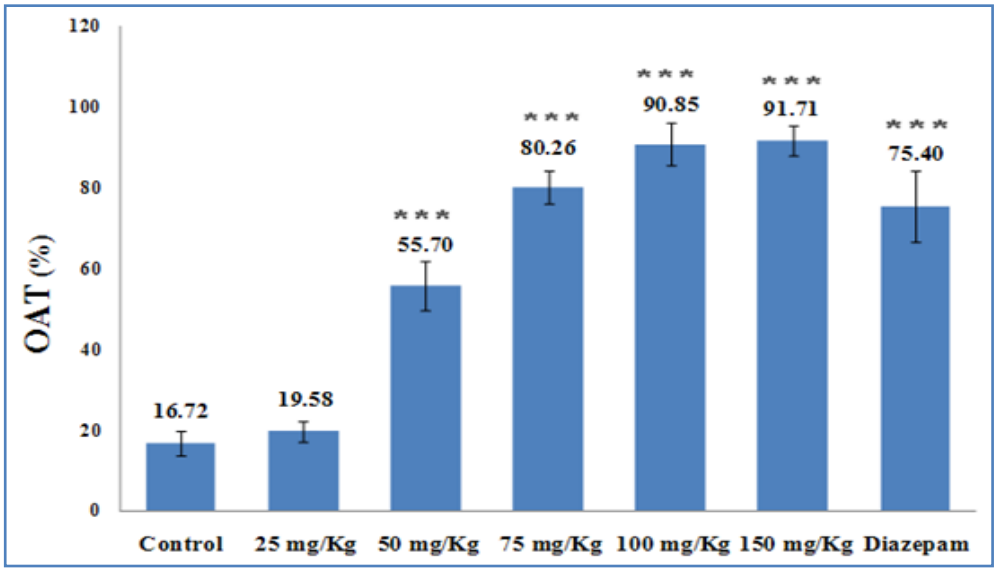

Figure 1. Comparison of the average OAT, which is plotted based on the Mean ± SEM.
*P <005, **P <0.01, and ***P <o.oo1

According to the chart (2) , the maximum value of OAE (the number of open arm entries) was observed in a dose of 150 mg/kg (77.22%), the next maximum values were observed in the group receiving a dosage of 75 mg/kg and the group receiving diazepam, respectively. The lowest level (28.7%) was observed in dose of 25 mg/kg.

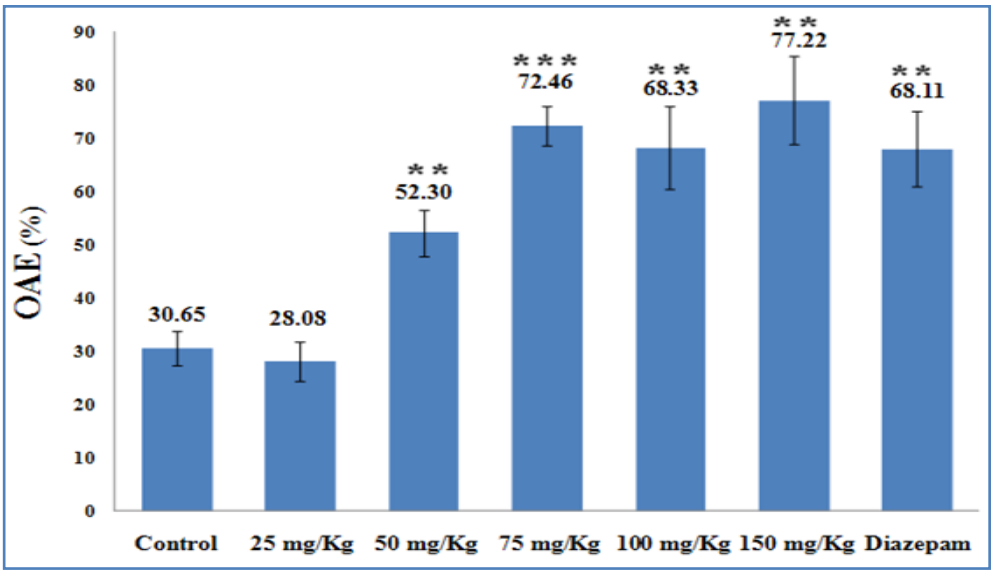

Figure 2. Comparison of the average OAE, which is plotted based on the Mean ± SEM.
*P <005, **P <0.01, and ***P <o.oo1

Considering diagram (3), the maximum value of the variable CAT (time spent in the closed arm) was observed in the control group (83.27%) and then in the groups receiving doses of 50 and 25 mg/kg. The minimum value (29.8%) was observed in the group receiving a dose of 150 mg/kg. So the maximum anxiety was observed in the control group, and the minimum level of anxiety was observed in group receiving a dose of 150 mg/kg.

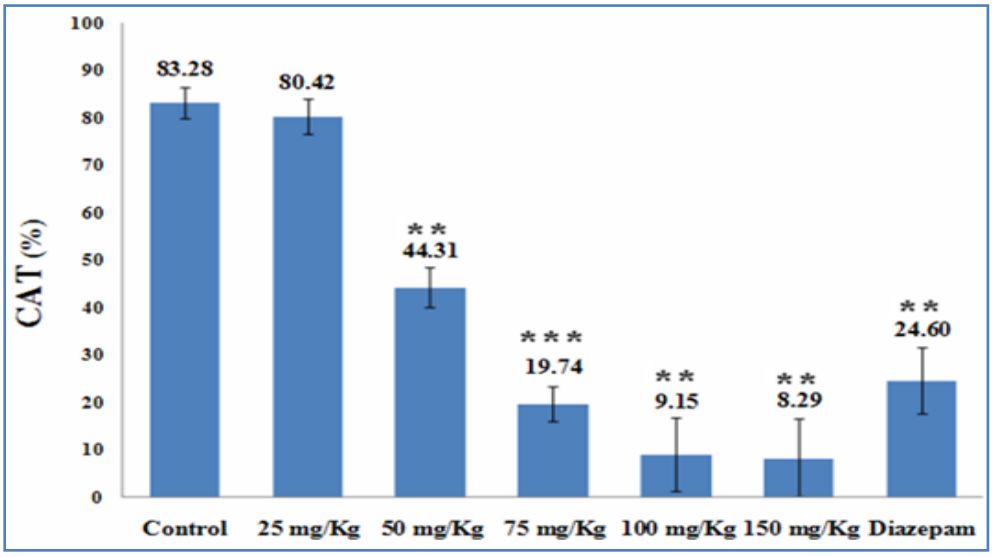

Figure 3. Comparison of the average CAT, which is plotted based on the Mean ± SEM.
*P <005, **P <0.01, and ***P <o.oo1

According to the diagram (4), the maximum value of CAE (number of closed arm entries) was observed in a dose of 25 mg/kg (71.92%) and then in a dose of 50 mg/kg. The lowest value (22.77%) was observed in a dose of 150 mg/kg.

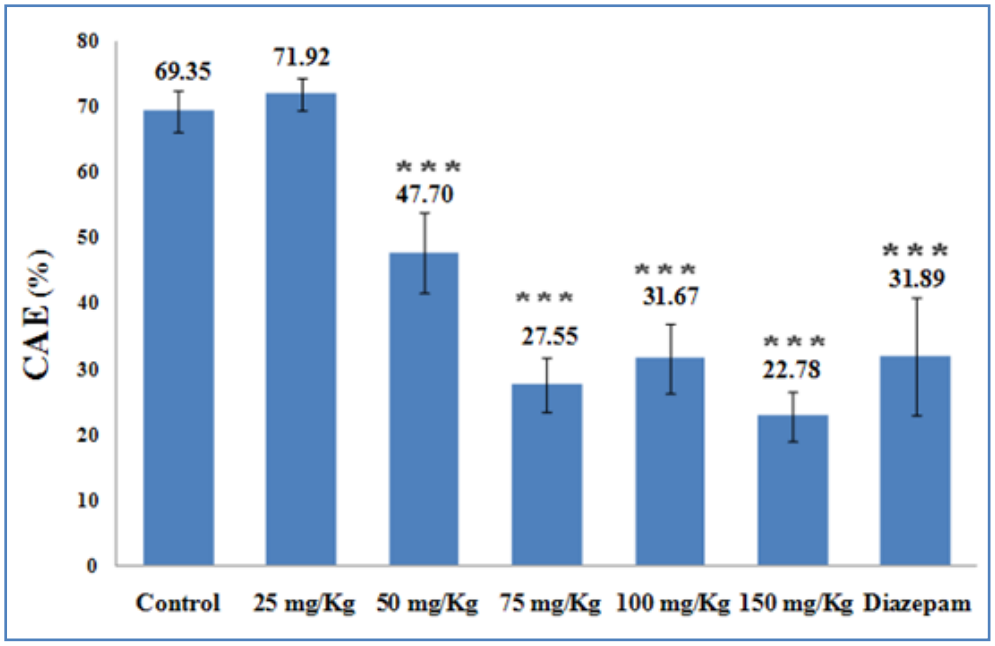

Figure 4. Comparison of the average CAE, which is plotted based on the Mean ± SEM.
*P <005, **P <0.01, and ***P <o.oo1

According to the diagram (5), the maximum locomotor activity was observed in a dose of 50 mg/kg (10.83 times) and the minimum level was observed in a dose of 150 mg/kg, i.e. with an increase in the dosage of extract, the values of these variable declined and rats had less traveling between the two arms.

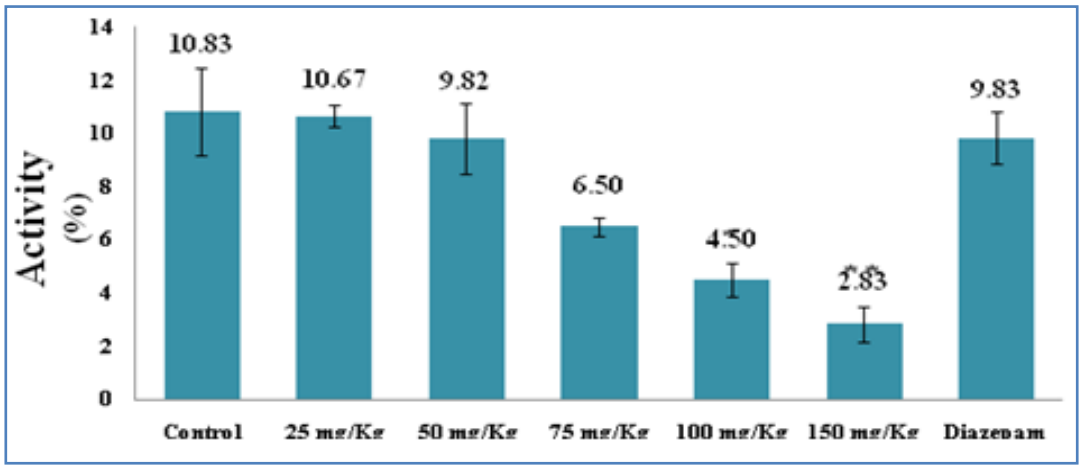

Figure 4: Comparison of the average locomotor activity, which is plotted based on theMean ± SEM.
*P <005, **P <0.01, and ***P <o.oo1

Considering the mean values of the variables and since the mean variable was lower in a dose of 25 mg/kg, it can be concluded that the level of anxiety decreased with an increase in the use of the extract or diazepam the level of anxiety decreased. In a dose of 50 mg/kg, there was an increase in OAT values with an

increase in the use of extract or drug there was an increase in OAT values, while there was a decrease in anxiety. However, increasing the doses above 75 mg/kg (i.e. the doses of 100 and 150 mg/kg or while using diazepam) for the treated samples did not make any significant change in OAT variable. Therefore, the best dosage of winter cherry root extract in wistar rats is 75 mg/kg, and increasing the dosage will cause no significant change.

Conclusion

Anxiety disorders are one of the most common mental disorders and are the major common cause of patients' admission to psychiatric clinics. Although several different medicinal and non-medicinal methods have been developed to control anxiety, yet there are many different people are still suffering people suffer from anxiety for a variety of reasons. It seems that there is a long way away facing mankind to control this personal and social problem (8). It is quite clear for everybody that traditional medicine and medicinal plants play a special role in treating diseases. The effective ingredients in medicinal plants are accompanied by other substances which make a biological balance and therefore they are not accumulated in the body and they have fewer side effects (9). Diazepam, as a benzodiazepine drug, on the one hand has proven soothing effect on the central nervous system and on the other hand it is considered as an anxiolytic drug. Diazepam causes palliative and anxiolytic effects through an interaction with GABA receptors in brain especially the brain, especially the midbrain reticular system, causes palliative and anxiolytic effects (10).

Bozidan dose-dependent treatment leads to a reduction in the activity of glutathione peroxidase and inhibits lipid peroxidation and protein peroxidation induced by copper in small mice. Accordingly, it can be effective in maintaining physical and mental health. Furthermore, the results of clinical and laboratory studies on animal models showed that this plant can be effective in treating anxiety and cognitive disorders (3). The study conducted in Pharmacology ward, Health Science Center of University of Texas indicated that extracts of this plant has a GABA-like activity; so, it is probably is effective in reducing anxiety. The study found that the winter cherry root extract prevents specific binding of GABA (3H) and TBPS (35s). An extract concentration of one mg completely hinders the binding.

In operational studies of assessing 36CL flow in spinal neurons of mammals it was found that root extract of winter cherry plant increased 36CL flow in the absence of GABA. Such an effect is inhibited by bicuculine picrotoxin and is reinforced by diazepam. These results indicate that the extracts contain an element which has a GABA-like activity. In another study, anti-depressants and anti-anxiety effect of bioactive glycowithanolides (WSG), extracted from WS roots were examined on rats and the results were compared with that of benzodiazepine lorazepam (0.5 mg/kg) and tricyclic imipramine (10 mg/kg) (4). It is probable that the extract acts on benzodiazepine receptors binding to GABA A receptors and causes an anxiolytic effect. To prove this, it is necessary to separate effective material of the plant and to conduct specific researches on each of these materials using other animal models to discriminate its sedative and anti-anxiety mechanisms. Hence, it requires further study to identify the chemical structure of the effective ingredients of the plant. The results showed that winter cherry root extract has anxiolytic effects while it reduces the locomotor activity. Plant extract at a dose of 75mg/kg has the same effect as diazepam, though it reduces locomotor activity.

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