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Research Article

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Anxiolytic and hypnotic effect of *Zizyphus jujube* mill aqueous extract in mice

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ABSTRACT

Anxiolytic disorders have high prevalence in society and many medications such as benzodiazepines and serotonin inhibitors are used to treat anxiolytic disorders nowadays. To overcome the side effects of synthetic drugs, further research needs to be conducted on natural ingredients. This study was conducted to determine the effect of aqueous extract of jujube on sleep duration and anxiety level of mice. In this experimental study, 80 male albino mice weighing 30 to 25 grams were used. They were randomly assigned to eight groups (6 experimental groups and two control groups, each group contains10 mice). To assess sleep period, behavioral method (Angel) was used and animals were divided into three experimental groups and one control group. To assess anxiety level, animals were divided into three experimental groups and one control group, and elevated plus maze was used in this regard. Standard indices of anxiety assessment (the number of entry and time spent in open arm) were examined and recorded for 5 minutes by observing them. Three doses of aqueous extract of Jujube (200-400-600 mg / kg)were injected to experimental groups, and saline was injected intraperitoneally in control group. the results showed that the aqueous extract of jujube at dose of 600 increases sleep period considerably compared with control group. (P <0.05). In addition,400 and 600 doses of aqueous extract of jujube significantly increased the number of entry (p <0.05) and time spent (p <0.05) in elevated plus maze open arm compared with control group. This study showed that aqueous extracts of jujube increases sleep period and reduces anxiety levels in mice

Key words: the aqueous extract of jujube, sleep period, antianxiety, mice

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INTRODUCTION

Natural anxiety is an adaptation - emotional response to stressful physiological, psychological and social stimuli that every person experiences it is his life (1 and 2). Pathological anxiety is the most common Psychological disorders leading to disrupted daily life and human suffering. To treat it, benzodiazepines and barbiturates are used (3). Anxiety is an unpleasant and vague and fear feeling that has unknown origin. It includes uncertainty, helplessness and physiological arousal associated with one or more physical feelings such as nausea, chest tightness, palpitations, sweating, and headache (4). It is in the process of mediating this phenomenon, multiple recipients such as serotonin, GABA, catecholamines, and sex hormones are involved (5). Most of conducted studies have been on mechanism of Neurotransmitter of GABA, serotonin and neuropeptide, while new studies propose the role of adenosine, cholecystokinin and neurosteroids in the development of anxiety (6). Selective serotonin reuptake inhibitors and some beta-adrenergic blockers are used to treat anxiety (7). Due to the side effects of these drugs, natural methods of traditional medicine have been used widely (8). One of the natural Vegetable compounds is jujube. Jujube is a plant belonging to Rhamnaceae family and its scientific name is Zizyphus jujube mill. This plant is a tree and its height is 2 to 8 meters, which sometimes it can be over 12 meters. It has high resistance to drought. Some varieties of it have thorn, while some other varieties of it are without thorn. Its leaves are small, beautiful, shaggy, and alternating transparent located on both sides of the branches, serrated, consisting three petals, in both sides of each petiole on each shoot, they are papillae in the shape of sturdy thorns. Then, they become wooden, and they remain permanently on the branches. It has small flowers with short peduncle colored yellowish green. It has tiny separate petals, 5 pistils. Its fruits known as jujube has transparent reddish color to the shaft (ripe fruit), and it is as big as an edible olive (8). Jujube has been used as medicinal plant since old days and it had been used in East Asia in the treatment of

diseases such as liver disorders, anemia; and dyspnea (9). Terpenoids, flavonoids and alkaloids compounds have been isolated from fruit of the jujube plant. Additionally, a type of phenyl glycoside compound known as jujufnoeid is also obtained by fruit of jujube. Investigations have shown that this plant has active compounds having inhibitory effect on histamine release and activating the Cholinesterase II and I and Cyclooxygenase. In addition, it has sitotoksiti effects. Jujube has higher values of mosilage, Malic acid, citric acid and sugar, protein substances and organic solutes, vitamin C, and minerals (9, 10).With respect to applying the jujube in traditional medicine as a sedative, this study was conducted for laboratory examining of anxiolytic and hypnotic effects of aqueous extract of jujube on the small laboratory mice.

EXPERIMENTAL SECTION

Animals:

In this experimental study, 80 male albino mice were used, weighting 25-30 g. they were kept in special cages and environmental conditions in an optimal temperature of approximately 22 ° c for 12 hours in light and 12 hours in darkness. That accessed to food and water freely.

Plant extract preparation

Fresh jujube was prepared from orange orchards of TabasGolshan city located in South Khorasan Province and it was identified and confirmed scientifically by Botanical expert in BirjandPayam Noor University. Flowers were dried and powdered by mill. The resulting powder was placed in the oven and dry matter was used for the preparation of medicine. 500 mg of effective dry matter was dissolved in 50 mL of saline solution. Then, for possible sediment of matters of powder that was not dissolved, the tested was centrifuged at 3000 rpm for 5 minutes and then it was passed through the filter, the main solution substance was obtained. To make other solutions with doses 200, 400, 600 mg/ kg doses dilution was carried out by saline. Due to lack of support of using Jujube to assess the level of anxiety and sleep, the considered doses were selected in several doses based on a pilot study.

Sleep assessment method using behavioral analysis (Angel)

In this method, animals were placed special covered with soft and tiny wires. The cage was placed on plastic bags tires filled with water, connected with each other by an interface, and one side of it was connected to converter. Other side of it was connected to physiograph. Before the test begins, the animal was allowed to stay for half an hour in this state so that fear and anxiety to be disappeared and be familiar with environment. Physiograph was turned on and animal movement was recorded for 30 minutes, and animal's waking was recorded. In this way, total sleep time can be measured. It should be noted that throughout the experiment, environmental conditions including light, temperature, etc. were kept constant. In this study, the aqueous extract of jujube was intraperitoneally injected at doses of 200 400 600 mg per kilogram of body weight to three group of animals immediately before assessment of sleep period (before putting animal within the cage), and saline was injected for fourth group (control group containing 10 mice).

Anxiety assessment method

To assess the anxiety, device called elevated plus maze (Elevated Plus Maze, EPM), as the standard model to assess the level of anxiety in rodents, was used. The device is made of wood and consists of two open arms (each 5 × 50 cm) and two closed arm (each 40 × 5 × 50 cm), and a central pan (5 x 5 cm), so that open arms are located in front of each other the closed arms also are in front of each other. They are 50 cm above the room floor. This experimental model to assess anxiety is unconditional and requires no animal learning. In this experiment, the jujube extract at doses of 200 400 600 mg per kg of body weight was intraperitoneally injected for three experimental groups (each containing 10 mice) and saline at same volume was injected for control group (containing 10 mice). Thirty minutes after injection, each mouse was separately placed for five minutes in a black box with made of plexiglass at size of 40 x 40 in 30 so that exploratory activity of animal to be increased. Then, to assess the level of anxiety, the animal was placed in elevated plus maze (on and exploratory activities of number of entries into the open arms and the time spent on open arms were assessed and recorded. It is worth noting that the increase in the number of entry to open arms and time spent in them are considered as reduced anxiety in mice. In addition, a judgment was performed on significant difference of anxiety level in the way that if time of both indices (entry to open arm and time spent in them) increases or decreases in line with each other and at least one of them has significant difference with control group, anxiety level change will be considered as significant.

RESULTS

The results showed that the aqueous extract of jujube at doses of 600 increases significantly sleep period compared with the control group. ($P < 0.05$). In addition, two doses of 400 and 600 of aqueous extract of jujube significantly

increased the number of entry ($p < 0.05$) and time spent ($p < 0.05$) in open arm of elevated plus maze compared to control group.

Experimental groups	(minute) Sleeping period	Number of entry to open arm	Percentage of time spent in open arm
Control group	5/21±0/12	2/1±0/31	41±0/21
group 1 (dose 200)	**7/1**±0/12	3/2±0/11	45±0/33
Group 2 (dose 400)	**7/8**±0/**8**	5/9±0/21***	68±3/51***
Group 3 (dose 600)	**13/1**±1/32***	7/2±0/28***	77±4/01***

DISCUSSION

The study showed that aqueous extract derived from jujube increased sleep period and increases the time spent in the elevated open arm. It also increased the frequency of open arm entries.

Therefore, it can be suggested as sedative and hypnotic effects of jujube have been mentioned in traditional medicine, compounds in the extract of this plant haveanxiolytic and hypnotic effects. Research shows that jujube ingredients include compounds such as, epigenin, spinosin, sanjoinine, and a variety of flavonoids. Many herbal extracts with same compounds showed similar sedative effects (11, 12, 13). Research shows that these compounds activateGABAergic system. Gamma-aminobutyric acid (GABA) is known as an inhibitory transmitter in brain neural synapses, secreted mainly through nerve endings in the spinal cord or basal ganglia in the brain. GABA is one the nerve inhibitory chemical intermediates acting through increasing the nerve membrane permeability to chlorine ion (14). On the other hand, GABAergic neurons are brain neurons related to sleep (15) so that the induction of physiological sleep after injection of GABA in the area thalamocortical area has been reported in various studies (16). Other studies have shown that binding of benzodiazepines $GABA_A$ receptors may induce sleeping. Sanjoinine that is one of main components of studied extract leads to simultaneous and collective reduction in the activity of the neurons of the central nervous system. This compound is able to bind $GABA_A$ receptors and lead to releasing of neurotransmitter GABA. Therefore, it can be said that sanjoinine will lead to anti-anxiolytic and anti-hypnotic effect by impact on GABAergic system through the suppression of central nervous system. Spinosin is also able to increase the releasing $GABA_A$ by binding to GABA receptor. As neural mediator of GABA plays an important role in inhibiting the brain neurons, it can be explained that anti-anxiolytic and anti-hypnotic effects of jujube relates to this issue. On the other hand, research shows that there are many flavonoid derivatives acting as ligands for GABAA receptor in the central nervous system. They lead to suppressive activities in the central nervous system of mice by binding to medications like benzodiazepines (17). For this reason, flavonoids, that have interactional ability with $GABA_A$receptorin the central nervous system(18),are known as herbal benzodiazepines (19). In addition, flavonoids, as sanjoinine and the sanjoinine have anti-anxiolytic and anti-hypnotic effects by binding $GABA_A$ receptor and releasing GABA. Since activation of GABAergic system and releasing of Gamma-aminobutyric acid reduce anxiety and sleep, it can be said that anti-anxiolytic and anti-hypnotic effects of jujube is due to activation of this system.

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