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Comparative Effect of Saffron Extract and Alprazolam on Reducing Anxiety in the Mice

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ABSTRACT

Anxiety disorders are of the most common types of mental disorders, and yet anti-anxiety medications are also of numerous side effects. The aim of this study is to investigate the possibility of replacing Alprazolam with saffron extract in reducing anxiety. In this study 48 adult mice were used in 6 groups, including extracts with amounts of 50, 100 and 200 mg/kg, alprazolam, anxiety and control. The control group received no intervention or drugs, and anxiety in both main groups of anxiety and appetite was affected by stress, hence, their anxiety and appetite was estimated and recorded without taking any drugs. The alprazolam group received doses of 0.5 mg/kg and 1 hour after the injection, anxiety was induced to the mice by dark boxes and related anxiety and appetite behaviors were studied. In the anxiety group, intraperitoneal injection of hydro-alcoholic extract of saffron in doses of 50, 100 and 200 mg/kg for each mouse was applied to 3 Extract Experimental groups. Then each mouse's anxiety level was recorded using the elevated plus-maze, and results were analyzed using ANOVA and Duncan's statistical tests. Saffron extract at doses of 50, 100 and 200 mg/kg of mice's body weight -compared to the anxiety group- meaningfully increased the duration of animal presence at open branches of the plus-shaped maze, which represents a decrease in anxiety. The 200 mg/kg dose of saffron extract compared to that of alprazolam, and doses 50 or 100 of the extract were more effective in reducing anxiety and loss of appetite. We can conclude that dose-dependent hydro-alcoholic saffron extract is effective in reducing anxious reactions and could be a viable alternative to alprazolam in the treatment of anxiety.

Key words: Anxiety, saffron, alprazolam, Plus-maze, Laboratory mice

INTRODUCTION

Anxiety disorders are the most common classes of mental disorders. Women with lifetime prevalence of 30.5% compared to men with a lifetime prevalence of 19.2%, are more prone to be suffering from an anxiety disorder [1].

Anxiety plays a direct role on people's health. Animals that are deliberately exposed to stress, show symptoms such as sleep and eating disorders, ulcers and lowered immune, unless stress is taken off them. This phenomenon increases heart disease and other illnesses [2].

Anxiety is a mental state or intense arousal which its main characteristics are: fear, doubt and excessive worry. In people who suffer from anxiety disorders, fear is much greater than that of threat or danger. That means there is no reasonable proportion to the severity of the threat and fear, and people are constantly in a state of fear and anxiety which its cause is yet unknown [3]. Clinical characteristics of different types of anxiety disorders such as anxiety is normal and of the same type but the difference is in their duration and intensity [4].

According to studies, three major medium-nerves which have some connection on animals and their reactions to anxiety treatment include: norepinephrine, serotonin and GABA. GABA's role in anxiety disorders is strongly

confirmed by the undisputed efficacy of Benzodiazepines in treating some anxiety disorders. These drugs alter the activity of GABA on the GABA receptor type [1].

Diazepam, Lorazepam, Alprazolam, Tryazolam and Estazolam, Benzodiazepines are classified among the most rapid absorption and high speed in reaching the maximum blood levels and starting to have an effect [5].

Alprazolam and other drugs used in anxiety disorders have many side effects. Some of them include the development of dependence, drug withdrawal syndrome (in case of drug withdrawal), state of drowsiness and lethargy [4]. Also Benzodiazepines withdrawal syndrome have symptoms of anxiety, insomnia, irritability, tremors and spasms [6].

Therefore, due to the increasing and continuous use of synthetic drugs, research and search for more effective drugs with fewer side effects has become part of the treatment program of anxiety disorders. One of the possibilities in this regard is the use of herbs, which have been used in the treatment of different diseases. Since, active ingredients in herbal remedies are associated with other materials of a biological equilibrium, they are not accumulated in the body and have no side effects or have fewer side effects and therefore, are significantly superior to chemical drugs, in this respect [7]. The miracle of herbs in ancient times has been the center of attention among Iranians. Some of these herbs were considered sacred because of their healing features [8].

Saffron is amongst the herbs for which traditional and modern medicine has mentioned different therapeutic effects. Saffron, is a perennial herb of the *Gladiola* family with a height of 10 to 30cm and a hard round bulb, fleshy and covered with a thin brown membrane. According to researchers, saffron extract in traditional medicine is used as an anti-anxiety, anti-depression, digestion facilitator, appetizer and sedative, and in traditional medicine of India it is used as an Adaptogen (an enhancer of body's resistance to stresses such as trauma, anxiety and fatigue), and due to the crocin, crocetin and safranal, it has anxiolytic properties [9].

Therefore, because of the side effects of chemical drugs, using herbal remedies can be a good alternative to chemical drugs for treating such diseases, which it yet needs further research on the effectiveness of herbs and medicinal plants. For economic reasons and other benefits of experiments on animals, including the genetic similarity of the mice with human genome, many studies, experiments and medical and biology laboratory sampling are carried out on laboratory mice [10]. As per a review of basic theoretical research and lack of scientific studies on the comparative impact of Saffron and Alprazolam in reducing anxiety, the aim of this study was to compare the effects of alcoholic extract of saffron with alprazolam in reduction of anxiety.

MATERIALS AND METHODS

In this experimental study, 48 female mice weighing approximately 25 to 30 grams were used. These animals were kept in standard cages made of polycarbonate with stainless steel mesh ceiling for 2 weeks in the same conditions and free access to food and water, natural photoperiod, 20 to 22 °C temperature and humidity of about 60 percent for sustainability.

They were given food and water without any restriction. Cage floors were carpeted with sawdust and changed and cleaned every other day. These conditions continued to be the same throughout the experiment. In this study, all animal care was based on guidelines of the Brazilian animal testing ethics and the committee of Animal Science Research of Vale do Paraíba University.

The mice were randomly divided into 6 groups of 8. Three experimental groups receiving saffron extract at doses of 50, 100 and 200 mg/kg of body weight. Alprazolam group included those that became insane after inducing anxiety by alprazolam in a dose of 5.0 milligrams per kilogram of body weight. Control group received no injection or medications and their behavior was monitored and recorded, and Anxiety group included examples that were in the black boxes under darkness stress and became anxious but no injection was done on them.

In order to prepare dried saffron extract, stamens of saffron was used. Alprazolam drug was administered at a dose of 5.0 mg/kg intraperitoneally. Treatment groups received fixed doses of the extract 55 minutes before the start of the test.

For assessment of anxiety an elevated plus-maze device, a standard model in assessing the level of anxiety in rodents, was used. The device is made of wood and consists of two open arms with dimensions of 50×10cm, and two blind arms with dimensions of 10×50×40cm.

Each two arms, face each other and are placed about 50cm above the floor. This model of anxiety testing is only experimental and training and teaching the animals is not required. The basis of assessment in this test, is designed to be on two instincts; one is the exploratory sense of rodents, and the other is avoidance of outdoor and bright areas. In this procedure the animal is more likely to spend their time in the blind arms.

Four parameters were determined by observation;

Ratio of open arm entries= (open arm entries)/ (open arm entries + blind arm entries) ×100

Ratio of open arm time = (open arm time)/ (open arm time + blind arm time) ×100

Movement activity= open arm entries + blind arm entries

A significant increase in the proportion of entries into open arms and the time ratio spent in open arms, plus no change in physical activity indicates a decrease in anxiety, in this test. However, the ratio of entries into open arms in proportion to the time spent in the open arms is of less sensitivity and importance in recording anxiety and anti-anxiety behaviors of animals [11]. Collected data was then analyzed using the Statistical Package for the Social Sciences and statistical tests of Analysis of Variance (ANOVA) and Duncan's tests.

RESULTS AND DISCUSSION

The results of these tests, including time spent in open arms, a 5-minute physical activity and average percentage of open arm entries in different treatment groups were measured and evaluated after statistical analysis. Table 1 shows the results of this study.

Table1: Comparison of Average and Standard Deviation resulted from comparative effect of hydro-alcoholic saffron extract and Alprazolam in the reduction of anxiety

group	Ratio of open arm entries		Proportion of time spent in the open arms		Movement activity	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
Control	48.6068	2.32520	39.2038	5.54072	14.75	1.909
Anxiety	39.5820	7.62521	27.2488	9.59069	9.38	4.207
Alprazolam	45.4855	4.12672	29.2900	5.05542	10.75	4.334
Saffron doses of 50 mg per kg of body weight	45.0397	8.80089	33.8427	7.97480	12.13	2.800
Saffron doses of 100 mg per kg of body weight	44.1600	10.55296	30.7083	7.62918	13.75	3.991
Saffron doses of 200 mg per kg of body weight	52.1429	4.41601	44.0916	15.59362	11.13	3.796

It can be concluded that in samples of this study, the average of entry into the open arms, time spent in open arms and activities inside the open arms for experimental groups which had received saffron extract, was significantly more than the average entry, time spent and activity in open arms for the anxious group. The results also show the group that received an extract dose of 200 mg/kg on these scales (entry, stay and activity in the open arms) had better averages compared with the other control and alprazolam groups.

Based on the data obtained, the results showed that the hydro-alcoholic extract of saffron crocus at a dose of 50, 100 and 200 mg/ kg increased the time animals spent in the open arms of the plus maze, which is assessed as an indicative of a reduction in anxiety levels, also doses of 50, 100 and 200 mg/kg caused an increase in animal's physical activity in the elevated plus-maze.

Since, an increase in the number of entries to open arms and the time spent in there is considered as an indicator of reduced anxiety, judging the significant difference in the level of anxiety tells us that if both the number of open arm entries and the time spent increases or decreases at the same time and direction, or at least one of them is significantly different from the control group, this is considered as a significant change in the anxiety level.

Respectively, due to the fact that none of the groups showed no significant difference in the proportion of open arm entries, it can be concluded that saffron is of anxiolytic effects and reduce anxiety reactions. Doses 50 and 100 of saffron were similar to alprazolam in reducing anxiety, and no significant differences in the results of saffron doses with control and Alprazolam group can be seen.

In addition, the 200mg dose of the extract was more effective than doses 50, 100 and better than alprazolam in reducing anxiety. In a comparison done between results of this study and that of other studies related to anxiety variables, results of this hypothesis could be seen as consistent with results of previous findings.

In an experimental study, Ghaffari, Hatami and Dehghan (2013), investigated the effects of hydro-alcoholic extract of saffron on oxidative stress markers and concluded that this plant is a powerful antioxidant, which modifies the stress indices in the hippocampus of mice and consequently reduces their anxiety [12]. Furthermore, the findings are consistent with results of this research. With regards to a dose-dependent effect of saffron hydro-alcoholic extract with alprazolam on reducing anxiety on the lab mice no similar work has been done so far. According to the surveys by Kianbakht (2008) on the pharmaceutical aspect of saffron, having a crocin component in the formulation of saffron crocus, also with respect to studies by Hoseinzadeh, et. al. (2003) about the effects of crocin component on norepinephrine reuptake inhibition and direct impact of disruptions on norepinephrine neurotransmitter in the emergence of anxiety disorders [9].

CONCLUSION

The results of this study showed the efficacy of three effective doses of the saffron extract (dependent doses), respectively.

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