



Comparing the Effects of Olive Leaves and Alprazolam on Stress Reduction of Mice with Type 2 Diabetes

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Abstract Olive leaves have antidepressant, antihypertensive, anti-diabetes, antioxidant, analgesic and anti-inflammatory properties. Current study was carried out to compare the effects of olive leaves and alprazolam on stress reduction of mice with type 2 diabetes. Sixty mice in the weight range of 25 to 30 were divided into six groups: control, diabetes, alprazolam and 50, 100, and 200mg/kg of olive leaves' extract. All groups except control were induced diabetes three days before the test by intraperitoneal injection of 50mg/kg streptozotocin (STZ). After diagnosing the diabetes, groups were evaluated by plus evaluated maze. Open and closed arms entries and open and closed arms times were measured as anxiety indices. Obtained data were analyzed using SPSS₂₂ program. Olive leaves extract increased open arm time in 50 and 100mg/kg doses which indicates anxiety reduction. Also, movement activities of mice were significantly increased in these doses. According to results, olive extract in 50 and 100mg/kg doses can be a good replacement for alprazolam to reduce diabetic patients' anxiety.

Keyword: olive leaves, stress, alprazolam, type2 diabetes, plus evaluated maze, mice

Introduction

Anxiety in general is a factor that affects the individual's physical and psychological balance and reduces the efficacy of him in various aspects of life [1]. Any anxiety can have damaging effects on body performance. Stress is a fact in everyday life. People often experience stress in their lives, depending on their living conditions including shopping, sales, and even illnesses, etc. [2]. Also, stress is a physiological response to biological stressors (surgery trauma and infection) and psychological stressors such as worries, fears and social tensions caused by new jobs or increasing family responsibilities [3].

Anxiety can be from diabetes reasons and also diabetes can increase the stress of diabetic patients. Stressful events of life have significant positive relationship with weak control of diabetes and small daily stresses are associated with poor metabolic control, even more than important stress [4]. Diabetes is a chronic metabolism disease which affects health and life quality of patients in physical, social, and psychosocial prosperity aspects [5]. In type 2 diabetes which includes 90-95% of diabetic patients, the body is resistant to insulin function; it means that body produces insulin, but its cell receptors are resistant to insulin and do not allow insulin to enter cells [6].

The effects of stressful events on causing and developing diabetes type 2 have been explained in different ways. Physiological stresses make accurate measurable changes in sympathetic- parasympathetic balance and activates hypothalamus-pituitary- adrenal axis which causes exocrine gland secretions, too much obesity and diabetes type 2



in people who have been in danger of disease. Increase in catecholamines and glucocorticoids levels following the activation of the hypothalamus-pituitary-adrenal axis can also play a role in reducing glucose tolerance [7].

Physical and psychosocial stress excites hormone-neural pathways especially hypothalamus-pituitary-adrenal axis. Catecholamine and glucocorticoid axes affect performance and structure of specific tissues which secrete cytokinin. This increases glucagon production and decreases recycling or breaking sugar in muscular environments. Cytokinin causes oxidative stress and inflammation via Interleukin 6 which leads to insulin resistance and cardiovascular complications [8].

Benzodiazepines are a group of prescribed drugs which are mainly used to reduce anxiety [9]. Alprazolam like other benzodiazepines as benzodiazepine receptors agonists affect the membrane of the nerve cells and facilitate the entry of GABA or increase its activity and increase the entry of chloride ions into cells [9]. This drug reduces blood sugar by decreasing stress or facilitating insulin secretion [10]. Considering that hormones like catecholamine and cortisol can increase blood sugar of patients at the time of stress, the role of alprazolam in reducing blood sugar can be prohibiting these hormones [11].

In addition to the mentioned benefits, anti-stress drugs including alprazolam have side effects such as disturbance in balance and coordination, disturbance in consciousness, sleepiness, fatigue, restlessness, and alertness, decreased libido, increased or decreased appetite, weight gain and weight loss, and speech impairment [9]. Although these drugs have high security level and the latest types have also more specific effects, but side effects such as drug resistance and quitting symptoms are inevitable [12]. Therefore, research for effective drugs with less side effects are still ongoing. Effective matters of herbal medicines are associated with other matters and are balanced, thus, will not be accumulated in body and don't have side effects or have few side effects. Therefore, they are superior to chemical drug [13]. Olive is a shrub with permanent green leaves, which has about five-meter height in wild form. Fruits and leaves are useable parts of plant. Leaves are opposite, long, elliptical, sharp, green on the upper surface, and bright green on the lower surface [14].

Olive leaves have been used from ancient time to cure fever, malaria, High blood pressure, gout, diabetes and atherosclerosis. Experimental studies have showed that the extract of olive leaves has anti-oxidant, anti-adrenal, hypolipic, hypotensive, anti-diabetic, anti-microbial, anti-tumor, anti-inflammatory, analgesic and anti-inflammatory properties [14]. The aim of this study was to compare the effects of olive leaves and alprazolam on stress reduction of mice with type 2 diabetes.

Materials and Methods

Sixty mature mice in the weight range of 25 to 30g were used. Animals were kept in a temperature and humidity Controlled room. Samples had free access to food and water, 12:12hour's light-darkness cycle for 10 days. 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

- Control group which did not receive any injection (n=10)
- Alprazolam group: samples of this group received 1.2mg/kg of alprazolam after diabetes induction(n= 10)
- Experimental groups: received 50,100and 200mg/kg of plants extract after diabetes induction(n=30)

Preparing the extract

Plant was chopped and then powdered by mill. 100g of this powder was weighed and placed in a sterilized erlen plus 40cc of ethyl alcohol. Erlen was shaken for few minutes and after sealing the door was kept for 48 hours in a cool environment.

Whatman paper was used to filter the extract. So that, paper was weighed at first, then extract was passed through the paper. Whatman paper and residual powder were dried using an oven at 50 °C for 90minutes. Weight of dried powder and paper were measured. This extract was used to make desired doses [15].



Diabetes induction

Streptozotocin (STZ) drug was used to enforce diabetes. Drug was injected intraperitoneal at single dose of 50mg/kg in cold physiological saline. Diabetes symptoms appeared after 72 hours as: weight loss, fatigue, overeating, urinating and motion less.

Drug and extract amount

Alprazolam was injected intraperitoneal (1.2mg/kg). Extracts doses were used 50minutes before the test.

Anxiety evaluation

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.

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:

open arm entries= open arm entries/ (open arm entries+ closed arm entries) * 100

open arm time= open arm time / (open arm time + closed arm time) * 100

movement activity= open arm entries+ 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 [16].

Obtained data were analyzed at two descriptive and inferential levels. Average and standard deviation were calculated in descriptive level whereas one-way variance analysis plus Tukey test were used for inferential. Data were analyzed using SPSS 22 program.

Results and Discussion

Open arm entries, open arm time and movement activity of different treatment groups were measured and analyzed statistically. Results are presented in Table 1.

Table 1: Measured variables of treatment groups

Group S Indices	Open arm entries		Open arm time		Movement activity	
	mean	Standard deviation	mean	Standard deviation	mean	Standard deviation
Alprazolam	0.53	0.018	0.56	0.048	14	4.37
Diabetes	0.47	0.016	0.34	0.031	14.37	4.37
Control	0.50	0.001	0.49	0.065	11.5	2.77
50mg/kg	0.55	0.023	0.596	0.056	10.12	2.85
100mg/kg	0.54	0.033	0.689	0.052	8.12	1.72
200mg/kg	0.78	0.023	0.879	0.045	8.23	1.23

According to results, extract in 100 and 200mg/kg doses increased open arm time in proportion to control and diabetes groups significantly. This shows anxiety reduction in diabetic mice of these groups. Also, these groups decreased movement activity significantly.

200mg/kg dose was identified as a useful dose. Since increase in open arm entry and open arm time are considered as stress reduction indices, it can be considered as a significant change in the level of stress.[17].

Therefore, the extract of olive leaves is effective for reducing stress of type 2 diabetes doses dependently. Previous studies have shown that this plant has many therapeutic properties for fever, malaria, high blood pressure, gout, diabetes and atherosclerosis [14].



In a study titled anti-diabetes activity and the improvement of oxidative stress in alloxan-induced diabetes of mice, olive leaves reduced diabetes and improved oxidative stress of animals [18]. In another study titled the effects of hydro alcoholic extract of olive leaves on gonadotropins, sexual hormones and spermatogenesis of diabetic male rats, the extract reduced side effects of diabetes on gonadotropins, sex hormones and spermatogenesis and improved pituitary-testicle hormone axis after diabetes induction [19]. In a study about the effects of intraventricular injection of olive leaf extract on pentylenetetrazole-induced seizures in male rats, olive leaves showed anti-seizure properties dose dependently [20].

Conclusion

Results of current study showed that the extract of olive leaves could reduce stress of mice with type 2 diabetes but to prove this and identify the effective compounds, more studies are necessary. Therefore, it is proposed to study wide range of extract doses and also compare this extract with other anti-anxiety drugs.

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