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Abstract

The aim of this study was to investigate the antidepressant effects of grape seed oil in male mice (n=60) using tail suspension and forced swim tests. Mice were divided to six groups of 10 in each group. Group I: Intraperitoneally receiving normal saline; Group II: Received normal saline & stress; Group III-V: Injected (i.p.) with different (60, 120, and 240 mg/kg) doses of grape seed oil; and Group VI: Received fluoxetine (10 mg/kg). Grape seed oil in 60 mg/kg significantly decreased immobility time in tail suspension and forced swim tests under chronic unpredictable stress compared to groups under chronic stress receiving normal saline and grape seed oil (120 and 240 mg/kg). Grape seed oil (60 mg/kg) significantly improved the motor balance in mice under chronic stress. It had no significant effect on serum corticosterone level. Grape seed oil can improve depression symptoms in mice.

Introduction

Depression is a multifactorial, chronic, and life-threatening disorder with high prevalence rate worldwide. People with major depression have been reported to have different levels of oxidative stress and decrease in antioxidant enzymes (Sarandol et al., 2007). Studies have indicated that some antidepressants can reduce certain oxidative stress markers and cause increase in internal antioxidants. Currently, there are almost 30 different types of antidepressants. Basically, these drugs cause certain adverse effects due to their effects on various neuromediators. Now-a-days, researchers are considering natural, plant-based compounds due to numerous adverse effects of chemical antidepressants (Rabiei and Rabiei, 2017; Aslam and Sultana, 2015).

Grape seed oil is prepared by squeezing and refining of fresh grape seed and its nutritional properties are comparable to those of olive oil. Chemically, there are high amounts of essential fatty acids and fat soluble vitamins in this oil. This oil is sodium and calcium-free

and the amount of its unsaturated fatty acids may reach to 90%. Grape seed oil is often produced from the wastes of the grapevine or fermentation beverage factories, and has many properties (Movahed and Ghavami, 2007).

Grape seed oil contains high concentrations (68-76%) of linoleic acid. Linoleic acid is an essential fatty acid of the human body and is nutritionally essential for functioning of tissues and maintenance of the body. Because grape seed oil naturally has no cholesterol, it is helpful in reducing the risk of heart diseases and other circulatory disorders (Ismail et al., 2015).

Grape seed oil contains proanthocyanidine compounds that are considered a group of bioflavonoid anti-oxidants. These compounds reduce the risk of cardiovascular diseases, stroke, and cancer, and most importantly, decrease LDL and increase HDL. These properties are unique to grape seed oil (Beveridge et al., 2005). Grape seed oil contains 60-120 mg vitamin E, one of the most potent natural antioxidants. It is not, therefore,



necessary to add artificial antioxidants such as butylated hydroxytoluene and tert-butylhydroquinone to grape seed oil. Grape seed oil, as with other vegetable oils, such as palm oil or coconut oil, contains natural tocopherols. These compounds have a much higher antioxidant potential than tocopherols. Therefore, despite being highly unsaturated, grape seed oil is stable against oxidation due to above mentioned stabilizing compounds (Beveridge et al., 2005).

Given harmful effects of chemical drugs, endeavours have been started to seek out sedative and antidepressant medications with fewer side effects, and in this regard, the production of herbal medicines has been expanded. It is also determined that if a plant-based medication is used with other compounds in that plant, it causes less side effects. Considering the beneficial effects of grape seed oil on the nervous system, we investigated its antidepressant effects *in vitro*.

Materials and Methods

Laboratory animals

Male mice (n=60) weighing 25 ± 30 g were used. The mice were maintained in the animal house of the Shahrekord University of Medical Sciences under 24°C temperature and 12 hours dark/light cycle with free access to water and food. In addition, each mouse was tested only once.

Induction of depression and grouping

In this study, depression was induced by exposure to chronic stress. According to the standard method of Kumar et al. (2011), mice were exposed to one of the stressful factors below daily for 3 weeks (Kumar et al., 2011):

A: Swimming in cold water (12°C) for 5 min; A1: Swimming in water with room temperature ($23 \pm 2^\circ\text{C}$) for 10 min; A2: Swimming in water with room temperature ($23 \pm 2^\circ\text{C}$) for 15 min; B: Tail pinch (30 sec); B2: Tail pinch (60 sec); B3: Tail pinch (90 sec); C: Water and food deprivation (24 hours); D: Overnight awakening; E: No stress.

Mice were divided to six groups of 10 each: Group I: Intraperitoneally received normal saline; Group II: Received normal saline and stress; Groups III-V: Intraperitoneally injected with different (60, 120, and 240 mg/kg) doses of grape seed oil; and Group VI: Received fluoxetine (10 mg/kg).

Tail suspension test

In tail suspension test used to assess the depression levels, metal bars of 70 cm high are used, a 50 cm rope is longitudinally stretched between them, and mouse tail is closed by the rope and the mouse is hanged up

from its tail. The test begins with jerking mice. When mouse becomes completely inactive and immobile and exhibits no reaction, it is considered immobility. Total duration of tail suspension test, as with forced swim test, is 6 min and the first 2 min is specified to animal's adaptation to the apparatus. The immobility time (s) in the following 4 min was recorded by chronometer. All tests of variables were recorded by one person (Gupta et al., 2016).

Forced swim test

This animal test is one of the most reliable and common tests for evaluation of depression. Based on learned helplessness of Martin seligman, if animal is exposed to chronic stress and fails to escape, it gradually loses its hope to escape, stops its activity and mobility, and becomes frustrated and immobile. To measure the immobility time, all time intervals when mouse is immobile are recorded within a specified duration. Extended immobility time represents depression and its decrease does the efficacy of treatment with antidepressant. In this test, a glass container that has a length of 25 cm, a width of 12 cm, and a height of 15 cm is filled with 25°C water and the mouse from a 20-cm distance to the water surface is slowly placed in the water. Conventionally, lack of paddling is considered immobility. All chronological measurements were conducted by one person. Total forced swim test lasts for 7 min and the first 3 min are specified for adaptation to test condition and therefore immobility time during this period is not recorded. All tests of variables were recorded by one person (Gupta et al., 2016).

Rotarod test

Rotarod apparatus was used to assess balance maintenance and resistance to movement. This apparatus has a rotator with rotation speed of 4,000 rpm. This apparatus has a belt and rotation speed is adjusted by moving it on the place of belt position. The mice were first placed on the rotarod roller rod and trained to move on it based on the main protocol (speed: 10 rpm and acceleration: 7 rpm^2), and the balance test was performed 30 min later. All mice were placed on the rotarod 1 hour after injection of the extract. The period when mouse could maintain its balance and resist against the movement of the rotarod was considered resistance time. The maximum test time for each mouse was considered 300 sec (Rabiei et al., 2016).

Measuring plasma corticosterone level

Blood samples (0.5 to 0.8 mL) were taken 1 hour after conducting the behavioral tests, and the plasma corticosterone levels were measured (Harati et al., 2014).

Data analysis

Statistical analysis was conducted using SPSS 16. To investigate the significant difference between the groups, one-way ANOVA and then Duncan's test were

used. Data expressed as mean \pm standard deviation and $p < 0.05$ was considered significance level.

Results

Effect of grape seed oil on immobility time in tail suspension test

Figure 1 illustrates the effect of intraperitoneal injection of fluoxetine and different doses of grape seed oil on immobility time in tail suspension test. As illustrated, the immobility time in the group under stress receiving normal saline was significantly higher than that in the control group ($p < 0.05$). Immobility duration significantly increased in the group under stress receiving grape seed oil (240 mg/kg) compared to the other groups ($p < 0.05$). There was no significant difference in immobility time between the groups under stress receiving normal saline and those injected with grape seed oil (120 and 240 mg/kg) ($p < 0.05$). Intraperitoneal injection of grape seed oil (60 mg/kg) significantly decreased immobility time compared to normal saline and grape seed oil (120 and 240 mg/kg) in the mice under stress ($p < 0.05$).

Intraperitoneal injection of fluoxetine (10 mg/kg) significantly decreased the immobility time compared to normal saline and grape seed oil (60, 120, and 240 mg/kg) in the mice under stress ($p < 0.05$).

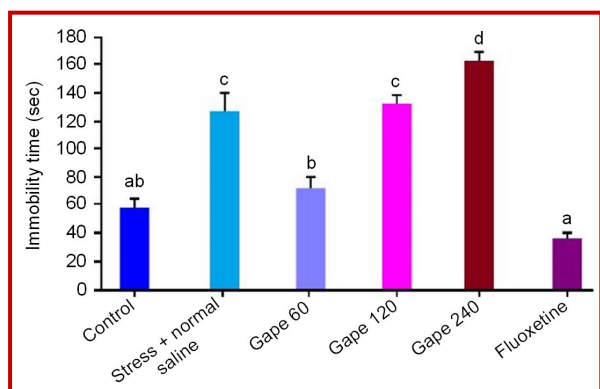


Figure 1: Effect of intraperitoneal injection of fluoxetine and different doses of grape seed oil on immobility time in tail suspension test. a: Significant difference compared 'b', 'c' and 'd'; b: Significant difference compared 'a', 'c' and 'd'; c: Significant difference compared 'a', 'b' and 'd'; d: Significant difference compared to 'a', 'b' and 'c' ($p < 0.05$); ab: No significant difference compared to 'a' and 'b'

Effect of grape seed oil on immobility time in forced swim test

Figure 2 illustrates the effect of intraperitoneal injection of fluoxetine and different doses of grape seed oil on the immobility time in forced swim test. As illustrated, the immobility time in group under chronic stress receiving normal saline was significantly higher than

that in the control group ($p < 0.05$). As illustrated, there was no significant difference among the groups under chronic stress receiving 120 and 240 mg/kg doses of grape seed oil and the group under chronic stress receiving normal saline ($p < 0.05$). Intraperitoneal injection with fluoxetine (10 mg/kg) significantly decreased the immobility time compared to normal saline and grape seed oil (120 and 240 mg/kg) in the mice under stress ($p < 0.05$). Intraperitoneal injection of grape seed oil (60 mg/kg) significantly decreased the immobility time compared to normal saline and grape seed oil (120 and 240 mg/kg) in the mice under stress ($p < 0.05$).

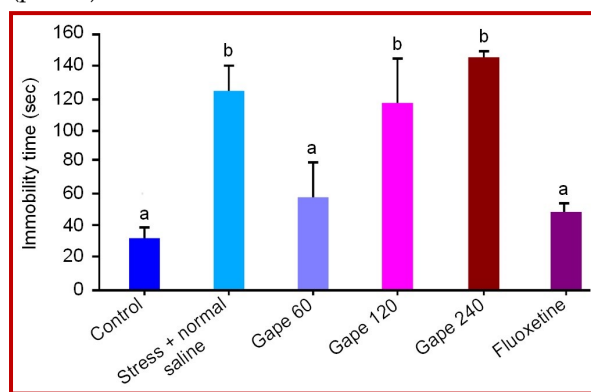


Figure 2: The effect of intraperitoneal injection of fluoxetine and different doses of grape seed oil on immobility time in forced swimming test; difference is considered significant at $p < 0.05$

Psychomotor coordination by rotarod treadmill apparatus

The ability to maintain balance and resist movement was investigated using rotarod (Figure 3). Resistance to rotation in groups under stress receiving fluoxetine and grape seed oil (60 mg/kg) was significantly higher than that in the normal saline group ($p < 0.05$). There was no significant difference in the time of resistance to rotation between group under stress receiving grape seed oil (120 and 240 mg/kg) and group under stress treated with normal saline. The time of resistance to rotation was significantly higher in the control group than the group under chronic stress.

Effect of grape seed oil on serum corticosterone level

Figure 4 shows the results related to the effect of grape seed oil on serum corticosterone levels in different groups. The serum corticosterone levels were significantly higher in the group under chronic unpredictable stress compared to the control group ($p < 0.05$). Treatment with grape seed oil (60 and 120 mg/mL) and fluoxetine caused no significant effect on serum corticosterone levels in mice under chronic unpredictable stress ($p > 0.05$). Serum corticosterone levels decreased in the group receiving grape seed oil

(240 mg/mL), but did not significantly change compared to the control group and the group under chronic unpredictable stress.

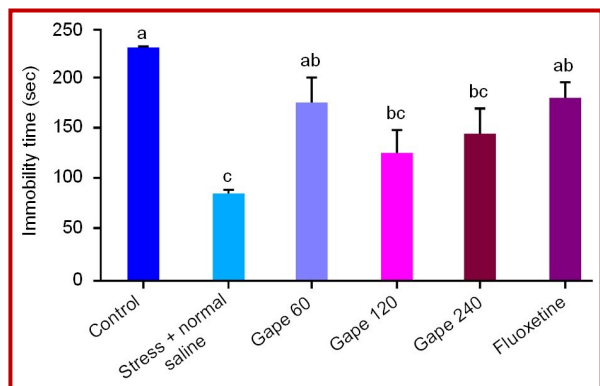


Figure 3: Effect of intraperitoneal injection of fluoxetine and different doses of grape seed oil on balance maintenance and resistance to movement

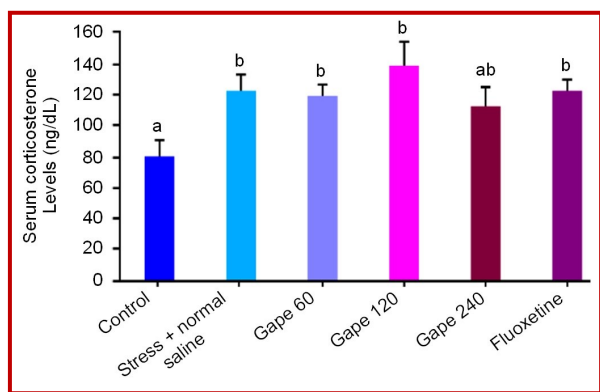


Figure 4: Effect of intraperitoneal injection of different doses of fluoxetine and grape seed oil on serum corticosterone levels

Discussion

This study investigated the effect of grape seed oil (60, 120 and 240 mg/kg) on the symptoms of depression induced by chronic unpredictable stress in mice. In addition, this study evaluated the effects of grape seed oil on motor coordination and serum corticosterone levels in mice under chronic stress. According to the results, immobility time in forced swim test and TST in control group was significantly lower than under-stress group. Injection of grape seed oil (60 mg/kg) to mice under chronic stress significantly decreased immobility time in forced swim test and tail suspension test compared to groups under chronic stress receiving normal saline and grape seed oil (120 and 240 mg/kg). In this study, injection of fluoxetine (10 mg/kg) to mice under chronic stress significantly decreased the immobility time compared to the group under chronic stress receiving normal saline. Moretti et al. consistently showed that intraperitoneal injection of fluoxetine (10

mg/kg) to mice under chronic unpredictable stress significantly decreased the immobility time in forced swim test and tail suspension test (Moretti et al., 2012). Kumar et al. (2011) also reported positive effects of fluoxetine on mild and chronic unpredictable stress (Kumar et al., 2011).

Studies have shown that most symptoms of chronic unpredictable stress-induced depression occur due to the impairment of neuroendocrine, antioxidant defense, and inflammatory systems (Eren et al., 2007; Mao et al., 2009; Marks et al., 2009; You et al., 2011). A study by Schaalán et al. (2011), reported neuroendocrine impairment and significant increase in serum adrenocorticotropic and corticosterone levels in mice exposed to chronic unpredictable stress (Schaalán and Nassar, 2011). Mao et al. (2009) reported increase in serum corticosteroids levels in mice under chronic unpredictable stress (Mao et al., 2009). A study by Grundmann et al. (2010) has shown chronic stress-induced depression in mice caused increase in serum adrenocorticotropic and corticosterone levels (Grundmann et al., 2010).

In Marks et al study, frequent administration of corticosterone led to significant increase in depressive-like behaviors in forced swim test in mice. These behaviors were independent of changes in motor activity or muscular strength (Marks et al., 2009). Chronic stress-induced depression, in our study, caused significant increase in serum corticosterone levels. The administration of grape seed oil (60, 120, and 240 mg/kg) did not have a significant effect on serum corticosterone levels, although this extract in 240 mg/kg concentration decreased its level yet insignificantly; therefore, it seems that other mechanisms are involved in antidepressant effects of grape seed oil. The role of inflammatory mechanisms in chronic unpredictable stress-induced depression has been reported in different studies.

In a study, mice were exposed to chronic unpredictable stress, and results showed increased serum levels of tumor necrosis alpha (TNF α) (Schaalán and Nassar, 2011). It was reported that the expression of pro-inflammatory cytokines such as TNF α , interleukin-6, and interleukin-1 beta significantly increased in the brain and spleen of mice under chronic stress, and expression of anti-inflammatory cytokines such as TGF- β and interleukin-10 significantly decreased (You et al., 2011).

Chronic stress-induced depression increased the levels of inflammatory markers, interleukin-6, and TNF α (Grundmann et al., 2010). Given the anti-inflammatory effects of grape seed oil confirmed in previous studies, it can be argued that grape seed oil can significantly inhibit depression due to chronic stress through decreasing the levels of inflammatory mediators.

In an experimental study, in mice treated with carbon tetrachloride through grape seed oil, brain TNF α and interleukin-6 levels decreased significantly (Ismail et al., 2015). The effect of procyanidine of grape seed on corticosterone-induced ear edema and carrageena-induced foot swelling has been demonstrated. Procyanidins of grape seed significantly decreased nitric oxide level, nitric oxide synthase activity, and TNF α , interleukin-1 beta, and prostaglandin E2 levels (Li et al., 2000).

The role of oxidative stress has been demonstrated in UCS-induced depression in different studies. In Schaalan et al study, chronic unpredictable stress was associated with decrease in antioxidant activity of superoxide dismutase and glutathione reductase, and a decrease in glutathione levels in mice hippocampus. In addition, it was observed that lipids peroxidation and lactate dehydrogenase levels increased in the hippocampus of mice exposed to chronic unpredictable stress (Schaalan and Nassar, 2011). Induction of depression using chronic stress model significantly reduced activity of glutathione peroxidase in mouse brain cortex. The induction of depression also reduced the levels of glutathione and vitamin C in the cortex and increased lipids peroxidation (Eren et al., 2007).

Increase in proteins peroxidation in the frontal lobe of the brain, hippocampus, and striatum caused increase in lipid peroxidation in the cerebellum and corpus striatum, and decrease in activity of superoxide dismutase in the frontal lobe of the brain, hippocampus, and striatum of mice exposed to chronic unpredictable stress (Lucca et al., 2009). In a study by Grundmann et al. (2010), chronic stress-induced depression caused decrease in antioxidant capacity and activity of glutathione per-oxidase, superoxide dismutase and catalase in the hippocampus and hypothalamus (Grundmann et al., 2010). Given the antioxidants effects of grape seed oil in different studies, it seems that grape seed oil significantly inhibits chronic unpredictable stress-induced depression through decreasing the oxidative stress markers in the brain.

In the study, proanthocyanidine, vitamin C, vitamin E, succinate, and beta-carotene of grape seed significantly inhibited lipid peroxidation and oxidative stress of brain cells' DNA by oxygen free radicals (Bagchi et al., 1998).

There was significant protective effects of grape seed against the oxidative damage of DNA in the brain neuronal cells using ethanol (Guo et al., 2007).

Grape seed oil significantly increased the activity of superoxide dismutase, catalase, glutathione peroxidase, and brain glutathione content in mice treated with carbon tetrachloride (Ismail et al., 2015). Treatment of grape seed oil-receiving mice with tetrachloride signifi-

cantly decreased their brain malondialdehyde levels.

In addition, the protective effects of vitamin C against chronic unpredictable stress-induced depression was reported. The mechanism of antidepressant effects of ascorbic acid is due to the reduction of oxidative stress parameters (Moretti et al., 2012).

Vitamin E reduces the levels of depression in women with periodic chest pain by decreasing the oxidative stress markers (Momeni, 2012). As grape seed oil contains high amounts of vitamin E and C, it seems that reduction of oxidative stress parameters is one of the main involved mechanisms in antidepressant effects of this oil; however, it is recommended to investigate this assumption by measuring the levels of oxidative stress markers in additional studies.

Conclusion

Administration of grape seed oil caused antidepressant effects and significantly increased immobility time in forced swim test and tail suspension test in depressed mice. In addition, grape seed oil increased motor coordination of mice. It seems that antidepressant effects of grape seed oil extract are due to antioxidant and anti-inflammatory mechanisms.

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Ethical Issue

The study was approved by the Ethical Committee of the Shahrekord University of Medical Sciences.

Conflict of Interest

Authors declare no conflict of interest

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