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Research Article

**COMPARING THE EFFECT OF HAWTHORN'S EXTRACT
AND CHLORDIAZEPOXIDE ON REDUCING ANXIETY IN
LABORATORY MICE****Khatereh Mandanizadeh¹, Mehrdad Modaresi^{2*}, Ilmaz Sajjadian³**¹ Dept. of Psychology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, IRAN² Dept. of Physiology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, IRAN³ Dept. of Psychology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, IRAN**Abstract:**

Anxiety is a natural feeling which is experienced in threatening situations and can affect the physiology of the nervous system. The aim of this study was to compare the effect of hawthorn and chlordiazepoxide on reducing anxiety in mice. Sixty female mice in the weight range of 25 to 30g were divided into six groups of control, anxiety, chlordiazepoxide and 50, 100, and 200 mg/kg doses of hawthorn extract. After receiving the last dose, Anxiety was induced by the dark box and then was evaluated using plus evaluated maze. Animals were placed in a box with black walls for five minutes. After that, samples were transferred to plus elevated maze and open arms entries and time were recorded as anxiety indices. Hawthorn extract increased open arm time significantly in 100 and 200 mg/kg doses in proportion to control and drug. Also, these doses increased movement activity significantly. According to results, hawthorn extract reduced the anxiety dose dependently, so that 50 mg/kg dose could not show better performance than chlordiazepoxide whereas 100 and 200 mg/kg doses can be proposed as a good replacement for this drug to reduce anxiety reflexes.

Keywords: *hawthorn, anxiety, chlordiazepoxide, mice****Corresponding Author:****Mehrdad Modaresi,**

Associated Prof., Department of Physiology,

Isfahan (Khorasgan) Branch,

Islamic Azad University, Isfahan, Iran.

Tel: +98(913)2074854 **Fax:** +983135354060**Email:** mehrdad_modaresi@hotmail.com**Address:** Jey St., Arghavanieh, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, IRAN

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INTRODUCTION:

Anxiety is an unpleasant ambiguous feeling which comes with one or more physical sensations, like worry, chest tightness, palpitations, transpiration, headache, and so on. Anxiety is basically used for response of a person against a threatening situation; a situation under the influence of intense exacerbations (whether external or internal) which person is not able to control it [1]. Anxiety is a fear that occurs by endangering one of principle values of personal life and sometimes is useful because encourages people to be more serious in their life and act to change and grow. Anxiety becomes a problem when it occurs in response to safe events or in the form of an unrealistic fear of weakness in doing things [2].

Although a degree of anxiety is necessary for person's adaption, but enhanced anxiety even daily anxiety which is left out by some psychologists and diagnostic criteria for mental illnesses can cause significant barriers to the realization of individual potentialities, and lead to many mental disorders [3]. Many treatments have been proposed to treat or reduce anxiety amount. One of these methods is drug therapy. Benzodiazepines which are the most important antianxiety medications also have anticonvulsant, hypnotic-sedative, and muscle-relaxant properties [4]. Chlordiazepoxide is one of frequently used benzodiazepine drugs which are useful for curing anxiety and insomnia. This is the first drug from benzodiazepines family which was discovered in 1957 and released to market in 1960 [5].

This tablet has side effects such as any other drug. Prolonged sleepiness, dizziness, headache, irritability, weakness and fatigue, constipation, dry mouth, nausea, vomiting, itching, decreased blood pressure, blurred vision and tinnitus. There are many side effects of this pill during pregnancy [6]. Other treatments for anxiety are herbal treatments. Herbal or traditional medicine dating to the time of the ancient Greeks was one way of treating different illnesses for years on end [7]. Aromatic plants and herbal products have been used worldwide as natural additives for medicinal purposes because they have been accepted by consumers as natural additives [8, 9].

Hawthorn is belonging to Rosacea family and is a shrub height of about 3 to 10 meters with egg-shaped felted leaves. The bottom of the leaf is usually divided into deep parts and a serrated. The fruit is fleshy, round, reddish-yellow, with a diameter of 1.5 to 2 centimeters which has several cores and a sour and sweet taste [10]. Hawthorn's extract has flavonoid compounds such as catechin, epicatechin,

rutin, quercetin, anthocyanins, chlorogenic acid and vitamin C [1].

Prescribing hawthorn's extract improves heart performance and reduces the extent of the infarction region in the experimental model of ischemic reperfusion in rats by applying a protective effect against oxidative stress caused by free oxygen radicals [11].

Given that there is no scientific research about the effects of this plant on anxiety in spite of herbal medicine recommendations, this experiment was carried out to compare the effect of hawthorn and chlordiazepoxide on reducing anxiety of laboratory mice.

MATERIALS AND METHODS:

Sixty female mice in the weight range of 25-30g were kept in standard cages made from polycarbonate with lattice steel ceilings. Animals were kept for two weeks to adapt to environment. Samples had free access to food and water, 20-22 °C temperature and 60% humidity. Cage floor were covered with sawdust which were replaced every 2 days. In the present study, ethical principles were observed in accordance with the rules of support and maintenance of laboratory animals and statements of animal researches committee, Vale do Paraiba University.

Treatment groups were:

- Control group: no injection (n=10)
 - Anxiety group: anxiety was created, but no injections were made (n=10)
 - Chlordiazepoxide group: 1.2 mg/kg of drug was injected intraperitoneal (n=10)
 - Experimental groups: received 50, 100, and 200 mg/kg of hawthorn's extract intraperitoneal (n=30)
- Hawthorn was cut to small pieces and grinded by mill. One hundred grams of this powder was weighed by digital weigh and poured into a sterilized Erlenmeyer flask plus 80cc of ethyl alcohol. After 8 hours the extract was filtered by using whatman paper [12]. Obtained extract was used to prepare 50, 100 and 200 mg/kg doses. Treatment groups received the prescribed extract at specified doses, 50 minutes before the test.

To evaluate the anxiety, Plus Elevated Maze was used. This is the standard model for evaluating the anxiety which includes two open arms (50*10cm) and two closed arms (50*40*10cm). Open and closed arms were opposite and about 50 cm higher than room floor [13].

This model is experimental and doesn't need training. The base of this test is searching sense and instincts

of rodents. Four parameters are measured in this test: open and closed arm entries, and the time that animal stays in each arm. Percentage of open arm entries (OAE%), percentage of open arm times (OAT%) and movement activity are calculated via following formula:

$$\text{Open arm entries} = \frac{\text{open arm entries}}{\text{open arm entries} + \text{closed arm entries}} * 100$$

$$\text{Open arm time} = \frac{\text{open arm time}}{\text{open arm time} + \text{closed arm time}} * 100$$

$$\text{Movement activity} = \text{open arm entries} + \text{closed arm entries}$$

Significant increase in the percentages of open arm entries and open arm time plus no change in movement activity shows anxiety reduction in this test. OAE parameter is less sensitive than OAT parameter for evaluating anxiety and anti-anxiety behaviors of animal.

Obtained data were analyzed using SPSS program, variance analysis and Duncan's test.

RESULTS AND DISCUSSION:

Obtained results of open arm time, movement activity per minutes and open arm percentage are presented in Table 1.

Table1. Experimental variables of various treatment groups

group indices	Open arm entries ratio		Open arm time ratio		Movement activity	
	mean	Standard deviation	mean	Standard deviation	mean	Standard deviation
50mg/kg	0.449	0.086	0.338	0.089	17.6	8.47
100mg/kg	0.59	0.047	0.523	0.103	29.4	8.38
200mg/kg	0.638	0.081	0.588	0.121	24.5	3.92
Chlordiazepoxide	0.495	0.078	0.547	0.22	32	4.29
Control	0.589	0.058	0.678	0.072	32	5.27
Anxiety	0.23	0.065	0.164	0.039	18.4	3.44

Figure 1 shows that hawthorn extract in 100 and 200 mg/kg doses increased open arm time to the control level ($p < 0.01$).

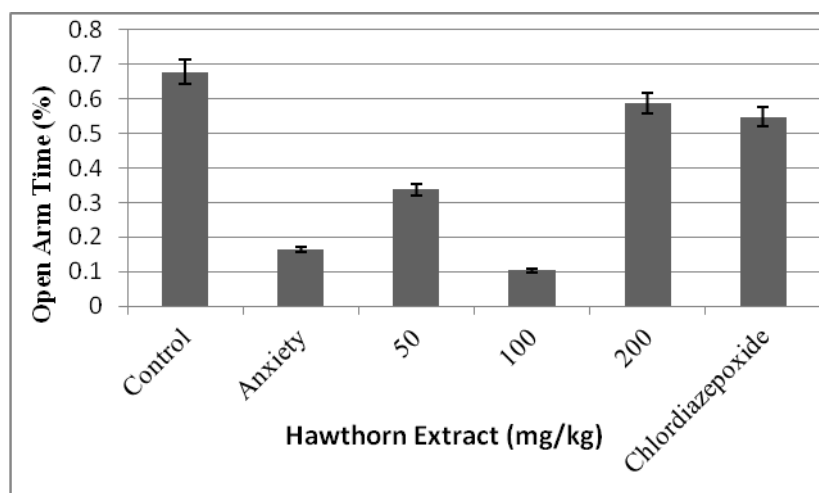


Figure 1. The comparison of open arm time (OAT%) ratio in anxiety, control, chlordiazepoxide and three experimental groups

Figure 2 shows increment in movement activity of 100 and 200mg/kg doses and chlordiazepoxide group which were about control group ($p < 0.01$).

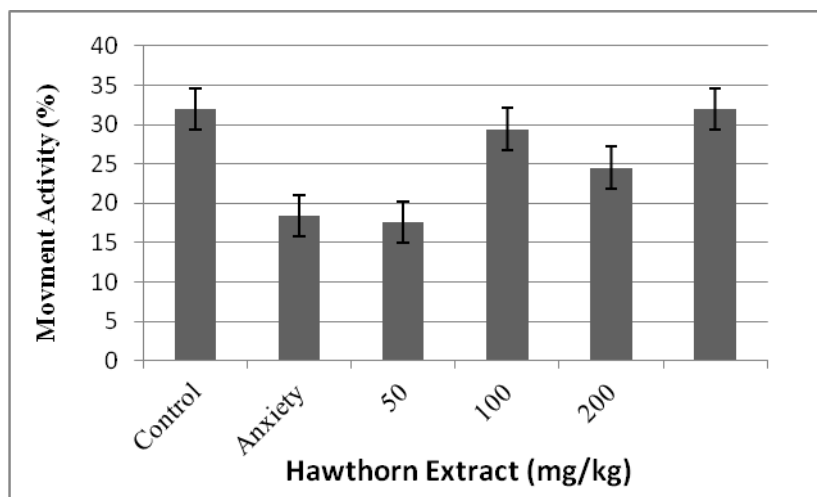


Figure 2. The comparison of movement activity ratio in anxiety, control, chlordiazepoxide and three experimental groups

According to Figure 3, 100 and 200 mg/kg doses were not significantly different from control group in open arm entries percentage.

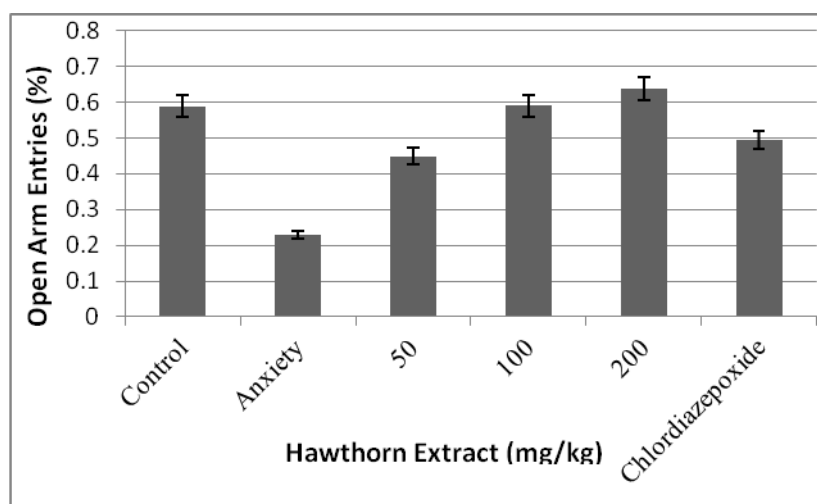


Figure 3. The comparison of open arm entries (OAE%) ratio in anxiety, control, chlordiazepoxide and three experimental groups

In current study the effects of hawthorn's extract and chlordiazepoxide were compared on anxiety treatment of laboratory mice. Results showed that hawthorn's extract in desired doses is an effective strategy to alleviate anxiety in an animal model. There is no similar study about comparing the hawthorn's extract and chlordiazepoxide.

Results showed that hawthorn in 200mg/kg dose increased open arm time which is considered as an index of anxiety controlling. Also, this dose increased movement activity. Increased open arm entry and open arm time are considered as anxiety improvement indicator. Significant change of anxiety

level is evaluated by simultaneous increase or decrease of open arm entry and open arm time or at least significant difference of one of them with control group, therefore, since none of groups were significantly different in open arm entry, we can conclude that hawthorn has anti-anxiety effects and reduces anxiety reflexes.

Pair comparisons of means showed that 200mg/kg dose was significantly ($p < 0.01$) different from anxiety and chlordiazepoxide groups in open arm entry but not from control group. Also, this group was significantly ($p < 0.01$) different from anxiety group in open arm ratio but not from control and

chlordiazepoxide groups. Movement activity of this group was not significantly different from anxiety, control and chlordiazepoxide groups. According to results, 200mg/kg dose increased open arm entry and movement activity of animal in plus evaluated maze.

Results of this study are in agreement with other studies which some of them had definite doses. Researcher compared mitagnus plant and vitamin E on women with periodic chest pain and observed significant differences between groups [14]. Shahinfar *et al.* (2016) compared cinnamon and oral diazepam on anxiety before orthopedic surgery and reported that cinnamon reduced anxiety and can be used as a premedication drug [15].

Brozin *et al.* (2015) in a study compared oxazepam and passion flower drop in a blinded clinical trial and showed that the plant had obvious better reducing effect although the dose of drug was not determined [16]. Hawthorn has tranquilizer properties. Capsules of this plant reduce heart palpitations, neurological states, and irritability or extreme feelings and adjust heart beats. Therefore, it causes more relaxation, less anxiety and comfortable sleep.

CONCLUSIONS:

According to results, hawthorn can control anxiety but more studies are necessary to prove this claim and determine effective compounds of this plant. So, it is proposed to study wider range of extract doses and also compare this extract with other anti-anxiety drugs.

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CONFLICT OF INTEREST

Authors claim that there is no conflict of interest.

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