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Lycium barbarum L. (goji berry) fruits improve anxiety, depression-like behaviors, and learning performance: the moderating role of sex

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Abstract: The aim of this research was to investigate the effects of the methanol extract obtained from *Lycium barbarum* fruits on anxiety, depression-like behaviors, and spatial memory in Wistar albino rats. A total of 28 rats were selected, randomly assigned to four experimental groups, and tested by means of the open field and elevated plus maze tests for anxiety-like behaviors, the forced swim test for depression-like behaviors, and the Morris water maze test for spatial memory. The findings demonstrated that in the open field, *L. barbarum*-administered rats spent more time at the center and showed more mobility and velocity than controls. In the elevated plus maze, *L. barbarum*-administered rats spent more time in the open arms, spent less time in the closed arms, and showed more mobility and velocity. In the Porsolt test, *L. barbarum*-administered rats showed less immobility. In the Morris water maze, *L. barbarum*-administered rats took more time to find the platform. However, females were better at finding the platform than males. The methanol extract of *L. barbarum* fruits decreased anxiety and depression-like behaviors and interacted with sex on spatial memory.

Key words: *Lycium barbarum* (goji berry), anxiety, depression, spatial memory, Wistar albino rat (*Rattus rattus*)

1. Introduction

Lycium barbarum L. (goji berry), a member of the family Solanaceae, has received a great deal of research attention because of its rich contents as well as its beneficial effects on health (Wang et al., 2010; Yao et al., 2011), such as impacts on aging, neuroprotection, well-being, fatigue, energy metabolism, diabetic control, the immune system, antitumor activity, and antioxidant activity (Amagase and Farnsworth, 2011; Song and Xu, 2013). Such beneficial effects of *L. barbarum* are usually attributed to high amounts of polysaccharides (LBPs), flavonoids, phenolic acids, and carotenoids in its content (Endes et al., 2015).It also includes essential oils, vitamins (A, B, and C), amino acids, mineral elements (K, P, Ca, Mg, Fe, and Na), and betaine (Potterat, 2010; Endes et al., 2015).

Despite these benefits, its effects on behavioral processes remain open to investigation because of a lack of controlled experiments on this matter. Thus, we investigated the effect of the methanol extract obtained from *L. barbarum* fruits on anxiety, depression-like behaviors, and spatial memory in Wistar albino rats because anxiety and depression are the most common psychological problem with a lifetime prevalence of 16.6% (Ramsawh et al., 2010) and 17%–20% (Cryan et al., 2002), respectively. Moreover, spatial memory, which is affected by various factors such as sex, hormones, cues, training, and emotions (Hawley et al., 2013), is also an essential part of our ability to recognize our environment and its spatial orientation. Just like humans, rats use spatial memory to learn the location of food at the end of a maze or another given place (Packard and McGaugh, 1996). For the treatment or improvement of these behaviors, new drugs and herbal treatment are sought since the side effects of current drugs are still a great problem for scientists and professionals.

The aim of the present study was to investigate how behaviors such as anxiety, depression, and learning are affected by the methanol extract of *L. barbarum* fruits. If *L. barbarum* fruits are beneficial to health (e.g., effects on antiaging activity, neuroprotection, and general well-being), it should also have antianxiety and antidepressant activities. This study was carried out with both male and female albino rats since females are reported to have more hormonal activities and attentional properties than males (Meyers-Levy, 1989). We also investigated whether or not sex modulates the effect of the methanol extract

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of *L. barbarum* fruits on anxiety, depression, and spatial learning performance. To test these issues, the rats were put into open field and elevated plus maze tests for anxiety-like behavior, a forced swim test for depression, and the Morris water maze test for spatial memory.

Given the fact that previous studies overlooked the relationships among anxiety, depression-like behaviors and spatial learning behavior (Karakas et al., 2011; Coskun et al., 2012), we also tried to investigate for the first time the role of the methanol extract of *L. barbarum* on anxiety, depression, and spatial memory in a longitudinal fashion. In these above-mentioned studies as well as the present research, all animals were tested for these behaviors within the span of 3 weeks. Despite the fact that this interval can be regarded as a long enough time period for eliminating carryover effects, one may wonder whether or not there may be carryover effects for both control and treatment conditions. Besides this plausible carryover effect, the nature of the relationships among these variables is of great importance. Thus, we separately conducted relationship analyses for control and treatment conditions in order to test whether or not these issues were meaningful.

2. Materials and methods

2.1. Plant material and extract preparation

The dried fruits of *L. barbarum*, produced by Bolu Kalite Yem Sanayi A.Ş., were originally imported from China and sold under the name of Naturol (code number: TR 14K00007). They were ground and weighed. The powdered fruits (30 g) were extracted with 300 mL of methanol (MeOH) in a hot water bath at 35 °C for 16–24 h and then filtered. The extraction solvent was then evaporated under low pressure at a temperature not higher than 38 °C using a rotary evaporator. The extraction solvent was evaporated successfully and crude methanolic extracts of *L. barbarum* were obtained and stored in refrigerator at 4 °C. The yield of extract (w/w) was 30% (yield % = weight of extract / powdered plant sample × 100).

2.2. Animal care

Adult Wistar albino rats (200–250 g; 14 males and 14 females) were acquired from the Center for Experimental Animals and Applied Research at Abant İzzet Baysal University, Bolu, Turkey. The procedures in this study were carried out in accordance with the standard animal scientific procedure and were approved by the Institutional Animal Care and Use Committee (ethical code number: 2014/35). For all rats, plastic cages (16 × 31 × 42 cm), which were exposed to 200 lx light, were used with pine shavings as bedding. They were maintained at 12 h of light and 12 h of darkness with lights off at 1800 hours. Food pellets and tap water were accessible ad libitum. Ambient temperatures in the animal facilities were held constant at 22 ± 2 °C in air-ventilated rooms.

2.3. Experimental method

In the present study, a total of 28 adult rats were selected and randomly assigned to four experimental groups [control males (n = 7), *L. barbarum*-administered males (n = 7), control females (n = 7), and *L. barbarum*-administered females (n = 7)]. Each rat received 50 mL of fresh drinking water that included 50 mg of *L. barbarum* methanol extract every day ad libitum from the 1st day to the end of the experiment (30 days). In the control group, animals received the same amount of fresh drinking water to obtain the same stress conditions as in the experimental groups. Anxiety-like behaviors, depression-like behaviors, and spatial memory of Wistar rats were measured by means of open field and elevated plus mazes, forced swimming, and the Morris water maze test, respectively.

2.3.1. Open field

The open field consisted of an arena of 80 cm × 80 cm with 40-cm-high walls; it is a commonly used test in order to investigate anxiety-like behaviors. This test has two main areas: a plain and an illuminated arena. A video camera was fixed above the arena, recording behaviors into the Ethovision video-tracking system (Noldus Ethovision, Version 6, the Netherland; Commat Ltd. Şti., Ankara, Turkey). This system recorded all the parameters of anxiety-like behaviors such as distance, time on the edge, time in the center, frequency on the edge, frequency in the center, mobility, and velocity among the different areas of the arena (Karakas et al., 2011).

2.3.2. Elevated plus maze

The elevated plus maze in a plus-sign configuration 55 cm off the floor, which has been used as a test for acrophobia or elevated anxiety. It consisted of two open and two closed 10-cm-wide arms. The closed arms were enclosed by 41-cm-tall black Plexiglas. All animals were tested by means of the procedure described by Karakas et al. (2011) and Kaya et al. (2011). Videotapes with Observer Software (Ethovision XT, Noldus Ethovision, Version 6) were recorded and analyzed for all anxiety-like behaviors (including distance, duration in the open arms, frequency in the closed arms, mobility, and velocity).

2.3.3. Forced swim test (Porsolt test)

Depressive-like responses were measured by means of the Porsolt test, which consists of an opaque cylinder tank (24 cm in diameter, 53 cm in height) filled with 17 cm of water kept at 28 °C. Swimming behavior was recorded on video for 5 min. A video camera was mounted above the arena, recording behavior into the Ethovision video-tracking system (Noldus Ethovision, Version 6) that provided a variety of depression-like behaviors including mobility time, mobility frequency, total distance travel, immobility time, rotation, and velocity (Karakas et al., 2011).

2.3.4. Morris water maze

Spatial memory was measured by using the Morris water maze, which consisted of a circular galvanized steel maze (1.5 m in diameter and 60 cm in depth), which was filled with 40 cm of water kept at 28 °C and rendered opaque by the addition of a nontoxic, water-soluble dye. The maze was surrounded by many visual cues external to the maze (e.g., the experimenter, ceiling lights, a rack, pictures). Locations of such cues were fixed throughout the period of testing. There were four equally divided quadrants in the pool. In one of the quadrants, a platform (2.0 cm below the water surface, 10 cm in diameter) was submerged centrally and fixed in position, which was kept constant throughout the acquisition trials. All the procedures in this test were similar to the procedure described by Karakas et al. (2011). A video camera was fixed above the arena. All spatial learning behaviors (distance, time on the edge, time in the center, frequency on the edge, frequency in the center, and immobility among the different areas of the arena) were recorded by a means of the Ethovision video-tracking system (Noldus Ethovision, Version 6).

2.4. Statistical analyses

Data were analyzed by two-treatment (*L. barbarum* treatment and control) × two-sex (female and male) ANOVA analysis with between-subject design and correlational analyses using SPSS 22.0. Data are presented as mean ± SEM after back-transformation from ANOVA results.

3. Results

3.1. Open field measurements

3.1.1. Total distance traveled in the open field

A significant effect of sex was detected on the total distance traveled in the open field ($F_{(1,50)} = 23.14$, $P = 0.0001$, $\eta^2 = 0.34$), with higher scores for females (M = 2337.15) than males (M = 1858.82). *L. barbarum* treatment effect was found for the total distance traveled in the open field ($F_{(1,50)} = 6.96$, $P = 0.01$, $\eta^2 = 0.12$), with a higher mean of subjects in the treatment group (M = 2229.13) than the control (M = 1966.84) (Figure 1a). This means that the subjects in the treatment groups were less anxious than those in the control groups.

3.1.2. Time spent at the edge of the open field

A significant effect of treatment was found for the time spent at the edge of the open field ($F_{(1,50)} = 4.35$, $P = 0.04$, $\eta^2 = 0.08$). The subjects in the treatment groups (M = 4.56) spent less time at the edge of the open field than controls (M = 4.68) (Figure 1b), indicating that the subjects in the treatment groups were less anxious than the controls.

3.1.3. Time spent at the center of the open field

A significant effect of treatment was found for the time spent at the center of the open field ($F_{(1,50)} = 4.35$, $P = 0.04$, $\eta^2 = 0.08$), with higher scores for subjects in the treatment groups (M = 0.44) than controls (M = 0.32) (Figure 1c).

3.1.4. Total entry to the edge of the open field

A significant effect of treatment was found for the entrance frequency to the edge of the open field ($F_{(1,52)} = 3.73$, $P = 0.059$, $\eta^2 = 0.07$), with higher total entry of the subjects in the treatment groups (M = 8.50) than the controls (M = 6.27). The interaction effect between sex and treatment was significant ($F_{(1,52)} = 3.73$, $P = 0.059$, $\eta^2 = 0.007$). Females were more anxious in treatment conditions (M = 9.57) than control conditions (M = 5.14), but males had the same level of anxiety in both treatment (M = 7.43) and control (M = 7.43) conditions (Figure 1d).

3.1.5. Total entry to the center of the open field

A significant effect of treatment was found for the entrance frequency to the center of the open field ($F_{(1, 52)} = 3.98$, $P = 0.051$, $\eta^2 = 0.071$), with higher entry of subjects in the treatment groups (M = 8.14) than controls (M = 5.93) (Figure 1e).

3.1.6. Mobility in the open field

A significant effect of sex was found for mobility in the open field ($F_{(1,50)} = 8.67$, $P = 0.05$, $\eta^2 = 0.15$), with higher mobility scores for males (M = 4.999) than females (M = 4.996). The main effect of treatment was significant on the mobility in the open field ($F_{(1,50)} = 5.94$, $P = 0.02$, $\eta^2 = 0.11$), with higher mobility of subjects in the treatment groups (M = 4.998) than controls (M = 4.996).

3.1.7. Velocity in open field

A significant effect of sex was found for velocity in the open field ($F_{(1,50)} = 8.67$, $P = 0.05$, $\eta^2 = 0.15$). Males (M = 371.97) showed less velocity than females (M = 468.08). The main effect of treatment was significant on the velocity in the open field ($F_{(1,50)} = 6.85$, $P = 0.01$, $\eta^2 = 0.12$), with a higher mean of subjects in the treatment groups (M = 446.05) than controls (M = 394.01) (Figure 1f).

3.2. Elevated plus maze measurements

3.2.1. Total distance traveled in the elevated arm maze

The main effect of sex was significant on the total distance traveled in the elevated plus maze ($F_{(1,50)} = 45.09$, $P = 0.0001$, $\eta^2 = 0.47$), with a higher mean of females (M = 1162.28) than males (M = 842.65) (Figure 2a).

3.2.2. Time spent in closed arms

A significant effect of *L. barbarum* administration was found for the time spent in closed arms ($F_{(1,50)} = 10.55$, $P = 0.002$, $\eta^2 = 0.17$). The subjects in treatment groups (M = 3.68) spent less time in the closed arms than the controls (M = 4.33) (Figure 2b).

3.2.3. Total entry to closed arms

A significant effect of sex was found for the entrance frequency to the closed arms ($F_{(1,50)} = 17.83$, $P = 0.0001$,

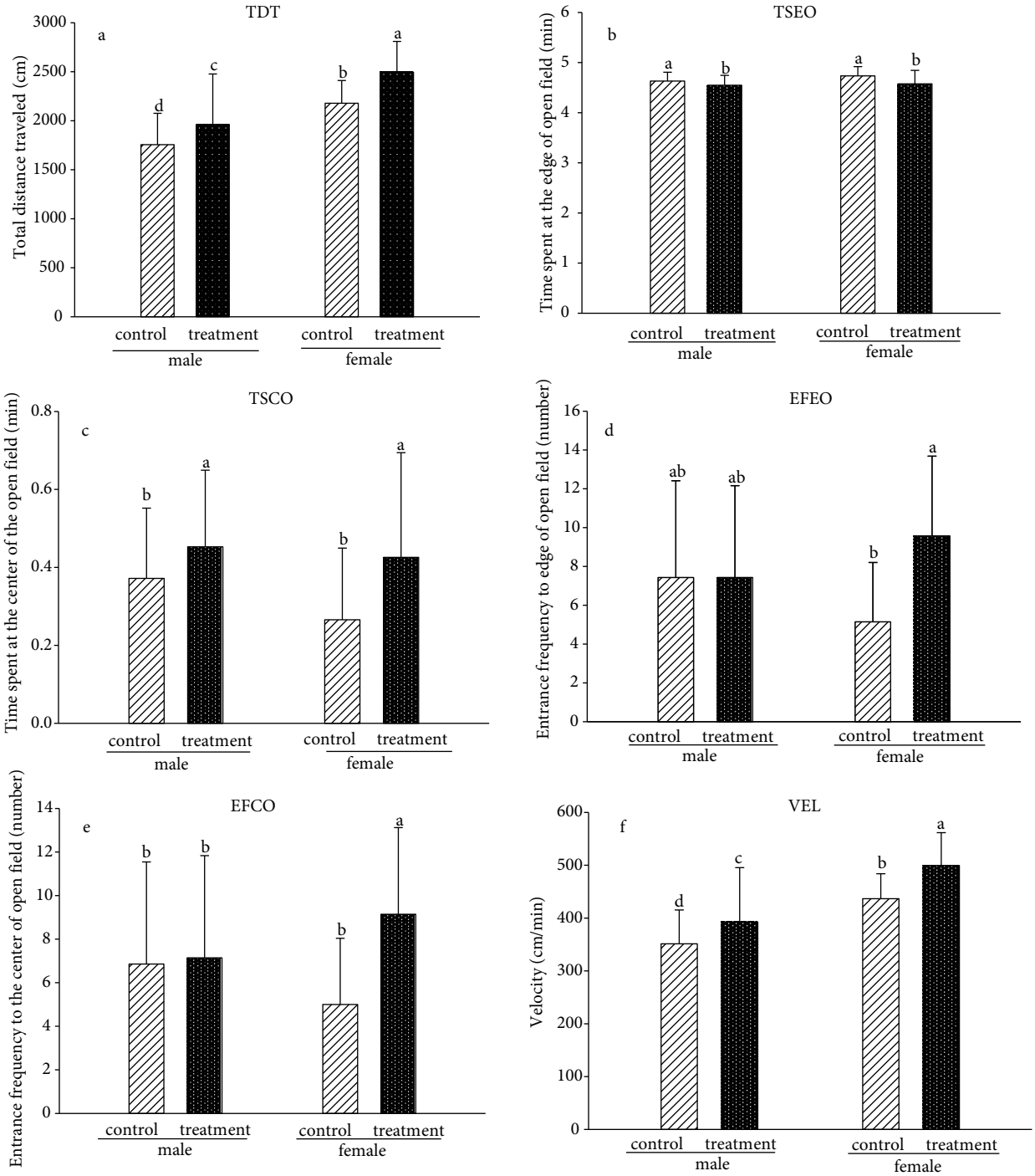

Figure 1. (a) Total distance traveled on the open field (TDT), (b) time spent at the edge of the open field (TSEO), (c) time spent at the center of the open field (TSCO), (d) total entries to the edge of the open field (EFEO), (e) total entries to the center of the open field (EFCO), and (f) velocity in the open field (VEL) are represented for open field measurements. Right striated bar represents rats exposed to the methanol extract of *L. barbarum* (n = 14); dotted striated bar represents control conditions (n = 14). a, b, c, d: mean values with the same letters above columns are not significantly different (P > 0.05).

η^2 = 0.26), with a higher entry of females (M = 7.36) than males (M = 5.35). The main effect of treatment was significant on the entrance frequency to the closed arms (F (1, 50) = 7.15, P = 0.01, η^2 = 0.13), with higher entry of the subjects in the treatment groups (M = 6.99) than controls (M = 5.71). An interaction effect between sex and *L.*

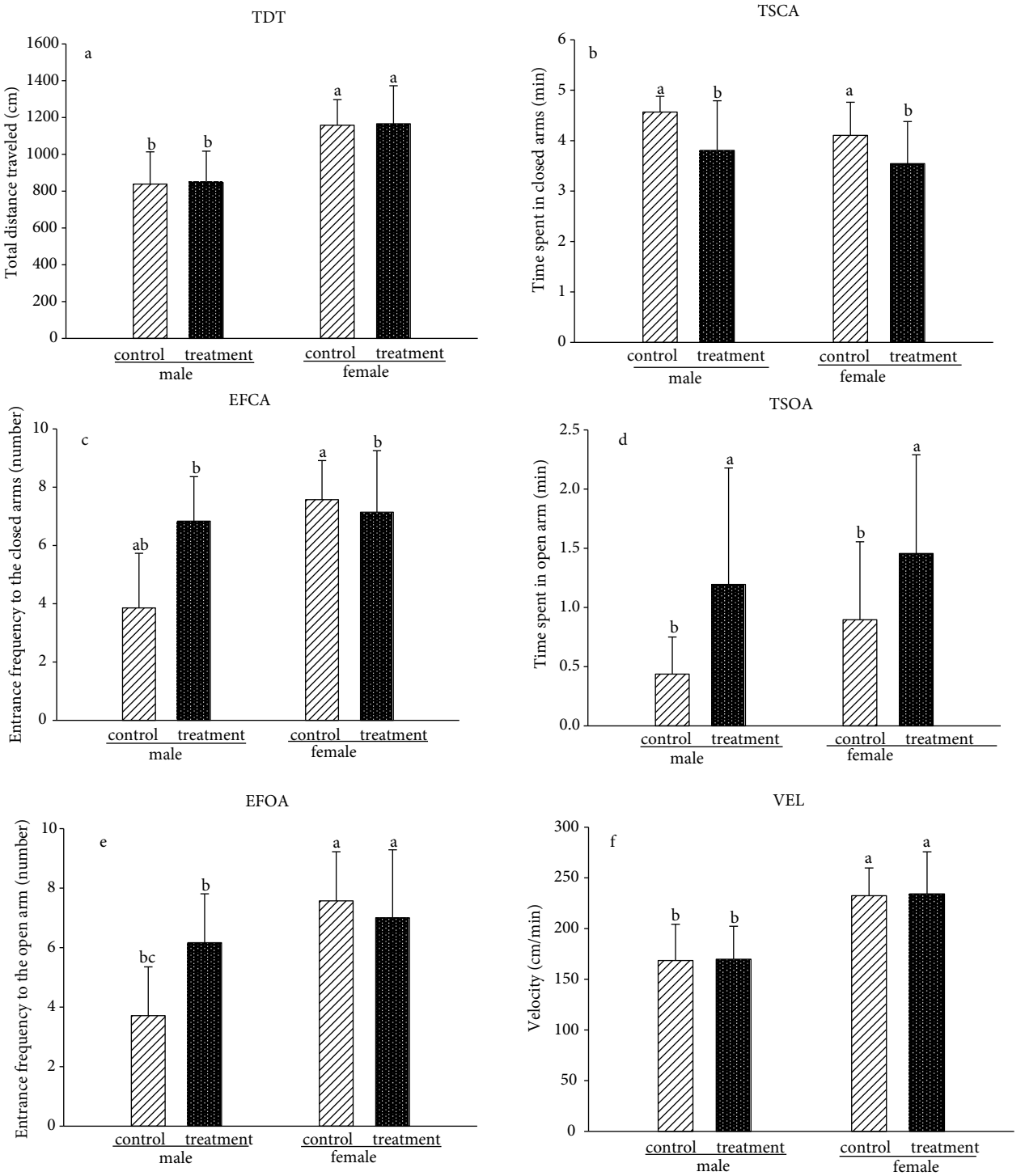

Figure 2. (a) Total distance traveled in the elevated arm maze (TDT), (b) time spent in closed arms (TSCA), (c) total entries to closed arms (EFCA), (d) time spent in open arm (TSOA), (e) total entries to open arm (EFOA), and (f) velocity (VEL) in elevated plus maze are represented for elevated plus maze measurements. Right striated bar represents rats exposed to the methanol extract of *L. barbarum* (n = 14); dotted striated bar represents control conditions (n = 14). a, b, c: mean values with the same letters above columns are not significantly different (P > 0.05).

barbarum administration was significant (F (1,50) = 12.77, P = 0.001, η^2 = 0.20). This means that males were more anxious in treatment conditions (M = 6.83) than control conditions (M = 3.86), but females were less anxious in treatment conditions (M = 7.14) than control conditions (M = 7.57) (Figure 2c).

3.2.4. Time spent in open arms

A significant effect of *L. barbarum* administration was found for the time spent in open arms (F (1, 50) = 10.55, P = 0.002, η^2 = 0.17), with higher scores of the subjects in the treatment groups (M = 1.33) than controls (M = 0.67) (Figure 2d).

3.2.5. Total entry to open arms

A significant effect of sex was found for the entrance frequency to the open arms (F (1,50) = 22.01, P = 0.0001, η^2 = 0.31), with a higher total entry of females (M = 7.29) than males (M = 4.94). An interaction effect between sex and *L. barbarum* administration was also significant (F (1,50) = 9.15, P = 0.004, η^2 = 0.16). Males were less anxious in treatment conditions (M = 6.17) than control conditions (M = 3.71), but females had the same level of anxiety in both treatment (M = 7.00) and control (M = 7.57) conditions (Figure 2e).

3.2.6. Mobility in elevated plus maze

A significant effect of sex was found for mobility (F (1,50) = 5.72, P = 0.02, η^2 = 0.10). Females (M = 4.986) were less mobile than males (M = 4.990). An interaction effect between sex and *L. barbarum* administration was also significant (F (1,50) = 4.76, P = 0.03, η^2 = 0.09). As can be seen, males were less anxious in treatment conditions (M = 4.989) than control conditions (M = 4.992), but females had the same level of anxiety in both treatment (M = 4.989) and control (M = 4.984) conditions.

3.2.7. Velocity in elevated plus maze

A significant effect of sex was found for the velocity (F (1,50) = 45.10, P = 0.0001, η^2 = 0.47). Males (M = 169.07) showed less velocity than females (M = 233.29) (Figure 2f).

3.3. Forced swim test (Porsolt) measurements

3.3.1. Total distance traveled in the Porsolt test

A significant effect of treatment was found for the total distance traveled (F (1,24) = 39.48, P = 0.0001, η^2 = 0.62), with higher performance of the subjects in the treatment groups (M = 1878.34) than controls (M = 1237.67) (Figure 3a), indicating that subjects in treatment conditions were less depressive than those in the control conditions.

3.3.2. Immobility

A significant effect of *L. barbarum* treatment was found for immobility (F (1,24) = 37.45, P = 0.0001, η^2 = 0.61). The subjects in the treatment groups (M = 0.78) were less immobile than controls (M = 1.51) (Figure 3b). This means that the subjects in treatment conditions were less depressive than those in control conditions.

3.3.3. Mobility

A significant effect of treatment was found for mobility (F (1,24) = 26.56, P = 0.0001, η^2 = 0.53) with higher mobility of the subjects in the treatment groups (M = 3.78) than the controls (M = 2.91) (Figure 3c).

3.3.4. Velocity

A significant effect of sex was found for velocity (F (1,50) = 8.52, P = 0.005, η^2 = 0.15), with a higher velocity of females (M = 455.91) than males (M = 506.45). The main effect of treatment was significant on velocity (F (1,50) = 4.05, P = 0.05, η^2 = 0.08), with a higher mean of the subjects in the treatment groups (M = 448.22) than controls (M = 414.13) (Figure 3d).

3.5. Water maze measurements

3.5.1. Immobility

An interaction effect between sex and *L. barbarum* treatment was significant (F (1,50) = 4.06, P = 0.05, η^2 = 0.8). As can be seen, females were less immobile in treatment conditions (M = 0.099) than control conditions (M = 0.221), but males were more immobile in treatment conditions (M = 0.123) than control conditions (M = 0.040) (Figure 4a).

3.5.2. Velocity in the water maze

The main effect of sex was significant on velocity (F (1,50) = 4.40, P = 0.04, η^2 = 0.08). Males (M = 1679.65) showed more velocity than females (M = 1515.39). The main effect of treatment was significant on velocity in the open field (F (1, 50) = 8.88, P = 0.004, η^2 = 0.15). The subjects in the treatment groups (M = 1714.18) were faster than controls (M = 1480.85) (Figure 4b).

3.6. Correlation analysis

Analyses were conducted in terms of velocity since it was a common parameter for all behaviors and free from all situational constraints. Concerning control conditions, velocity scores in the elevated plus maze were positively highly correlated with those in the open field test (r = 0.50, P < 0.01). These scores in the elevated plus maze and open field test were not significantly correlated with those for depression (r = 0.24, P > 0.05 and r = –0.15, P > 0.05, respectively). However, the scores in the elevated plus maze and open field test were significantly negatively correlated with those for spatial learning (r = –0.39, P < 0.05 and r = –0.56, P < 0.01, respectively). Moreover, the scores for depression were negatively correlated with those for spatial learning (r = –0.38, P < 0.05).

As for the treatment condition (i.e. methanol extract of *L. barbarum*), velocity scores in the elevated plus maze were not correlated with those in the open field test (r = 0.15, P > 0.01). These scores in the elevated plus maze were significantly correlated with those for depression (r = 0.44, P < 0.01), whereas the scores in the open field were not correlated with those for depression (r = –0.19, P >

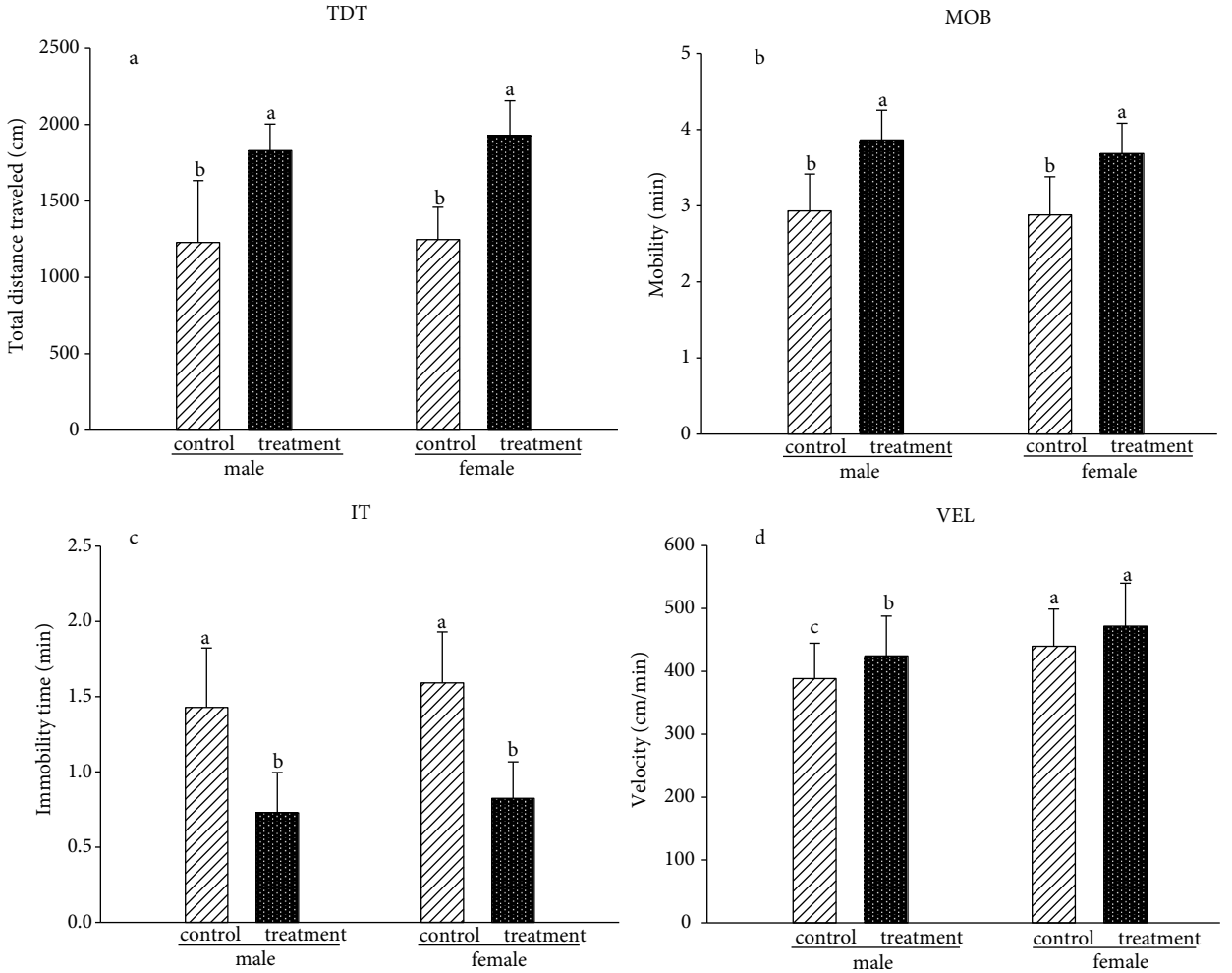

Figure 3. (a) Total distance traveled in the Porsolt test (TDT), (b) immobility (IT), (c) mobility (MOB), and (d) velocity (VEL) in the forced swim test are represented for forced swim test (Porsolt) measurements. Right striated bar represents rats exposed to the methanol extract of *L. barbarum* (n = 14); dotted striated bar represents control conditions (n = 14). a, b: mean values with the same letters above columns are not significantly different (P > 0.05).

0.05). The scores in the elevated plus maze and open field test were also not significantly correlated with those for spatial learning (r = 0.30, P > 0.05 and r = 0.09, P > 0.05). Moreover, the scores for depression were not significantly correlated with those for spatial learning (r = –0.21, P > 0.05) in the treatment condition.

4. Discussion

4.1. Anxiety-like behaviors

In the open field test (Pyter and Nelson, 2006; Benabid et al., 2008), *L. barbarum* was found to reduce anxiety in this study. Specifically, our findings showed that the subjects treated with *L. barbarum* traveled greater distances in the open field, spent more time at the edge of the open field, spent more time at the center of the open field, less frequently entered the edge of the open field, more frequently entered the center of the open field, showed more mobility, and showed more velocity than those in control conditions. In the elevated plus maze (Dawson and Tricklebank, 1995), *L. barbarum* also reduced anxiety. Specifically, the subjects receiving *L. barbarum* spent more time in the open arms, spent less time in the closed arms, were more mobile, and more frequently entered the closed arms than the controls.

Taken together, the findings of the present study show that in both the open field and the elevated plus maze *L. barbarum* reduced anxiety. This finding is consistent with previous research indicating that LBP-standardized juice reduced levels of anxiety (Amagase and Nance, 2008). These findings can be explained from at least three perspectives. First, since the methanol extract of *L. barbarum* included polysaccharide, this polysaccharide may increase the level of serotonin or melatonin, which in turn may decrease anxiety. The evidence for this comes

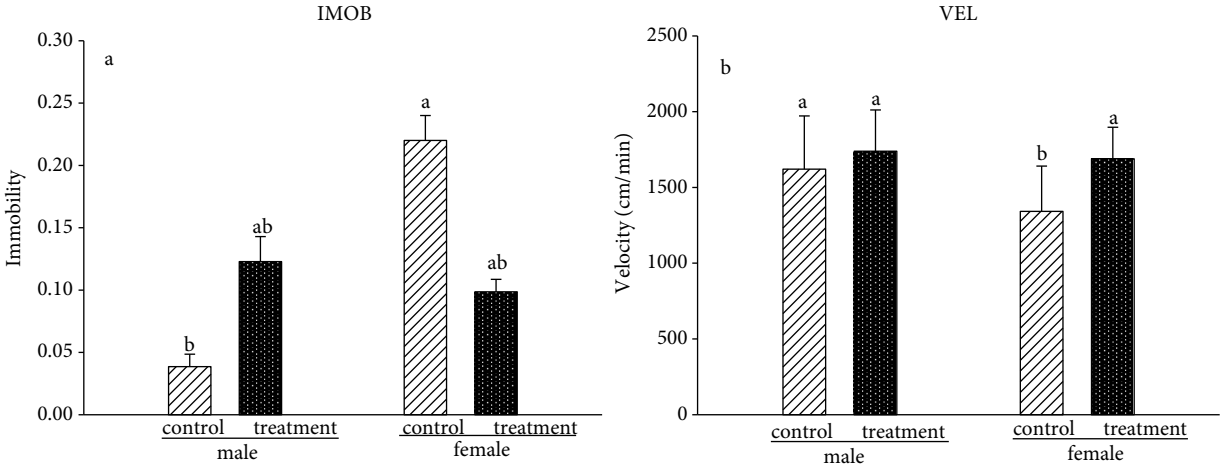

Figure 4. (a) Immobility (IMOB) and Velocity (VEL) in water maze are represented for water maze measurements. Right striated bar represents exposured the methanol extract of *L. barbarum* (n = 14); Dotted striated bar represents control condition (n = 14). a, b, c mean-values with the same letters above columns are not significantly different (P > 0.05).

from previous studies indicating that melatonin (Karakas et al., 2011) and serotonin (Parks et al., 1998; Sarnyai et al., 2000) decrease anxiety levels. Second, polysaccharide may stimulate the dendrite patterns in the hippocampus. There is strong evidence that polysaccharides enhance the network of dendrites in the hippocampus (Zhang et al., 2012) that is closely related to emotions such as anxiety and prevented hippocampal volume reduction (Gao et al., 2014). Third, β-carotene, one of the important components of *L. barbarum*, may decrease anxiety levels. There is some evidence for the beneficial effects of β-carotene in decreasing anxiety and increasing memory (Song and Xu, 2013). Future studies should compare the effects of LBPs with those of polysaccharides and β-carotene on anxiety-like behaviors and spatial memory.

In the current research a significant interaction effect between sex and *L. barbarum* treatment on anxiety indicated that females outperformed males in both the open field and elevated plus maze. For instance, we found that females benefitted from the methanol extract of *L. barbarum* more than males in terms of total entrances to closed arms and total entrances to open arms. This outcome may be due to two factors: selectivity and estradiol effects. According to the selectivity hypothesis, females tend to process all information in the environment, whereas males tend to focus on only what is most important in the environment (Meyers-Levy, 1989). Searching for all information may be difficult for females. The methanol extract of *L. barbarum* may facilitate information processing and thereby may lower the level of anxiety. Second, estradiol level of females may contribute to low levels of anxiety. Even though there is no direct support for this, studies on spatial learning showed that varied estradiol levels may differently affect the learning performance of female Wistar rats (Wide at al., 2004; Daniel, 2006). Future studies should examine the effects of estradiol levels in females with different levels of the methanol extract of *L. barbarum*.

4.2. Depression-like symptoms

We evaluated the depressive-like responses (learned helplessness) of the subjects in a forced swim test (Porsolt et al., 1977) and found that the subjects receiving *L. barbarum* were less depressive than the controls.

This outcome was in line with previous research findings (Zhang et al., 2012) that demonstrated that LBP treatment significantly decreased the immobility time of stressed rats in a forced swimming test. Zhang et al. (2012) explained this outcome from the view that LBP may stimulate dendrite patterns in the hippocampus. We also speculate that this outcome may be due to the increase in levels of melatonin or serotonin, which are the two important neurotransmitters in reducing depression levels. Another plausible factor may be the increased level of energy metabolism (Coskun et al., 2012). The methanol extract of *L. barbarum* may increase glucose levels of blood, which in turn leads to high energy metabolism. We observed that subjects exposed to the methanol extract of *L. barbarum* were more mobile controls.

4.3. Spatial memory performance

In this experiment the behaviors related to spatial memory (i.e. time to find the platform, time spent in the correct quadrant, entrance frequencies to the correct and other quadrants, mobility, and velocity) were measured by the Morris water maze (Hooge and De Deyn, 2001), in which an animal tries to find a hidden platform 2 cm below the water.

In the present study it was found that in the Morris water maze the subjects receiving *L. barbarum* showed

more velocity and were less immobilized among females but more immobilized among males than in control condition. This means that the methanol extract of *L. barbarum* is more beneficial to spatial learning than control conditions and females are better at spatial memory than males. The finding that the methanol extract of *L. barbarum* is beneficial to spatial learning is consistent with a recent finding (Chen et al., 2014) of some beneficial effects of LBPs on learning and memory in rats. In line with this finding, this research also demonstrated that the methanol extract of *L. barbarum* increases spatial learning memory, especially for females. This effect may be modulated by the hippocampus. Research demonstrated that LBPs enhanced cell proliferation in the hippocampus. It is well known that the hippocampus may play an important role in searching and navigating in the environment (Ekstrom et al., 2003). The second finding, that females are better at spatial memory than males, is also consistent with previous research findings indicating that females are better at recognizing tasks than males (Sutcliffe et al., 2007).

Correlational analyses showed a significant positive relationship between the elevated maze and open field and a negative relationship between these anxiety measures with spatial learning, as well as a negative relationship between depression and spatial learning in control conditions. This clearly provides evidence for the fact that the methanol extract of *L. barbarum* plays an important role as an effective agent in eliminating significant relationships among anxiety, depression, and spatial learning. We also found that the relationship between the elevated plus maze and depression was stronger in treatment conditions than in controls. This may suggest that velocity in the elevated plus maze can be transferred to that in the Porsolt test via the methanol extract of *L. barbarum*. This outcome may be due to closed and narrow spaces in both tests. The methanol extract of *L. barbarum* may be more time-resistant in closed and narrow areas than open and wider areas. Obviously, future studies are needed for the investigation of underlying reasons for this strong relationship.

In conclusion, the present experiment provides, for the first time, evidence for the beneficial effect of the methanol extract of *L. barbarum* on low levels of anxiety and depression-like behaviors. This study also makes a contribution to the understanding of the interaction effect between treatment and sex, indicating that females seem to benefit from the methanol extract of *L. barbarum* more than males in terms of anxiety and depression-like behaviors as well as spatial learning behavior.

Future studies should include a research paradigm in which secondary metabolites such as polysaccharides, polyphenols, and β-carotene can be separated and isolated from the crude methanol extract of *L. barbarum* to better understand the underlying mechanism of its role in affective and learning behaviors. In conclusion, the findings of this study suggest that the methanol extract of *L. barbarum* decreases the level of anxiety and depression-like behaviors and increases spatial learning behavior in Wistar albino rats and that females benefit from it more than males in terms of these behaviors.

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