ABANT IZZET BAYSAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

THE EFFECTS OF GOJI BERRY (*LYCIUM BARBARUM* L.) FRUITS ON ANXIETY, DEPRESSION, AND MEMORY

MASTER OF SCIENCE

KADİR SAĞLAM

BOLU, JUNE 2015

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APPROVAL OF THE THESIS

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June 08, 2015

Prof. Dr. Duran KARAKAŞ ……

Director, **Graduate School of Natural and Applied Sciences**

To my family and ALPER KARAKAŞ

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

KADİR SAĞLAM

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ABSTRACT

THE EFFECTS OF GOJI BERRY (*LYCIUM BARBARUM* L.) FRUITS ON ANXIETY, DEPRESSION, AND MEMORY
MSC THESIS
KADİR SAĞLAM
ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: ASSIST. PROF. DR. FATMA PEHLİVAN KARAKAŞ)
(CO-SUPERVISOR: PROF. DR. HAMİT COŞKUN)
BOLU, JUNE 2015

Some plant species have been used as herbal medicine and functional food for the thousand years. One of these plants is *Lycium barbarum* L. (Goji berry) that belongs to the family Solanaceae. It can nourish liver and kidney, brighten eyes. The aim was to investigate how the behaviors, such as anxiety, depression and learning would be affected by methanol extract of *L. barbarum* fruits. In the present study, the effects of the methanol extract obtained from *Lycium barbarum* fruits on anxiety, depression-like behaviors, and spatial memory in Wistar albino rats were tested. A total of twenty eight adult rats were selected and randomly divided into the four experimental groups [I. control-male (n=7), II. *L. barbarum* administration-male (n=7), III. control-female (n=7) and IV. *L. barbarum* administration-female (n=7)]. The rats were tested by a 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 of the present experiment demonstrated that in the open field, the methanol extract of *L. barbarum* fruits administrated rats spent more time at the center, showed more mobility and velocity than control ones. In the elevated plus maze, the methanol extract of *L. barbarum* fruits administrated rats spent more time in the open arms, spent less time in the closed arms, showed more mobility and velocity. In Porsolt test, the methanol extract of *L. barbarum* fruits administrated rats showed less immobility (helplessness). In the Morris water maze, the methanol extract of *L. barbarum* fruits administrated rats spent more of the time to find the platform. However, females were better at finding to the platform than males. In conclusion, the methanol extract of *L. barbarum* fruits decrease anxiety and depression-like behaviors. On the other hand, the methanol extract of *L. barbarum* fruits interacted with sex on spatial memory.

KEYWORDS: *Lycium barbarum* (goji berry), Anxiety, Depression, Spatial memory, Wistar Albino Rat (*Rattus rattus*)

ÖZET

KURT ÜZÜMÜ (*LYCIUM BARBARUM*) MEYVESİNİN ANKSİYETE, DEPRESYON VE HAFIZA ÜZERİNE OLAN ETKİLERİ

YÜKSEK LISANS TEZI

KADİR SAĞLAM

ABANT İZZET BAYSAL ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ

BİYOLOJİ ANABİLİM DALI

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BOLU, HAZİRAN - 2015

Bazı bitkiler binlerce yıldan beri bitkisel ilaç ve besin kaynağı olarak kullanılmaktadır. Bu bitkilerden biri olan *Lycium barbarum* (Goji berry) bitkisinin Solanaceae familyasına ait olduğu, göze parlaklık verdiği, karaciğeri ve böbrekleri beslediği bilinmektedir. Çalışmanın amacı *L. barbarum* meyvesinden elde edilen metanol özünün, anksiyete, depresyon ve öğrenme gibi davranışlar üzerine nasıl bir etkisinin olduğunun incelenmesidir. Bu çalışmada *L. barbarum* meyvesinin metanol özütünün anksiyete, depresyon ve öğrenme üzerine olan etkileri Wistar Albino sıçanlar üzerinde test edilmiştir. I. Kontrol erkek (n=7), II. *L. barbarum* uygulanan erkek (n=7), III. kontrol dişi (n=7), IV. *L. barbarum* uygulanan dişi (n=7) olmak üzere toplam 24 yetişkin sıçan rastgele seçilerek dört gruba ayrılmıştır. Deney hayvanlarına; anksiyete için açık alan ve yükseltilmiş artı labirent testi; depresyon için zorunlu yüzme testi ve öğrenme için ise Morris su havuzu testi uygulanmıştır. Yapılan çalışmanın bulguları; açık alanda *L. barbarum* meyvesinin metanol özütü uygulanan deneklerin kontrol koşuluna göre merkezde daha fazla zaman geçirdiklerini, daha hareketli ve daha hızlı olduklarını göstermiştir. Yükseltilmiş artı labirentte *L. barbarum* meyvesinin metanol özütü uygulanan deneklerin kontrol koşuluna göre açık kolda daha fazla, daha hızlı ve daha hareketli oldukları kapalı kolda daha az zaman geçirdikleri gözlemlenmiştir. Porsolt testinde *L. barbarum* meyvesinin metanol özütü uygulanan deneklerin kontrol koşuluna göre daha az çaresizlik göstermektedir. Morris su testinde ise *L. barbarum* meyvesinin metanol özütü uygulanan denekler kontrol koşuluna göre platformu daha uzun sürede bulmuşlardır. Bununla birlikte dişiler erkeklere göre platformu daha kolay bulmaktadır. Çalışmanın sonuçlarına göre *L. barbarum* meyvesinin metanol özütü anksiyete ve depresyon belirtilerini düşürmektedir. Öte yandan *L. barbarum* meyvesinin metanol özütü uzaysal öğrenme üzerinde cinsiyet ile ortak etki göstermektedir.

ANAHTAR KELİMELER: *Lycium barbarum* (Goji berry), Anksiyete, Depresyon, Uzaysal hafıza, Wistar Albino Sıçan (*Rattus rattus*)

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LIST OF ABBREVIATIONS ANDSYMBOLS

EFCA : Total Entry to Closed Arms

EFCO : Total Entry to the Center of the Open Field

EFCQ : Entrance Frequently to the Correct Quadrant

EFEO : Total Entry to the Edge of the Open Field

EFOA : Total Entry to Open Arm

IT : Immobility Time

LBP : *Lycium Barbarum* Polysaccharide

MT : Mobility Time

TDT : Total Distance Travelled

TSCA : Time Spent in Closed Arms

TSCO : Time Spent at the Center of the Open Field

TSCQ : Time Spent in the Correct Quadrant

TSEO : Time Spent at the Edge of the Open Field

TSFP : Time Spent to Find the Platform

TSOA : Time Spent in Open Arm

VEL : Velocity

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1. INTRODUCTION

1.1 Goji Berry

Human beigns have used many plants for feeding, drinking, treatment, cleaning and other situations for thousand years. One of the these plants is *Lycium barbarum* L.(also known as, goji berry, wolf berry) that is a perennial, deciduous shrub growing in northwest China and the Mediterranean region. It grows fastly and has a deep, well-developed root system which is tolerant to drought and cold and has extensive adaptability (Chang and So, 2008; Chen et al., 2004). Colour of *L. barbarum* fruit is orange-red. Taste of *L. barbarum* fruits are sweet and *L. barbarum* fruits are harvested in late summer–early autumn and sun-dried. *L. barbarum* is a member of the Solanaceae family which includes peppers, potatoes, tomatoes and eggplants. *L. barbarum* can nourish brighten eyes, liver and kidney, reduce serum lipids and blood glucose, reduce senescing, immuno-modulate, and male fertility-facilitate (Gan and Zhang, 2003). It has been used as a customary Chinese functional food and herbal medicine for approximately 2500 years (Shi et al., 1997). *L. barbarum* plays important role in China economy, with the fruits (Gouqiziin Chinese) having high contents of *L. barbarum* polysaccharides (LBP), carotenoids and flavonoids (Wang et al., 2010; Yao et al., 2011). For other substances, *L barbarum* includes alkaloids, vitamins, trace elements amino acids, and fat. Previous studies have showed useful effects of *L. barbarum* related to aging, general wellbeing, neuroprotection, metabolism/energy expenditure, immunomodulation, glaucoma, cytoprotection, fatigue/endurance, anti-tumor effects, diabetic control and anti-oxidant activity, (Amagase and Farnsworth, 2011; Amagase and Nance, 2008; Bensky and Gamble, 1993; Bryan et al., 2008; Chang and But, 2001; Chen et al., 2012, Potterat, 2010; Zhu, 1998). *L. barbarum* extract prevented glutamate toxicity, proposing that it might decelerate dementia progression (Ho et al., 2009). *L. barbarum* extract also prevented homocysteine toxicity, a risk factor, for Alzheimer's disease (Ho et al., 2010). Flavonoids which are the active ingredients of *L. barbarum* can make easy cough, reduce cholesterol in blood, expand coronary artery, and scavenge free radical

effect (Dong et al., 2009; Qian, 2004; Wang et al., 2010). Different *Lycium* species fruits have complicated herb formulae in traditional medicines, in a 6–18 g/100 g ratio as dried material. On the upshot of decoction, scientific references define 5–15 g/100 mL of goji, equal to 25–120 g of fresh fruits (Amagase and Farnsworth, 2011; Amagase et al., 2009; Anonymous, 2010). Researchers set a advised amount/volume of 30 mL of decoction four times in every day (total 120 mL/day), which is equal to approximately 150 g of fresh fruits (Ionica et al., 2012; Potterat, 2010). Recent studies also have demonstrated that many of the world's longest living people eat *L. barbarum* fruit on a daily basis, suggesting it may be one of the world's most strong anti-aging foods (Yu et al., 2005).

Figure 1.1. Dried fruits of *L. barbarum*

1.1.1 Botany

Approximately 70 species of *Lycium* are growed in separate and distincted areas spread in temperate to subtropical parts of South America, North America, Australia, southern Africa, and Eurasia (Bryan et al., 2008, Fukuda et al., 2001). *L. barbarum* grows up to 3 m high, that has green-gray leaves are lanceolate, alternate

and progressively narrow to the petiole. *L barbarum* has 1 to 3 axillary, radical flowers. Colour of the goji berry flower is violet or purple with a 5-lobed margin (PDR, 2007). *L. barbarum* fruit is 3–8 mm in diameter, 6–20 mm in length, orange to dark red (Japanese Pharmacopoeia, 2006). *L. barbarum* naturally plants in Asia, largely in northwest China (Bensky and Gamble, 1993; PDR, 2007; Wu, 2005; Zhu, 1998). *L. barbarum* fruits are harvested in the late summer and early autumn (PDR, 2007; Zhu, 1998).

1.1.2 Chemical Contents and Biological Activities of *L. barbarum*

L. barbaum is used as a traditional functional food and herbal medicine. For supporting these traditional particularities, previous researches have specified that one important extract from *L. barbarum* fruit, namely polysaccharides (LBP) including effects on anti-aging, increased metabolism, neuroprotection effects, diabetics control, anti-fatigue, anti-oxidant activities, anti-cancer effects and cytoprotection properties (Bensky and Gamble, 1993; Bryan et al., 2008; Chang and But, 2001; Potterat, 2010; Zhu, 1998).

Nowadays, fruits of *L. barbarum* are known as "superfruits" with very rich nutrient contents (Endes et al., 2015; Song and Xu, 2013). It plays as an important role in enhancing of vision, age-related diseases (atherosclerosis, neurodegeneration and diabetes) and prevention of aging, boosting immune system and prevention of cancer development (Chang and So, 2008; Gan et al., 2003; Ha et al., 2005; Song and Xu, 2013). These beneficial effects have been attributed to the presence of high amount of polysaccharides (LPB), flavonoids (myricetin, quercetin, kaempferol and rutin), phenolic acids (chlorogenic acid, p-coumaric acid and caffeic acid) (Potterat, 2010) and carotenoids (e.i. β-carotene, zeaxanthin) (Endes et al., 2015). The fruits of *L. barbarum* also included essential oil (i.e. linoleic acid, hexadecanoic acid, myristic acid, β-elemene and ethylhexadecanoate) (Potterat, 2010), vitamins (A, B and C), amino acids, elements (K, P, Ca, Mg, Fe, and Na) and betaine (Endes et al., 2015).

The antioxidant activity of *L. barbarum* was found to be related to phenolic compounds and β-carotene (Qian et al., 2004; Song and Xu, 2013). The β-carotene also accounts for the large portion of carotenoid in *L. barbarum* (Liu et al., 2008;

Song and Xu, 2013). Other antioxidants present naturally in *L. barbarum* are flavonoids, polyphenols, lignans, including tannins, and some other simple phenolic compounds (Song and Xu, 2013). The LBPs (23 % of extract) show quantitatively the most significant part of components in the fruit of *L. barbarum*. The betaine that is an alkaloid from *L. barbarum* fruits provides to decrease anxiety, increases spatial memory, supports muscle growth and guard against fatty liver diseases (Song et al., 2008; Song and Xu, 2013). Many significant biochemical processes lean upon methylation, including the metabolism of DNA, lipids, and neurotransmitters (Stampfer and Malinow, 1995). It is well known that methylation capability of human body decreases with age, therefore the decline of methylation causes to the aging process. The betaine alkaloid addition, hence, provides prevention utility against the aging process (Zhu et al., 1998).

1.2 Anxiety

Anxiety, an unpleasant emotional/psychological and physiological state, that produce life threating results in response to stress factors. Anxiety disorders state a public health problem with a time of life prevalence of 16.6 % (Somers et al., 2006) and in the pediatric populations, the prevalence of anxiety disorders generally ranged from 5 to 18% (Ramsawh et al., 2010).

1.3 Depression

Depression is the most common psychological problem in today world. It is a state of low mood and unwillingness to activity that can negatively affect a person's thoughts, feelings and/or sense of general well-being, and some conducts (i.e., social behaviors, learning behaviors, and etc.). The widespread presence of depression in population range from 17 to 20 % with major outcomes for society (Kessler et al., 1994) and possible event of depression episode at any age from childhood to elderly. Depressive disorders are defined by behavioral, psychological and physiological changes, anhedonia and guilt, bearing feelings of hopelessness, suicidal opinion, distortion of appetite and sleep as well as cognitive function (Cryan et al., 2002).

1.4 Spatial Memory

Spatial memory is an essential portion of our ability to identify our environment and its spatial orientation. Human spatial memory system is just like an animal memory system in a way that a person's spatial memory is required to navigate around a knowing city, all of a piece a rat's spatial memory is needed to learn the location of food at the end of a maze. Navigation behaviors include different cognitive strategies as well as biologically interacting brain structures (McDonald and White, 1994; Packard and McGaugh, 1996). The spatial learning performance of rodents is also affected by cue salience, emotional status, hormonal profile, biological sex, and training level (Chang and Gold, 2004; Hawley et al., 2011; Hawley et al., 2012; Hawley et al., 2013; Kanit et al., 2000, Korol et al., 2004; Wingard and Packard, 2008).

2. OBJECTIVES

The purpose of the present study was to investigate how anxiety, depression and learning are affected by the methanol extract of *L. barbarum* fruits. Some studies have suggested the effects of *L. barbarum* fruits on anti-aging, neuroprotection, metabolism/energy expenditure, well-being, fatigue, diabetic control, anti-oxidant activity, immune system modulation, anti-cancer properties, glaucoma and cytoprotection. But effects the methanol extract of *L. barbarum* fruits on anxiety, depression and memory has not been investigated yet to best of our knowledge in the literature. If *L. barbarum* fruits are beneficial to such various health parameters, it is reasonable that it should also have anti-anxiety and anti-depressant outcomes. From this standpoint, one can speculate that the subjects being received *L. barbarum* fruits should have less anxiety and depression than those with control one. However, no controlled research has been conducted on humans because of some ethical concerns and application difficulties. Therefore a controlled research is needed for illuminating the effects of *L. barbarum* fruits on anxiety, depression, and learning performance as well as illuminating underlying mechanisms of it. This study was carried out with male and female albino rats. We also investigated whether or not gender modulates the effect of the methanol extract of *L. barbarum* fruits on anxiety, depression, and spatial learning performance. To test these issues mentioned above, the animals were tested by elevated plus maze and open field tests for anxiety-like behavior, forced swim test for depression and Morris water maze test for spatial memory.

3. MATERIALS AND METHODS

3.1 Plant Material and Extract Preparation

The dried fruits of *L. barbarum* were bought from herbal store in Bolu / TURKEY. The dried fruits of *L. barbarum* were grinded with grinding machine. The powdered 30 g fruits were extracted with 300 ml methanol (MeOH) in water bath at 40 ºC for 16-24 hours (see Figure 3.1.) and then filtered (see Figure 3.2.). The methanol is commonly used solvent because it is effective polar modifier (Hamburger et al., 2004). Then extraction solvents were evaporated under low pressure at a temperature not higher than 40 °C using rotary evaporator machine (see Figure 3.3.). The MeOH in extraction solvent was evaporeted and concentrated successfuly and sticky extract of *L. barbarum* were obtained in plastic tube (see Figure 3.4.). Following process then 1 gr extract of *L. barbarum* were mixed with 1000 ml water completely so methanol (MeOH) extract of *L. barbarum* 1 mg/ml (1000 ppm) solution was prepared in daily during to experiment (see Figure 3.5.).

Figure 3.1. Water bath

Figure 3.2. Filtration apparatus

Figure 3.3. Rotary evaporatory

Figure 3.4. The methanol extract of *L. barbarum*

Figure 3.5. The methanol extract of *L. barbarum* 1 mg/ml (1000 ppm) solution

3.2 Animal Care

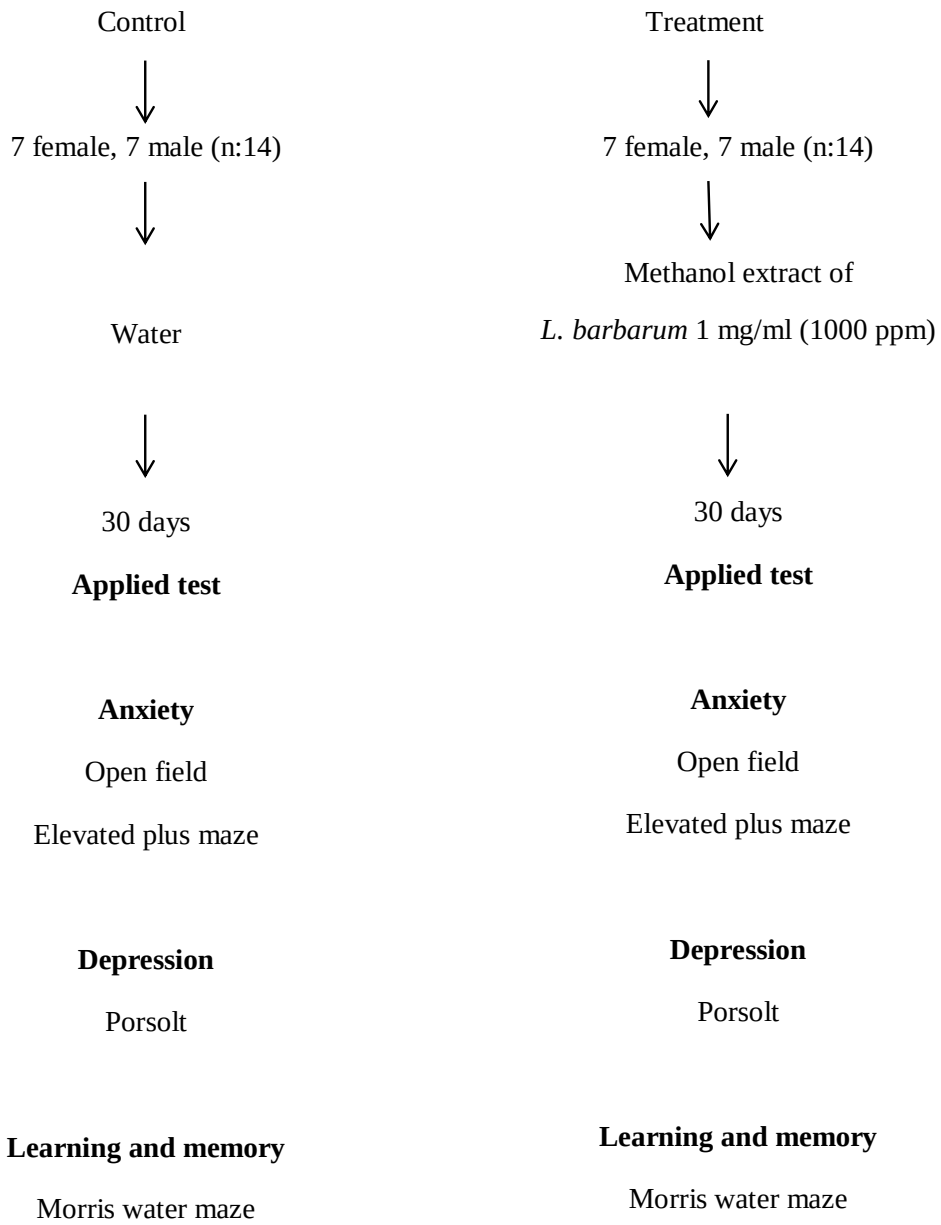
In this study, adult male and female Wistar albino rats (200 – 250 g) were obtained from the Abant Izzet Baysal University Animal Research Center (see figure 3.6). The Wistar albino rats were preserved in plastic cages (16x31x42 cm) with pine shavings used as bedding. Water and food pellets were reachable ad libitum. All cages exposed to 200 lux light. All lighting that controlled by automatic programmable timers was provided by the cool-white fluorescent tubes. Ambient temperatures in the animal facilities were held constant at 22 ± 2 °C in air-ventilated rooms. The procedures in this study were carried out in conformity with the Animal Scientific procedure and approved by the Institutional Animal Care and Use Committee.

Figure 3.6. Wistar albino rat

3.3 Experimental Protocol

In the present study, a total of twenty eight adult rats were selected and randomly divided into the four experimental groups [I. control-male (n=7), II. *L. barbarum* administration-male (n=7), III. control-female (n=7) and IV. *L. barbarum* administration-female (n=7)]. The methanol extract of *L. barbarum* 1 mg/ml (1000 ppm) solution were diluted with a fresh drinking water daily and were applied every day at ad libitum between 14 - 16 h starting from the 1st day to the end (30 days) of the experiment. In control group, animals were applied with the same quantity of the fresh drinking water to obtain the same stress conditions with the experiments groups (see experimental protocol). Anxiety-like behavior, depression-like behavior, and spatial memory of Wistar albino rats were measured by a means of open field, elevated plus maze, force swimming and Morris water maze test, respectively.

Experimental Protocol



3.4 Open Field

The open field has been the most widely used test in animal psychology. The Open-field test is occured in 80 cm × 80 cm arena with 40 cm high walls. For this test, rats were put into a plain and illuminated set and their attitude is mostly appertained as a basic index of whole behavior. The anxiety-like behavior of animals was recorded on video for 5 minutes in this test. In this study, a video camera (Gkb CC-28905S, Commat LTD.ŞTİ. Ankara/Turkey) was affixed upper the set, recording behavior into the Ethovision videotracking system (Noldus Ethovision, Version 6, Netherland; Commat LTD.ŞTİ. Ankara/Turkey) that ensured a variation of behavioral measures with the inclusion of total distance travelled, time spent in the edge, entrance frequency to the edge, time spent in the center, entrance frequency to the center, velocity and mobility among the different areas of the platform (see Figure 3.7.).

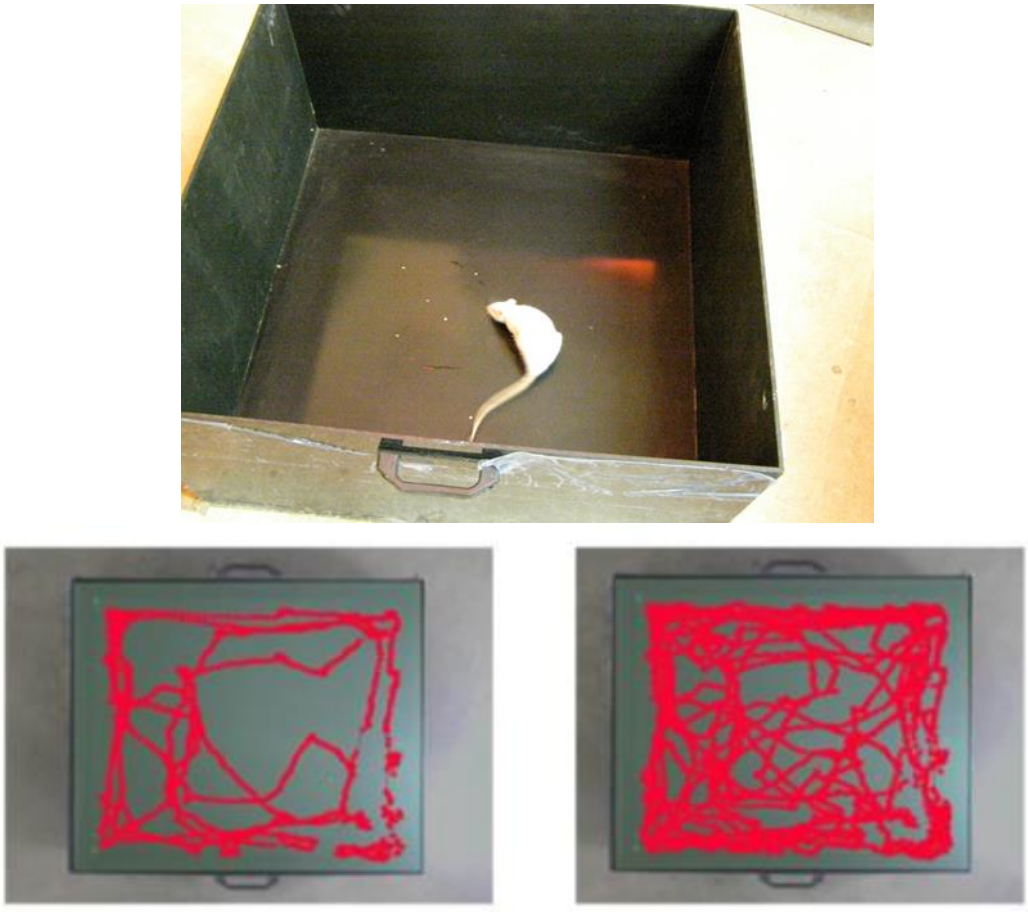


Figure 3.7. Open field apparatus and Ethovision video tracking system

3.5 Elevated Plus Maze

The elevated plus maze has been used test in animal psychology The elevated plus maze consisted of the two closed, covered by 41 cm tall black Plexiglas, and two open 10 cm wide arms in a plus-sign configuration 55 cm off the floor. All arms were enclosed with contact paper to forestall the animals from sliding off. Each animal was put into one of the closed arms and allowed to move freely on the elevated plus maze. Testing period was 5-min that was recorded from above the elevated plus maze. In this study, a video camera (Gkb CC-28905S, Commat LTD.ŞTİ. Ankara/Turkey) was affixed upper the set, recording behavior into the Ethovision videotracking system (Noldus Ethovision, Version 6, Netherland; Commat LTD.ŞTİ. Ankara/Turkey) for total distance travelled, time spent in the open arm, time spent in the closed arms, entrance frequency in the closed arms, entrance frequency in the open arm, mobility duration, and velocity. The rats were conceived to have entered an arm when all four paws crossed onto the arm (see Figure 3.8.).

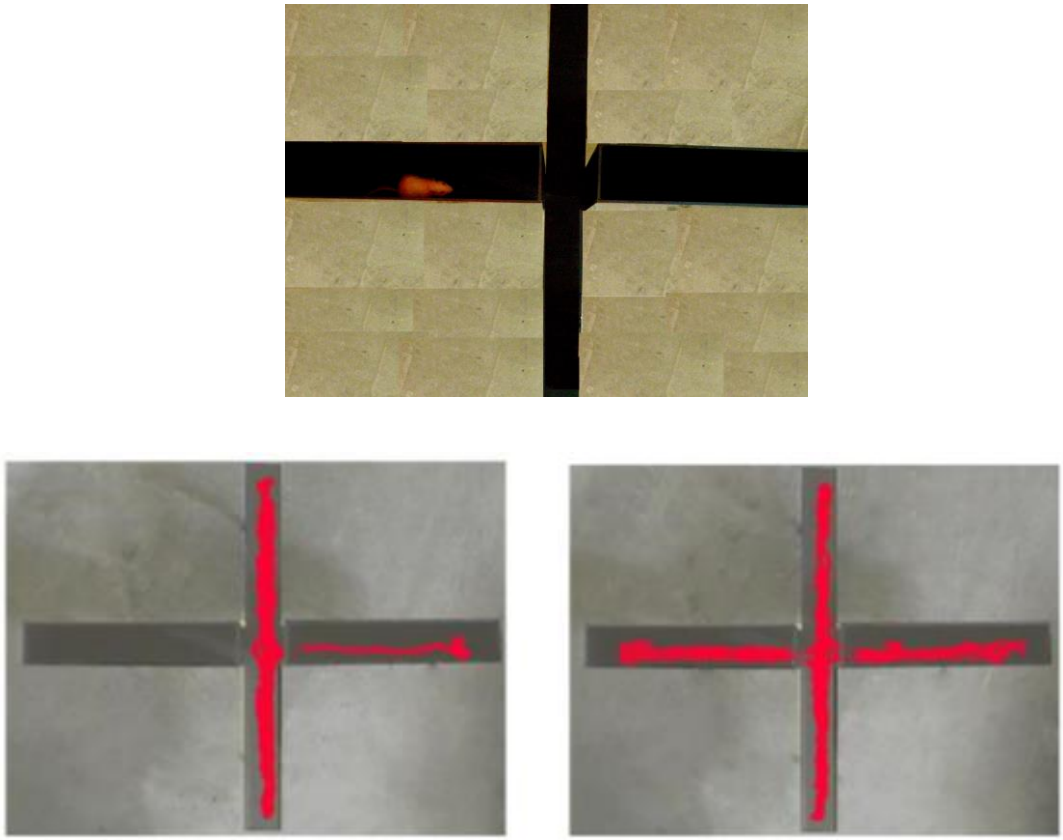


Figure 3.8. Elevated plus maze and Ethovision video tracking system

3.6 Forced Swim Test (Porsolt)

The Forced Swim Test (Porsolt) has been used test in animal psychology to appraise depressive- like symptons. The animals were put into porsolt which consist of an opaque cylinder tank (24 cm diameter, 53 cm height) (see Figure 3.9.) which was filled with 17 cm deep water kept at 28 °C. Swimming behavior was recorded on video for 5 minutes. In this experiment a video camera (Gkb CC-28905S, Commat LTD.ŞTİ. Ankara/Turkey) was affixed above the arena, recording behavior into the Ethovision video tracking system (Noldus Ethovision, Version 6, Netherland; Commat LTD.ŞTİ. Ankara/Turkey) that provided avariety of behavioral measures including total distance travelled, mobility time, immobility time, mobility frequency, rotation, and velocity.

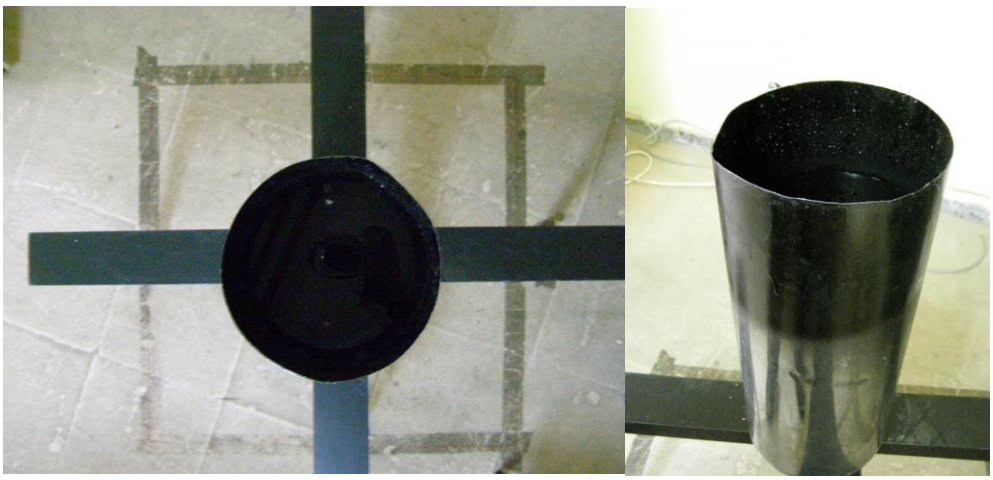


Figure 3.9. Porsolt Apparatus

3.7 Morris Water Maze

The Morris water maze has been used for testing the spatial memory. It is a circular, galvanized steel maze (60 cm in depth and 1,5 m in diameter) (see Figure 3.10.). Water was filled into the Morris water maze until 40 cm deep and kept at 28 °C. The Morris water maze tank was rendered opaque by the addition of a non-toxic, water soluble dye. The Morris water maze tank was established in a wide quiet test room, enclosed by some visual cues external to the maze (e.g. the experimenter, lights, pictures, rack, ceiling etc.). The cues can be seen from within the maze and

could be used by the rats for spatial orientation. The cues were maintained same location throughout the period of testing. A video camera mounted to the ceiling over the center of the Morris water maze was used for monitoring and recording movements of the rats.

There were the four equally divided quadrants in the pool. In one of the quadrants, a platform (10 cm in diameter, 2.0 cm below water surface) was dipped and mounted. The animals executed the four trials per day for the four consecutive days (16 trials). In the swimming trials, each individual rat was affranchised kindly into the water at a chosen quadrant except for the one that contained the hidden platform for facing an extra maze cue. The rat swam and learned how to find the hidden platform within 60 s. The escape latency which is the time elapsed before the rat reached the platform in each trial, is a measure of acquisition of spatial navigation. After reaching, the rat was allowed to stay on the platform for 15 s and was then taken back into the cage. During the inter-trial intervals, animals were kept in a dry home cage for 60 s.

The video camera recordings were got on the fifth day of the experiment. The rat had to swim until it climbed onto the platform submerged underneath the water. The location of platform was fixed in the same position relative to the distal cues. The time to reach the platform (latency in seconds) and time passed in the correct quadrant, total distance travelled, total entry to the correct quadrant, velocity, and immobility duration were measured as the parameters of the spatial memory.

In this study a video camera (Gkb CC-28905S, Commat LTD.ŞTİ. Ankara/Turkey) was mounted above the arena, recording behavior into the Ethovision videotracking system (Noldus Ethovision, Version 6, Netherland; Commat LTD.ŞTİ. Ankara/Turkey) that provided assortment of behavioral measures including total distance travelled, time spent in the center, time spent in the edge, total entrance frequency in the center, total entrance frequency in the edge, and immobility among the different areas of the arena. All animals were then returned to the reproduction and exhibition colonies.

Figure 3.10. Morris water maze and Ethovision video tracking system

3.8 Statistical Analyses

In the study, data were analyzed using SPSS (SPSS Statistical Software, SPSS Inc., Los Angeles, CA, USA, Ver. 22.0). Data were analyzed by 2 treatment (treatment and control) X 2 sex (female and male) ANOVA analysis with the between subject design. Values were considered statistically significant at $p \leq 0.05$. Data are presented as MEAN ± SEM after back transforming from ANOVA results.

4. RESULTS AND DISCUSSIONS

4.1 RESULTS

4.1.1 Open Field Measurements

4.1.1.1 Total Distance Travelled on the Open Field (TDT)

The main effect of sex was significant on the total distance travelled in the open field, $F(1, 50) = 23.14$, $p = 0.0001$, $\eta^2 = 0.34$. Females ($M = 2337.15$) travelled more distance than males ($M = 1858.82$). This means that females were less anxious than males.

The main effect of treatment was significant on the total distance travelled in the open field, $F(1, 50) = 6.96$, $p = 0.01$, $\eta^2 = 0.12$. The subject in the treatment ($M = 2229.13$) condition more travelled than control ($M = 1966.84$) condition. This means that the subject in treatment condition was less anxious than subject in control condition (see Figure 4.1.).

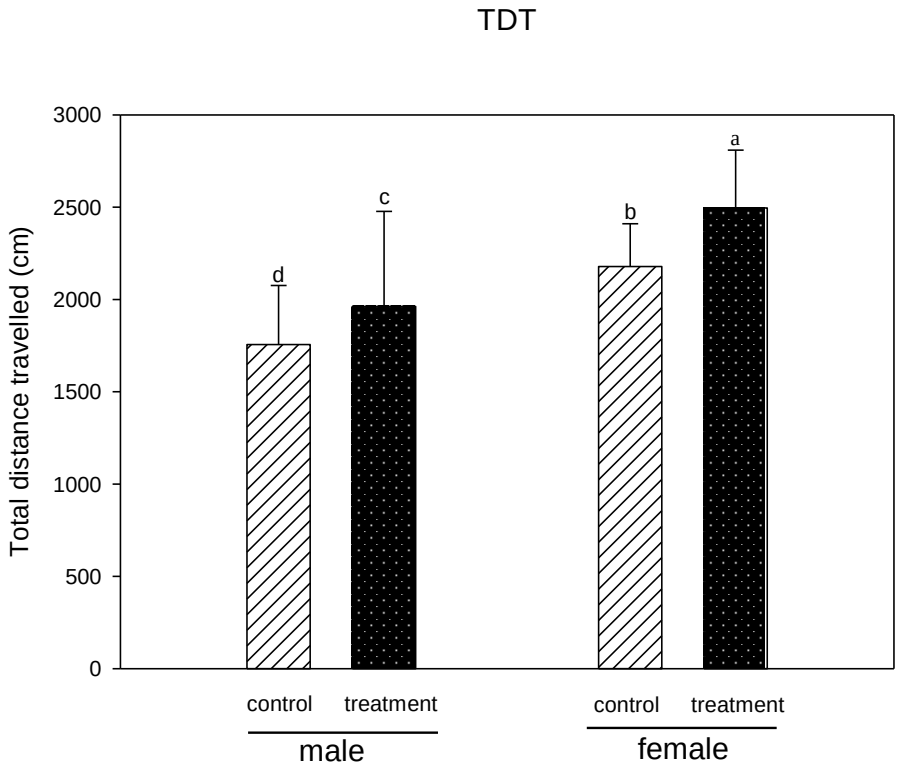


Figure 4.1. Mean (S.E.M.) total amount distance travelled (TDT). Dotted striated bar represents applied the methanol extract of *L. barbarum* (n:14); Right striated bar represents control condition (n:14). Mean-values with the same letters within vertical columns are not significantly different ($p > 0.05$).

4.1.1.2 Time Spent at the Edge of the Open Field (TSEO)

The main effect of treatment was significant on the time spent at the edge of the open field, $F(1, 50) = 4.35$, $p = 0.04$, $\eta^2 = 0.08$.

Subject in the treatment ($M = 4.56$) condition spent less time at the edge of open field than control ($M = 4.68$) condition. This means that the subject in treatment condition was less anxious than subject in control condition (see Figure 4.2.).

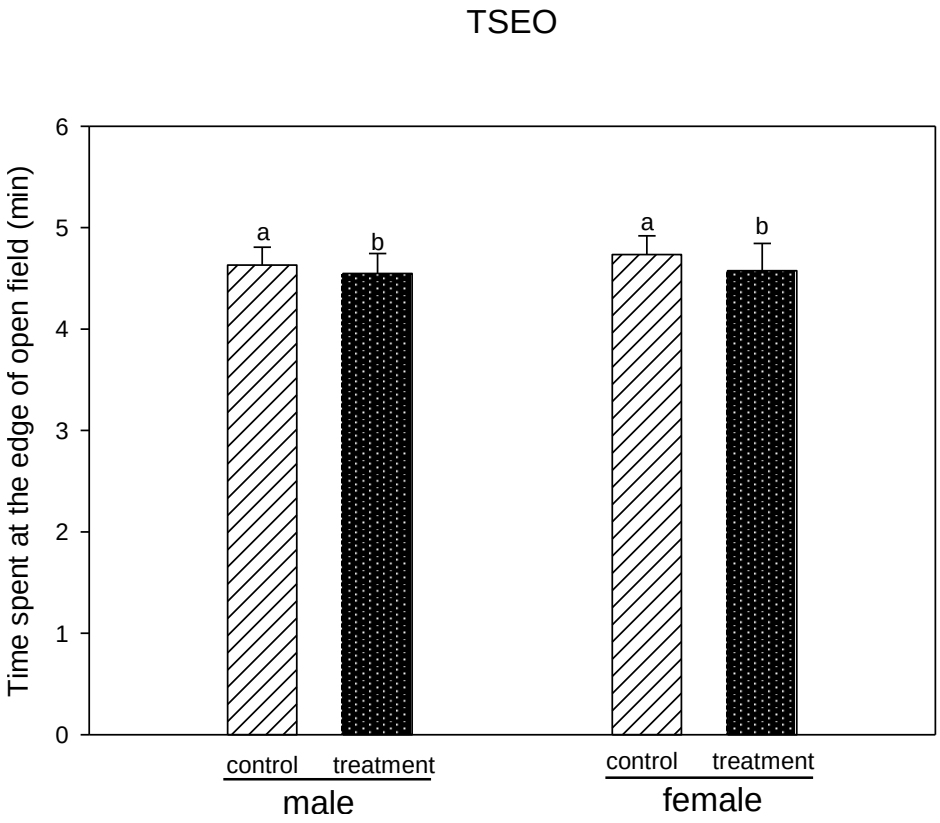


Figure 4.2. Mean (S.E.M.) time spent at the edge of the open field (TSEO). Mean-values with the same letters within vertical columns are not significantly different ($p>0.05$).

4.1.1.3 Total Entry to the Edge of the Open Field (EFEO)

The main effect of treatment was significant on the entrance frequency to the edge of open field, $F(1, 52) = 3.73$, $p = 0.059$, $\eta^2 = 0.07$.

Subject in the treatment ($M = 8.50$) condition more frequently to enter the edge of open field than control ($M = 6.27$) condition. This means that the subject in treatment condition was more anxious than subject in control condition.

An interaction effect between sex and treatment was significant, $F(1, 52) = 3.73$, $p = 0.059$, $\eta^2 = 0.007$. As can be seen in graph; females were more anxious in treatment condition ($M = 9.57$) than control condition ($M = 5.14$) but males had the same level of anxiety in both treatment ($M = 7.43$) and control ($M = 7.43$) condition (see Figure 4.3.).

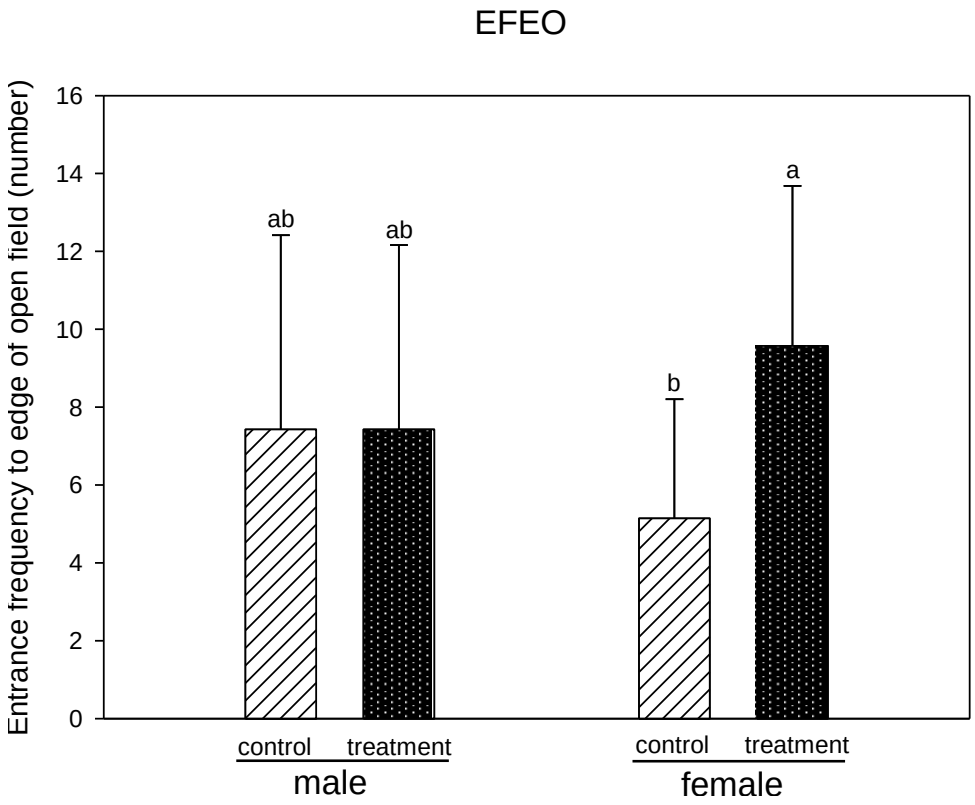


Figure 4.3. Mean (S.E.M.) the frequency of entries to the edge in the open field (EFCO). Mean-values with the same letters within vertical columns are not significantly different ($p>0.05$).

4.1.1.4 Time Spent at the Center of the Open Field (TSCO)

The main effect of treatment was significant on the time spent at the center of the open field, $F(1, 50) = 4.35$, $p = 0.04$, $\eta^2 = 0.08$.

Subject in the treatment ($M = 0.44$) condition spent more time at the center of open field than control ($M = 0.32$) condition. This means that the subject in treatment condition was less anxious than subject in control condition (see Figure 4.4.).

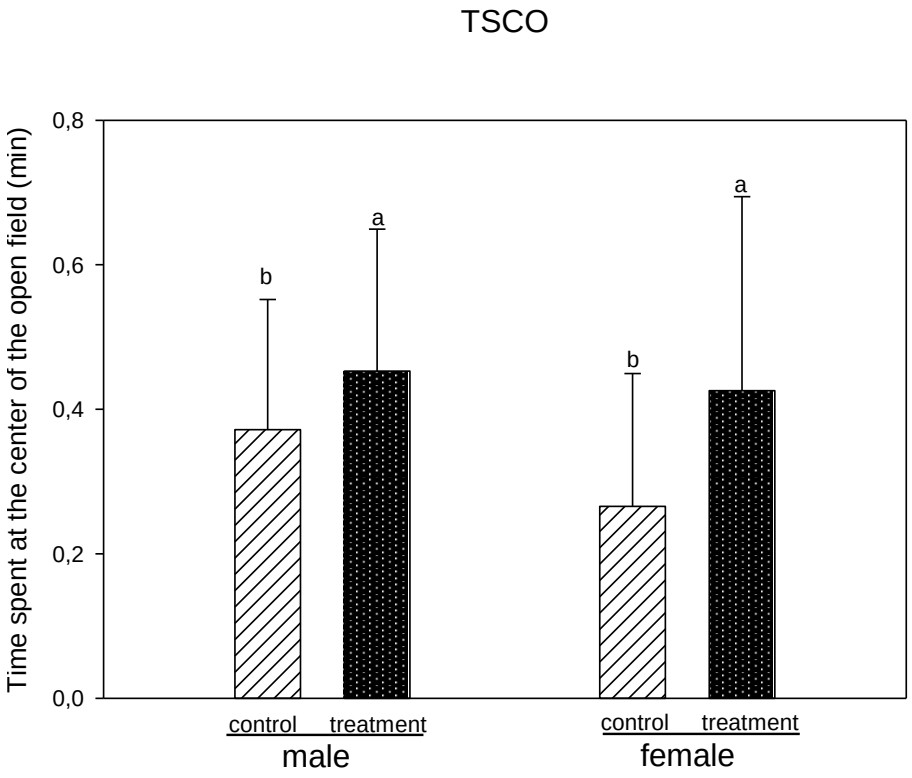


Figure 4.4. Mean (S.E.M.) time spent at the center of the open field (TSCO). Mean-values with the same letters within vertical columns are not significantly different ($p>0.05$).

4.1.1.5 Total Entry to the Center of the Open Field (EFCO)

The main effect of treatment was significant on the entrance frequency to the center of open field, $F(1, 52) = 3.98$, $p = 0.051$, $\eta^2 = 0.071$.

Subject in the treatment ($M = 8.14$) condition more frequently to enter the center of open field than control ($M = 5.93$) condition. This means that the subject in treatment condition was less anxious than subject in control condition (see Figure 4.5.).

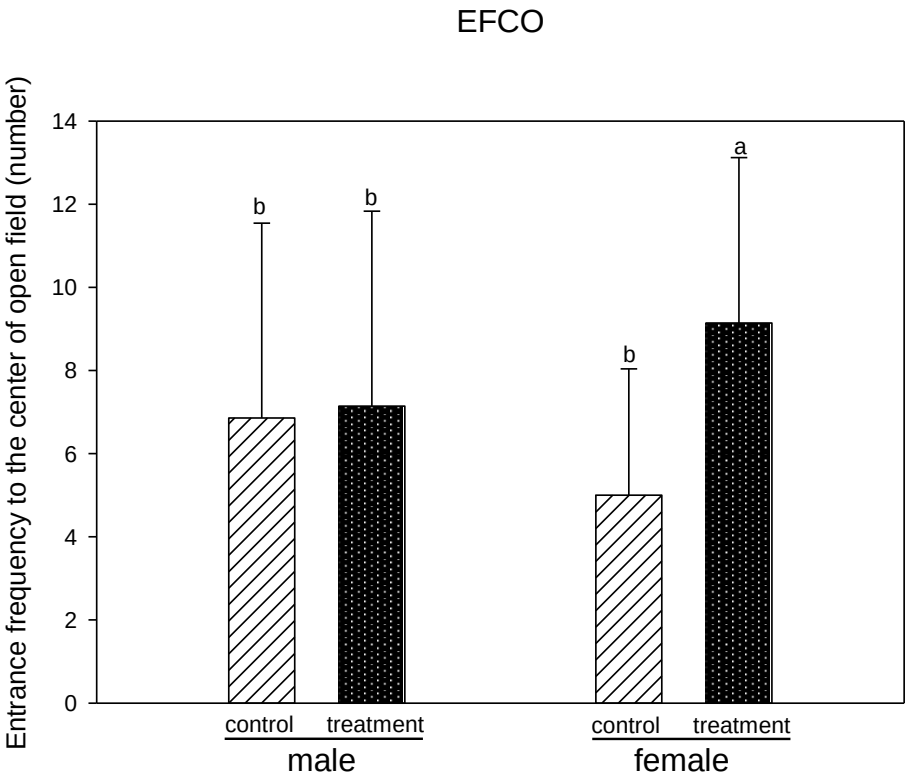


Figure 4.5. Mean (S.E.M.) the frequency of entries to the center in the open field (EFCO). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.1.6 Mobility in Open Field (MT)

The main effect of sex was significant on the mobility in the open field, F (1, 50) = 8.67, p = 0.05, $\eta^2 = 0.15$.

Males (M = 4.999) were more mobile than females (M = 4.996). This means that males were less anxious than females.

The main effect of treatment was significant on the mobility in the open field, F (1, 50) = 5.94, p = 0.02, $\eta^2 = 0.11$.

Subject in the treatment (M = 4.998) condition was more mobile than control (M = 4.996) condition. This means that the subject in treatment condition was less anxious than subject in control condition (see Table 4.1.).

Table 4.1. The mobility scores of treatment and control groups in open field.

Sex	Control	Treatment
Male	4.998 ± 0.004^{ab}	5.000 ± 0.00^{a}
Female	4.997 ± 0.005^{b}	4.998 ± 0.004^{b}

Mean-values with the same letters are not significantly different ($p>0.05$).

4.1.1.7 Velocity in Open Field (VEL)

The main effect of sex was significant on the 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 ($M = 468.08$). This means that males were more anxious than females.

The main effect of treatment was significant on the velocity in the open field, $F (1, 50) = 6.85$, $p = 0.01$, $\eta^2 = 0.12$.

Subject in the treatment ($M = 446.05$) showed more velocity than control ($M = 394.01$) condition. This means that the subject in treatment condition was less anxious than subject in control condition (see Figure 4.6.)

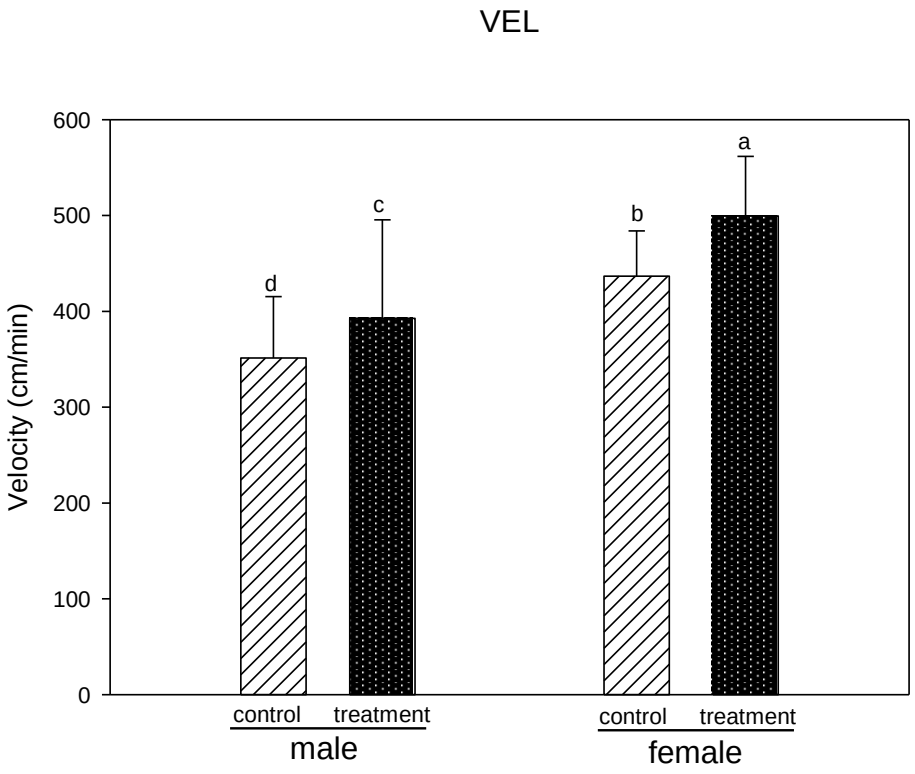


Figure 4.6. Mean (S.E.M.) the velocity of the subjects in the open field (VEL). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.2 Elevated Plus Maze Measurements

4.1.2.1 Total Distance Travelled on the Elevated Plus Maze (TDT)

The main effect of sex was significant on the total distance travelled in the elevated plus maze, F (1, 50) = 45.09, p = 0.0001, $\eta^2 = 0.47$.

Females (M = 1162.28) travelled more distance than males (M = 842.65). This means that females were less anxious than males (see Figure 4.7.)

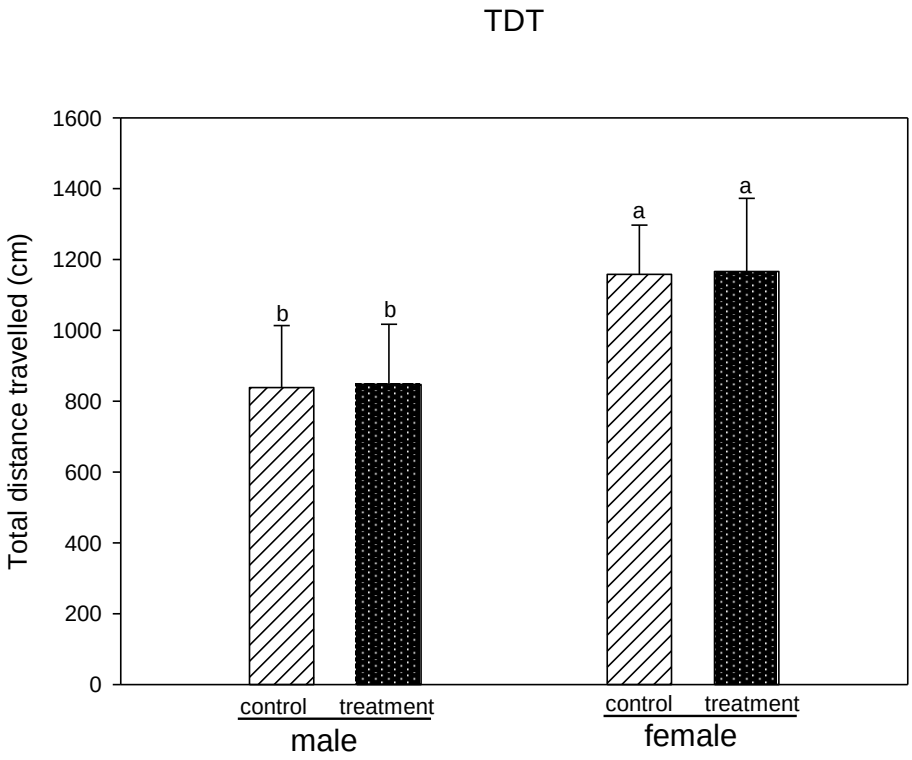


Figure 4.7. Mean (S.E.M.) Total distance travelled on the elevated arm maze (TDT). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.2.2 Time Spent in Closed Arms (TSCA)

The main effect of treatment was significant on the time spent in closed arms, $F(1, 50) = 10.55$, $p = 0.002$, $\eta^2 = 0.17$.

Subject in the treatment (M = 3.68) condition spent less time in closed arms than control (M = 4.33) condition. This means that the subject in treatment condition was less anxious than subject in control condition (see Figure 4.8.).

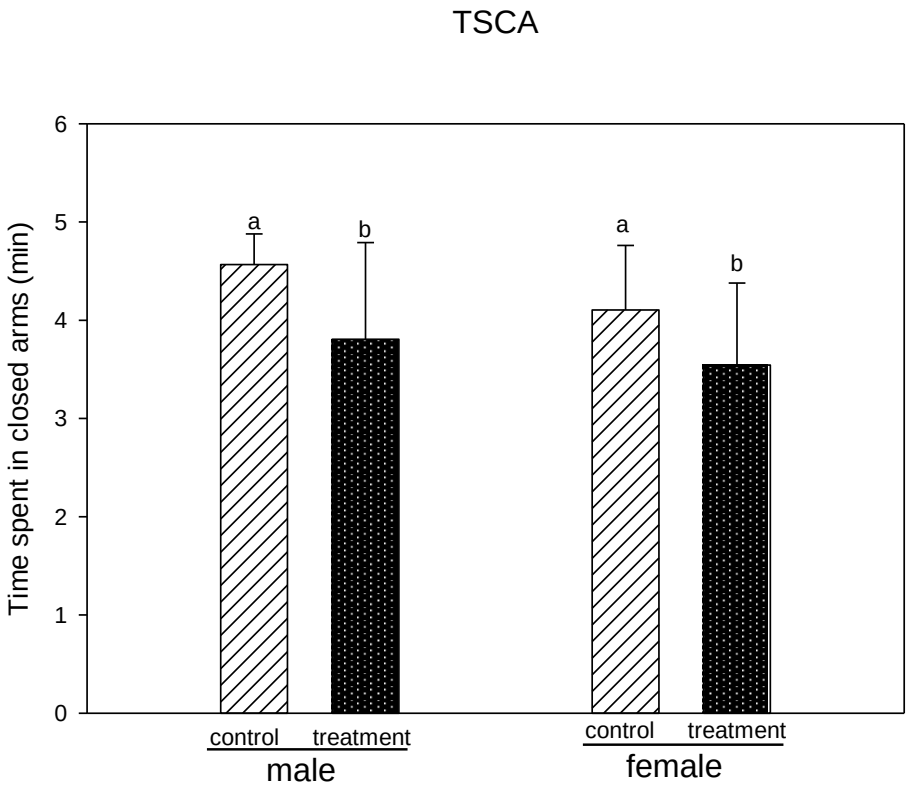


Figure 4.8. Mean (S.E.M.) Time spent in the closed arms on the elevated plus maze (TSCA). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.2.3 Total Entry to Closed Arms (EFCA)

The main effect of sex was significant on the entrance frequency to the closed arms, F (1, 50) = 17.83, p = 0.0001, η^2 = 0.26.

Females (M = 7.36) travelled more frequently to enter the closed arms than males (M = 5.35). This means that females were more anxious than males.

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.

Subject in the treatment (M = 6.99) condition more frequently to enter the closed arms than control (M = 5.71) condition. This means that the subject in treatment condition was more anxious than subject in control condition.

An interaction effect between sex and treatment was significant, F (1, 50) = 12.77, p = 0.001, η^2 = 0.20. As can be seen in graph; males were more anxious in treatment condition (M = 6.83) than control condition (M = 3.86) but females were less anxious in treatment (M = 7.14) than those in control (M = 7.57) condition (see Figure 4.9.).

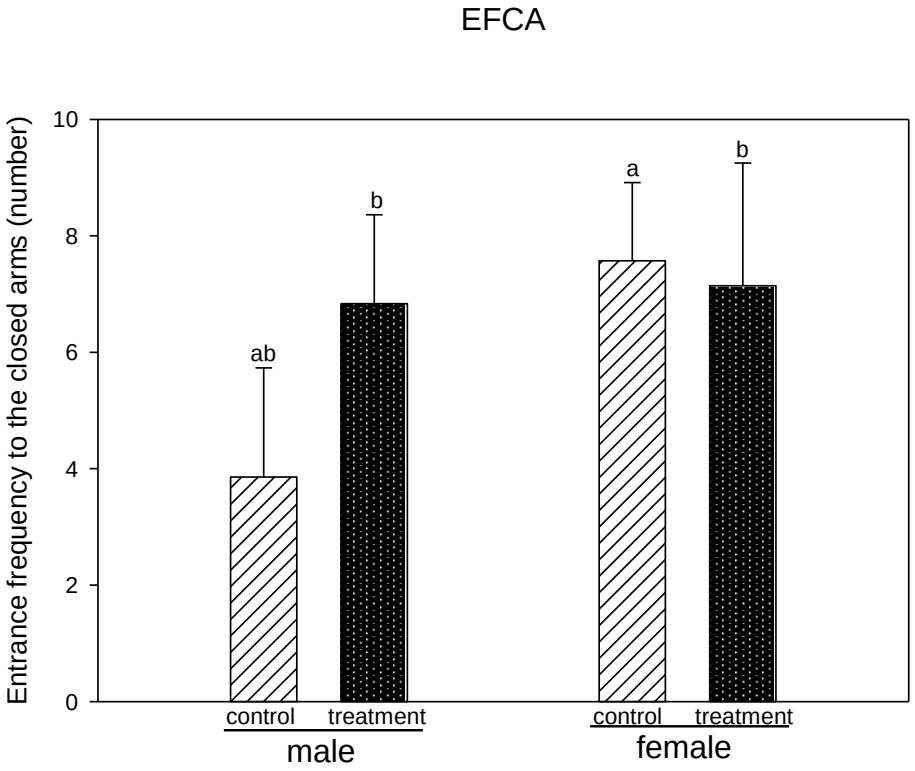


Figure 4.9. Mean (S.E.M.) Total entry to closed arms on the elevated plus maze (EFCA). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.2.4 Time Spent in Open Arm (TSOA)

The main effect of treatment was significant on the time spent in open arm, F (1, 50) = 10.55, p = 0.002, η^2 = 0.17.

Subject in the treatment (M = 1.33) condition spent more time in open arm than control (M = 0.67) condition. This means that the subject in treatment condition was less anxious than subject in control condition (see Figure 4.10.).

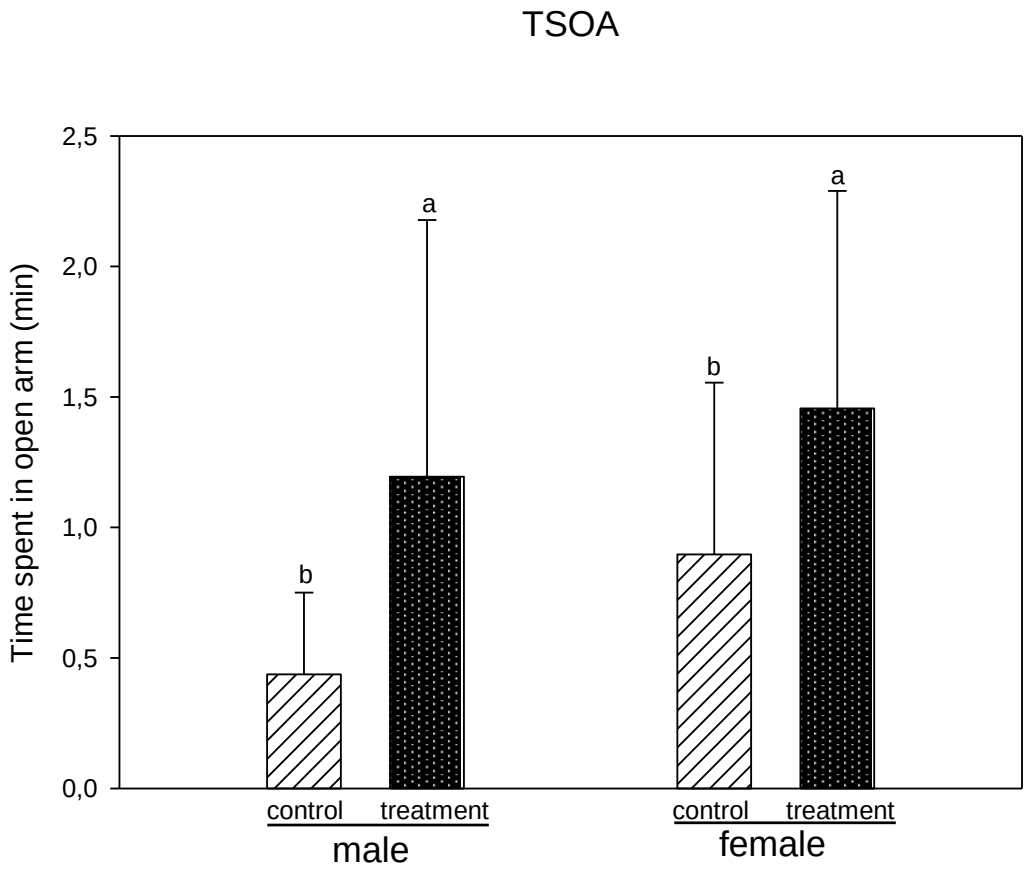


Figure 4.10. Mean (S.E.M.) Time spent in the open arm on the elevated plus maze (TSOA). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.2.5 Total Entry to Open Arm (EFOA)

The main effect of sex was significant on the entrance frequency to the open arm, F (1, 50) = 22.01, p = 0.0001, η^2 = 0.31.

Females (M =7.29) travelled more frequently to enter the open arm than males (M = 4.94). This means that females were less anxious than males.

An interaction effect between sex and treatment was significant, F (1, 50) = 9.15, p = 0.004, η^2 = 0.16. As can be seen in graph; males were less anxious in treatment condition (M = 6.17) than control condition (M = 3.71) but females had the same level of anxiety in both treatment (M = 7.00) and control (M = 7.57) (see Figure 4.11.).

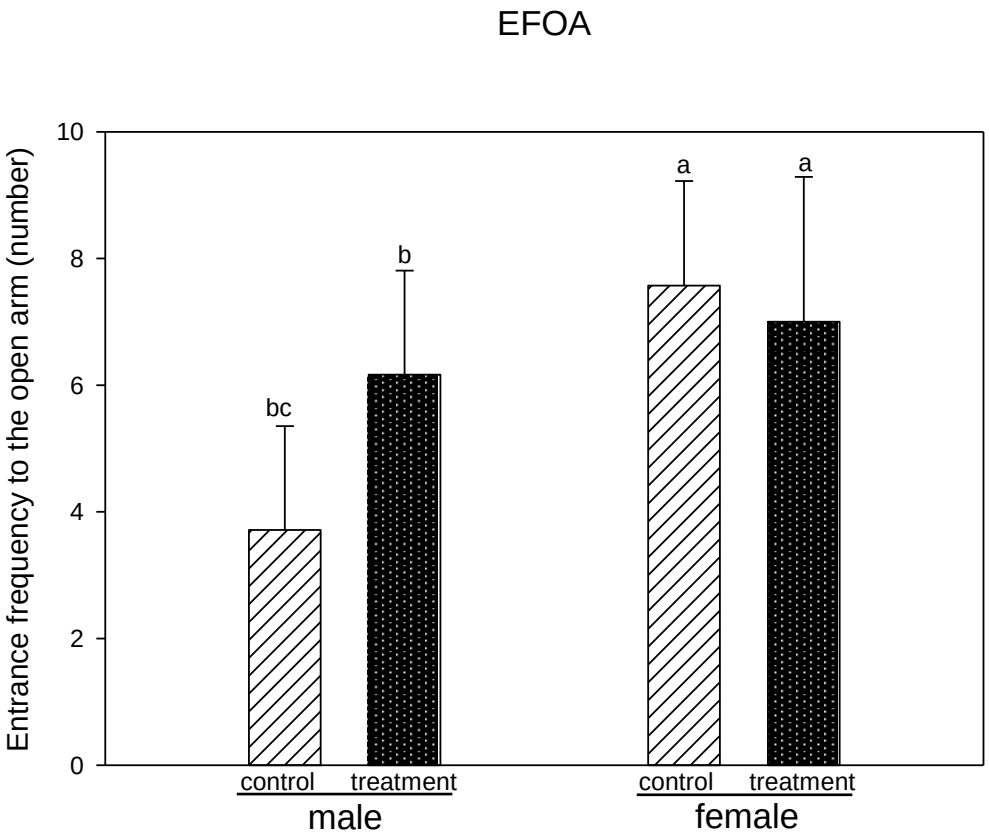


Figure 4.11. Mean (S.E.M.) Total entry to open arm on the elevated plus maze (EFOA). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.2.6 Mobility in Elevated Plus Maze (MT)

The main effect of sex was significant on the mobility, F (1, 50) = 5.72, p = 0.02, $\eta^2 = 0.10$.

Females (M = 4.986) were less mobile than males (M = 4.990). This means that females were more anxious than males.

An interaction effect between sex and treatment was significant, F (1, 50) = 4.76, p = 0.03, $\eta^2 = 0.09$. As can be seen in graph; males were less anxious in treatment condition (M = 4.989) than control condition (M = 4.992) but females had the same level of anxiety in both treatment (M = 4.989) and control (M = 4.984) (see Table 4.2.).

Table 4.2. The mobility scores of treatment and control groups in elevated plus maze.

Sex	Control	Treatment
Male	4.993 ± 0.005^{a}	4.988 ± 0.007^{ab}
Female	4.984 ± 0.011^{b}	4.989 ± 0.004^{ab}

Mean-values with the same letters are not significantly different ($p>0.05$).

4.1.2.7 Velocity in Elevated Plus Maze (VEL)

The main effect of sex was significant on the velocity, $F(1, 50) = 45.10$, $p = 0.0001$, $\eta^2 = 0.47$.

Males ($M = 169.07$) showed less velocity than females ($M = 233.29$). This means that males were more anxious than females (see Figure 4.12.).

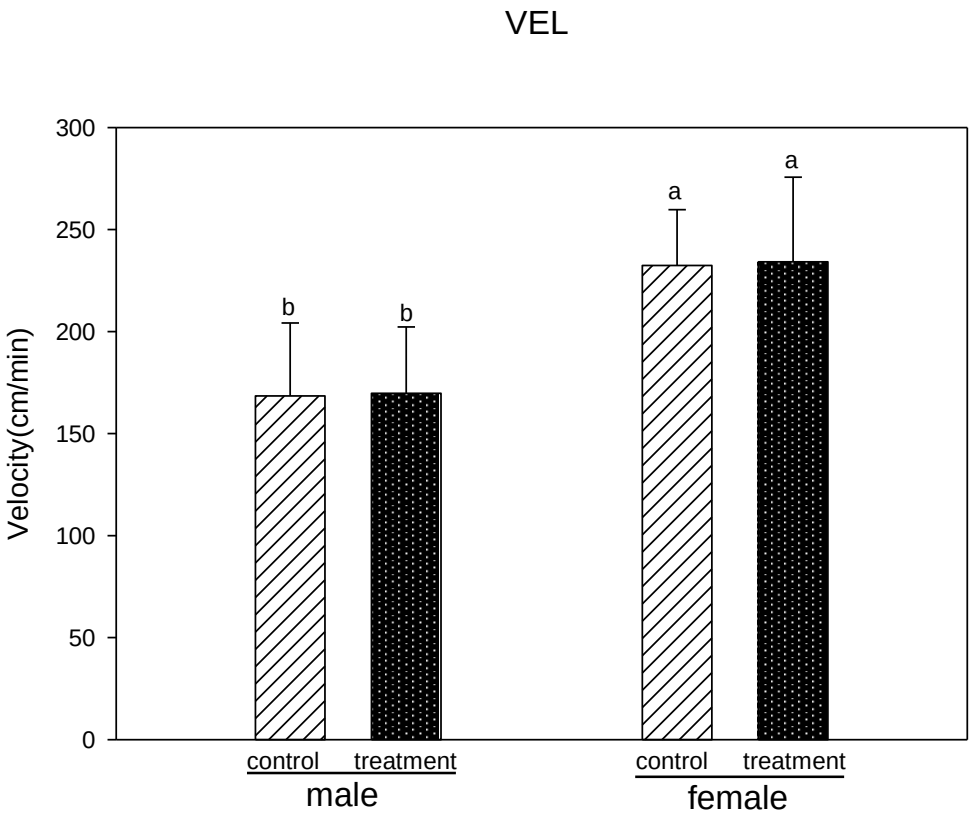


Figure 4.12. Mean (S.E.M.) Velocity in elevated plus maze (VEL). Mean-values with the same letters within vertical columns are not significantly different ($p>0.05$).

4.1.3 Forced Swim Test (Porsolt) Measurements

4.1.3.1 Total Distance Travelled on the Porsolt (TDT)

The main effect of treatment was significant on the total distance travelled, $F(1, 24) = 39.48$, $p = 0.0001$, $\eta^2 = 0.62$.

Subject in the treatment ($M = 1878.34$) condition more travelled than control ($M = 1237.67$) condition. This means that the subject in treatment condition was less depressive than subject in control condition (see Figure 4.13.).

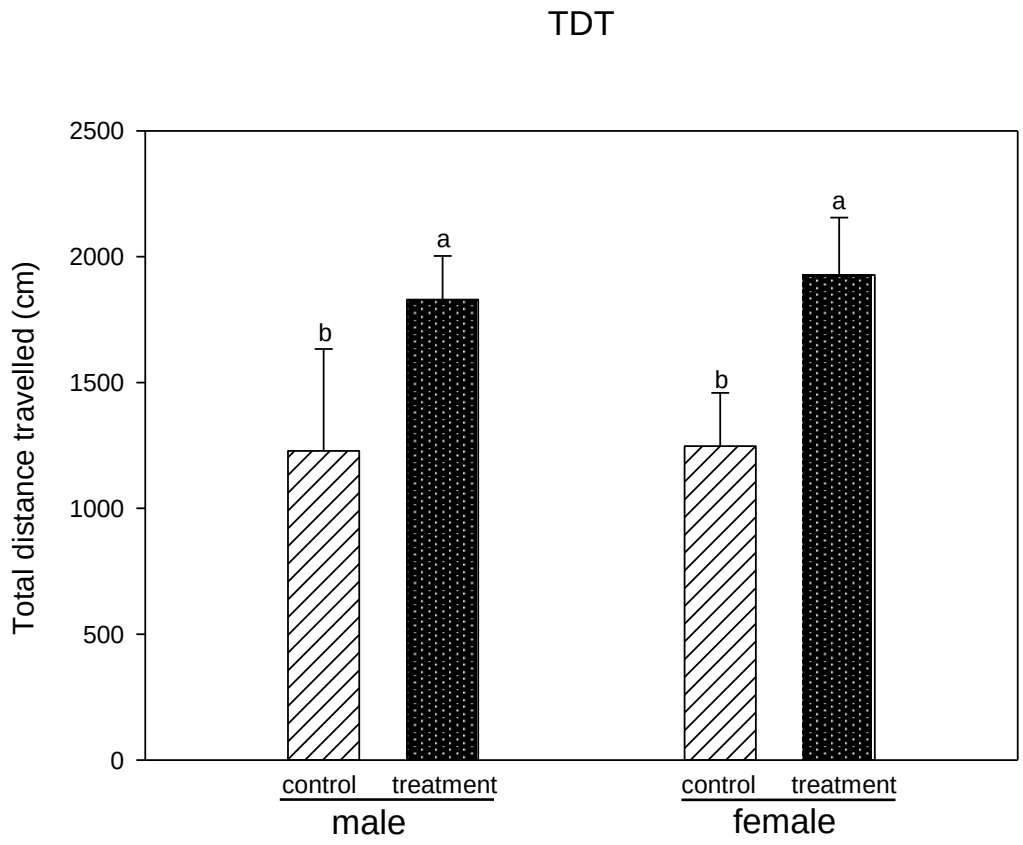


Figure 4.13. Mean (S.E.M.) Total distance travelled on the porsolt (TDT). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.3.2 Immobility (IT)

The main effect of treatment was significant on the immobility, F (1, 24) = 37.45, p = 0.0001, $\eta^2 = 0.61$.

Subject in the treatment (M = 0.78) condition were less immobile than control (M = 1.51) condition. This means that the subject in treatment condition was less depressive than subject in control condition (see Figure 4.14.).

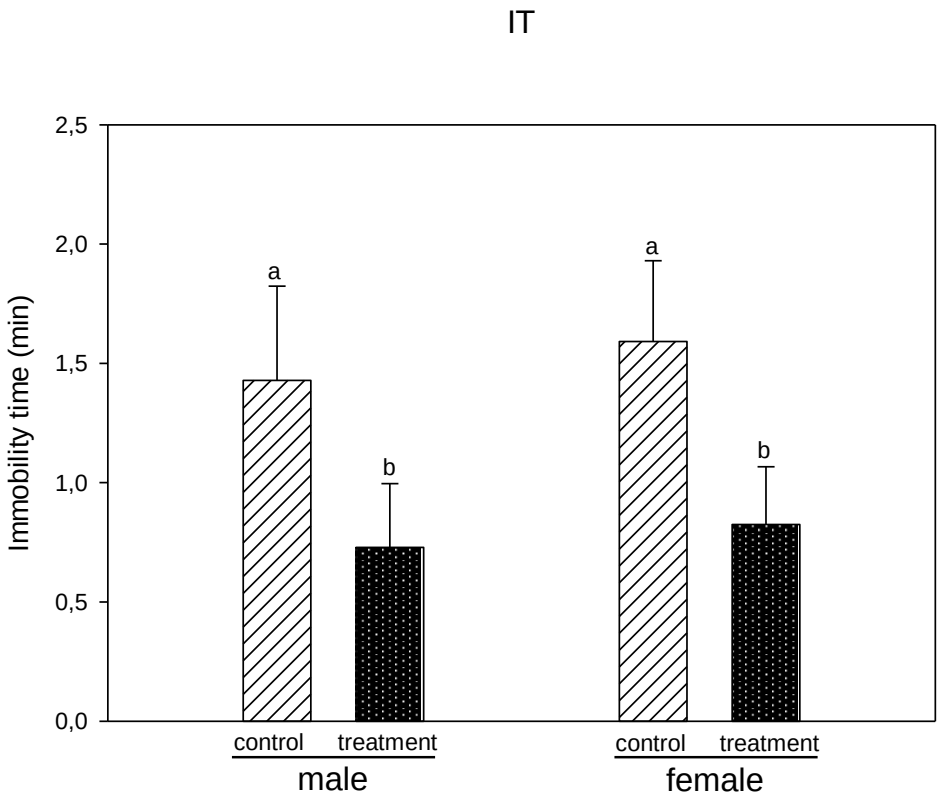


Figure 4.14. Mean (S.E.M.) Immobility duration on the porsolt (IT). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.3.3 Mobility (MOB)

The main effect of treatment was significant on the mobility, F (1, 24) = 26.56, p = 0.0001 η^2 = 0.53.

Subject in the treatment (M = 3.78) condition were more mobile than control (M = 2.91) condition. This means that the subject in treatment condition was less depressive than subject in control condition (see Figure 4.15.).

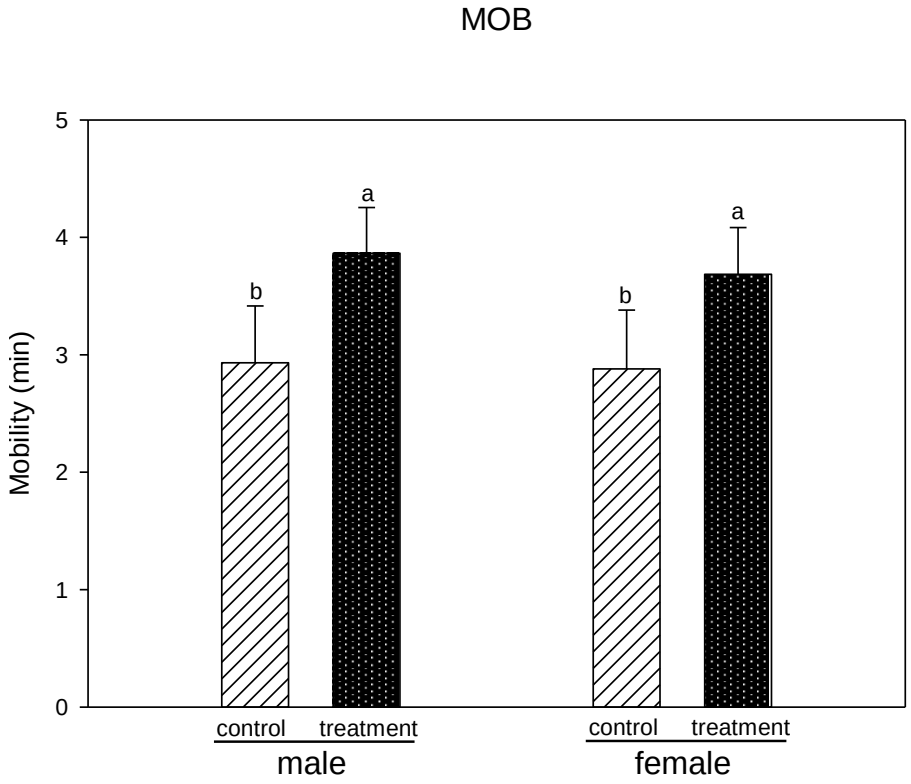


Figure 4.15. Mean (S.E.M.) Mobility duration on the porsolt (MOB). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.3.4 Velocity (VEL)

The main effect of sex was significant on the velocity, F (1, 50) = 8.52, p = 0.005, η^2 = 0.15.

Females (M = 455.91) showed more velocity than males (M = 506.45). This means that females were less depressive than males.

The main effect of treatment was significant on the velocity, F (1, 50) = 4.05, p = 0.05, η^2 = 0.08.

Subject in the treatment (M = 448.22) condition showed more velocity than control (M = 414.13) condition. This means that the subject in treatment condition was less depressive than subject in control condition (see Figure 4.16.).

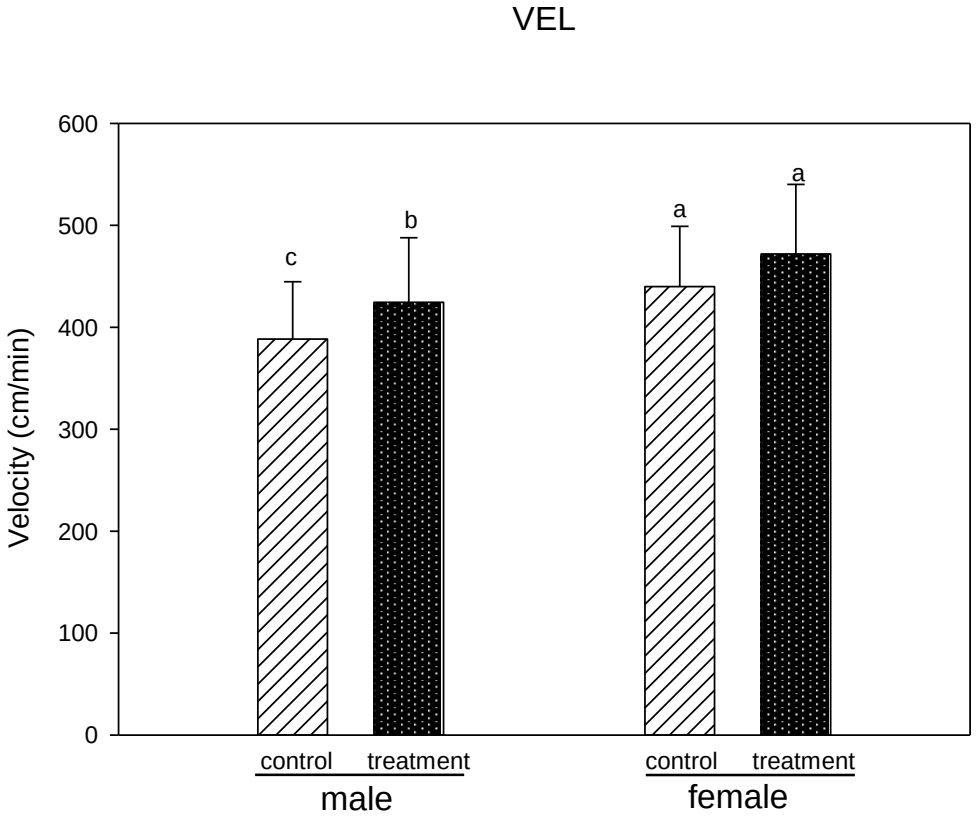


Figure 4.16. Mean (S.E.M.) Velocity on the porsolt (VEL). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.4 Water Maze Measurements

4.1.4.1 Total Distance Travelled (TDT)

No significant effects related to the treatment condition and as well as the interaction effect between treatment and sex were found to be significant at $p > 0.05$.

4.1.4.2 Time Spent to Find the Platform (TSFP)

No significant effects related to the treatment condition and as well as the interaction effect between treatment and sex were found to be significant at $p > 0.05$.

4.1.4.3 Time Spent in the Correct Quadrant (TSCQ)

No significant effects related to the treatment condition and as well as the interaction effect between treatment and sex were found to be significant at $p > 0.05$.

4.1.4.4 Entrance Frequently to the Correct Quadrant (EFCQ)

No significant effects related to the treatment condition and as well as the interaction effect between treatment and sex were found to be significant at $p > 0.05$.

4.1.4.5 Immobility (IT)

An interaction effect between sex and treatment was significant, $F(1, 50) = 4.06$, $p = 0.05$, $\eta^2 = 0.8$. As can be seen in graph; females were less immobile in treatment condition ($M = 0.099$) than control condition ($M = 0.221$) but males were more immobile in treatment condition ($M = 0.123$) than control condition ($M = 0.040$) (see Figure 4.17.).

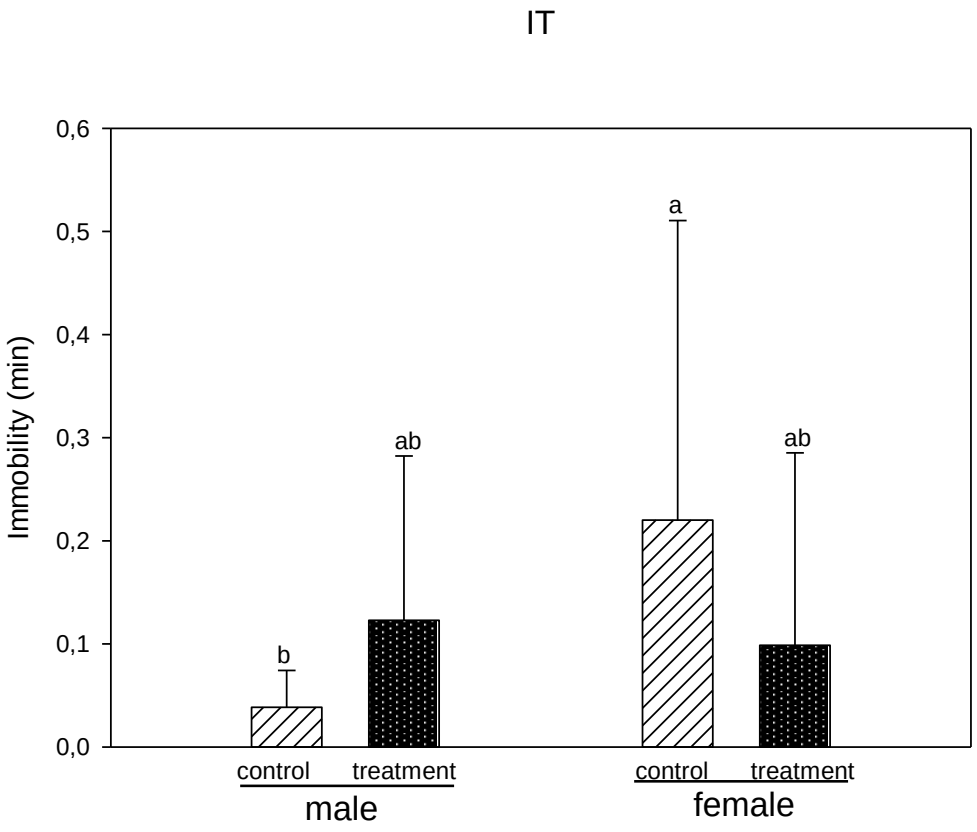


Figure 4.17. Mean (S.E.M.) Immobility on the water maze (IT). Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.1.4.6 Velocity on the Water Maze (VEL)

The main effect of sex was significant on the 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 the velocity in the open field, F (1, 50) = 8.88, p = 0.004, η^2 = 0.15.

Subject in the treatment (M = 1714.18) condition were faster than control (M =1480.85) condition (see Figure 4.18.).

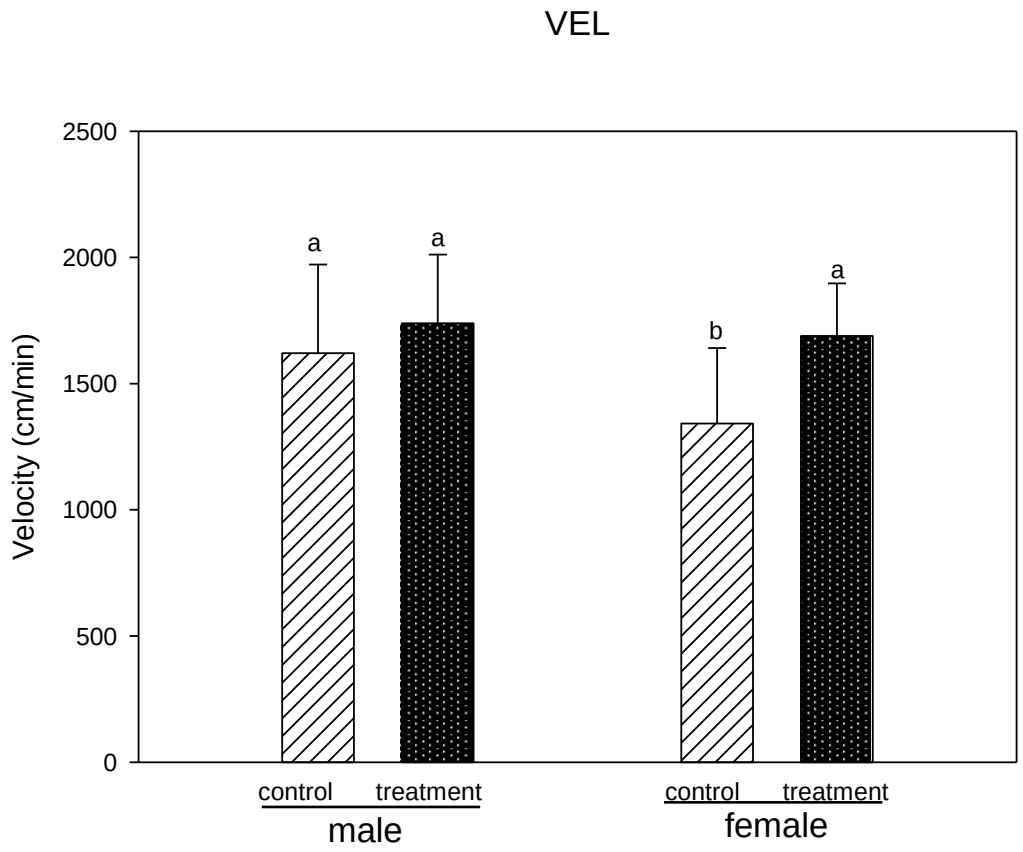


Figure 4.18. Mean (S.E.M.) Velocity on the water maze (VEL). Mean-values with the same letters within vertical columns are not significantly different ($p>0.05$).

4.2 DISCUSSION

4.2.1 Anxiety-Like Behaviours

The open field measures the anxiety like behaviors (i.e. the time spent in the center of the open field versus time spent at the edge of the open field, mobility and so on) in rodents (Benabid et al., 2008; Pyter and Nelson, 2006). In this test, if the level of anxiety of the animal is high, for instance the total distance traveled, mobility and velocity are decreasing and the number of the entries to the edge of the open field is increasing.

In open field and elevated plus maze tests *L. barbarum* reduces anxiety. In the present study it was found that in the open field, the subjects in the treatment of

L. barbarum administration; a) travelled more distance in open field, b) spent more time at the edge of the open field, c) spent more time at the center of the open field, d) were less frequently to enter edge of the open field, e) were more frequently to enter center of the open field, f) showed more mobility; and e) showed more velocity than those in the control condition.

The elevated plus maze has been used test to measure the anxiety like behaviors (Dawson and Tricklebank, 1995). For this maze, if the level of anxiety of the animal is high, the total distance traveled, mobility and velocity are decreasing and the number of the entries to closed arms is increasing. In the present study, it was found that in the elevated plus maze, the subjects in the high treatment of *L. barabarum* administration; a) spent more time in the open arms, b) spent less time in the closed arms, c) were more mobile d) were more frequently to enter the closed arms than those in the control condition.

In this study, the findings of the present study, in general, showed that in both open field and elevated plus maze, *L. barbarum* reduced level of anxiety. This finding was consistent with the previous research finding indicating that LBP-standardized juice reduced level of anxiety (Amagase, H. and Nance, D. M., 2008). In their study, Amagase and Nance (2008) led subjects to orally consume *L. barbarum* fruit in a form of LBP-standardized juice by healthy adults for 14 days. They measured body weight, body–mass index, blood pressure, pulse rate, and visual acuity for before and after study. It was stated that LBP-standardized juice not only reduced level of anxiety but also reduced feelings of fatigue, backache, stress, weakness, headache, impaired concentration, unreasonable worry, shortness of breath, (Amagase, H. and Nance, D. M., 2008). But in present study, the methanol extract of *L. barbarum* was applied to Wistar albino rats for 30 days and used animal behavior test (open field and elevated plus maze), whereas in the former study it was applied to 14 days. Even though there have been some differences in methodology (i.e., days of application), both study showed similar finding: the methanol extract of *L. barbarum* lowered anxiety level. These findings can be explained from at least three perspectives. First, since the methanol extract of *L. barbarum* included polysaccharide, this polysaccharide may increase some neurotransmitters such as serotonin and melatonin. In other words, polysaccharide may increase the level of

serotonin or melatonin, which, in turn, may decrease anxiety. The evidence comes from the previous studies indicating that melatonin decrease anxiety levels (Karakas et al., 2011). Also, most studies showed that serotonin decreased anxiety levels (Parks et al., 1998; Sarnyai et al., 2000). However, in this experiment, the serotonin levels of wistar albino rats were not obtained. Therefore the future studies should examine this plausible explanation. Second, polysaccharide may stimulate the dendrite patterns in hippocampus. There is strong evidence that polysaccharide enhanced the network of dendrites in hippocampus that is closely related to emotions such as anxiety (Zhang et al., 2012). The other evidence also comes from the study on the effects of LBP on the anxiety and space memory in rats who experienced posttraumatic stress disorder, indicating that LBP improved the hippocampal neurons and prevented the hippocampus volume reduction (Gao et al., 2014). Third, β-carotene, one of the important components of *L. barbarum*, may decrease anxiety level. There are some evidence for the beneficial effect of β-carotene on anxiety and memory. Put it in detail, the betaine that is an alkaloid from *L. barbarum* fruits were found to be effective in decreasing anxiety and increasing memory (Song et al., 2008; Song and Xu, 2013). Taken together, the future studies should compare the effects of LBP with those of β-carotene on anxiety like behaviors.

This experiment also demonstrated a significant interaction effect between sex and treatment on anxiety. This effect means that females outperform males in both open and elevated plus maze. For instance, we found that females in contrast to males were less anxious in treatment than those in control condition in terms of the total entrance to closed arms and the total entrance to the open arm. In short, females did get more benefit from the methanol extract of *L. barbarum* than males. This outcome may be due to two factors: selectivity and estradiol effects. According to selectivity hypothesis, females tend to process all information in the environment, whereas males tend to focus on only what is more important in the environment (Meyers- Levy, 1989). Searching for all information may be difficult processes for females. The methanol extract of *L. barbarum* may facilitate the information processing and thereby may lower the level of anxiety. Second, estradiol level of females may contribute to the low level of anxiety. Even though there is no direct support for this explanation, studies on spatial learning showed that estradiol levels such as low and high levels may differently affect learning performance of female

Wistar albino rats (Wide at al., 2004; Daniel, 2006). The future studies should examine the effects of estradiol levels in females with the different levels of the methanol extract of *L. barbarum*.

4.2.2 Depression-Like Symptoms

We evaluated the depressive- like responses (learned helplessness) of the subjects in forced swim test (Porsolt et al., 1977) consisted of an opaque cylinder tank which was filled with water. The total distance travelled, mobile duration, immobile duration and velocity are frequently used parameters measured in forced swim test in the literature. Completely, the findings from the forced swim test showed that the subjects in the treatment condition were less depressive than those in the control condition.

This finding was in line with the previous research finding (Zhang et al., 2012). In the previous study, Zhang and others (2012) examined the effects of LBP on depression in an animal model for two weeks. The results showed that LBP application significantly increased the mobility time in Porsolt swimming test, a test for the level of depressive behaviors. Even though these two studies showed a similar finding that *L. barbaum* reduces depression-like behavior, the present study is different from the previous study in some ways. First in the present study, the methanol extract of *L. barbarum* and water were applied to albino rats for 30 days, however, medium dose (40 mg/kg) or severe dose (50 mg/kg) of LBP were applied to albino rats for 21 days in previous study. Zhang et al. (2012) explained this outcome from the view that LBP may stimulate the dendrite patterns in 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 the depression levels. Secondly, another plausible factor may be the increased level of energy metabolism (Coskun et al., 2012). The methanol extract of *L. barbarum* may increase energy metabolism. We observe that the subjects who were exposed to the methanol extract of *L. barbarum* were more mobile than control ones. This observation was also confirmed by the results of the current experiment.

4.2.3 Spatial Memory Performance

Morris water maze is popularly used for spatial memory (Hooge and De Deyn, 2001). The goal of the animal by using some cues around the experimental zone was to find a hidden platform 2 cm below the water level. Some parameters discuss in method section were measured by means of video tracking system.

In the present study it was found that in the Morris water maze, the subjects in the treatment of *L. barbarum* administration; a) showed more velocity b) less immobiled in females but more immobiled in males than control condition. This means that the methanol extract of *L. barbarum* is more beneficial to spatial learning than control one and females are better at spatial memory than males. The finding that the methanol extract of *L. barbarum* is more beneficial to spatial learning than control one is consistent with a more recent finding (Chen et al., 2014). Chen et al., (2014) reported the beneficial effects of LBPs on spatial memory in scopolamine (SCO)-treated rats. In previous study, rats were divided into three groups: received distilled water and subcutaneous saline; distilled water and SCO; LBPs and SCO for 28 days and applied morris water maze. But in present study, the methanol extract of *L. barbarum* and water were applied to rats for 30 days. Like the methanol extract of *L. barbarum* in present study, LPB reduced time to find the platform in previous study. This effect may be modulated by hippocampus. Chen et al., (2014) demonstrated that LBP enhanced cell proliferation in hippocampus. It is well known fact that hippocampus may play important role in searching environment and navigating in the environment (Ekstrom et al., 2003). The second finding that females are better at spatial memory than males are also consistent with the previous research findings indicating that females are better at recognizing task than males (Sutcliffe et al., 2007).

There are some limitations in this study. In this experiment only one dose of the methanol extract of *L. barbarum* was applied. Thus the future studies should examine the different doses of it (i.e., low, high, or high doses) and application methods (gavage, intraperiotonal, or introcerbroventricular). For instance, intracebroventricular administration of the methanol extract of *L. barbarum* into hippocampus may prove a more robust evidence for whether or not cell proliferation

in hippocampus is evident. Second, the different application duration (i.e., acute an one week, two week, three week, and two months daily application of the methanol extract of *L. barbarum*) should be examined in order to see how much it is effective and in what ways it is influential.

5. CONCLUSION

The aim of the present study was to examine whether or not the methanol extract of *L. barbarum* is effective in reducing anxiety and depression like behaviors and in enhancing spatial learning performance. The present experiment did provide for the first time evidence for the beneficial effect of the methanol extract of *L. barbarum* on anxiety and depression like behaviors as well as spatial learning. This study also made contribution to the understanding interaction effect between treatment and sex on affective behaviors and learning. In particular, females seemed to benefit from the methanol extract of *L. barbarum* more than males on anxiety and depression like behaviors as well as spatial learning behavior.

One should be kept in mind that in general the methanol extract of *L. barbarum* was investigated. However, the methanol extract of *L. barbarum* includes secondary metabolites such as polysaccharide, polyphenols and β-carotene, and etc. These secondary metabolites can be separated and isolated from the crude methanol extract of *L. barbarum* and these isolated components of it should be investigated in a single research paradigm with the methanol extract of *L. barbarum* as to understand underlying mechanism of it on 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 these behaviors.

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