ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

THE EFFECTS OF THE *LYCIUM BARBARUM* L. POLYSACCHARIDES ON THE ANXIETY, DEPRESSION AND LEARNING BEHAVIORS OF THE OVARIECTOMIZED RATS

MASTER OF SCIENCE

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June 15, 2017

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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.

BİHTER GÖKÇE BOZAT

ABSTRACT

THE EFFECTS OF THE *LYCIUM BARBARUM* L. POLYSACCHARIDES ON THE ANXIETY, DEPRESSION AND LEARNING BEHAVIORS OF THE OVARIECTOMIZED RATS
MSC THESIS
BİHTER GÖKÇE BOZAT
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(CO-SUPERVISOR: PROF. DR. HAMİT COŞKUN)
BOLU, JUNE 2017

To investigate the effects of the *Lycium barbarum L.* polysaccharides (LBP) on anxiety, depression and learning behaviors in ovariectomized female rats using the open field, elevated plus maze, Porsolt and Morris water maze tests. Two weeks after ovariectomy operations, rats were divided into seven major groups: control [distile water, 3 mL/kg, oral gavage (o.g), per day], low dose of LBP (20 mg/kg, 3 mL/kg, o.g., per day), high dose of LBP (200 mg/kg, 3 mL/kg, o.g., per day), 17 beta estradiol (1 mg/kg, 3 mL/kg, o.g., per day), diazepam (1 mg/kg, 3 mL/kg, o.g., per day), imipramine (2,5 mg/kg, 3 mL/kg, o.g., per day), donepezil (1 mg/kg, 3 mL/kg, o.g, per day) and two minor group within the each major group: pseudo ovariectomized rat (SHAM) and overiectomized (OVX) rat groups. The treatments were applied for 30 consecutive days and then serum and brain tissue sample of all rats were collected. Biochemical [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), malondialdehyde (MDA) and 17 beta estradiol (17β-ES)] and immunohistochemical [brain drived neutrophic factor (BDNF), serotonin receptor (SER) and apoptosis] analysis of the samples were performed. The results of the behavioral experiments demonstrated that the high dose and low dose of LBP administrated rats less anxious, less depressive and more memory performances than control groups. Biochemical study findings showed that serum of the high dose of LBP administrated rats showed high level SOD, low level of MDA. Immunohistochemical study findings demonstrated that high dose of LBP and drugs administrated groups had high level of serotonin receptor and BDNF positive cell and, low level of TUNEL positive cell. In conclusion, high dose of LBP treatments decrease anxiety and depression-like behaviors and increase learning and memory performance by increasing antioxidant enzyme activities and hippocampal SER, and BDNF neurotransmitter levels and decreasing TUNEL positive cell count of ovariectomized female rats.

KEYWORDS: Goji berry, LBP, Ovariectomy, Anxiety, Depression**,** Learning

ÖZET

KURT ÜZÜMÜ POLİSAKKARİTLERİNİN, OVEREKTOMİLİ SIÇANLARIN ANKSİYETE, DEPRESYON VE ÖĞRENME DAVRANIŞLARI ÜZERİNE ETKİLERİ
YÜKSEK LİSANS TEZİ
BİHTER GÖKÇE BOZAT
ABANT İZZET BAYSAL ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ
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BOLU, HAZİRAN - 2017

Lycium barbarum L. polisakkaritlerinin (LBP) overektomili dişi sıçanlarda anksiyete, depresyon ve öğrenme davranışları üzerine olan etkilerini incelemek için, açık Alan, yükseltilmiş artı labirent, Porsolt ve Morris su tankı testleri kullanılmıştır. Overektomi operasyonundan iki hafta sonra, sıçanlar 7 ana gruba (tedavi): kontrol [distile su, 3 mL/kg, oral gavaj (o.g.), günlük], düşük doz LBP (20 mg/kg, 3 mL/kg, o.g., günlük), yüksek doz LBP (200 mg/kg, 3 mL/kg, o.g., günlük), 17 beta estradiol (1 mg/kg, 3 mL/kg, o.g., günlük), diazepam (1 mg/kg, 3 mL/kg, o.g., günlük), imipramine (2,5 mg/kg, 3 mL/kg, o.g., günlük), donepezil (1 mg/kg, 3 mL/kg, o.g., günlük) ve her grup kendi içinde iki alt gruba (operasyon): sham (yalancı overektomili sıçan) ve overektomili (ovx) sıçan gruplarına ayrılmışlardır. Tedaviler 30 gün uygulandıktan sonra tüm sıçanların serum ve beyin doku örnekleri toplanmıştır. Biyokimyasal [superoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPX), malondialdehit (MDA) ve 17-β estradiyol (17-β ES)] ve immunohistokimyasal [beyin türevli nötrofik faktör (BDNF), serotonin reseptör (SER) and apoptoz] analizleri yapılmıştır. Davranış çalışmasının bulguları, yüksek ve düşük doz LBP uygulanan sıçanların daha az kaygılı ve daha az depresif olduğunu ve öğrenme performansının yüksek olduğunu göstermektedir. Biyokimyasal çalışma bulgularında, yüksek doz LBP uygulanan sıçanların serumları yüksek SOD ve düşük MDA seviyesi göstermektedir. Immunohistokimyasal çalışma bulguları, yüksek doz LBP ve ilaç tedavilerinin sıçanların yüksek seviye SER ve BDNF pozitif hücre ve düşük seviyede TUNEL pozitif hücreye sahip olduğunu göstermiştir. Sonuç olarak, yüksek doz LBP tedavisi, serum antioksidan enzim aktivitesini ve hipokampal SER ve BDNF seviyelerini artırarak, ayrıca TUNEL pozitif hücre sayısını azaltarak overektomili sıçanların anksiyete, depresyon gibi davranışlarını önemli ölçüde azaltmış ve öğrenme performanslarını arttırmıştır.

ANAHTAR KELİMELER: Kurt üzümü, LBP, Overektomi, Anksiyete, Depresyon, Öğrenme

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LIST OF ABBREVIATIONS AND SYMBOLS

LBP : *Lycium barbarum* Polysaccharides
DZ : Diazepam
17-β ES : 17 β Estradiol
HD-LBP : High dose of *L. barbarum* Polysaccharides
LD-LBP : Low dose of *L. barbarum* Polysaccharides
DW : Distilled Water
IMP : Imipramine
DNP : Donepezil
SHAM : Pseudo Surgery
OVX : Ovariectomy
BDNF : Brain Drived Neutrophic Factor
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
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
O_2 : Oxygen Atom
OH : Hydroxyl Radical
H_2O_2 : Hydrogen Peroxide
ROS : Reactive Oxygen Radicals
MDA : Malondialdehyde
ABTS : (2,2-azinobis-6-s(3-ethylbenzothiazoline sulfonic acid)

CA1 : Cornu Ammonis 1

BDNF : Brain Drived Neutrophic Factor

SER : Serotonin Receptor

BCL-2 : B-Cell Lymphoma 2

5HT : Serotonin

5HT2-A : Serotonin 2A

HPG axis : Hypothalamic-Pituitary-Gonadal Axis

FSH : Follicle Stimulating Hormone

LH : Luteinizing Hormone

GnRH : Gonadotrophin Releasing Hormone

ER-α : Estrogen Receptor α

ER-β : Estrogen Receptor β

GPER1 : G-Protein-Coupled Estrogen Receptor

OFT : Open Field Test

EPM : Elevated Plus Maze

FST : Force Swimming Test

WHO : World Health Organization

BDNF/ TrkB :Brain Derived Neutrophic Factor/ Tropomyosin Receptor KinaseB

MWM : Morris Water Maze Test

DNA : Deoxyribonucleic Acid

SOD : Superoxide Dismutase

CAT : Catalase

GPX : Glutathione Peroxidase

GST : Glutathione-S Transferase

GR : Glutathione Reductase

TUNEL : Terminal deoxynucleotidyl transferase d UTB Nick End Labelling

PBS : Phosphate Buffer Solution

TDT : Terminal deoxynucleotidyl Transferase

ELISA : Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay

DAB : 3,3’ Diamino benzidine

GABA : Gamma-Amino Butyric Acid

SSRI : Selective Serotonin Re-uptake Inhibitors

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1. INTRODUCTION

1.1 Menopause

Menopause is a physiological and endocrinological process that lead to a diminish in circulating levels of the female sex steroids naturally or after surgery. The pre-menopause (climacteric) is described as firstly decreased ovarian reserved and then increased mentstrual cycle disorder caused by importantly reduced in the level of folicle stimulating hormone (FSH) sensitive follicles (Daan and Fauser, 2015). During menopause, 17-β-estradiol (17-β ES) level are decreased and FSH level are incresed, thus several alterations in body are triggered. The decreased estrogen hormone level cause increament in gonadotropins such as luteinizing hormone (LH) through loss of feedback inhibition (Braden et al., 2010). During the post menopausal period on rat and women, effect of estrogen on gonadotropin-releasing hormone (GnRH) are lacked and thus the LH and GnRH suppression are reduced on the aged rat and women (post menopausal period) (Braden et al., 2010).

Healthy women's reproductive life span is avarage 36 years, with an age range varying between 40 and 60 years (Krashia et al., 2016). Steroid hormones can affect life of neurons and glial cell and regulation of brain functions (Brinton et al., 2015; Rettberg et al., 2014). Especially, after the menopause, changing of estrogen concentration in the central nervous system affects some brain region functions such as amygdala, prefrontal cortex and hippocampus. The physiological alterations in the brain regions affects the emotional, cognitive and behavioral aspects to women life such as anxiety and depression disorders and memory impairment (Berent-Spillsona et al., 2016; Rettberg et al., 2014). Many study showed that changing of estrogen level causes depression symptoms (Berent-Spillsona et al., 2016; Brinton et al., 2015; Freeman et al., 2014). Although hormonal fluctuation after menopause cause depression syptomps but few study suggested that another factors result in menopausal symptoms. These factors may be age, time spent after menopause or metabolic disorders such as diabetus, insülin resistance and metabolic syndrome (Berent-Spillsona et al., 2016; Freeman et al., 2014; Gibbs et al., 2012).

Although bilaterally surgical removal of ovaries has been applied for treatment in women with endometrial and ovarion cancer, but it has been applied for ovariectomy model in rodents. Ovariectomy has been used to clarify insufficiency of estrogen hormone and its metabolic results to rodents (Xu and Yu, 2002; Vukovic et al., 2014). Though women has menstruel cycle, rodents has estrous cycle. These cycles are also differed each other by timing of the cycles. Menstruel cycle occurs twenty-eight days but estrous cycle occurs nearly five days. Also, estrous cycle have four phases: proestrous, estrous, metaestrous and diestrous. In menopause, women have little and immature follicles but, rodents have mature follicle in ovarian reserve. In post menopausal period, women have decreased level of 17-β ES, progestrone and increased level of FSH and LH but rodents have nearly same level of 17-β ES (Koebele and Bimonte-Nelson, 2016; Lu et al., 1979). On the other hand, both of the two cycles have similar hormone fluctuations (Gerbarg and Brown, 2016). Hormonal changing has started with age in women and rodents. Hypothalamic-pituitary-gonadal axis (HPG axis) dysregulation, fluctuation of GnRH releasing and LH level caused by aged in women or rodents (Koebele and Bimonte-Nelson, 2016; Wise, 1982).

1.2 Estrogen

Estrogens are steroid hormones found in three form in the women body and arrange the menstrual cycle (Berent-Spillsona et al., 2016). These forms were estrone, 17-β ES, and estriol. One of the most important estrogen forms were 17-β ES. Estrogen is largely produced in the ovarian follicle by conversion of cholestrol to testesterone and androstenedione in the theca interna cells of ovaries, and subsequently conversion to estrone and estradiol in granulosa cells. In corpus luteum and placenta, estrogen can also be produced. In addition, estrogen produced in brain and it can pass through blood brain barrier (Berent-Spillsona et al., 2016). In daily, estrogen generated as high as 700 μg relying on the phase of the menstrual cycle (Krashia et al., 2016).

Estrogen interacts with intracellular by their receptor. Estrogen receptors were found in brain, heart, reproductive system organs, kidney, bone and salivary

glands (Buyuk et al., 2015). In addition, they were found in neurons, glial cells, cell membrane and cell nucleus. The receptors have two forms: estrogen receptor α (ER-α) and β (ER- β). Although these receptors have genomic functions but their location is different. ER- α was found mainly in mammary gland and in the uterus. ER- β was found especially in mitochondri and has many function in central nervous system, heart, immune system, urogenital tract, bones, kidneys, and lungs (Krashia et al., 2016). In addition to these receptors, there is a thirth non-genomic estrogen receptor in women body is G-protein-coupled estrogen receptor (GPER1). It was found in cell membrane especially in brain (Furakoshi et al., 2006; Prossnitz and Barton 2011).

Previous studies have showed that estradiol includes a phenol group and the group plays a role in removing hydroxyl radical (OH) in vitro (Mahieu et al., 2016). Estrogen has protective roles in woman body, lack of its protective roles during the menopause triggers many of diseases such as behavioral, hormonal and cognitive disorders (Vukovic et al., 2014).

1.2.1 Estrogen & Behavior

It is expected that by 2030, 47 million women will be undergoing menopause each year (Krashia et al., 2016). Quality of life and life expectancy are negatively affected by menopause because menopousal period can cause decreasing estrogen level. Morover, decreasing estrogen level caused behavioral disorders such as anxiety, depression, memory impairtment.

Anxiety is one of the most important mental disorders and it threated human life. It is a normal reaction to a stressor or threatening environments. The situmulants arises from general stimulus sensed as being potentially danger in the future. On the other hand, when anxiety becomes damning, it may be included the grading of an anxiety disorder (Kouvaros et al., 2016).

In neurobiological studies, rodents were mainly used for anxiety measurements. Anxiety in rodents were measured by using two important behavioral tests: the open field test (OFT) and elevated plus maze test (EPM) (Heredia et al., 2014). Firstly, OFT were discovered by Hall in 1932 (Hall, 1934; Hall and

Ballechey, 1932). In OFT, indicator of high level anxiety in rodents are avoiding of center arena of open field. Rodents are feeling safe in edge of open field (Heredia et al 2014). This behavior were named as thigmotaxis.

Secondly, EPM are usually used for anxiety behavior measurements to rodents. This test consist of elevated two open and two closed arms. Rodents are cautios in new or unknown environments and fear elevated or unsafe platform (Casarrubea et al., 2016). Rodents with low anxiety level tend to spend their most of the time in the open arms (Carobrez and Bertoglio, 2005; Casarrubea et al., 2016).

In many studies, benzodiapezine and selective serotonin reuptake inhibitors were used in treatments of anxiety disorders (Thompson et al., 2015). Especially, diazepam (DZ) is one of the mainly using anxiolytic drug for human or animal models (Ahir and Pratten, 2011; Guney et al., 1999). DZ has 0.1-1.5 therapeutic range in blood and it has half life of approximately 40-100 h. (Ahir and Pratten, 2011). It is reported that DZ decreased thigmotaxis behavior of rodents (Bruhwyler, 1990; Gentsch et al., 1987; Thompson et al., 2015). These drugs have many side effects thus herbal treatment were more preferred to enhancing anxiety, depression, and memory disorders.

World Health Organization (WHO) reported that depression is biggest health problems in the first five common illnesses in the World. It is a common chronic and mental disorder and it causes many symptoms in especially menopausal women because of decreasing estrogen levels in brain. This syptoms are sadness, fatigue, helplessness, worthlessness, weight gain or sleepless and withdrawal from social interactions. Previous studies have showed that depression induces decline of the volume of the many brain regions such as prefrontal cortex, hippocampus and amygdala (Hastings et al., 2004; Jindal et al., 2015; Koliatsos et al., 2004; Sheline et al., 1998). Considerable evidence in female rodents suggest that high levels of estrogen are related to variation in the some brain regions such as hippocampus, including decrease long term depression (Fonteles et al., 2016). Moreover, it has been suggested that depression is caused by changing in serotonin levels in the brain (Duman and Monteggia, 2006; Harmsa, 2016; Jindal et al., 2015). In many studies, animal model such as rodents is used and experience to forced swimming test for deppression behavior measurements (Rollema et al., 2009; Sequeira-Cordero et al.,

2014). This aparatus is a beaker filled with water at 25-28 °C. In the beginning of the Porsolt test, animal put this apparatus for 15 minutes. After 24 hour, the animal is taken in this aparatus for 5 minutes to record immobility time of the animal (Molendijka and Kloet, 2015).

Imipramine (IM) is largely utilized in treatment of depression. It has a function on central nervous system and also has immunosuppressive properties. It obstructs serotonin and norepinephrine reuptake (Andrade-Neto et al., 2016; Obonago et al., 2014). It is one of the tricyclic antidepressant and the antidepressants perform its function by inducing brain derived neutrophic factor/tropomyosin receptor kinase B (BDNF/TrkB) pathways (Balu et al., 2008; Han et al., 2016; Réus et al., 2011; Siuciak et al., 1997; Xu et al., 2002).

Ovarian hormones affect cognitive functions such as learning and memory (Khodabandehloo et al., 2013). Especially, estrogen plays a key role memory task and estrogen deficiency increased the risk of Alzheimer's disease (Khodabandehloo et al., 2013; Vearncombe and Pachana, 2009). A research has shown that low dose estrogen application improved learning and memory performance but, high dose estrogen distrupted it (Galea et al., 2001; Holmes et al., 2002; Khodabandehloo et al., 2013). Another study reported that estradiol valerate applying to ovx rats increased learning memory performance however, its applying to sham group decreased learning and memory (Khodabandehloo et al., 2013). It was considered that estrogen was protective against memory deficiency (Patki et al., 2013; Seeman, 1997; Walf and Frye, 2006; Walf et al., 2009). Another memory improving function of estrogen is increasing the concentration of acetylcholine transferase, thus it increases synthesizes acetylcholine (Vuković et al., 2014). Morris water maze test (MWM) is used testing spatial learning and memory in rodents (Belviranlı et al., 2013; Piber et al., 2016). In this test, rodents tries to learn location of a hidden platform in four concecutive trial days and than record time spent to finding the platform in fifth day. Decreased the recorded time showed increased memory and learning condition. This test depend on hippocampal cognitive function because rodents tries find platform, no find food or water (Singh et al., 2016).

Donepezil (DNP) is an acetylcholinesterase inhibitor drug and increases acetylcholine levels in brain thus it treat many cognitive distruptions such as

Alzheimer's disease in human and rodents (Mina et al; 2012; Wang et al., 2016; Winblad et al., 2012). It increases acetylcholine concentration in brain. DN was firstly used for treatment of Alzehemir's disease in vitro and in vivo (Fujiki et al., 2008). In the previous studies showed that DN enhanced learning and memory performance in rodents (Creeley et al., 2008; Rogers and Friedhoff, 1998; Sugimoto et al., 1995; Zhang et al., 2007). Although DN has beneficial effects on memory, it has side effects such as diarhea, anorexia, muscle convulsion and nausea.

However, declined estrogen level after menopause/ovariectomy may cause accumulation of oxidative stress in blood serum and thus it can cause changing of antioxidant enzymes concentrations and behavioral distruptions.

1.2.2 Behavior & Oxidative Stress and Antioxidant Defence Systems

Oxidative stress is an instability between free radical generation and inadequate antioxidant defense systems. Reactive oxygen species (ROS) containing superoxide ions, the hydroxyl radicals and hydrogen peroxide generating by aerobic metabolism and increased reactive oxygen species cause oxidative stress (Eraldemir et al., 2015).

During menopause transition, decreased ovarian hormones results in increasing production of reactive oxygen radicals and thus increased oxidative stress induces tissue or cell damages (Salli et al., 2016; Vuković et al., 2014). During the menopause, reduction in estrogen hormone level is associated with elevated oxidative stress, which occurs due to an instability between production and elimination of reactive oxygen species (ROS) via the antioxidant defense system (Park et al., 2016; Vuković et al., 2014). Estrogen diminishes oxidative stress in vitro and vivo (Unfer et al., 2015).

Especially, brain tissue demands more oxygen and has a restricted level of antioxidant capacity thus it is sensitive to oxidative stress induced by ROS (Asha Devi et al 2011; Belviranlı et al., 2013; Halliwell and Gutteridge 1985). Accumulating of oxidative stress in the brain tissue results in many neurodegenerative disorders due to damage proteins, lipids, and nucleic acids.

Moreover, oxidative stress causes damages and apoptosis of neurons because it especially disrupts proteins and deoxyribonucleic acid (DNA) in the brain (Valvassori et al., 2015). Furthermore, decreased level of antioxidant enzymes and increased level of oxidative stress caused oxidative DNA damage and apoptosis in cell (Cevik et al., 2015). Previous studies have indicated that oxidative stress cause decreased cognitive function with aged (Belviranlı et al., 2013; Markesbery, 1997).

Antioxidant systems are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) (Liu et al., 2015; Vuković et al., 2014). Oxidative damage level in the brain is detected by multiple biomarker meausrements such as enzymatic and non enzymatic antioxidants, cellular redox balance and lipid peroxidation (Rodriquez et al., 2013).

Reactive species are generously created throughout both physiological and pathological courses. Antioxidant system provides protection against many various diseases. These deseases cause increasing free radicals levels. Antioxidant scavenges free radicals, ensures cellular prevention and struggles against human diseases (Kushwara et al., 2014). When the antioxidant defence system is unstable, oxidative stress is produced, changing and damaging many molecules such as, lipids, proteins and nucleic acids (Ramirez-Exposito et al., 2014).

Antioxidant defense system includes many enzymes which deactivate free radicals in a body. These enzymes are SOD, CAT, GPx, glutation-S-transferase (GST), glutation reductase (GR). However, there are non enzymatic antioxidants such as tocoferol, retinol, glutation, ascorbic acid, melatonin, and etc. SOD and CAT transform the oxygen atom (O_2) into hyrdogen peroxide (H_2O_2) (Dilek et al., 2010). All aerobic cells include SOD enzyme and it was found also cytosol and mitochondria. Moreover, GPx plays a significant role in neuronal antioxidant system. Decreased glutathione level is related to increasing power of antioxidant system. In addition, GPx induces degredation of hydrogen peroxidase to water (Dilek et al., 2010; Kovacic and Somanathan, 2008).

Imbalance between ROS production and antioxidant defence system caused oxidative stress and thus, it cause increasing lipid peroxidation and tissue damage (Poli and Parola, 1997; Vucovic et al., 2014). Malondialdehyde (MDA) was occured

by lipid peroxidation and it was a good marker to detection oxidative stress level and tissue damage (Baeza et al., 2010; Comelekoglu et al., 2012; Del Rio et al., 2005). Previous studies showed that stress induced lipid peroxidation caused learning and memory distruption (Frlich and Riederer, 1995; Khodabandehloo et al., 2013) and lipid peroxidation level decreased because of the fact that estrogen was a powerful antioxidant (Niki and Nakano, 1990; Vucovic et al., 2014; Yoshino et al., 1987).

Balanced diet, sufficient intake of mineral and vitamins and other beneficial foods play an importent role in preventing menopause symptoms (Patki et al., 2013). Especially, antioxidants treated symptoms and distruption of menopause and post menopause (Selli et al., 2016). Estrogen was a power antioxidants in women (Cevik et al., 2015; Moorthy et al., 2015; Vucovic et al., 2014). Consumption of antioxidants protected women with menopause and post menopause against oxidative stress induced damages. For example, vitamin E and C was power antioxidant and their combined consumption was beneficial to menopausal women health (Jialal et al., 1990; Jialal et al 2001).

Many patients prefer herbal medicines to treat diseases because of side effects and harmful effects of some chemical drugs, The utilization of herbal therapy is extensive amongst suffers of mood and anxiety disorders. For example, *Melissa officinalis* (lemon balm), *Matricaria recutita* (chamomile) and *Humulus lupulus* (hops) are known anxiolytics and its mechanism of action has started to be explored (Gutierrez et al., 2014). It has been suggested that oxidant/antioxidant systems play a crutical role in the pathophysiology of depression (Jindal et al., 2015; Nikisch et al. 2005).

Evidences indicate that the estrogen arranges expression of the SOD and GPX (Unfer et al., 2015; Vucovic et al., 2015). Moreover, it has been shown that estrogen therapy prevents the reduce in serum total SOD activity in postmenopausal women (Unfer et al., 2015). The current studies indicate that a significance of the estrogen hormone for ideal brain working. Furthermore, extended estrogen deficiency induces neuronal cell death (Salli et al., 2016).

Phytoestrogens, vitamins, minerals, antioxidants, antidepressants and memory improver drugs were used as alternative estrogen replacement therapy (Kurter, 2003;

Borrelli and Ernst, 2010; Patki et al., 2013). It was reported that grape powder prevented anxiety, depression and memory disorders induced by oxidative stress (Patki et al., 2013). In previous studies, goji berry was one of the powerful antioxidants and studied for treatment of anxiety, depression and memory distruption studies.

1.2.3 *Lycium barbarum* Linnaeus (Goji Berry)

Lycium barbarum Linnaeus is also known as goji berry, wolfberry, super fruit, lycium fruits or fructus lycii. It belongs to genus Lycium of the family Solanaceae. The fruits are used in herbal medicine and health food for thousands of years in China, Southeast Asia, Europe, and North America. (Bilgic et al., 2015; Potterat, 2010; Wang et al., 2010; Wiest and Garcia-Tsao, 2005; Zheng et al., 2015).

The fruits are harvested in between August and September months and usually sun-dried (Bondia-Pons et al., 2014). It has red-orange and sweet fruits. In these days, many countries consume *L. barbarum* such as especially in Korea, Japan, and Europe. The fruits are popular beneficial foods in various forms such as soups, juice, wine, tea and a variety of solid foods (Amagase & Farnsworth, 2011; Xie et al., 2016). The consumption of berry fruits is well known to have a helpful impact on human health. For example, *L. barbarum* has many beneficial effects such as protection of eyesight, immunity, prevention of several metabolic disturbances including diabetes hyperlipidemia (Amagase and Farnsworth, 2011).

Morever, among the berries, goji berry fruits are composed of important dietary constituents at high concentration. The berry has many beneficial effects for human because of these dietary constituents such as flavonoids, phenolics, vitamins, caretonoids and polysaccharides. The possession of diverse functional chemical constituents and biological activities in the fruits is believed to be responsible for these beneficial effects (He et al. 2012; Magiera and Zareba, 2015; Yao et al. 2011). *L. barbarum* have flavonoids, phenolic acids, saccharides and organic acids. In a study showed that goji berry have many flavonoids such as 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 3-hydroxybenzoic acid, hippuric acid, caffeic acid, vanillic acid, 4-hydroxy-3-methoxyphenylacetic acid, 3-(2,4-dihydroxyphenyl)

propionic acid, p-coumaric acid, ferulic acid, (±)-catechin, (−)-epicatechin, rutin, quercitrin, hesperidin, neohesperidin, (±)-naringenin, hesperetin, chrysin, and pinocembrin (Magiera and Zareba, 2015). The goji berries have also phenolic compounds such as caffeic acid, p-coumaric acid, rutin, scopoletin, N-trans-feruloyl tyramine, N-cis-feruloyl tyramine, N-feruloyl tyramine dimer (Forino et al., 2016).

Goji berries have also carotenoids and especially mainly composed of zeaxanthin (Inbaraj et al., 2008). Although it has other phytochemicals at lower concentrations, beneficial effects of the fruit are provided by these phytochemicals. On the other hand, the fruit has fewer amount of malic acid than other berry fruits (Mikulic-Petkovsek et al., 2012). *L. Barbarum* have also non-proteinogenic amino acids such as taurine and γ-aminobutyric acid, as well as trimethylglycine (Cao et al., 2003; Wang et al., 1988). Furthermore, the fruit has both essential oil and fatty acid such as hexadecanoic acid, linoleic acid, myristic acid and ethylhexadecanoate (Altintas, 2006).

Morover, *L. barbarum* have also polysaccharides. The polysaccharides are main components of the fruit (Wang et al., 2009). The composition of *L. barbarum* polysaccharides consists mainly of 9 monosaccharides, mannose, rhamnose, galactose, xylose, arabinose, fucose and glucose, galacturonic acid, and glucuronic acid (Jin et al., 2013; Wang et al., 2009). Several animal studies indicates that LBP has in ocular neuroprotective, antioxidant, immunomodulator, hepatic pretection, and antitumor effects in animals (Chang and So, 2008 Cheng & Kong, 2011).

In many studies, researchers tent to antioxidant properties of LBP. Previous research showed that LBP indicated remarkable influences on scavenging ABTS (2, 2-azinobis-6-s (3-ethylbenzothiazoline sulfonic acid)), diphenylpicrylhydrazy and superoxide radicals (Zhang et al., 2014).

LBP importantly alleviated neuronal injury and obstructed lactate dehydrogenase release (Chen et al., 2012). Moreover, in the previous research showed that LBP enhanced the SOD and GPx enzyme activities, however it decreased their MDA content. In addition, LBP importatly deactivated the caspase-3 activation, thus LBP may be a hopeful applicant for neuronal apoptosis induced-neurodegenerative diseases (Teng et al., 2013). Moreover, in another study showed

that LBP prevented cognitive and memory disorders (Chin et al., 2014). The function of LBP on oxidative stress and apoptosis of hippocampal neurons provided that LBP have regulatory effect on memory (Xie et al., 2016).

In an experimental study, the influence of LBP on the spatial memory and oxidative stress in the hippocampus was investigated. The result of this study have shown that LBP administration standardized the increased oxidative stress induced by hypoxia. Furthermore, LBP applications intercepted the spatial memory deficit and improved the hippocampal neurogenesis induced by hypoxia (Lam et al., 2015). Moreover, another study indicated that the treatment of LBP developed increased level of apoptosis in the hippocampus (Gao et al., 2015).

1.2.4 Hippocampus

The nervous system is related with a lots of hormones and its specific receptors. Especially, steroid hormone such as estrogen, aldosterone, androgen, and corticosterone play a role in the nervous system function (Foy, 2001). In the brain, many types of regions are associated with cognitive behaviors. Hippocampus is one of the most important brain regions. Furthermore, decreased number of neurons in the hippocampus cause cognitive impairtment (Valvossori et al., 2015). Evidence shows that hippocampus is one of the most sensitive region to oxidative stress thus it has effective role in the anxiety, depression and especially learning-memory behaviors (Markham et al., 2002; Vouimba et al. 2000). Studies demonstrate that the antidepressant drugs treat patients via hippocampal neurogenesis (Hutton et al., 2015; Malberg et al., 2000; Sahay et al., 2011; Santarelli et al., 2003).

It also has receptors for steroid hormones such as estrogen. Morover, estrogen receptors influence many neurotransmission systems in many location, comprising the catecholaminergic, serotoninergic and gabaergic ones. These receptors are highly expressed in hippocampus and enhance the density of the dendritic spines in the neurons of the cornu ammonis 1 (CA1) area in this region (Braden et al., 2010; Gould et al 1990; Santos-Galduroz et al., 2010; Sherwin, 2006; Woolley et al 1990).

These behavioral distrupted caused by increasing oxidative stress and thus changing cognitive hormone levels or its receptor such as brain derived neutrophic factor (BDNF) and serotonin receptor (SER) in the hippocampus brain region (Selli et al., 2016; Suzuki et al., 2009).

1.2.5 Brain-Derived Neurotrophic Factor (BDNF)

Neurotrophic factors are involved in neurogenesis in the brain. BDNF is one of the neutrophic factors that the moderator protein enhances survival of peripheral neurons by inhibiting cellular apoptosis owing to arrangement of B cell lymphoa 2 (Bcl-2) family members (Bavithra et al., 2015; Belviranlı and Okudan, 2015; Valvassori et al., 2015). It also arranges many neuronal functions such as survival and migration of neurons, cell differentiation, growth of axons and dendrites and synapse formation (Eraldemir et al., 2015; Valvassori et al., 2015; Zhang et al., 2015).

Many studies have indicated that neurotrophic factors can play an important role as therapeutic agents in neuronal disorders. In the especially hippocampus region, the neurotrophin BDNF concentration level affects behavior. For example, decreasing in the BDNF concentration level causes increasing neuronal degeneration in hippocampus and thus causes behavioral impairtment such as anxiety, depression and learning (Belviranlı and Okudan., 2015; Hritcu and Gorgan, 2014; Jindal et al., 2015; Mendez-Davida et al., 2015). Moreover, several studies demonstrated that BDNF level in serum decreased in depressive patients (Belviranlı and Okudan et al., 2015). Another research has indicated that decreasing BDNF levels cause depressive patient on the other hand, antidepresant drugs provide increased BDNF level in the treated patients (Liu et al., 2015).

Accumulating of oxidative stress and alterations of BDNF level in hippocampus cause impairtment of learning and memory ability (Zhang et al., 2015). One previous research has reported that increment BDNF concentrations are connected a slower rate of cognitive decline in Alzheimer's disease patients (Zhang et al., 2015).

Further, the reports of several studies have indicated that the elevated blood oxidative stress may lead to neuronal impairment by decreasing hippocampal BDNF levels, causing depressive effects (Jindal et al., 2015) .

1.2.6 Serotonin Receptor (SER)

Serotonin is one of the important monoamine neurotransmitters and is produced in the raphe nuclei by the tryptophan hydroxylase 2. It also arranges many neuronal functions (Banerjee and Poddar, 2016; Knobelman et al., 2000; Roy and Poddar, 1990). It can bind its receptors. SERs are ligand-gated cation channel and are found in both pre- and post synapse. The SERs are expressed in the brain largely in presynaptic regions related to axons and nerve terminals. They are predominantly found in somatodendritic region of hippocampus and medial temporal lobe memory system (Pithia et al., 2016; Verdurand et al., 2016). There are approximately fifteen types of serotonin receptors has been reported and they are connected to seven SER families (Banerjee and Poddar, 2016; Kroeze et al., 2002). SERs also play a significant role in pain processing, integration of the vomiting reflex (emesis), sensory transmission, the reward system, anxiety control and gastrointestinal disorders. Moreover, SERs regulate different behaviors such as anxiety, depression and learning (Banerjee and Poddar, 2016). It is indicated that serotoneric neurons are largely found in all brain regions such as cortical, limbic, midbrain, hindbrain and brain stem regions (Berger et al., 2009). Previous studies showed that SERs level was decreased during aging serotonin-2A (5-HT2A), belonging to serotonin (5-HT) receptors' family, has been reported to associate with learning and memory behaviors, neuroendocrine functions, and sleep (Banerjee and Poddar, 2016).

2. AIM AND SCOPE OF THE STUDY

The purpose of the this study was to examine behavioral, biochemical and immunohistochemical effects of *L. barbarum* polysaccharides on ovariectomized female rats. Many studies have suggested the effects of *L. barbarum* polysaccharides have many beneficial effects on human or rodents such as anxious, anti deppressant, memory enhancer and antioxidant activity. However, effects of the *L. barbarum* polysaccharides on anxiety, depression and memory functions of ovariectomized rats has not been investigated yet. In our previous study, we investigated that the effects of *L. barbarum* methanol extract on anxiety, depression and memory performance of rats. We found that *L. barbarum* treatment decrease anxiety and depression and increase memory performances of rats. From these results, we hypothesized that how positive or negative effect of the fruit polysaccharides would have on anxiety, depression and memory behavior of ovariectomized rats. Moreover, we aim to examine under the biochemical and immunohistochemical mechanism of the behavioral findings. For this aim, we planned three studies.

First of all, we examined whether or not anxiolytic, antidepressant and memory enhancer effect of *L. barbarum* polysaccharides is evident on the anxiety, depression, and spatial learning performance of ovariectomized female rats. In the first study, we aim to observe whether or not ovariectomy operation will increase the level of anxiety, depression and memory dysfunctions. Than, 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.

The second study, we aimed to investigate under the biochemical mechanisms the behavioral changing of the rats. For this purpose, we tested SOD, CAT, GPX, MDA and 17-β estradiol serum level of ovariectomized rats. In previous study, LBP increased SOD level of rats but the effects of LBP on antioxidant enzymes level of ovarictomized rats has not been researched before. Thus, it was expected that LBP would increased SOD level. Alternatively because of ovariectomy LBP would not have an effect on SOD level.

The third study, we aimed to examine under the immunohistochemical mechanims of the behavioral changing of the rats. For this purpose, we collected brain tissue samples of all rats and investigated hippocampus region. Especially, hippocampal serotonin, BDNF and apoptotic cell count were observed. In literature, there was no information related with effects of LBP on hippocampal neurotransmitters level of ovariectomized rats.

3. MATERIAL AND METHOD

3.1 Animal

Female Wistar albino rats and body weight of 180-250 g, were purchased from the Department of Experimental Animal Center (Abant İzzet Baysal University) at nearly 60 days old were used (Figure 3.1.).

Total of three hundred female rats were used in this study. Seven days before the starts of the experiments, female rats were housed individually in plastic cages (40 × 50 × 20 cm) with a temperature-controlled environment (22 ± 2 °C), and a reversed light/dark (12/12 h) cycle (lights on at 8:00 AM, and lights off at 8.00 PM). Food and water were received *ad libitum* until the time of experiments. All applications were performed between 13:00 and 17:00 h in order to avoid potential circadian alteration (Citraro et al., 2015; Russo et al., 2013). Animals were fasted for 12 h prior to gavage except water. All behavioral procedures were performed during the light cycle. The procedures in this study were carried out in accordance with the Animal Scientific procedure and approved by the Institutional Animal Care and Use Committee. All efforts were made to diminish animal pain and to minimize the number of animal used (Pic-Taylor et al., 2015).

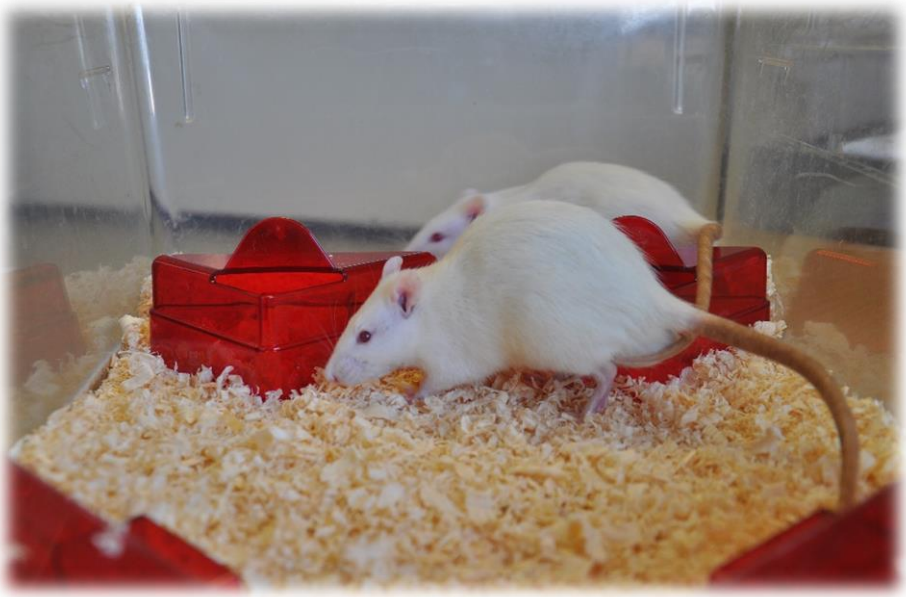

Figure 3.1. Wistar Albino Rats

3.2 Surgical Procedure

Ovariectomy (OVX) or false surgery (SHAM) operations were applied on two months old female Wistar rats. The female rats were anesthetized by intraperitoneal injection with a mixture of Ketamin/Ksilazin (4:1, 0.25 ml i.p.). OVX were applied over a midline abdominal incision (2 cm in length) in the linea alba, cauterization, crushing and ligating of the fallopian tubes and ovaries were gently bilaterally removed by cutting above the clamped area. However, the rats in the sham-operated group were exposed to same OVX process with the exception of ovary removal. After 10 days of recovery from their ovariectomies, the rats were divided into three main groups: anxiety, depression and learning, and started gavage applications for thirty consecutive days (Figure 3.2). All behavioral tests were commenced after the gavage applications.

Figure 3.2. Gavage application

3.3 Preparation of *Lycium barbarum* Polysaccharides

LBP was prepared as described in previous reports (Wang et al., 2016; Zhang et al., 2015). In short, fruits of *L. barbarum* purchased from Gojiform, Turkey (Figure 3.3.). Two hundred grams of dried fruits were crushed and extracted two times with 600 ml of chloroform:methanol (2:1) solution at 80 ºC (Figure 3.4.). Then, the mixture was filtrated and added 600 ml of 80 % ethanol twice to remove some colored materials; each extraction lasted 2 h (Figure 3.5.). Filtration was applied the second mixture and the dry residuals were extracted with hot water (80º C) for three times (2 h for each time) (Figure 3.6.). Reflux extraction was conducted with % 95 ethanol for precipitation at +4 ºC. 12 hour after the process, the precipitation was collected by centrifugation (3,400 rpm, 15 min) and washed with

pure ethanol and acetone (Figure 3.7.). After the washing, the precipitation was dried under reduced pressure (Figure 3.8.).

Figure 3.3. Dried goji berrry fruits

Figure 3.4. Extraction with hot shaking water bath

Figure 3.5. Extracted goji berry fruits

Figure 3.6. Filtration of goji berry extract

Figure 3.7. Evaporation of extraction solvent with rotary evaporator

Figure 3.8. Dried LBP extraction

3.4 Study of Anxiety Like Behaviors Experimental Groups

One hundred female Wistar rats were randomly distributed into two groups: OVX and SHAM. These groups were subdivided into the five treatment groups: low and high doses of LBP, 17-β ES, DZ and distilled water (DW). All of the

experimental groups were treated orally by gavage (3 ml/kg body weight) once a day during 30-day treatment.

LBP (200 mg/kg per day), LBP (20 mg/kg per day), 17-β ES (1 mg/kg per day) or DZ (1 mg/kg per day) were given to both OVX and SHAM groups, and administered orally for 4 weeks, based on previously reports (Bradley et al., 2011; Changbo and Zhaojun, 2012; Cui et al., 2010; Wu et al., 2010). The body weights of all groups were measured once a week until the last day of administration. At the end of all applications, behavioral functions were investigated after last application and using the open field and elevated plus maze tests for measurement of anxiety like behaviors. After the rats were euthanized by decapitation, blood samples and brain tissue specimens were collected.

3.4.1 Open Field Test (OFT)

OFT was performed after the the last day of treatments. A single rat was placed in the center of a black, Plexiglas square measuring 80 cm in length × 80 cm in width × 40 cm in height (Figure 3.9.). The subject discovered the different environment for 15 min in training session. After the training session, rat were exposed the test for 5 min in test session. During the test session, the time spent in center arena and edges were monitored by a video camera (Gkb CC-28905S, Commat LTD. ŞTİ., Ankara/Turkey) and recorded by a videotaped interfaced with EthoVision videotracking system-Noldus Ethovision, Version 6, Netherlands; Commat LTD. ŞTİ. Ankara/Turkey). The area was seperated into sixteen imaginary squares (10.75 × 10.75 cm) by the program, and center area was referred as the middle four squares. The apparatus was wiped clean with 70% ethanol to remove any animal scent (McDermott et al., 2015). During the test session, the frequency of the entry to center area, the number of center entries and time spent in center area was recorded for min and recorded data was calculated by program. Decreased activity indicates increased level of anxiety. Mean velocity and total distance moved in the test session were also recorded and analyzed by the program (Russo et al., 2013a).

Figure 3.9. Open field test

3.4.2 Elevated Plus Maze Test (EPM)

EPM is the most thoroughly utilized test to evaluate anxiety-like behavioral state. It is especially sensitive to anxiety-reducing drugs, such as anxiolytic agents (Walf and Frye, 2007). The structure of EPM apparatus has black color and it composed of two open arms which are crossed by two closed arms of equal size (55 × 10 cm) with 41-cm-high walls as previously described (Guimaraes et al., 2015) (Figure 3.10.). The maze was elevated to a height of 55 cm above floor level. Rats of each group were placed individually in the central area of the EPM to explore for five minutes. Time spent on open arms, time spent on closed arms, the number of open and closed arms entries were recorded by the EthoVision videotracking system-Noldus Ethovision (Gkb CC-28905S, Commat LTD. ŞTİ., Ankara/Turkey). After each test, the apparatus was cleaned with 70% ethanol between trials.

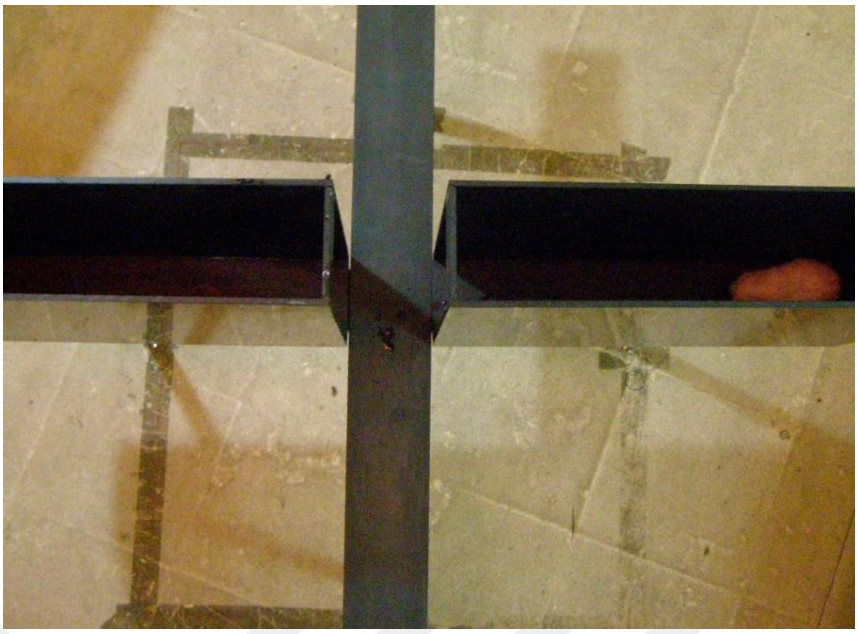

Figure 3.10. Elevated plus maze test

3.5 Study of Depression Like Behaviors Groups

One hundred female Wistar albino rats were arbitrarily seperated into two groups: OVX and SHAM. Each of these group was subdivided five different treatment groups: low and high doses of LBP, 17-β ES, IM and DW. All experimental groups were treated orally by gavage (3 ml/kg body weight) once a day during thirty-day treatment.

LBP (200 mg/kg per day), LBP (20 mg/kg per day), 17-β ES (1 mg/kg per day) or IMP (2.5 mg/kg per day) were given to different OVX and SHAM groups, and administered orally for 4 weeks, based on previously reports (Bradley et al., 2011; Changbo and Zhaojun, 2012; Cui et al., 2010; Wu et al., 2010). The body weights of all groups were measured once a week until the last day of administration. At the end of all applications, behavioural functions were investigated after last application and using the forced swimming tests for measurement of depression like behaviors. After the rats were euthanized by decapitation, blood samples and brain tissue specimens were collected.

3.5.1 Forced Swimming Test (FST)

The FST is mostly used to measure immobility time and evaluate depressive like behavior in rodents. (Figure 3.11.). The subject were gently dropped singly in a glass cylinder containing water that was maintained at 26–28 °C (the cylinder measured 24 cm in diameter and 53 cm in height and contained water to a depth of 40 cm). Following an initial 15-min training session in the water, the rat was removed and placed in its home cage. The following day, the animals were again put in the water for a 5-min session. In the test session, the total time of immobility (floating) were recorded by EthoVision videotracking system- Noldus Ethovision, Version 6, Netherlands; Commat LTD. ŞTİ. Ankara/Turkey (Guimaraes et al., 2015).

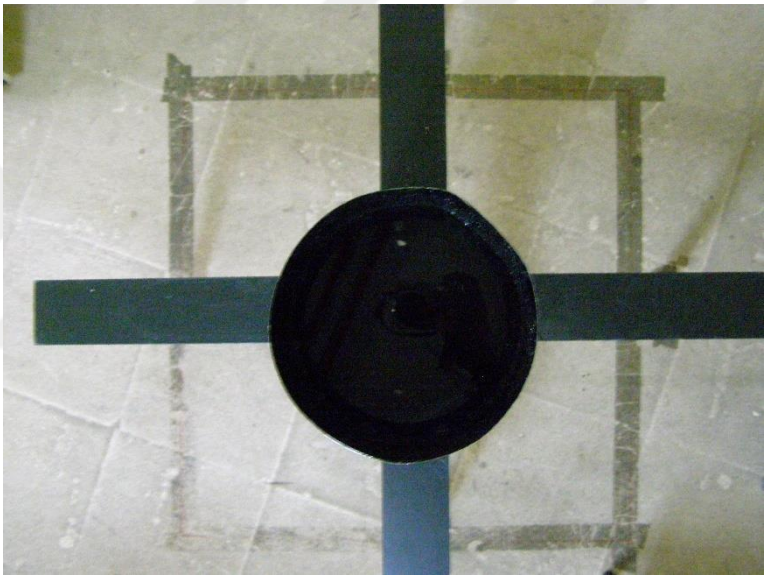

Figure 3.11. Force swimming test

3.6 Study of Learning Like Behaviors Groups

One hundred female rats were randomly seperated into main two groups: OVX and SHAM. These groups were subdivided into five treatment groups: low and high doses of LBP, 17-β ES, DNP and DW. All experimental groups were treated orally by gavage (3 ml/kg body weight) once a day during thirty day treatment. Rats were acclimatized to the environment for 1 week prior to OVX and sham surgeries.

LBP (200 mg/kg per day), LBP (20 mg/kg per day), 17-β ES (1 mg/kg per day) or DNP (1 mg/kg per day) were given to different OVX and SHAM groups, and administered orally for 4 weeks, based on previously studies (Amat-Froster et al., 2017; Bradley et al., 2011; Changbo and Zhaojun, 2012; Cui et al., 2010; Wu et al., 2010). The body weights of all groups were measured once a week until the last day of administration. At the end of all applications, behavioral functions were investigated after last application and using the Morris water maze test for measurement of learning and memory like behaviors. After the rats were euthanized by decapitation, blood samples and brain tissue specimens were collected (Markham et al., 2002).

3.6.1 Morris Water Maze Test (MWM)

The Morris water maze is an apparatus largely used for the experiment of spatial learning and memory behavior that rely on hippocampal function (Kaur et al., 2013). The water maze composed of a black circular tank, 1,5 m in diameter and 60 cm high, filled with 40 cm height of water, and 4 identical areas for the purpose of analysis. A square black escape platform 2 cm below the water level was situated at the centre of one of the four quadrant of the apparatus. 4 different colors and shapes of cues were ensured the animal (Figure 3.12.). The apparatus comprise of 4 consecutive trials for five days with a 1-h inter-trial interval. Rats were allowed to explore for 90 s. If it faled to find platform, they were gently guided to it for waiting 10 s. 1 day after the last training trial, each animal was exposed to the test trial. In test trial, rats were let to exploration for the hidden platform for 60 sec. When it found the platform, rats were allowed to stay for 5 seconds to let them observe the clue of around the platform. The spent times to find the platform on the fifth training trial were determined by EthoVision videotracking system-Noldus Ethovision, Version 6, Netherlands; Commat LTD. ŞTİ. Ankara/Turkey (Figure 3.13.).

Figure 3.12. Morris water maze

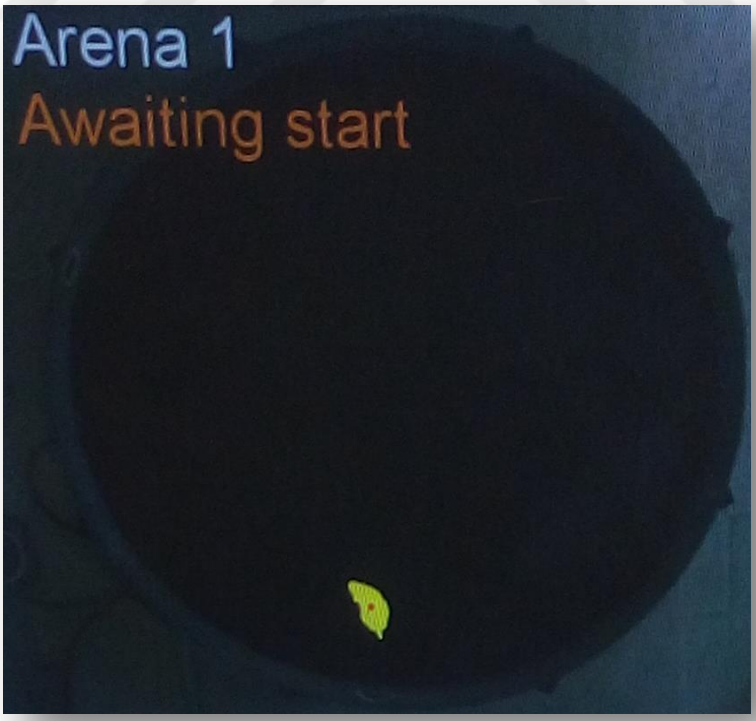

Figure 3.13. Morris water maze video tracking

3.7 Treatments

3.7.1 Distilled Water Treatment

Sixty female rats were randomly divided into 6 groups: anxiety-OVX/SHAM (n=20), depression OVX/SHAM (n=20) and learning-OVX/SHAM (n=20). Ten recovery days after the OVX and SHAM surgeries, all groups were orally fed with DW (3 ml/kg) by oral gavage for thirty consecutive days. After the injections, rats were exposed to behavioral test for mesurements of anxiety, depression and learning like behaviors.

3.7.2 Treatments of *Lycium barbarum* Polysaccharides (LBP)

One hundred twenty female rats were randomly divided into 12 groups: anxiety-OVX/SHAM (n=40), depression OVX/SHAM (n=40) and learning-OVX/SHAM (n=40). Ten recovery days after the OVX and SHAM surgeries, all groups were orally fed with low and high dose LBP by oral gavage for thirty consecutive days. Stock solutions were prepared by dissolving LBP in distilled water. Previous study reported that 200 mg/kg dose and 20 mg/kg of LBP was selected the high and low efective doses (Changbo and Zhaojun, 2012; Cui et al., 2010; Daendee et al., 2013; Wu et al., 2010). After the injections, rats were exposed to behavioral test for mesurements of anxiety, depression and learning like behaviors.

3.7.3 Treatment of 17-β Estradiol

Sixty female rats were randomly divided into 6 groups: anxiety-OVX/SHAM (n=20), depression OVX/SHAM (n=20) and learning-OVX/SHAM (n=20). Ten recovery days after the OVX and SHAM surgeries, all groups were orally fed with 17-β ES (Sigma) by gavage for thirty consecutive days. Stock solutions were prepared by dissolving 17-β ES in 100% ethanol and added to the DW. Final ethanol concentration were adjusted to 1 %. This concentration was indicated that it was a safe and effective way to generation uterotrophic responses in ovariectomized

subjects. (Carmel et al., 2015; Levin-Allerhand et al., 2003). Previous study reported that 1 mg/kg dose of estrogen was selected the minimum dose including anxiolytic effect (Daendee et al., 2013; Kalandadekanond-Thongsong et al., 2012). After the injections, rats were exposed to behavioral test for mesurements of anxiety, depression and learning like behaviors.

3.7.4 Treatments of Drugs

Diazepam (DZ), imipramine (IMP) and donepezil (DNP) were purchased from Sigma (St. Louis, USA). All drugs was dissolved in DW and administered by oral gavage (Kaur et al., 2013).

Sixty animals were divided into main three groups: anxiety, depression and learning-memory. Each of these groups were subdivided into two groups: OVX and SHAM. The first group is anxiety which treated with DZ for its anxiolytic effect. Ten days after the OVX and SHAM surgeries, DZ (1 mg/kg, 3 ml/kg per day) were given orally by gavage method for thirty consecutive days. The second group is depression. The group composed of thirty female rats and all rats were fed with IMP (2.5 mg/kg, 3 ml/kg per day). The drug were used for thirty following days after the recovery time of OVX and SHAM surgeries. The last group is learning and memory, administered with DNP (1 mg/kg, 3 ml/kg per day). It was applied to same procedure and timing with previous drug groups.

3.1 Blood Sampling and Biochemical Assays

All animals were anesthetized using ketamine hydrochloride (50 mg/kg body weight) and xylazine (10 mg/kg body weight) and sacrificed immediately after behavioral test. The systemic blood was collected by cardiac puncture method. The samples were centrifuged to obtain the serum to analyze the 17-β ES and oxidative stress biomarker estimations such as SOD, CAT, GPX and MDA using a commercial kit (Sunred) and a spectrophotometer (Da Silva et al., 2014).

3.1.1 Measurement of E2, SOD, CAT, GPX, MDA Serum Levels

The 17-β ES, SOD, catalase CAT activity and MDA content in the rats serum were measured according to the kit manufacturer's protocol as described previously using ELISA kit (Figure 3.14.) (Patki et al., 2013).

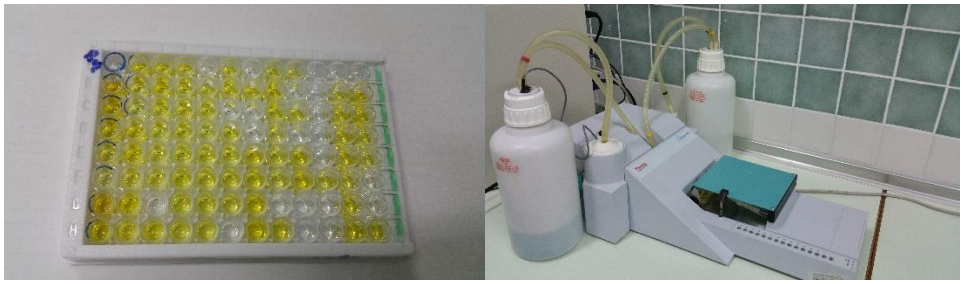

Figure 3.14. ELISA test

3.1.2 Antioxidant Enzymes Activity Quantification

3.1.2.1 Superoxide Dismutase Activity

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of rat SOD in samples. Add SOD to monoclonal antibody enzyme well which is pre-coated with rat SOD monoclonal antibody, incubation; then, add SOD antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add chromogen solution A, B the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the rat substance super oxide dismutase of sample were positively correlated. Activity was assessed by measuring the quantify of the inhibition of superoxide dismutase in a spectrophotometer at 460 nm, as previously described. Results were described as units of SOD/mg protein (Behr et al., 2010; Misra and Fridovich 1972).

3.1.2.2 Catalase Activity

The kit uses ELISA to assay the level of rat CAT in samples. Add CAT to monoclonal antibody enzyme well which is pre-coated with rat CAT monoclonal antibody, incubation; then, add CAT antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add chromogen solution A, B the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the rat substance CAT of sample were positively correlated (Behr et al., 2010).

3.1.2.3 Glutathione Peroxidase Activity

The kit uses a ELISA to assay the level of rat GPx in samples. Add GPx to monoclonal antibody enzyme well which is pre-coated with rat GPx monoclonal antibody, incubation; then, add GPx antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add chromogen solution A, B the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the rat substance super oxide dismutase of sample were positively correlated (Behr et al., 2010).

3.1.2.4 Malondialdehyde Levels

The kit uses a ELISA to assay the level of rat MDA in samples. Add MDA to monoclonal antibody enzyme well which is pre-coated with rat SOD monoclonal antibody, incubation; then, add MDA antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add chromogen solution A, B the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the rat substance super oxide dismutase of sample were positively correlated.

3.2 Tissue Collection and Immunohistochemical Analysis

3.2.1 Tissue Removing Procedure

After performing the OFT, EPM, FST and MWM, five rats from each group were randomly chosen for histological studies. After anesthesia with overdose of ketamin-ksilazin, perfusion–fixation was applied. The skulls of the animals were opened and the brain tissues were removed and placed in 10% neutral formaldehyde and were given code numbers.

After 72 hours, the brain tissues were divided into two lobes and each right lobe of brains were chosen to be analyzed. The tissues were many processed for analyses. Fistly, the tissues were wash and dehydrated in an increment alcohol series, cleared entirely a xylene series. Then, tissues immersed in liquid paraffin and embedding in paraffin blocks. The blocks were cut 5 μm thick using a microtome (Leica RM2125RT) and the aquired sections were brought from deparaffinization to the water and stained with haematoxylin and eosin for histopathological investigation with respect to the conventional photomicroscope (Olympus BX51) and photographed by using camera system (Olympus DP72) by two independent experienced histologists (Figure 3.15.) (Cevik et al., 2015).

Hematoxylin and eosin staining procedure:

1. Slides should be incubated for 30 minutes at 37°C.
2. Xylol – 2 min
3. Xylol – 2 min
4. Absolute alcohol – 1 min
5. Absolute alcohol – 1 min
6. 96% alcohol – 1 min
7. 96% alcohol – 1 min
8. Wash in water - 3 min
9. Haematoxylin – 3-5 min
10. Wash in water
11. Immersion in Acid alcohol – two times

12. Wash in water – 3-5 min
13. 80 % alcohol – 1-2 min
14. Eozin - 15 sec – 2 min
15. 96 % alcohol – 2 min
16. 96 % alcohol – 2 min
17. Absolute alcohol – 2 min
18. Xylol – 2 min
19. Xylol – 2 min

Histopathologic changes were examined in ten randomly selected hipocampus areas of five sections for each group. Evaluations and scoring of the histopathologic changes for hippocampus were level of SER, BDNF, and neuronal death (apoptosis) (Selli et al., 2016).

The levels of SER, BDNF and apoptosis in hippocampal tissue were measured by immunohistochemical staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining methods (Millipore) according to the manufacturer's instructions.

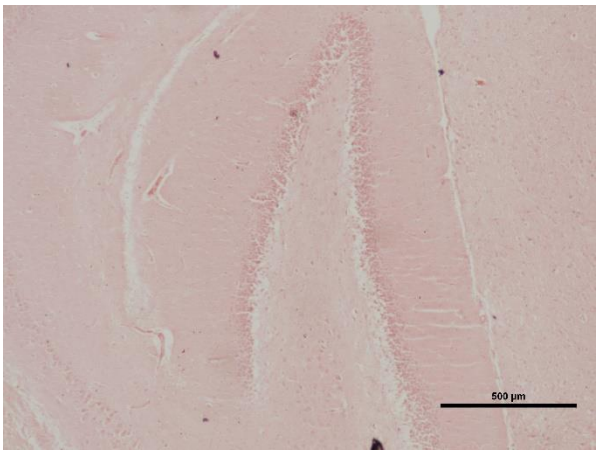

Figure 3.15. Hematoxylen and eosin staining of hippocampus (4X)

The protocol of detection of SER or BDNF by immun staining:

1. Slides should be incubated overnight at 37 °C.
2. First day: Washing
    a. Xylol for 5 min

b. Xylol for 5 min
c. Xylol for 5 min
d. 100% alcohol for 5 min
e. 96% alcohol for 3 min
f. 76% alcohol for 3 min
g. 50% alcohol for 3 min
h. DW for 5 min
i. PBS for 3 min

3. Pretreatment (Boiling)
    a. 10X Citrate Buffer for firstly 5 min at 360 W and secondly for 4 min at 360 W
    b. Waiting for 30 min at room temperature
4. Applying Peroxidase
    a. 3% Hydrogen Peroxide (H_2O_2)in PBS for 5 min at room temperature
    b. Wash with PBS for 5 min two times
    c. Dry specimens
5. Applying Serum-V Block for 10 min
    a. Dry specimens
6. Applying Primary Antibody Solution – overnight at 37 °C.
7. Second day:
    a. Wash with PBS for 5 min two times
    b. Dry Specimens
8. Applying Seconder Solution for 30 min
    a. Wash with PBS for 5 min two times
    b. Dry specimens
9. Applying UltraVision ONE HRP Polymer for 20 min
    a. Wash with PBS for 5 min two times
10. Applying DAB Plus Chromogen and DAB Plus Substrate Mixture for 1 min
    a. Wash two times in DW
11. Counterstaining with Hematoxylen and Eosin (3:1) for 1 min
    a. Wash two times in DW

12. 50% alcohol for 30 s
13. 70% alcohol for 30 s
14. 96% alcohol for 30 s
15. 100% alcohol for 1 min
16. Xylol for 1 min
17. Xylol for 1 min
18. Cover slip using a permanent mounting media.

Evaluation of immunohistochemistry

Immunohistochemistry SER and BDNF staining was identified as either negative or positive. Immunohistochemical positive staining was defined as the detection of brown chromogen on the edge of the hematoxylin-stained cell nucleus, distributed within the cytoplasm or in the membrane and evaluated as previously described (Musumeci et al., 2015b). Digitalpictures were taken with a digital camera (Canon, Japan) at 20×,40× and 63.5× x magnifications (Figure 3.16.).

The protocol of TUNEL staining:

1. Slides should be incubated overnight at 37 °C.
2. Deparaffinization
    a. Xylol for 5 min
    b. Xylol for 5 min
    c. 100% alcohol for 5 min
    d. 96% alcohol for 3 min
    e. 50% alcohol for 3 min
    f. Distille water for 3 min
    g. Phosphate Buffer Solution (PBS) for 3 min
3. Pretreatment (Boiling)
    a. 10X Citrate Buffer for firstly 5 min at 360 W and secondly for 4 min at 360 W
4. Waiting for 30 min at room temperature
5. Applying Peroxidase
    a. 3% H_2O_2 in PBS for 5 min at room temperature
    b. Wash with PBS for 5 min two times

6. Applying Equilibration Buffer
    a. Dry specimens
    b. Apply Equilibration Buffer (75 µl/5 cm^2)
    c. Incubate 10 sec at room temperature
7. Applying TDT Enzyme
    a. Dry sections
    b. Apply 55 µl/5cm^2 of TDT enzyme
    c. Incubate at 37 °C for 1 hour
8. Applying Stop/Wash Buffer
    a. Prepare a coplin jar containing stop wash buffer aqitate for 15 sec
    b. Incubate for 10 min
    c. Wash in 3 changes of PBS each for 1 min
    d. Dry sections
9. Applying Anti-Digoxigenin Conjugate
    a. 65 µl/5cm^2 of apply anti-digoxigenin conjugate
    b. Incubate for 30 min at room temperature
10. Washing in PBS
    a. Wash in 4 changes of PBS in a coplin jar for 2 min per wash at room temperature
11. Development of color in Proxidase Substrate
    a. Dry sections
    b. Apply 75µl/5cm^2 of Peroxidase Substrate
    c. Wait for 3 to 6 min at room temperature
12. Washing
    a. Wash in 3 changes of distiled water for 1 min each wash
    b. İncubate the slide in 3 changes of distilled water in a coplin jar for 5 minutes at room temperature
13. Counterstaining
    a. Counterstain in 0.5% (w:v) methyl green in a coplin jar for 10 min at room temperature (22 ± 2 °C)

b. Wash the specimen in 3 changes of distilled water in a coplin jar, dipping the slide 10 times each in the first and second washes, followed by 30 sec without agitation in the third wash
c. Wash the specimen in 3 changes of 100% *N*-Butanol in a coplin jar, dipping the slide 10 times each in the first and second washes, followed by 30 sec without agitation in the third wash

14. Mount Specimen
    a. Dehydrate the specimen by moving the slide through three jars of XYLENE, incubating for 2 min in each jar
    b. Remove the slides one at a time from the coplin jar. Gently tap the edge of the slide to drain, but do not allow the specimen to dry
    c. Mount under a glass coverslip in a mounting medium
15. View Under Microscope

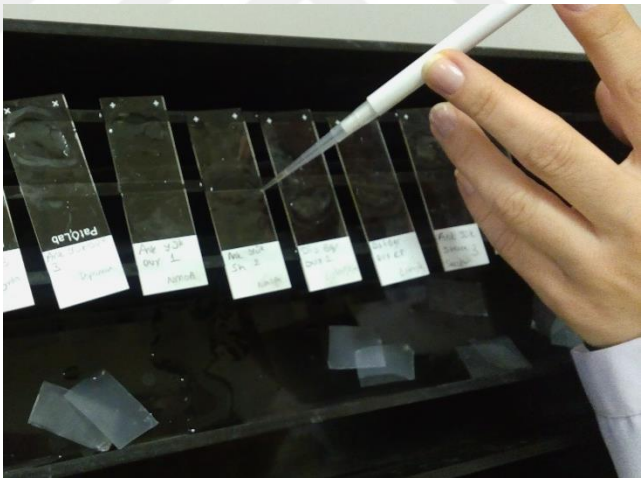

Figure 3.16. Immune staining

3.3 Statistical Analysis

Statistical analysis was performed using IBM SPSS 20.0 version for Windows (SPSS Statistical Software, SPSS Inc., Los Angeles, CA, USA, Ver. 19.0). Data were analyzed by 2 operations (sham and ovx) X 5 treatments (low and high doses of LBP, one of the drugs, 17-β ES, DW) ANOVA analysis with Tukey and

Bonferonni tests for post hoc multiple comparisons. The data are presented as the mean ± SEM. A P-value < 0.05 was considered to be statistically significant (Liu et al., 2015)

4. RESULTS AND DISCUSSION

4.1 RESULTS OF BEHAVIORAL STUDY

4.1.1 Result of Anxiety Study

4.1.1.1 Open Field Measurements

4.1.1.1.1 Total Distance Travelled on The Open Field (TDTO)

When all the groups studied, treatment had no significant effect on TDTO, $F(4, 81) = 1.84$, $p > 0.12$. Also, operation had no significant effect, $F(1, 81) = 2.05$, $p > 0.16$.

In addition, there was no significant interaction effect between treatment and operation groups, $F(4, 81) = 1.29$, $p > 0.27$ (Figure 4.1.).

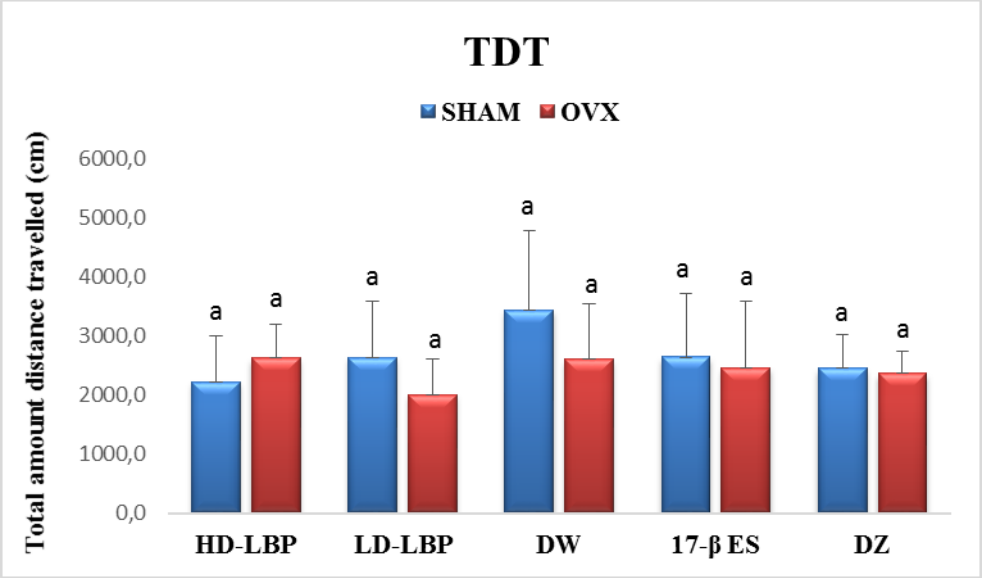

Figure 4.1. Mean (S.E.M.) total amount distance travelled (TDT-OF). Light blue color bar represents sham operated condition; red color bar represents ovariectomy operated condition. Mean-values with the same letters within vertical columns are not significantly different ($p > 0.05$).

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

Treatment condition had no significant effect on time spent at the edge of the open field, F (4, 81) = 1.31, p = 0.27.

Operation condition had significant effect on time spent at the edge of the open field, F (1, 81) = 17.47, p = 0.0001, η^2 = 0.18. The subjects in the sham operated groups spent less time at the edge of the open field than those in the ovx operated groups (M_{SHAM} = 4.77 < M_{OVX} = 4.91).

Interaction effect between treatment and operation was no significant, F (4, 81) = 1.18, p > 0.32 (Figure 4.2.).

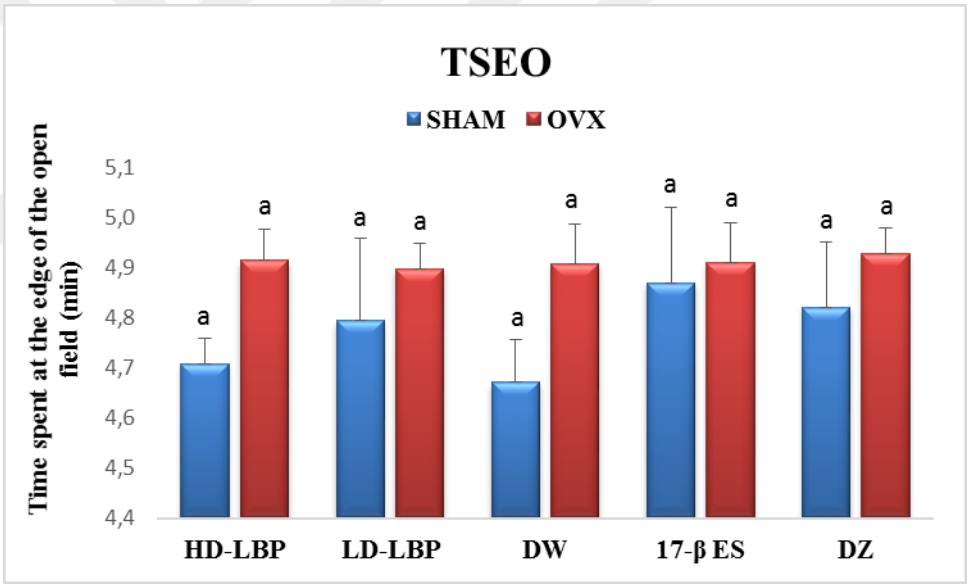

Figure 4.2. Mean (S.E.M.) time spent at the edge of the open field (TSEO-OF). Mean-values with the same letters within vertical columns are not significantly different (p > 0.05).

4.1.1.2 Time Spent at the Center of the Open Field (TSC)

Treatment condition had no significant effect on time spent at the edge of the open field, F (4, 81) = 1.25, p = 0.29.

Operation condition had also no significant effect on time spent at the edge of the open field, F (1, 81) = 1.59, p = 0.21.

The interaction effect between treatment and operation was no significant, F(4,81) = 1.20, p = 0.32 (Figure 4.3.).

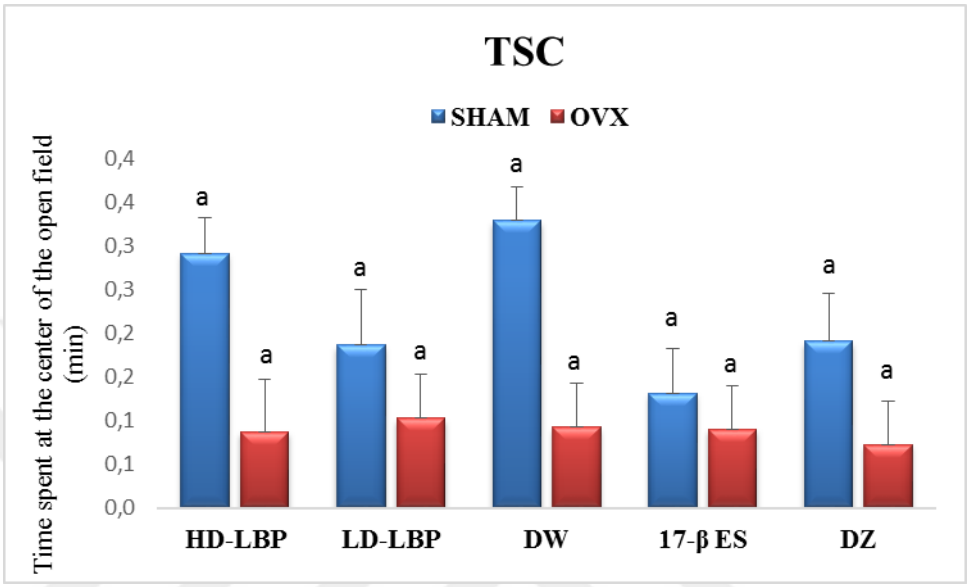

Figure 4.3. Mean (S.E.M.) time spent at the center of the open field (TSC-OF). Mean-values with the same letters within vertical columns are not significantly different (p > 0.05).

4.1.1.3 Mobility Duration

Treatment had significant effect on mobility time on the open field, F(4, 78) = 5.33, p = 0.001, η^2 = 0.21. The Bonferonni test showed that the subjects in the DW condition was more mobile than those in the other treatment conditions (M_{DW} = 0.009 > $M_{HD\text{-}LBP}$ = 0.003, $M_{LD\text{-}LBP}$ = 0.003, $M_{17\text{-}\beta\ ES}$ = 0.002, M_{DZ} = 0.003).

On the other hand, operation condition had no significant effect on mobility time on the open field, F (1, 78) = 0.48, p = 0.49.

The interaction effect between treatment and operation also was not significant, F(4, 78) = 0.78, p > 0.54 (Figure 4.4.).

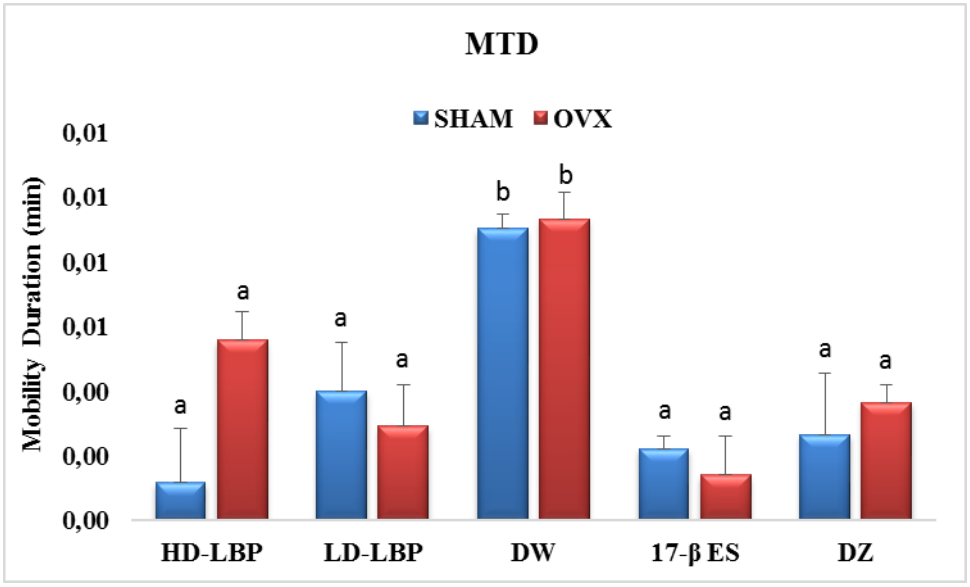

Figure 4.4. Mean (S.E.M.) the mobility duration of center in the open field (MTD-OF). Mean-values with the same letters within vertical columns are significantly different ($p < 0.05$).

4.1.1.4 Velocity (VEL)

Treatment condition had no significant effect on velocity in open field, $F(4, 81) = 1.82$, $p = 0.13$.

Also, operation condition had no significant effect on velocity in open field, $F(1, 81) = 3.09$, $p = 0.08$. Despite being marginal significant, the subjects in the SHAM group tend to be faster than those in OVX group.

The interaction effect between treatment and operation was not significant, $F(4, 81) = 1.23$, $p = 0.30$ (Figure 4.5.).

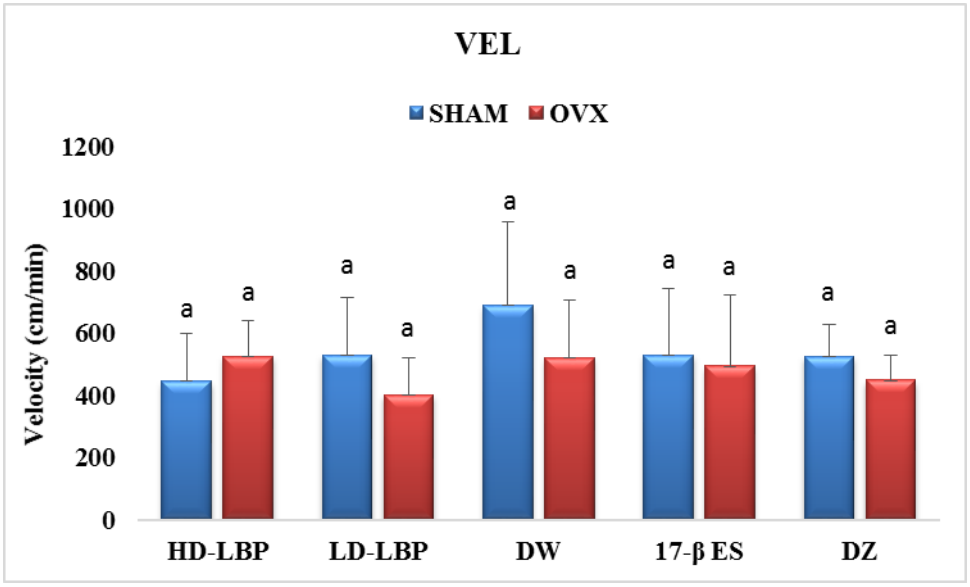

Figure 4.5. Mean (S.E.M.) the velocity of the subjects in the open field (VEL-OF). Mean-values with the same letters within vertical columns are not significantly different ($p > 0.05$).

4.1.1.5 Elevated Plus Maze Measurements (EPM)

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

Treatment condition had no significant effect on total distance travelled on the elevated plus maze, $F(4, 78) = 2.16$, $p = 0.08$.

However, the main effect of operation had a significant effect on total distance travelled on the elevated plus maze, $F(1, 78) = 5.26$, $p = 0.02$, $\eta^2 = 0.06$. The Bonferonni test showed that the subjects in the SHAM operation travelled more distance than those in the OVX operation ($M_{SHAM} = 1389.15 > M_{OVX} = 1216.40$). This means that subjects in SHAM operation condition were less anxious than those in the OVX operation condition.

The interaction effect between treatment and operation was marginally significant, $F(4,78) = 2.26$, $p = 0.07$. Despite being not significant, subjects in DW treated in the SHAM operated condition travelled more distance than those in the OVX operated condition, total distance travelled of the subjects in the HD-LBP treated, 17-β ES treated and DZ treated groups in the SHAM operated condition was

the same as those in the ovx operated conditions ($M_{HD\text{-}LBP\text{-}SHAM}$ = 1442.60, $M_{HD\text{-}LBP\text{-}OVX}$ = 1356.75; $M_{17\text{-}\beta\text{-}ES\text{-}SHAM}$ = 1120.17, $M_{17\text{-}\beta\ ES\text{-}OVX}$ = 1093.37; $M_{DZ\text{-}SHAM}$ = 1210.45, $M_{DZ\text{-}OVX}$ = 1330.31) (Figure 4.6.).

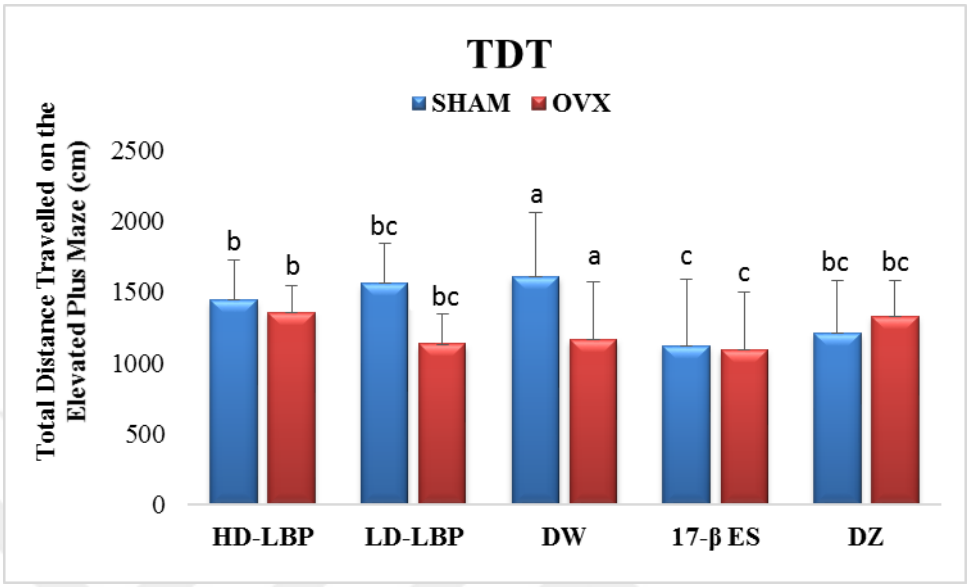

Figure 4.6. Mean (S.E.M.) total amount distance travelled (TDT-EPM). Mean-values with the same letters within vertical columns are significantly different ($p < 0.05$).

4.1.1.5.2 Time Spent in Closed Arms (TSCA)

The main effect of treatment had no significant on the time spent in closed arms, F (4, 78) = 1.49, p = 0.21.

The main effect of operation had also no significant on the time spent in closed arms, F (1, 78) = 1.41, p = 0.24.

The interaction effect between treatment and operation was not significant, F(4, 78) = 2.09, p = 0.09. Despite being not significant, subjects in the DW treated groups in sham operated condition spent less time in closed arms than those in the ovx operated groups, subjects in the HD-LBP treated, time spent in closed arms of the subjects in the HD-LBP treated, 17-β ES treated and DZ treated groups in the sham operated condition was close those in the ovx operated conditions ($M_{HD\text{-}LBP\text{-}}$

$_{SHAM}$ = 4.31, $M_{HD\text{-}LBP\text{-}OVX}$ = 4.09; $M_{17\text{-}\beta\text{-}ES\text{-}SHAM}$ = 4.32, $M_{17\text{-}\beta\ ES\text{-}OVX}$ = 4.40; $M_{DZ\text{-}SHAM}$ = 4.26, $M_{DZ\text{-}OVX}$ = 4.07) (Figure 4.7.).

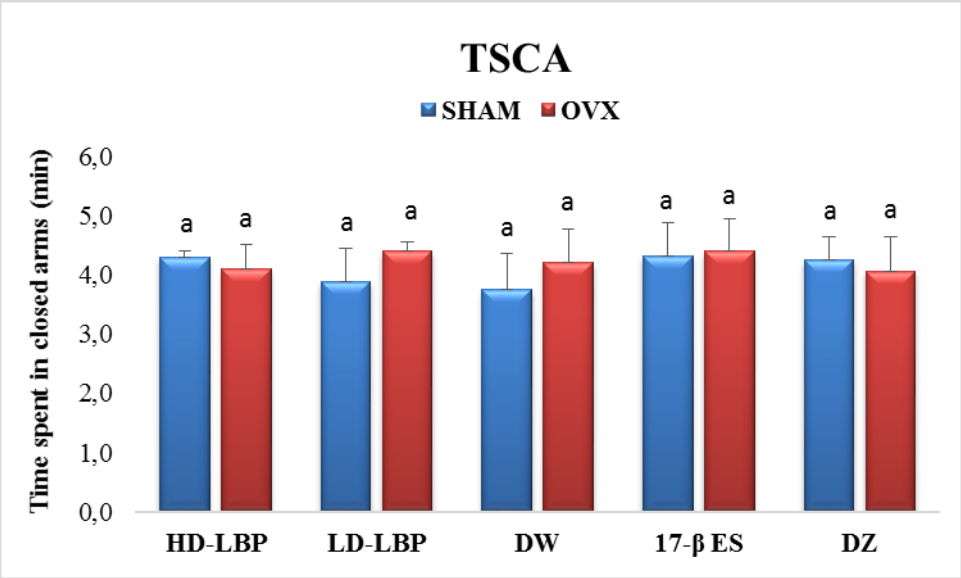

Figure 4.7. Mean (S.E.M.) Time spent in the closed arms on the elevated plus maze (TSCA-EPM). Mean-values with the same letters within vertical columns are not significantly different (p > 0.05).

4.1.1.6 Time Spent in Open Arms (TSOA)

The main effect of treatment was not significant on the time spent in open arm, F (4, 78) = 1.50, p = 0.21.

The main effect of operation was not significant on the time spent in open arm, F (1, 78) = 1.40, p = 0.24.

The interaction effect between treatment and operation was not significant, F (4, 78) = 2.10, p > 0.09. Although there was no significant interaction effect between treatment and operation group, the subjects in the DW treated groups in the sham operated condition spent more time in open arms than those in the ovx operated condition ($M_{DW\text{-}SHAM}$ = 1.25 > $M_{DW\text{-}OVX}$ = 0.80). However, the subjects in HD-LBP, 17-β ES and DZ groups in the sham operated condition tent to spend close time in the open arms than those in the ovx operated condition ($M_{HD\text{-}LBP\text{-}SHAM}$ = 0.69, $M_{HD\text{-}LBP\text{-}OVX}$ = 0.91; $M_{17\text{-}\beta\ ES\text{-}SHAM}$ = 0.68, $M_{17\text{-}\beta\ ES\text{-}OVX}$ = 0.60; $M_{DZ\text{-}SHAM}$ = 0.74, $M_{DZ\text{-}OVX}$ = 0.93) (Figure 4.8.).

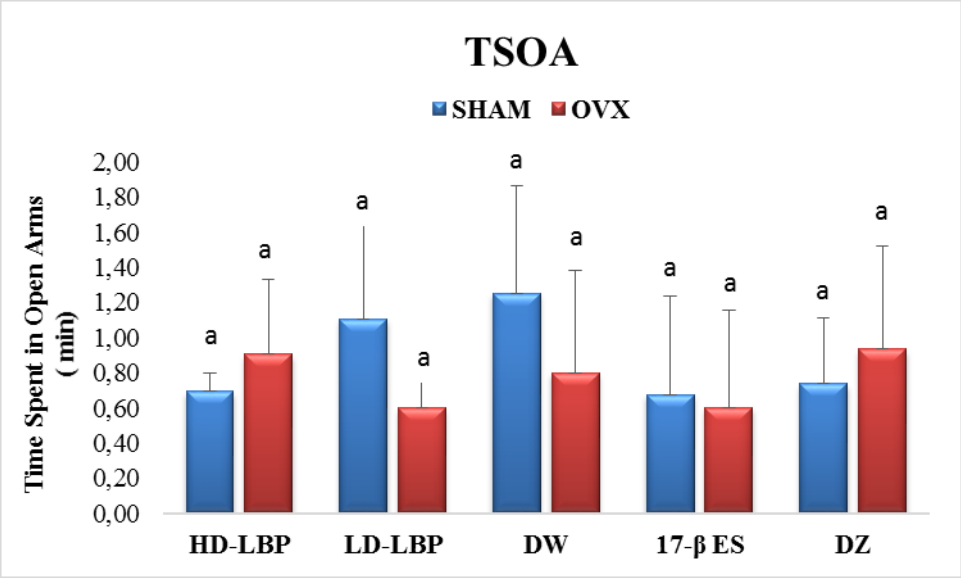

Figure 4.8. Mean (S.E.M.) Time spent in the open arm on the elevated plus maze (TSOA-EPM). Mean-values with the same letters within vertical columns are not significantly different ($p > 0.05$).

4.1.1.6.1 Mobility in Elevated Plus Maze (MT)

The main effect of treatment was significant on the mobility, $F(4, 73) = 4.69$, $p = 0.002$, $\eta^2 = 0.20$. The Bonferonni test showed that the subjects in the HD-LBP and LD-LBP treated groups ($M_{HD\text{-}LBP} = 0.04$ and $M_{LD\text{-}LBP} = 0.04$) were more mobile than those in the DW treated groups ($M_{DW} = 0.01$). This means that the subjects in the HD-LBP and LD-LBP treated groups were less anxious than those in the DW treated groups.

The main effect of operation was significant on the mobility duration in the elevated plus maze, $F(1, 73) = 12.20$, $p = 0.001$, $\eta^2 = 0.14$. The subjects in the sham operated groups were more mobile than those in the ovx operated groups ($M_{SHAM} = 0.04 > M_{OVX} = 0.02$).

The interaction effect between treatment and operation was significant, $F(4, 73) = 6.96$, $p > 0.0001$, $\eta^2 = 0.28$. The subjects in HD-LBP and LD-LBP treated groups in sham operated condition were more mobile than those in the ovx operated condition ($M_{HD\text{-}LBP\text{-}SHAM} = 0.06$, $M_{HD\text{-}LBP\text{-}OVX} = 0.01$; $M_{LD\text{-}LBP\text{-}SHAM} = 0.06$, $M_{LD\text{-}LBP\text{-}OVX} = 0.02$). In the 17-β ES and DZ treated groups, there was a little difference

between the sham and ovx operated conditions ($M_{17\text{-}\beta\ ES\text{-}SHAM}$ = 0.03, $M_{17\text{-}\beta\ ES\text{-}OVX}$ = 0.03; $M_{DZ\text{-}SHAM}$ = 0.03, $M_{DZ\text{-}OVX}$ = 0.03) (Figure 4.9.).

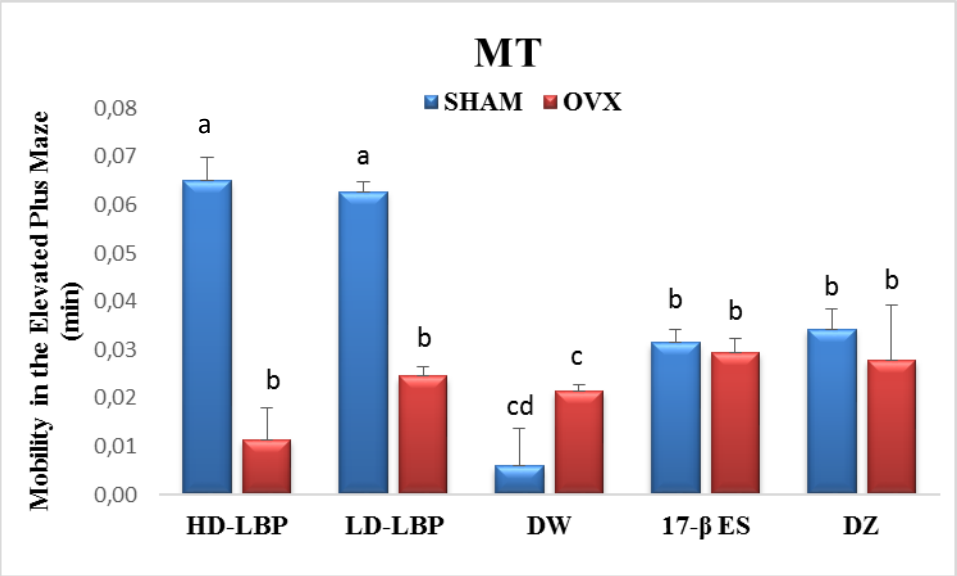

Figure 4.9. Mean (S.E.M.) Mobility time in the elevated plus maze (MT-EPM). Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.1.1.6.2 Velocity in the Elevated Plus Maze (VEL)

The main effect of treatment was not significant on the velocity, F (4, 78) = 1.54, p = 0.20.

The main effect of operation was significant on the velocity, F (1, 78) = 5.22, p = 0.02, η^2 = 0.06. The subjects in the sham operated groups were faster than those in the subjects in the ovx operated groups (M_{SHAM} = 280.70 > M_{OVX} = 246.00). This means that ovx operation caused the anxiety like behavior in the female rats.

The interaction effect between treatment and operation was not significant, F (4, 78) = 2.21, p = 0.07. Although subjects in DW treated groups in the sham operated condition was faster than those in the ovx condition ($M_{DW\text{-}SHAM}$ = 322.72 > $M_{DW\text{-}OVX}$ = 234.21), velocity of subjects in the HD-LBP treated, 17-β ES treated and DZ treated groups in the sham operated condition was close to those in the ovx operated

condition ($M_{HD\text{-}LBP\text{-}SHAM}$ = 289.25, $M_{HD\text{-}LBP\text{-}OVX}$ = 272.63; $M_{17\text{-}\beta\ ES\text{-}SHAM}$ = 232.42, $M_{17\text{-}\beta\ ES\text{-}OVX}$ = 228.97; $M_{DZ\text{-}SHAM}$ = 245.66, $M_{DZ\text{-}OVX}$ = 267.13) (Figure 4.10.).

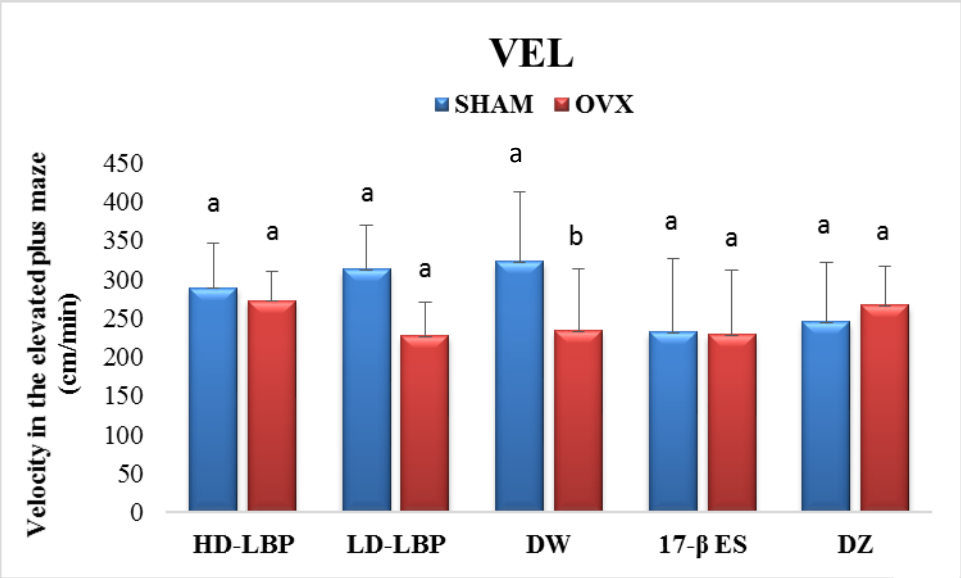

Figure 4.10. Mean (S.E.M.) Velocity in elevated plus maze (VEL-EPM). Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.1.2 Result of Depression Study

4.1.2.1 Forced Swim Test (Porsolt) Measurements

4.1.2.1.1 Total Distance Travelled on the Porsolt (TDT)

The main effect of treatment was significant on the total distance travelled, $F(4, 85) = 11.09$, $p = 0.0001$, $\eta^2 = 0.34$. The subjects in the IMP treatment groups travelled more distance than HD-LBP, LD-LBP and 17-β ES treatment groups (M_{IMP} = 2859.58, $M_{HD\text{-}LBP}$ = 2329.86, $M_{LD\text{-}LBP}$ = 2365.05 and $M_{17\text{-}\beta\ ES}$ = 2063.60). This means that IMP treatment condition were less depressive than those in HD-LBP, LD-LBP and 17-β ES treatment condition.

Hovewer, operation had significant effect on the total distance travelled parameter, $F(1, 85) = 22.81$, $p = 0.0001$, $\eta^2 = 0.21$. The subjects in the sham operated groups travelled more distance than those in the ovx oprated groups (M_{SHAM} =

2677.43 > M_{OVX} = 2258.99). This means that the subjects in sham operated groups were less depressive than subject in ovx operated.

The interaction effect between treatment and operation was not significant, F (4, 85) = 1.19, p = 0.30 (Figure 4.11.).

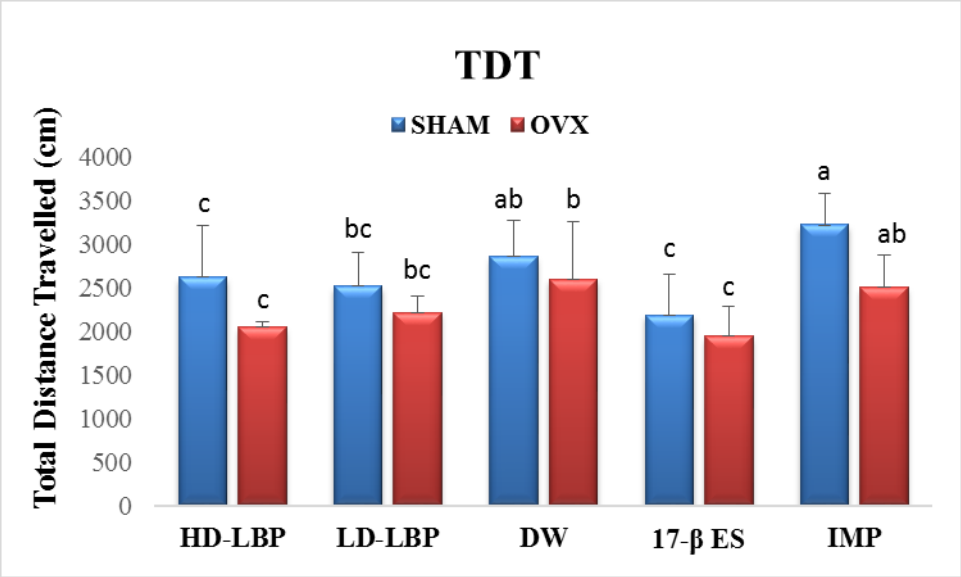

Figure 4.11. Mean (S.E.M.) Total distance travelled on the porsolt (TDT-FS). Mean- values with the same letters within vertical columns are significantly different ($p<0.05$).

4.1.2.1.2 Immobility Duration (ITD)

The main effect of treatment was significant on the immobility duration, F (4, 84) = 20.85, p = 0.0001, η^2 = 0.50. Bonferonni test showed that subject in the IMP treatment groups were less immobile than those in the HD-LBP, DW and 17-β ES treatment groups (M_{IMP} = 4.45 < $M_{HD\text{-}LBP}$ = 4.72 < M_{DW} = 4.77 < $M_{17\text{-}\beta\ ES}$ = 4.88). This means that the subject in IMP treatment condition was less depressive than those in the HD-LBP, DW and 17-β ES treatment condition.

The main effect of the operation was significant in the terms of immobility duration in porsolt, F (1, 84) = 41.31, p=0.0001 η^2 = 0.33. The subjects in the sham operated groups were less immobile than those in the ovx operated groups (M_{SHAM} = 4.59 < M_{OVX} = 4.79). This means that the subjects in the sham operated groups were less depressive than those in ovx operated groups.

The interaction effect between treatment and operation was significant, F (4, 84) = 3.99, p = 0.005, η^2 = 0.16. The subjects in the IMP treated groups in sham operated condition were less immobile than those in the ovx operated condition ($M_{IMP\text{-}SHAM}$ = 4.23 < $M_{IMP\text{-}OVX}$ = 4.68) (Figure 4.12.).

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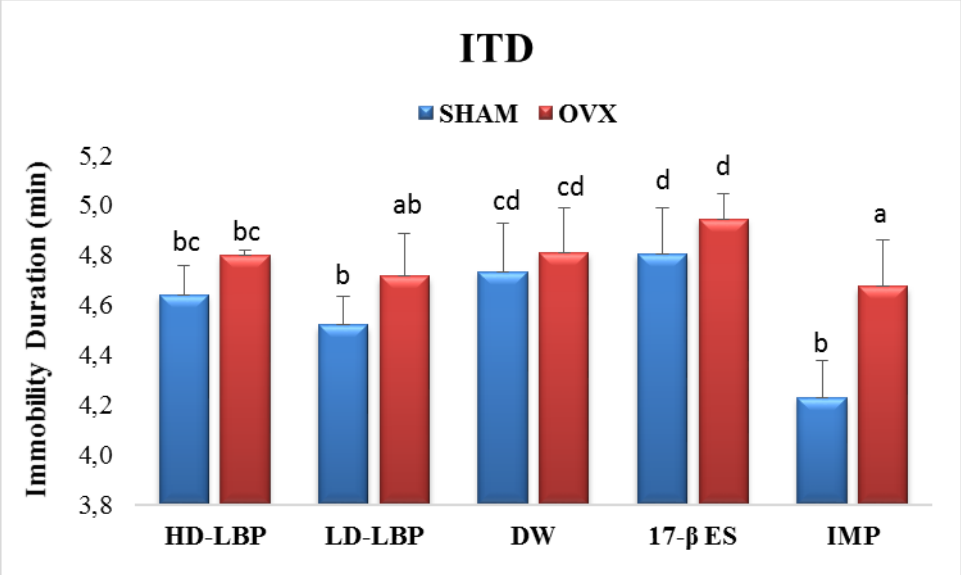

Figure 4.12. Mean (S.E.M.) Immobility duration on the porsolt (ITD-FS). Mean-values with the same letters within vertical columns are significantly different (p<0.05).

4.1.2.1.3 Velocity (VEL)

The main effects of treatments were significant on the velocity, F (4, 85) = 11.35, p = 0.0001, η^2 = 0.35. Bonferonni test showed that subject in the IMP treatment groups were faster than those in the HD-LBP, LD-LBP and 17-β ES treatment groups (M_{IMP} = 585.50 < $M_{LD\text{-}LBP}$ = 482.87 < $M_{HD\text{-}LBP}$ = 473.83 < $M_{17\text{-}\beta\ ES}$ = 410.62). This means that the subjects in IMP treatment condition was less depressive than those in the LD-LBP, HD-LBP and 17-β ES treatment condition.

The main effects of operations were also significant on the velocity, F(1, 85) = 22.90, p = 0.0001, η^2 = 0.21. The subjects in the sham operated groups were faster than those in the ovx operated groups (M_{SHAM} = 542.14 > M_{OVX} = 455.49). This means that sham operated groups were less depressive than ovx operated groups.

The interaction effect between treatment and operation was significant, F (4, 85) = 1.17, p > 0.32. The subjects in IMP treated groups in sham operated condition were faster than those in the ovx operated condition ($M_{IMP\text{-}SHAM}$ = 658.14 < $M_{IMP\text{-}OVX}$ = 512.86) (Figure 4.13.).

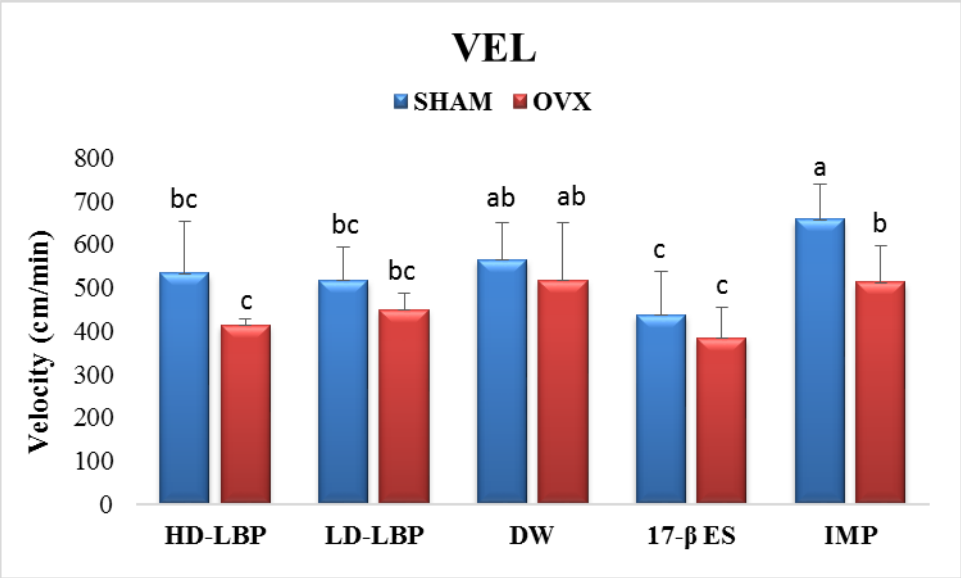

Figure 4.13. Mean (S.E.M.) Velocity on the porsolt (VEL-FS). Mean-values with the same letters within vertical columns are significantly difference (p < 0.05).

4.1.3 Result of Learning and Spatial Memory Study

4.1.3.1 Morris Water Maze Test Measurements

4.1.3.1.1 Total Distance Travelled (TDT)

The main effects of treatments were significant on the total distance travelled, F (4, 85) = 21.68, p = 0.0001, η^2 = 0.51. The Bonferonni test showed that the subjects in the DW treatment groups travelled more distance than those in the other treatment groups (M_{DW} = 339.60 > M_{DNP} = 199.16 > $M_{17\text{-}\beta\ ES}$ = 172.97 > $M_{HD\text{-}LBP}$ = 94.21 > $M_{LD\text{-}LBP}$ = 58.97) (Figure 4.14).

However, the main effects of operation was not significant on the total distance travelled, F(1, 85) = 1.73, p = 0.19. The subjects in the sham operated

groups were travelled less distance than those in the ovx operated groups (M_{SHAM} = 159.17 < M_{OVX} = 186.80).

The interaction effect between treatment and operation was not significant, F (4, 85) = 1.94, p > 0.11.

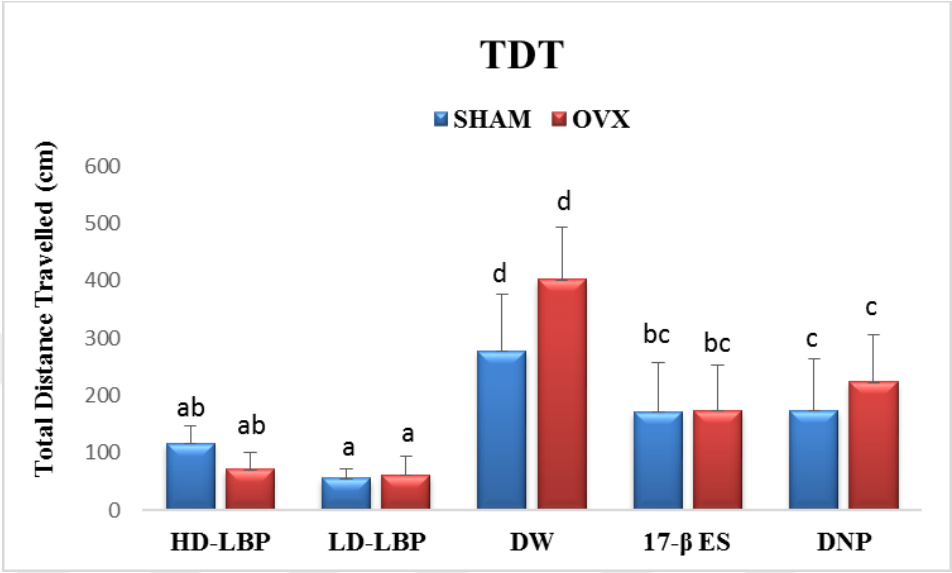

Figure 4.14. Mean (S.E.M.) Total distance travelled on the Morris Water Maze (TDT-MWM). Mean-values with the same letters within vertical columns are significantly different (p<0.05).

4.1.3.1.2 Time Spent to Find the Platform (TSFP)

The main effect of treatment was significant on time spent to find the platform, F(4, 85) = 17.90, p = 0.0001, η^2 = 0.46. The Bonferonni test showed that the subjects in the HD-LBP treated groups spent less time to find platform than those in the DW treated and DNP treated groups ($M_{HD\text{-}LBP}$ = 0.06 < M_{DNP} = 0.19 < M_{DW} = 0.25). This means that HD-LBP treatment had positive effect on spatial memory of the female rats.

The main effect of the operation was not significant on the time spent to find the platform, F(1, 85) = 1.29, p = 0.26.

The interaction effect between treatment and operation was not significant, F (4, 85) = 1.54, p > 0.20. The subjects in the DW treated groups in SHAM operated

condition spent less time to find platform than those in the OVX operated condition ($M_{DW\text{-}SHAM}$ = 0.20 < $M_{DW\text{-}OVX}$ = 0.30) (Figure 4.15.).

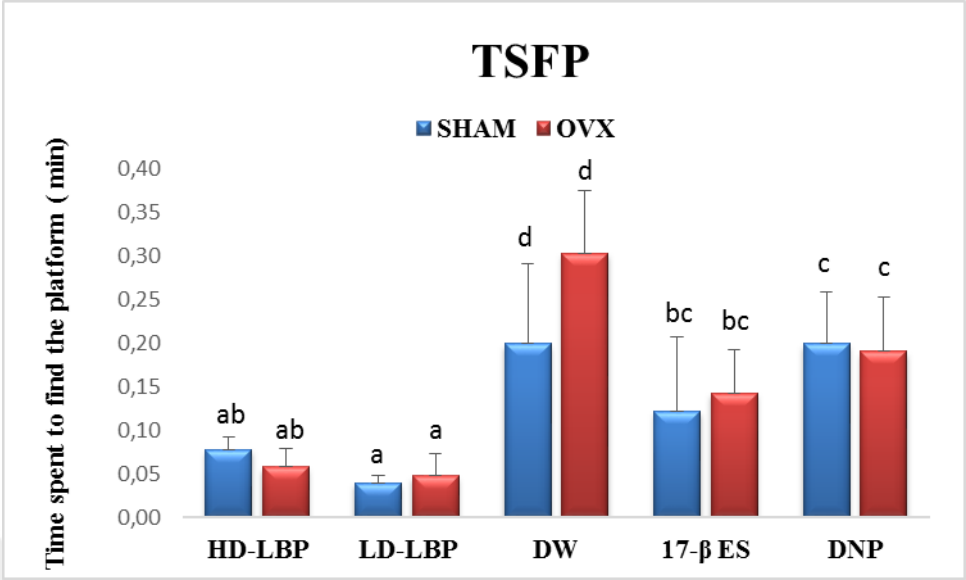

Figure 4.15. Mean (S.E.M.) Time spent to find the platform on the water maze (TSFP-WMW). Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.1.3.1.3 Time Spent in the Correct Quadrant (TSCQ)

The main effect of treatment was significant on time spent in the correct quadrant, $F(4, 85) = 17.90$, $p = 0.002$, $\eta^2 = 0.18$. The Bonferonni test showed that the subjects in the LD-LBP treated groups were spent less time in the correct quadrant than those in the DW treated ($M_{LD\text{-}LBP} = 0.03 < M_{Dw} = 0.08$). This means that LD-LBP treatment had a positive effect on spatial memory of the female rats.

The main effect of the operation was significant on the time spent in the correct quadrant, $F(1.85) = 3.58$, $p = 0.06$, $\eta^2 = 0.18$. The subjects in the SHAM operated groups were spent less time in the correct quadrant than those in the OVX operated groups ($M_{SHAM} = 0.04 < M_{OVX} = 0.06$)

The interaction effect between treatment and operation was not significant, $F (4, 85) = 3.67$, $p = 0.008$, $\eta^2 = 0.15$. The subjects in the DW treated groups in SHAM operated condition spent more time in the correct quadrant than those in the OVX operated condition ($M_{DW\text{-}SHAM} = 0.05 > M_{DW\text{-}OVX} = 0.04$) (Figure 4.16.).

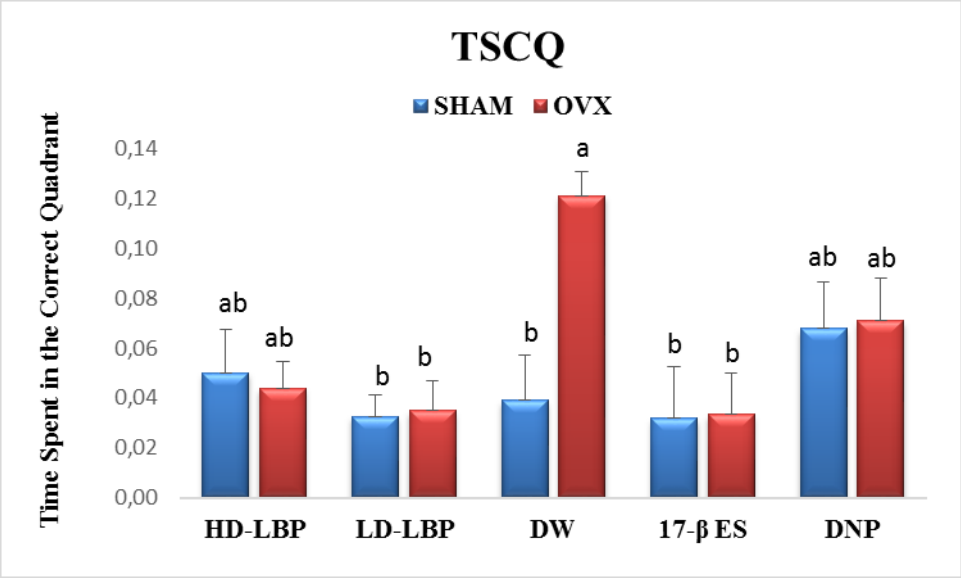

Figure 4.16. Mean (S.E.M.) Time spent in the correct quadrant on the Morris water maze (TSCQ-MWM). Mean-values with the same letters within vertical columns are significantly different ($p < 0.05$).

4.1.3.1.4 Velocity on the Water Maze (VEL)

The main effect of treatment was not significant on velocity, $F(4, 85) = 1.51$, $p = 0.21$.

The main effect of the operation was significant on velocity, $F(1, 85) = 4.84$, $p = 0.03$, $\eta^2 = 0.05$. The subject in the sham operated groups were faster than ovx operated groups ($M_{SHAM} = 1485.62 > M_{OVX} = 1359.46$).

The interaction effect between treatment and operation was not significant, $F(4, 85) = 0.29$, $p > 0.80$ (Figure 4.17.).

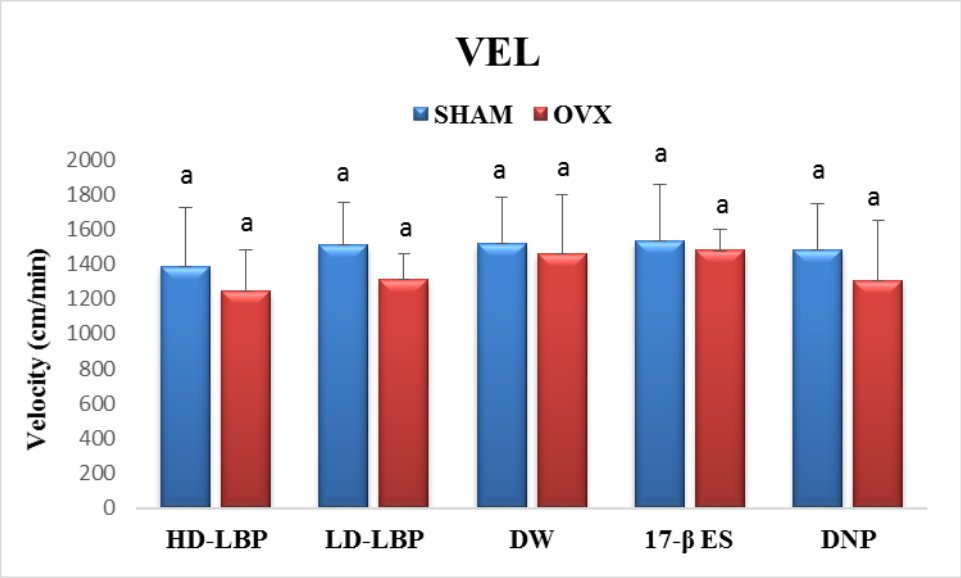

Figure 4.17. Mean (S.E.M.) velocity on the water maze (VEL-MWM). Mean-values with the same letters within vertical columns are significantly different ($p < 0.05$).

4.2 Results of Biochemical Study

4.2.1 Results of Anxıety Study

4.2.1.1 SOD Activity Measurements

The main effect of treatment was significant on SOD enzyme activity in the serum of rats, $F(4, 41) = 4.18$, $p = 0.006$, $\eta^2 = 0.29$. The serum of subjects in the HD-LBP treated groups had higher amount of SOD enzyme activity than those in the 17-β ES treated groups ($M_{HD\text{-}LBP} = 11.85 > M_{17\text{-}\beta\ ES} = -2.45$).

The main effect of the operation was not significant on SOD enzyme activity in the blood serum, $F(1, 41) = 1.21$, $p = 0.27$. Subject in the SHAM operated groups had higher amount of SOD enzyme activity than OVX operated groups ($M_{SHAM} = 6.38 > M_{OVX} = 3.76$).

The interaction effect between treatment and operation was not significant, $F(4, 41) = 2.03$, $p > 0.10$. The serum of the subjects in the HD-LBP and DZ treated

groups in the ovx operated condition had higher amount of the SOD enzyme activity than those in the sham operated condition. ($M_{HD\text{-}LBP\text{-}SHAM}$ = 9.57 < $M_{HD\text{-}LBP\text{-}OVX}$ = 14.13; $M_{DZ\text{-}SHAM}$ = 1.65 < $M_{DZ\text{-}OVX}$ = 5.68). On the other hand, there is a trend that 17-beta estradiol in SHAM group was higher than that in OVX group; however there were no significant differences in other conditions (Figure 4.18.).

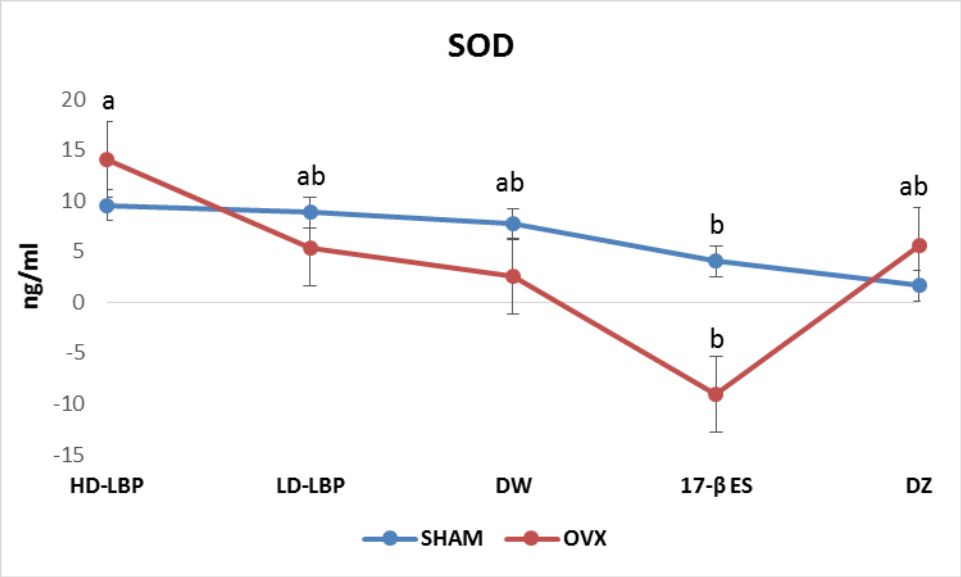

Figure 4.18. Mean (S.E.M.) SOD enzyme activity in anxiety study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.1.2 CAT Activity Measurements

The main effect of treatment was significant on CAT enzyme activity in the blood serum, $F(4, 41) = 11.31$, $p = 0.0001$, $\eta^2 = 0.53$. Blood serum of subjects in the 17-β ES treated groups had lower amount of CAT enzyme activity than those in the other treated groups ($M_{17\text{-}\beta\ ES} = 6.45 < M_{LD\text{-}LBP} = 43.15 < M_{DW} = 48.56 < M_{HD\text{-}LBP} = 49.52 < M_{DZ} = 57.63$).

The main effect of the operation was not significant on CAT enzyme activity in the blood serum, $F(1, 41) = 0.36$, $p = 0.50$.

The interaction effect between treatment and operation was not significant, $F(4, 41) = 1.91$, $p = 0.12$. Despite being not significant, SHAM subjects tend to have higher CAT activity than OVX group in HD-LBP, whereas OVX subjects had higher

CAT activity than SHAM group in LD-LBP. On the other hand, there were no significant differences for other conditions (Figure 4.19.).

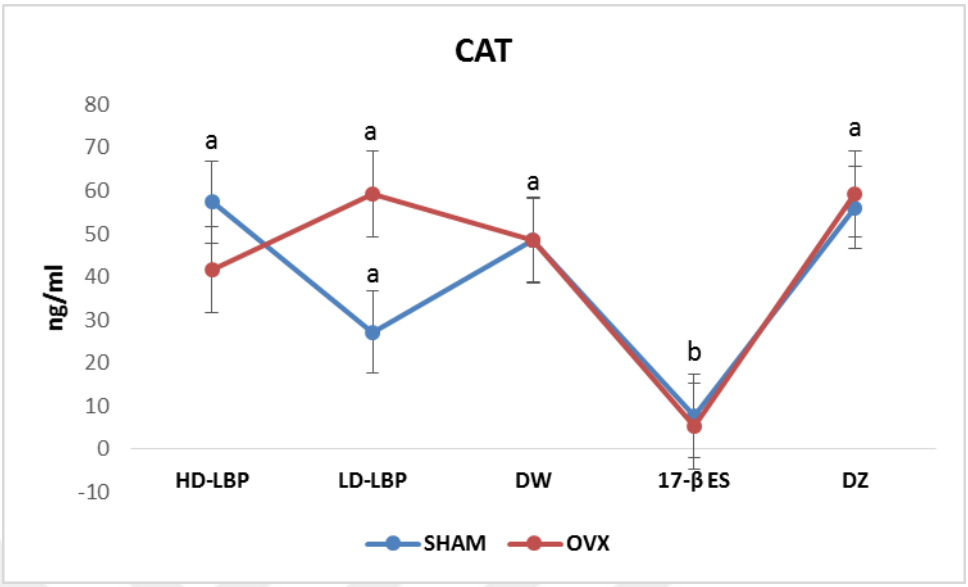

Figure 4.19. Mean (S.E.M.) CAT enzyme activity in anxiety study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.1.3 GPX Activity Measurements

The main effect of treatment was significant on GPX enzyme activity in the blood serum, $F(4, 41) = 43.89$, $p = 0.0001$, $\eta^2 = 0.81$. Blood serum of subjects in the 17-β ES treated groups had lower amount of GPX enzyme activity than those in the other treatment groups ($M_{17\text{-}\beta\ ES} = -5.25 < M_{DW} = 11.85 < M_{DZ} = 40.85 < M_{LD\text{-}LBP} = 45.08 < M_{HD\text{-}LBP} = 48.60$).

The main effect of the operation was not significant on GPX enzyme activity in the blood serum, $F(1, 41) = 0.64$, $p > 0.43$.

The interaction effect between treatment and operation was not significant, $F(4, 41) = 2.05$, $p > 0.10$ (Figure 4.20.).

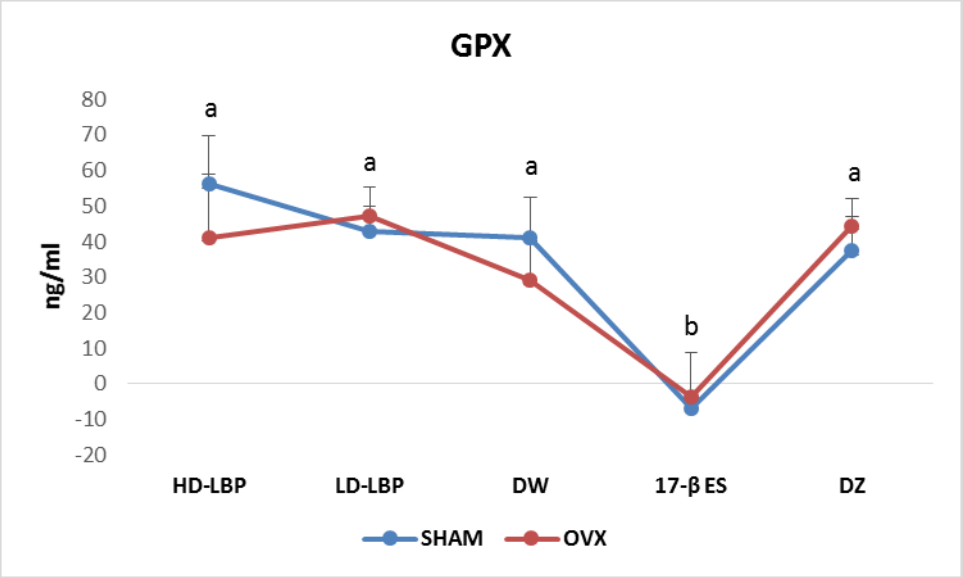

Figure 4.20. Mean (S.E.M.) GPX enzyme activity in anxiety study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.1.4 17- β Estradiol Measurement

The main effect of treatment was not significant on 17-β ES enzyme activity in the blood serum, $F(4, 41) = 0.14$, $p > 0.97$.

The main effect of the operation was also not significant on 17-β ES enzyme activity in the blood serum, $F(1, 41) = 0.01$, $p > 0.91$.

In addition, the interaction effect between treatment and operation was not significant, $F(4, 41) = 0.70$, $p > 0.60$ (Figure 4.21.).

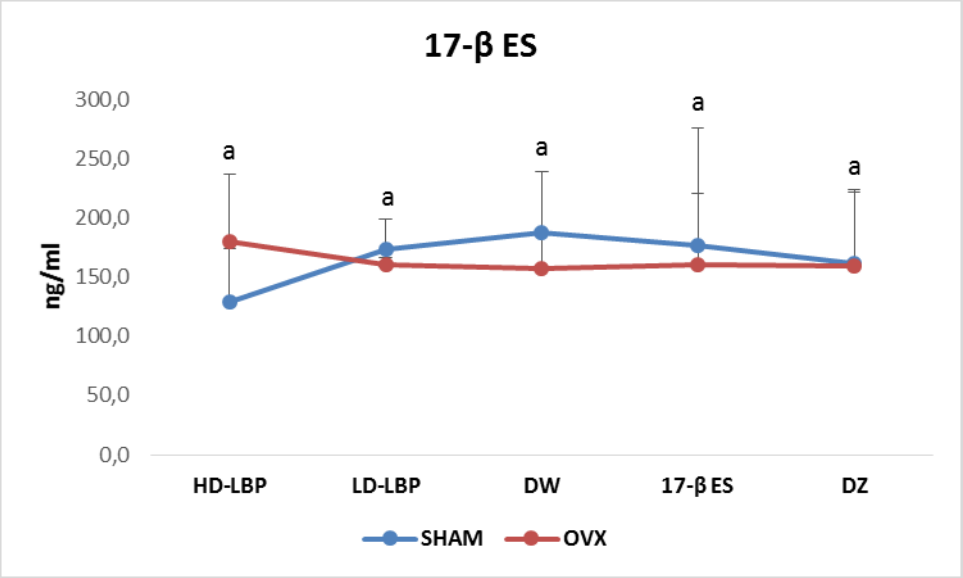

Figure 4.21. Mean (S.E.M.) 17 β ES level in anxiety study. Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.2.1.5 MDA Activity Measurements

The main effect of treatment was also not significant on MDA enzyme activity in the blood serum, $F(4, 41) = 0.73$, $p > 0.58$.

The main effect of the operation was not significant on MDA enzyme activity in the blood serum, $F(1, 41) = 0.03$, $p > 0.86$.

The interaction effect between treatment and operation was significant, $F(4, 41) = 2.90$, $p = 0.03$. Although the level of the MDA activity in the serum of the subjects in the HD-LBP treated in the SHAM operated condition had lower than those in the OVX operated condition ($M_{HD\text{-}LBP\text{-}SHAM} = -2.74 < M_{HD\text{-}LBP\text{-}OVX} = 4.04$), the level of the MDA activity in the serum of the subjects in the LD-LBP treated in the SHAM operated condition had higher than those in the OVX operated condition ($M_{LD\text{-}LBP\text{-}SHAM} = 5.47 > M_{LD\text{-}LBP\text{-}OVX} = -1.06$) (Figure 4.22.).

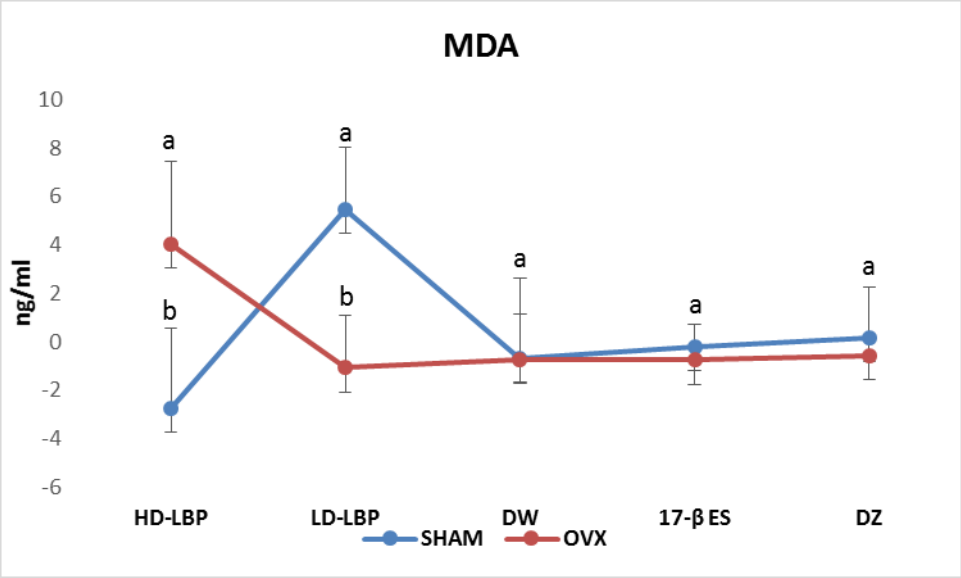

Figure 4.22. Mean (S.E.M.) MDA level in anxiety study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.2 Result of Depression Study

4.2.2.1 SOD Activity Measurements

The main effect of treatment was significant on SOD enzyme activity in the blood serum, $F(4, 47) = 4.16$, $p = 0.006$, $\eta^2 = 0.26$. Blood serum of subjects in the HD-LBP treated, 17-β ES treated and IMIP treated groups had higher amount of SOD enzyme activity than those in the DW treated groups ($M_{17\text{-}\beta\ ES} = 8.10 > M_{HD\text{-}LBP} = 7.86 > M_{IMIP} = 7.63 > M_{DW} = -5.85$).

The main effect of the operation was not significant on SOD enzyme activity in the blood serum, $F(1, 47) = 0.41$, $p = 0.50$.

An interaction effect between treatment and operation was not significant, $F(4, 47) = 0.95$, $p = 0.44$ (Figure 4.23.).

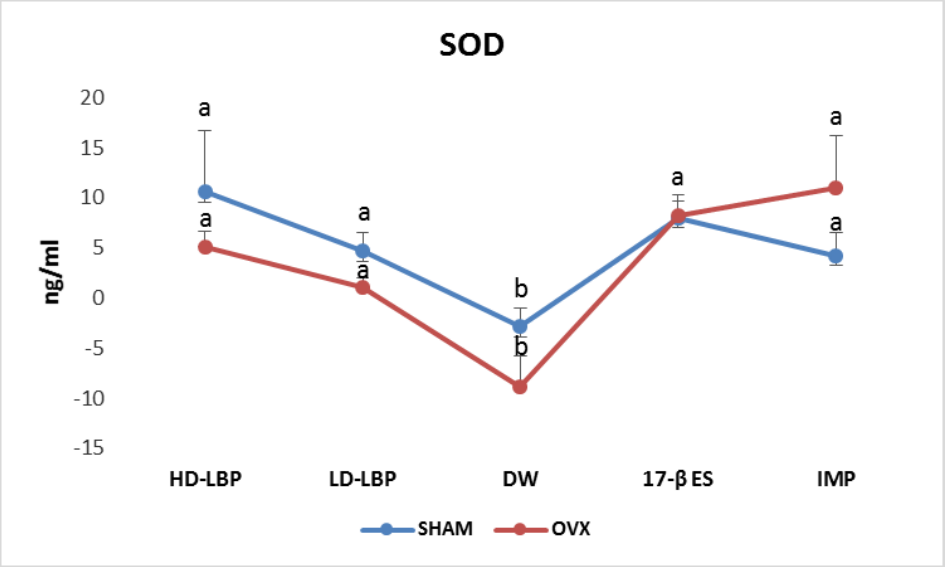

Figure 4.23. Mean (S.E.M.) SOD enzyme activity in depression study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.2.2 CAT Activity Measurements

The main effect of treatment was significant on CAT enzyme activity in the blood serum, $F(4, 48) = 27.30$, $p = 0.0001$, $\eta^2 = 0.69$. The blood serum of subjects in the 17-β ES treated groups had lower amount of CAT enzyme activity than those in the other treated groups ($M_{17\text{-}\beta\ ES} = 10.61 < M_{IMIP} = 68.83 < M_{DW} = 69.16 < M_{HD\text{-}LBP} = 69.33 < M_{LD\text{-}LBP} = 73.71$).

The main effect of the operation was significant on the CAT enzyme activity in the blood serum, $F(1, 48) = 5.00$, $p = 0.03$, $\eta^2 = 0.09$. The subject in the SHAM operated groups had higher amount of CAT enzyme activty than OVX operated groups ($M_{SHAM} = 63.56 > M_{OVX} = 53.10$).

The interaction effect between treatment and operation was not significant, $F(4, 48) = 1.79$, $p = 0.15$ (Figure 4.24.).

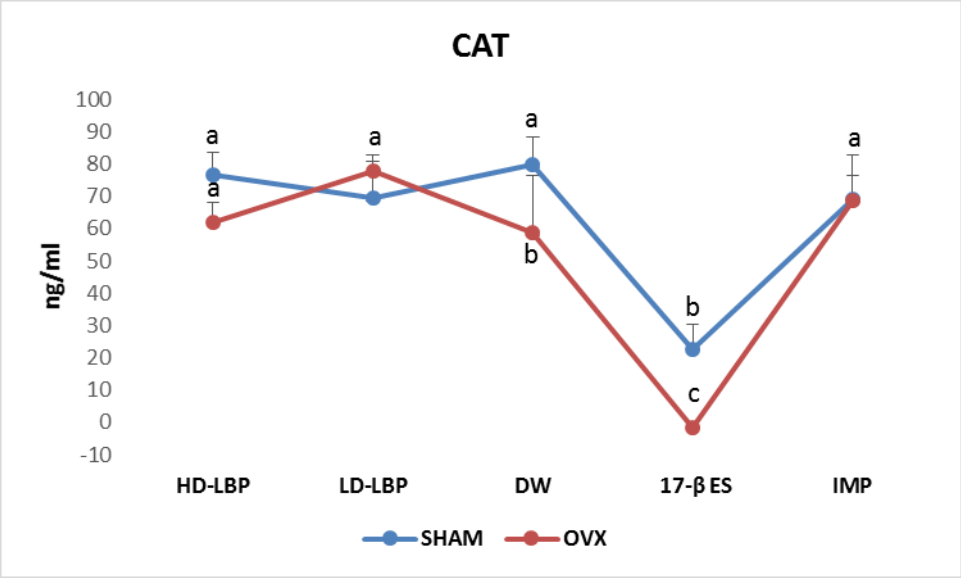

Figure 4.24. Mean (S.E.M.) CAT enzyme activity in depression study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.2.3 GPX Activity Measurements

The main effect of treatment was significant on GPX enzyme activity in the blood serum, $F(4, 48) = 4.03$, $p = 0.007$, $\eta^2 = 0.25$. Blood serum of subjects in the 17-β ES treated groups had lower amount of GPX enzyme activity than those in the DW treated groups ($M_{17\text{-}\beta\ ES} = 5.81 < M_{DW} = 32.89$).

The main effect of the operation was not significant on GPX enzyme activity in the blood serum, $F(1, 48) = 0.001$, $p = 0.96$.

The interaction effect between treatment and operation was significant, $F(4, 48) = 4.91$, $p = 0.002$, $\eta^2 = 0.29$. Although level of the GPX activity in the serum of the subjects in the HD-LBP treated in the SHAM operated condition had lower amount of GPX enzyme activity than those in the OVX operated condition ($M_{HD\text{-}LBP\text{-}OVX} = 41.03$, $M_{HD\text{-}LBP\text{-}SHAM} = 5.91$), the amount of the GPX activity in the serum of the subjects in the LD-LBP treated in the SHAM operated condition had higher amount of GPX enzyme activity than those in the OVX operated condition ($M_{LD\text{-}LBP\text{-}OVX} = 10.31$, $M_{LD\text{-}LBP\text{-}SHAM} = 39.83$) (Figure 4.25.).

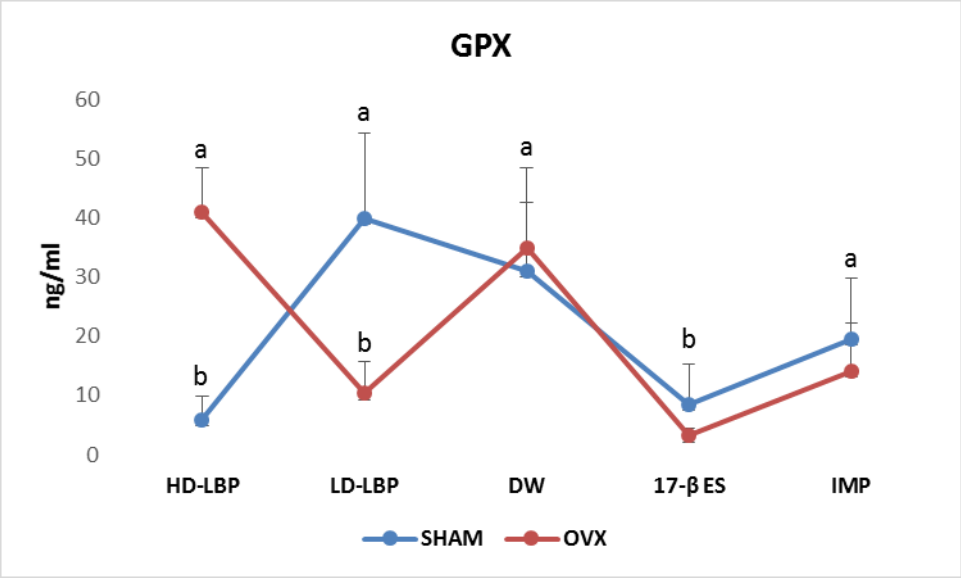

Figure 4.25. Mean (S.E.M.) GPX enzyme activity in depression study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.2.4 17-β Estradiol Measurements

The main effect of treatment was not significant on 17-β ES enzyme activity in the blood serum, $F(4, 48) = 0.28$, $p > 0.89$.

The main effect of the operation was not significant on 17-β ES enzyme activity in the blood serum, $F(1, 48) = 0.65$, $p > 0.43$.

The interaction effect between treatment and operation was not significant, $F(4, 48) = 1.10$, $p = 0.37$ (Figure 4.26.).

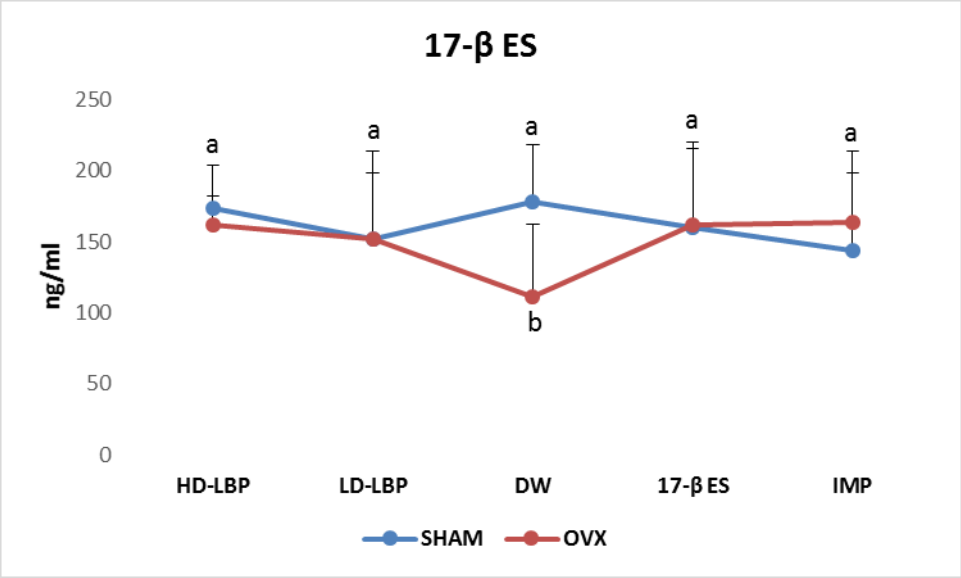

Figure 4.26. Mean (S.E.M.) 17-β ES level in depression study. Mean-values with the same letters within vertical columns are significantly different (p>0.05).

4.2.2.5 MDA Activity Measurements

The main effect of treatment was significant on MDA enzyme activity in the blood serum, $F(4, 48) = 5.50$, $p = 0.001$, $\eta^2 = 0.31$. Blood serum of subjects in the HD-LBP treated groups had lower amount of MDA enzyme activity than those in the LD-LBP treated groups ($M_{HD\text{-}LBP} = 1.13 < M_{LD\text{-}LBP} = 1.08$). In addition, serum of subjects in the DW and HD-LBP treated groups had lower amount of MDA enzyme activity than those in the IMP treated groups ($M_{DW} = -1.3 < M_{HD\text{-}LBP} = 1.13 < M_{IMP} = 2.99$).

The main effect of the operation was not significant on MDA enzyme activity in the blood serum, $F(1, 48) = 0.000$, $p > 0.99$.

The interaction effect between treatment and operation was not significant, $F(4, 48) = 0.48$, $p = 0.75$ (Figure 4.27.).

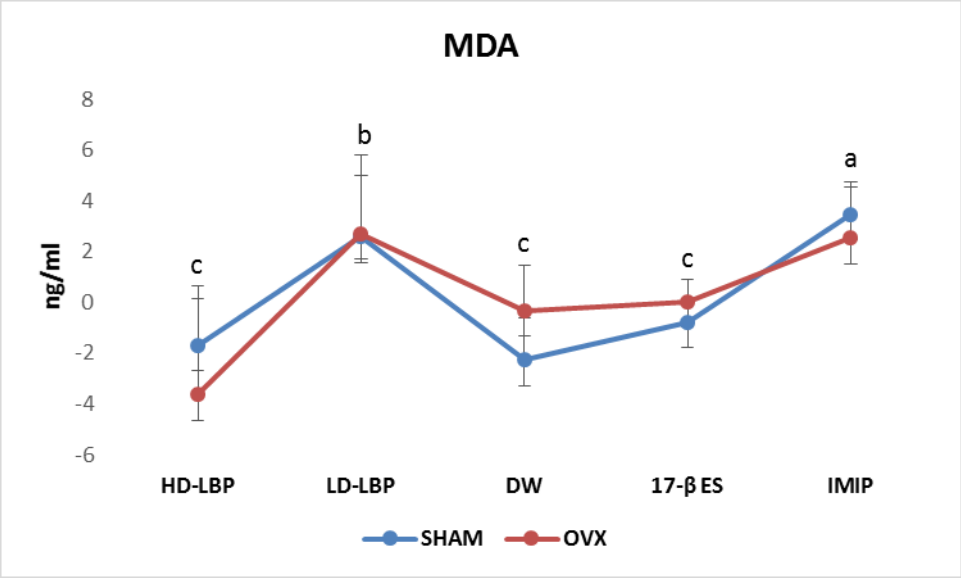

Figure 4.27. Mean (S.E.M.) MDA level in depression study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.2.6 Results of Learning and Memory Study

4.2.2.7 SOD Activity Measurements

The main effect of treatment was significant on SOD enzyme activity in the blood serum, $F(4, 51) = 5.04$, $p = 0.002$, $\eta^2 = 0.28$. Blood serum of subjects in the HD-LBP treated and 17-β ES treated groups had higher amount of SOD enzyme activity than those in the LD-LBP treated groups ($M_{HD\text{-}LBP} = 6.29 > M_{17\text{-}\beta\ ES} = 6.18 > M_{LD\text{-}LBP} = -8.12$).

The main effect of the operation was significant on SOD enzyme activity in the blood serum, $F(1, 51) = 7.84$, $p = 0.007$, $\eta^2 = 0.13$. The subjects in the SHAM operated groups had lower amount of SOD enzyme activity than those in the OVX operated groups ($M_{SHAM} = -5.62 > M_{OVX} = 2.45$).

The interaction effect between treatment and operation was not significant, $F(4, 51) = 1.00$, $p = 0.40$ (Figure 4.28.).

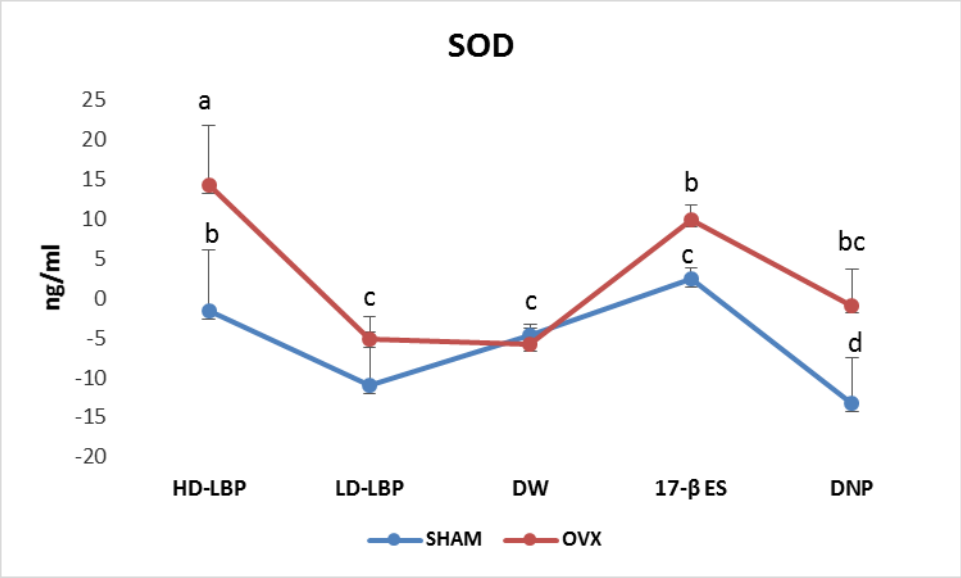

Figure 4.28. Mean (S.E.M.) SOD enzyme activity in learning study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.2.8 CAT Activity Measurements

The main effect of treatment was not significant on CAT enzyme activity in the blood serum, $F(4, 51) = 2.27$, $p = 0.07$. Despite being not significant, DNP treated groups had lower CAT ctivity than other groups.

The main effect of the operation was not significant on CAT enzyme activity in the blood serum, $F(1, 51) = 3.17$, $p = 0.08$. Despite being not significant, SHAM operated group had higher CAT activity than OVX operated groups.

The interaction effect between treatment and operation was not significant, $F(4, 51) = 2.27$, $p = 0.11$. Despite being not significant, the level of the CAT activity in the serum of the subjects in the HD-LBP treated in the SHAM operated condition had lower than those in the OVX operated condition, the level of the CAT activity in the serum of the subjects in the LD-LBP, 17-β ES and DNP treated in the SHAM operated condition had higher than those in the OVX operated condition ($M_{\text{HD-LBP-SHAM}} = 24.57 < M_{\text{HD-LBP-OVX}} = 34.48$; $M_{\text{LD-LBP-SHAM}} = 36.20 > M_{\text{LD-LBP-OVX}} = 23.93$, $M_{\text{17-β ES-SHAM}} = 29.79$, $M_{\text{17-β ES-OVX}} = 14.85$; $M_{\text{DNP-SHAM}} = 26.42 > M_{\text{DNP-OVX}} = -3.47$) (Figure 4.29.).

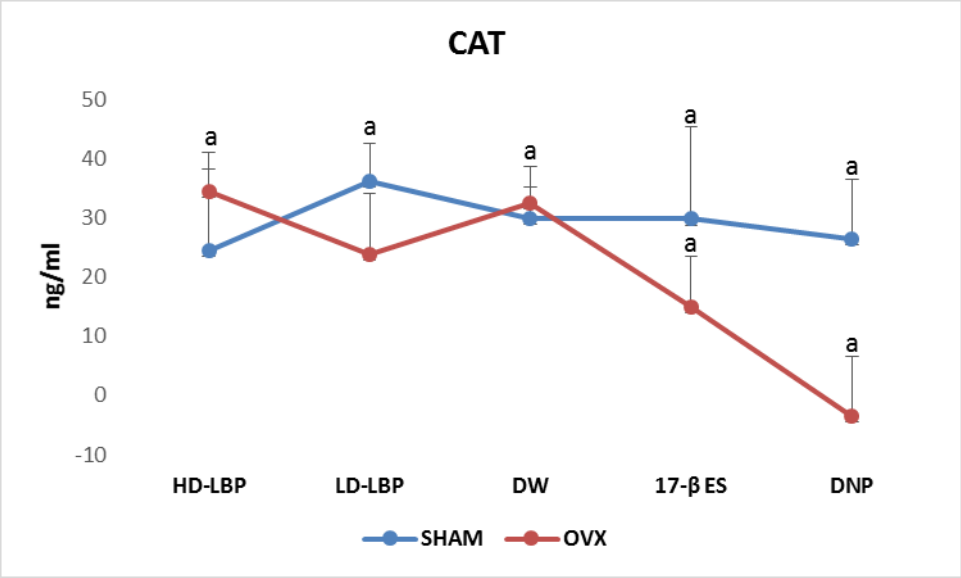

Figure 4.29. Mean (S.E.M.) CAT enzyme activity in learning study. Mean-values with the same letters within vertical columns are not significantly different ($p>0.05$).

4.2.2.9 GPX Activity Measurements

The main effect of treatment was not significant on GPX enzyme activity in the blood serum, $F(4, 51) = 2.07$, $p = 0.09$. Despite being not significant, the subjects in DNP treated groups tend to have higher GPX activity than other treatment groups.

The main effect of the operation was not significant on GPX enzyme activity in the blood serum, $F(1, 51) = 0.98$, $p = 0.33$. Despite being not significant, SHAM subjects tend to have higher GPX activity than OVX group.

An interaction effect between treatment and operation was not significant, $F(4, 51) = 1.32$, $p = 0.28$. Despite being not significant, sham subjects in DW and LD-LBP groups tend to have higher GPX activity than other sham groups ($M_{LD\text{-}LBP\text{-}SHAM} = -1.22$, $M_{LD\text{-}LBP\text{-}OVX} = -4.87$; $M_{DW\text{-}SHAM} = 0.15 > M_{DW\text{-}OVX} = 12.99$), whereas the level of the GPX activity in the serum of the subjects in the HD-LBP treated, 17-β ES and DNP treated in the sham operated condition was close those in the ovx operated condition ($M_{HD\text{-}LBP\text{-}SHAM} = -9.26$, $M_{HD\text{-}LBP\text{-}OVX} = -8.17$, $M_{17\text{-}\beta\ ES\text{-}SHAM} = -6.39$, $M_{17\text{-}\beta\ ES\text{-}OVX} = -4.27$, $M_{DNP\text{-}SHAM} = 0.99$, $M_{DNP\text{-}OVX} = 2.29$) (Figure 4.30.).

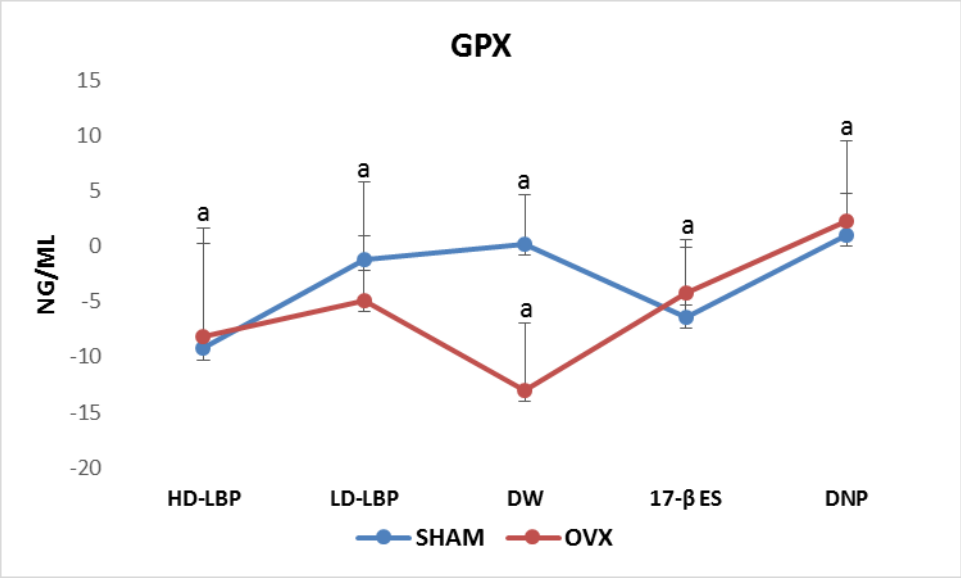

Figure 4.30. Mean (S.E.M.) GPX enzyme activity in learning study. Mean-values with the same letters within vertical columns are not significantly different ($p<0.05$).

4.2.2.10 17-β Estradiol Measurements

The main effect of treatment was not significant on 17-β ES level in the blood serum, $F(4, 50) = 2.43$, $p = 0.06$. Despite being not significant, sham subjects tend to have higher 17-β ES level than ovx group.

The main effect of the operation was not significant on 17-β ES level in the blood serum, $F(1, 50) = 1.01$, $p = 0.32$.

An interaction effect between treatment and operation was not significant, $F(4, 50) = 0.55$, $p = 0.70$. (Figure 4.31.)

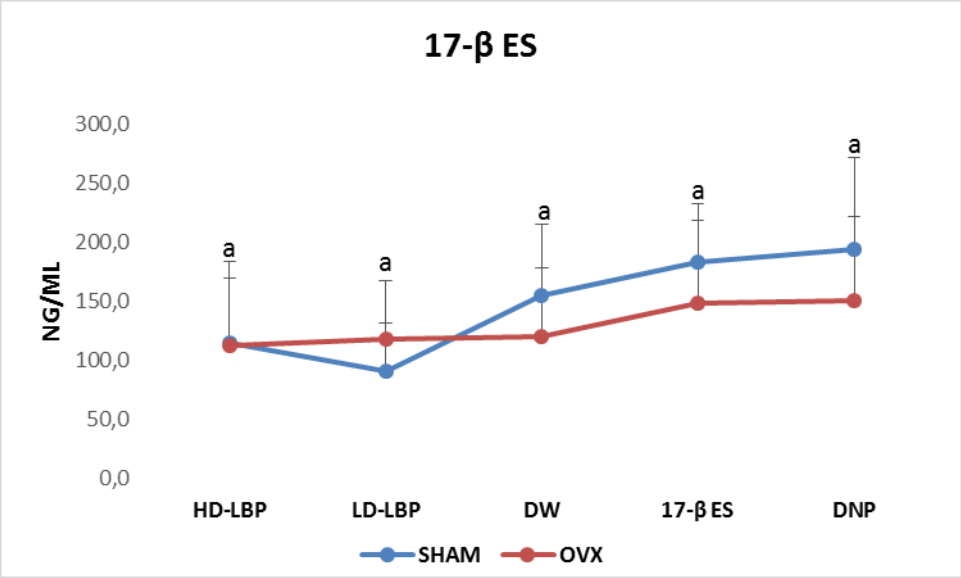

Figure 4.31. Mean (S.E.M.) 17-β ES level in learning study. Mean-values with the same letters within vertical columns are not significantly different (p>0.05).

4.2.2.11 MDA Measurements

The main effect of treatment was not significant on MDA enzyme activity in the blood serum, $F(4, 51) = 1.00$, $p = 0.41$.

The main effect of the operation was not significant on MDA enzyme activity in the blood serum, $F(1, 51) = 0.00$, $p = 0.96$.

An interaction effect between treatment and operation was not significant, $F(4, 51) = 0.87$, $p = 0.49$ (Figure 4.32.).

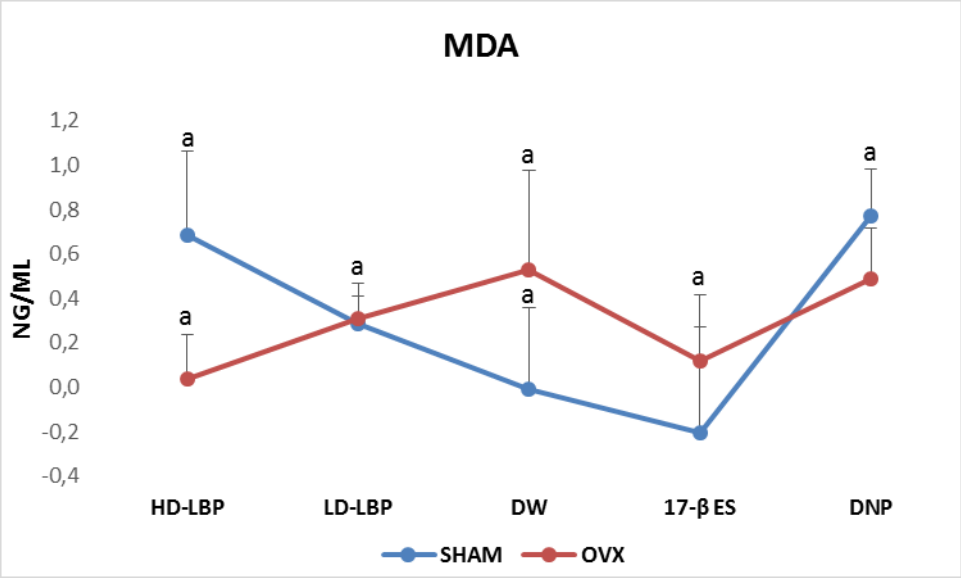

Figure 4.32. Mean (S.E.M.) MDA level in learning study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

4.2.3 Histological Results

4.2.3.1 Anxiety Study Results

4.2.3.1.1 Result of the SER Positive Cells Count

The main effect of treatment was significant on SER positive cell count in the hippocampus region, $F(4, 40) = 68.92$, $p = 0.001$, $\eta^2 = 0.87$. The hippocampus region of subjects in the HD-LBP treated groups had higher count of SER positive cell than those in the othe treated groups. DW treatment and 17-β ES treatment had least number of SER positive cells (Figure 4.33.).

The main effect of the operation was significant on SER positive cell count in the hippocampus region, $F(1, 40) = 10.23$, $p = 0.003$, $\eta^2 = 0.20$. SHAM operated groups had more number of SER positive cell than OVX operated groups (Figure 4.34.).

The interaction effect between treatment and operation was significant, $F (4, 40) = 15.10$, $p = 0.001$ $\eta^2 = 0.60$. HD-LBP treated groups had more SER positive

cells than other groups in SHAM operated condition, whereas DW treated groups had lower SER positive cells than other treatment groups in OVX condition (Figure 4.35-4.37.).

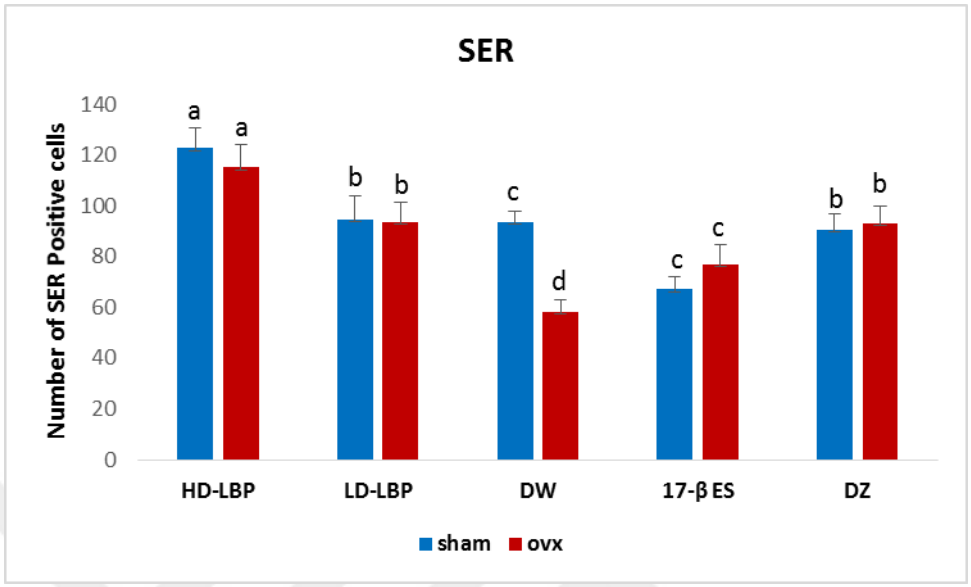

Figure 4.33. Mean (S.E.M.) Number of SER positive cells of HD-LBP and LD-LBP treated rats in anxiety study. Mean-values with the same letters within vertical columns are significantly different ($p < 0.05$).

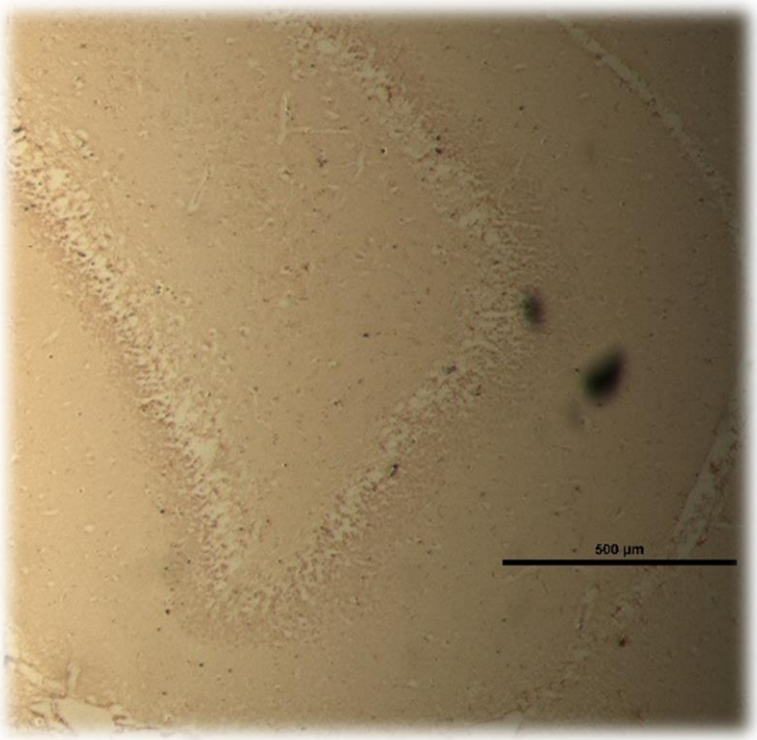

Figure 4.34. Immune staining of the hippocampus area in anxiety study (SER)

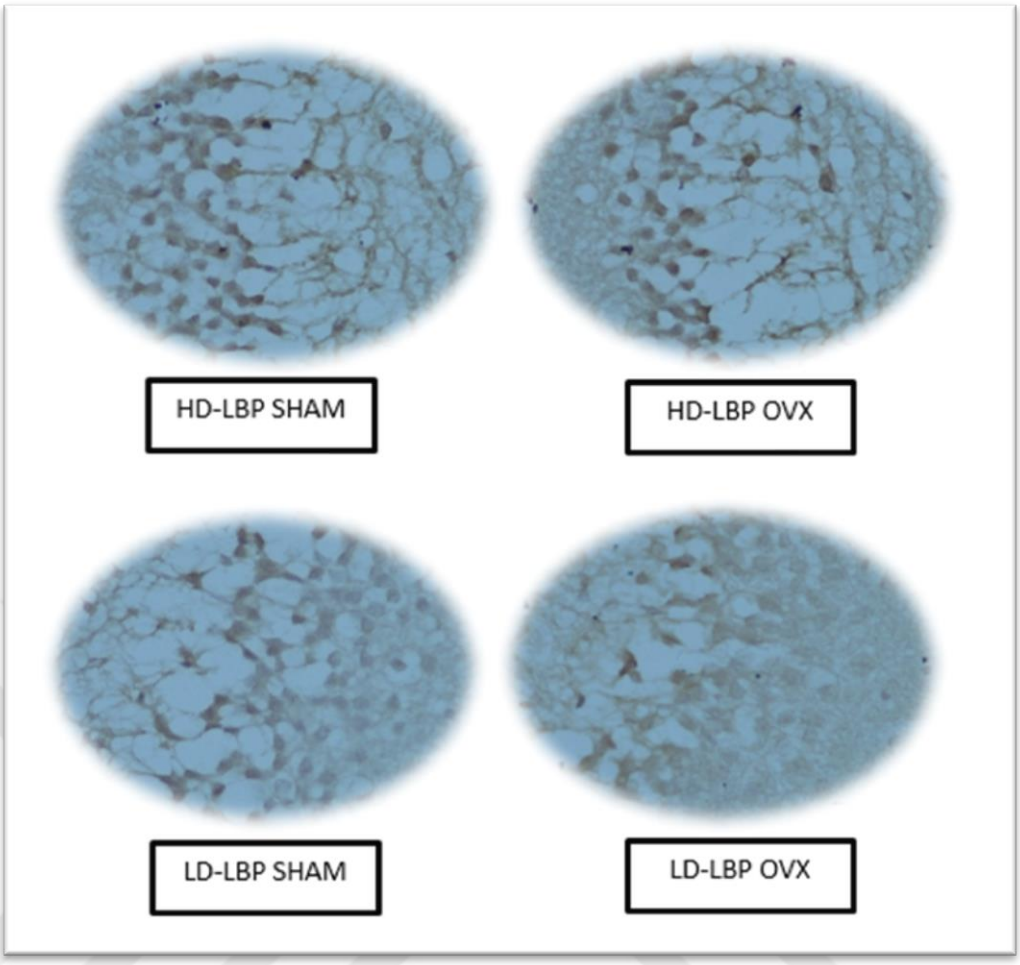

Figure 4.35. Immune staining of hippocampal SER positive cells of HD-LBP and LD-LBP treated rats in anxiety study (40X)

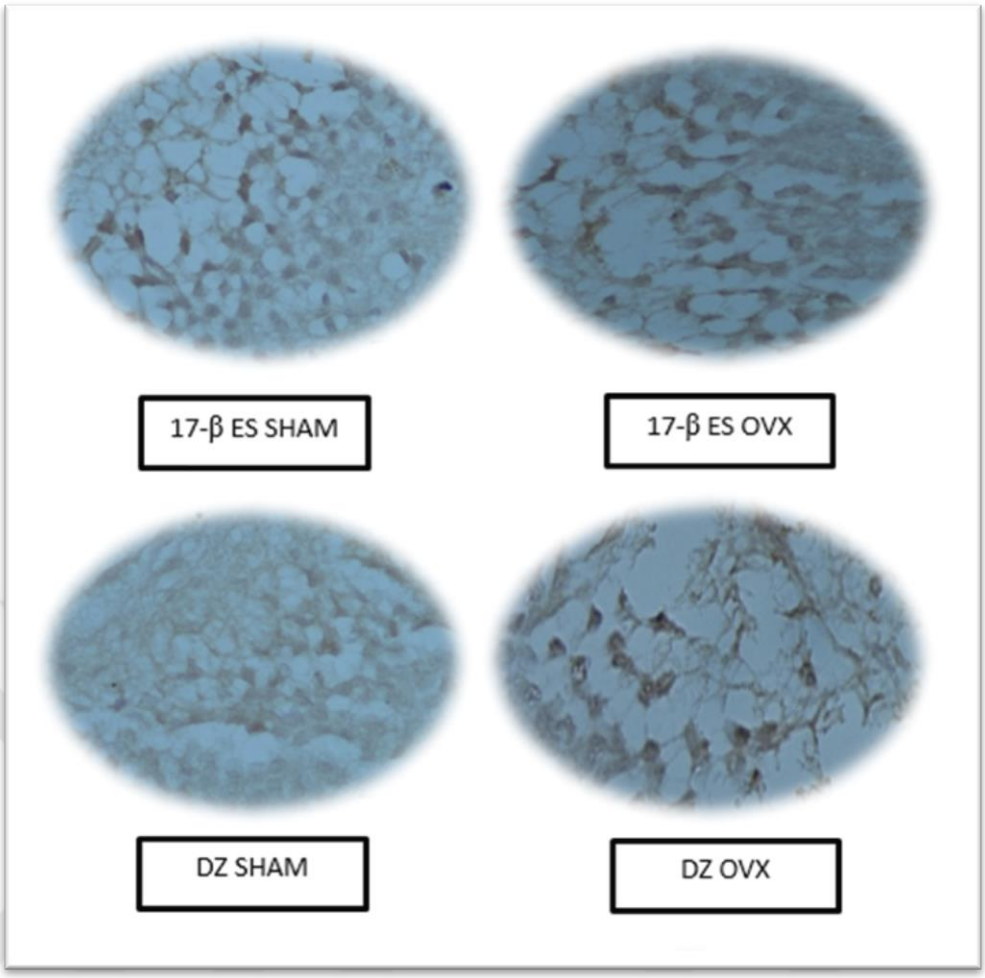

Figure 4.36. Immune staining of hippocampal SER positive cells of 17-β ES and DZ treated rats in anxiety study (40X)

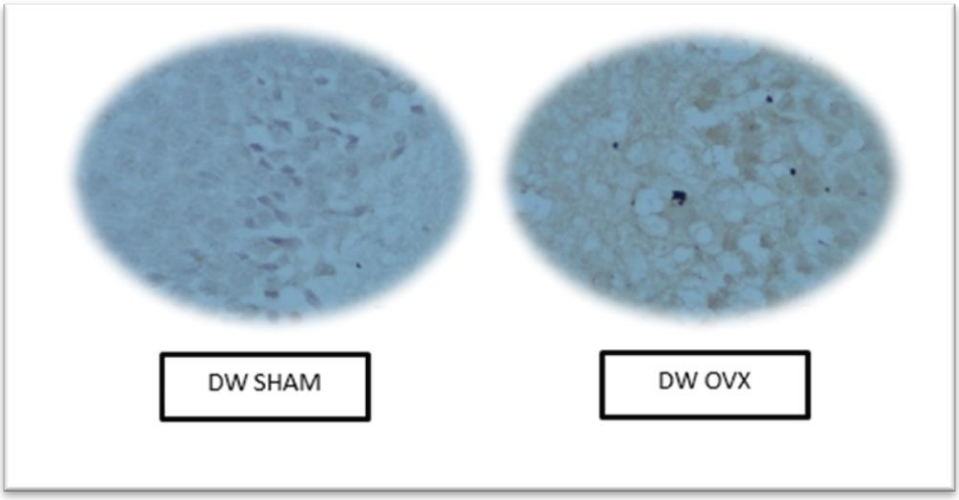

Figure 4.37. Immune staining of hippocampal SER positive cells of DW treated rats in anxiety study (40X)

4.2.3.1.2 Result of the BDNF Positive Cells Count

The main effect of treatment was significant on BDNF positive cell count in the hippocampus region, $F(4, 40) = 64.32$, $p = 0.001$, $\eta^2 = 0.87$. The hippocampus region of subjects in the HD-LBP treated groups had more number of BDNF positive cells than those in the other treated groups. LD-LBP tratment groups and DZ treatment groups had similar number of BDNF positive cells. DW treatment and 17-β ES treatment had similar and least number of BDNF positive cells (Figure 4.38.)

The main effect of the operation was significant on BDNF positive cell count in the hippocampus region, $F(1, 40) = 6.84$, $p = 0.013$, $\eta^2 = 0.15$. SHAM operated groups had more number of BDNF positive cell than OVX operated groups (Figure 4.39.).

The interaction effect between treatment and operation was significant, $F (4, 40) = 11.96$, $p = 0.001$ $\eta^2 = 0.54$. HD-LBP treated groups had more BDNF positive cells than other treatment groups in SHAM operated condition, whereas DW treated groups had lower BDNF positive cells than other treatment groups in OVX condition (see Figure 4.38.). DW treated groups in SHAM operated condition had more number of BDNF positive cell than OVX operated groups, whereas there were no significant differences between ovariectomy and sham groups in other treatment condition (Figure 4.40-4.42).

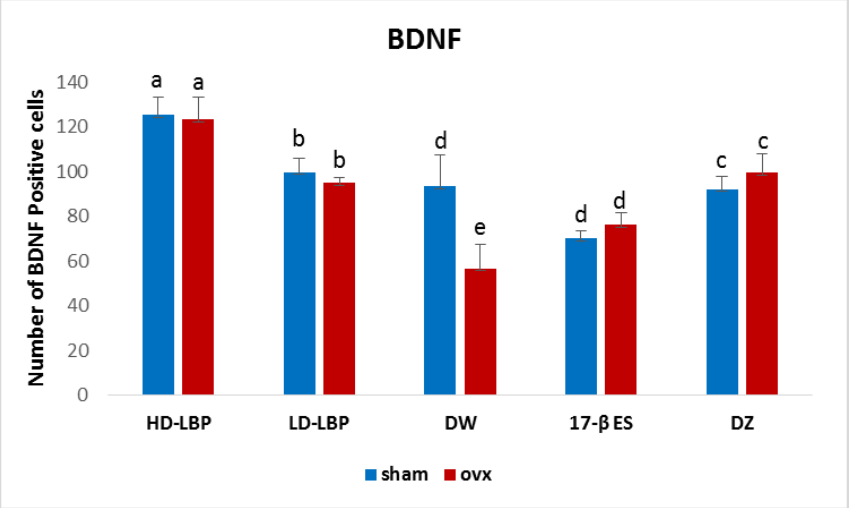

Figure 4.38. Mean (S.E.M.) Number of BDNF positive cells in anxiety study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

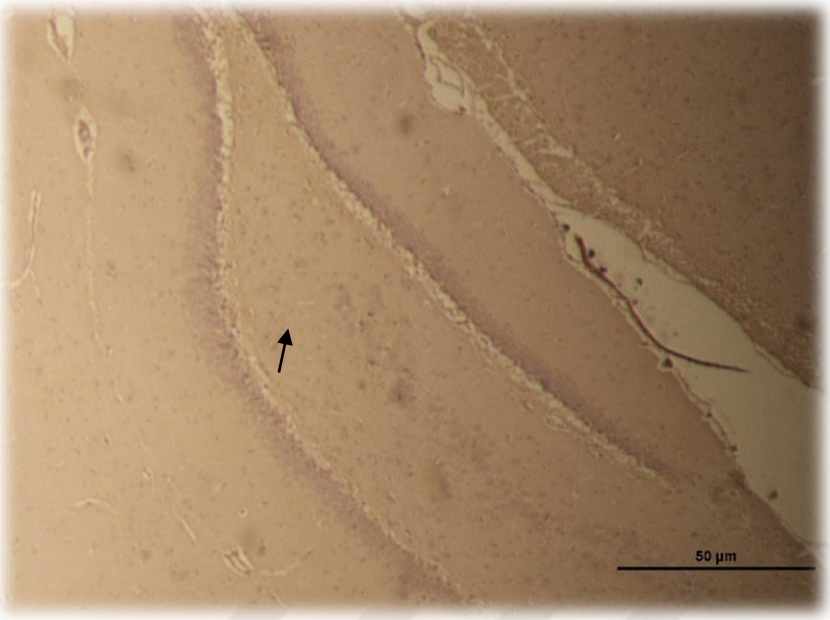

Figure 4.39. Immune staining of the hippocampus area in anxiety study (BDNF-4X)

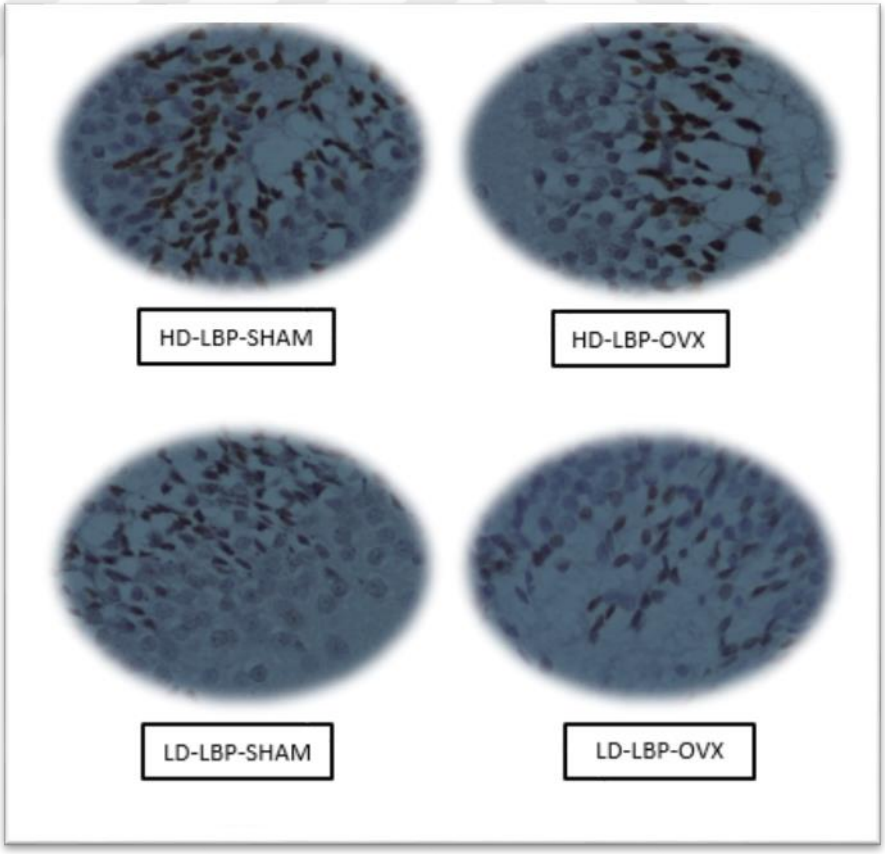

Figure 4.40. Immune staining of hippocampal BDNF positive cells of HD-LBP and LD-LBP treated rats in anxiety study (40X)

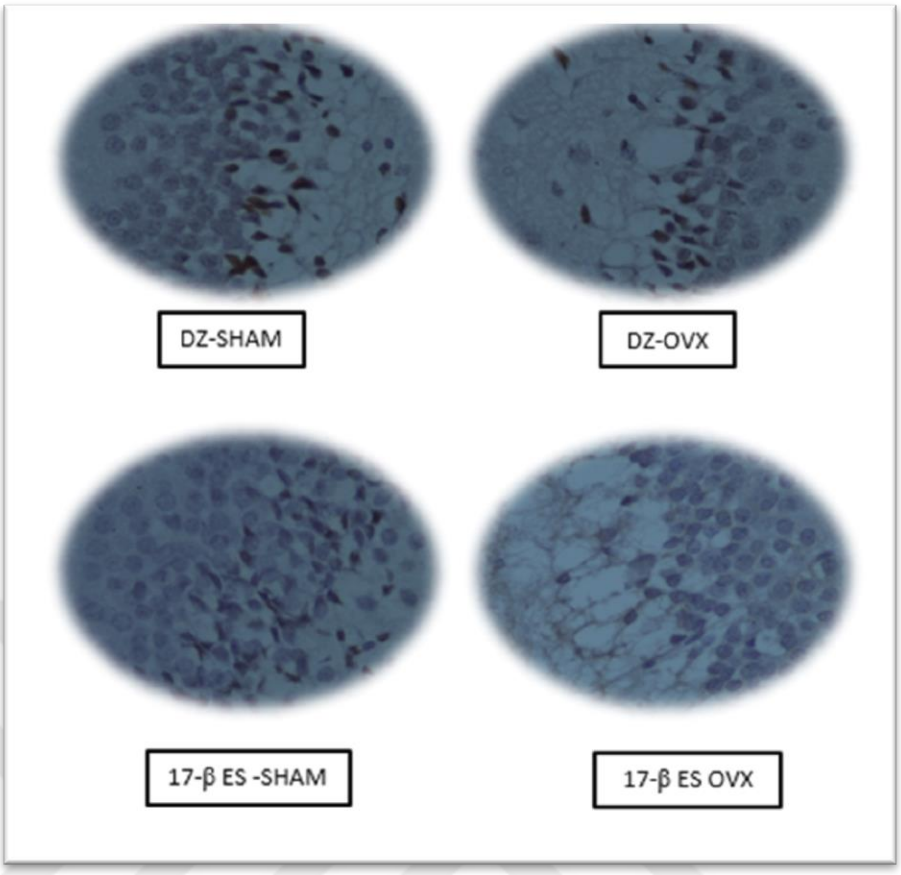

Figure 4.41. Immune staining of hippocampal BDNF positive cells of 17-β ES and DZ treated rats in anxiety study (40X)

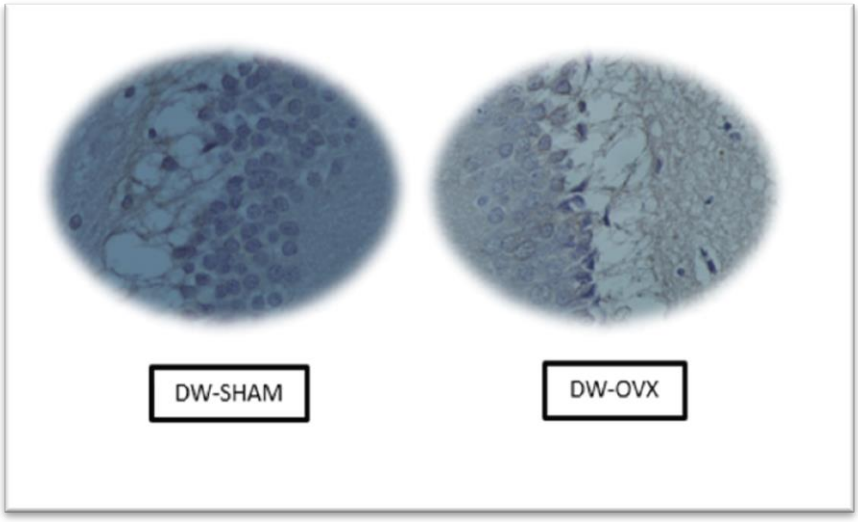

Figure 4.42. Immune staining of hippocampal BDNF positive cells of DW treated rats in anxiety study (40X)

4.2.3.1.3 Result of the TUNEL Positive Cells Count

The main effect of treatment was significant on apoptotic cell count in the hippocampus region, F(4, 40) = 22.86, p = 0.001, ηƨ2 = 0.70. The hippocampus region of subjects in the HD-LBP treated groups and LD-LBP trated groups had lower count of apoptotic cell than those in the other treated groups. DW trated groups showed most number of TUNEL positive cells (Figure 4.43.).

The main effect of the operation was significant on TUNEL positive cell count in the hippocampus region, F(1, 40) = 52.47, p = 0.001, η^2 = 0.57. SHAM operated groups had fewer TUNEL positive cells than OVX operated groups.

The interaction effect between treatment and operation was significant, F (4, 40) = 23.46, p = 0.001 η^2 = 0.70. HD-LBP treated groups had lower TUNEL positive cells than other groups in SHAM operated condition, whereas DW treated groups had more TUNEL positive cells than other treatment groups in OVX condition. Sham operated groups had lower number of TUNEL positive cells than overectomy group in DW and LD-LBP treatment conditions, however there were no significant differences between ovariectomy and sham groups in other treatment condition (Figure 4.44-4.46.)

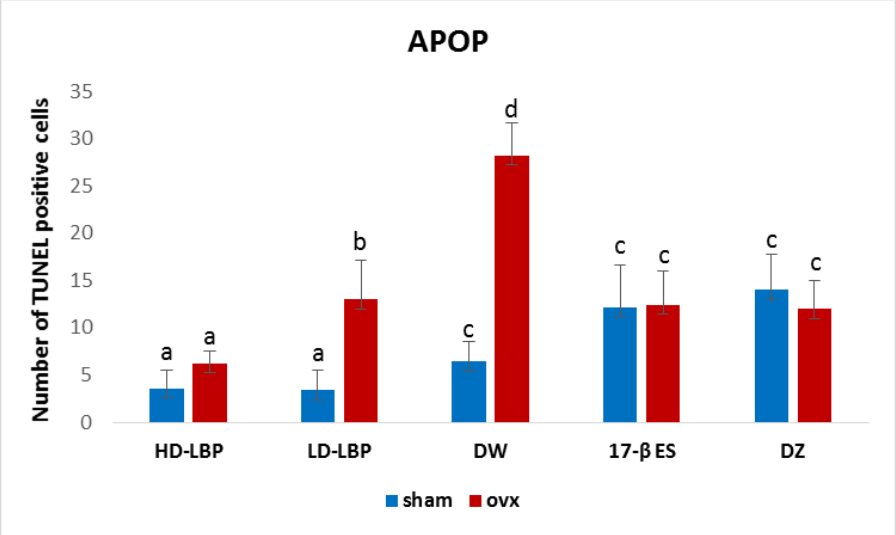

Figure 4.43. Mean (S.E.M.) Number of TUNEL positive cells in anxiety study. Mean-values with the same letters within vertical columns are significantly different (p<0.05).

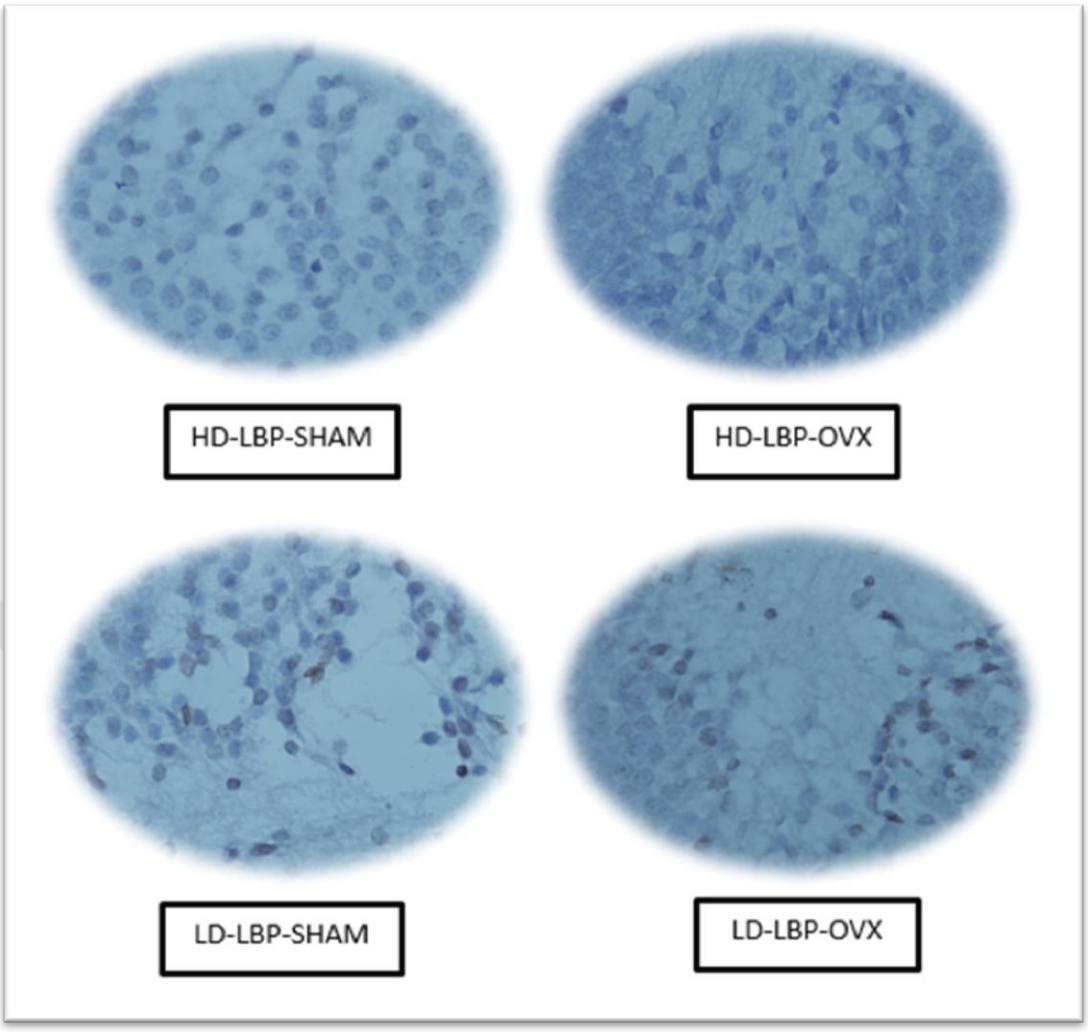

Figure 4.44. Immune staining of hippocampal TUNEL positive cells of HD-LBP and LD-LBP treated rats in anxiety study (40X)

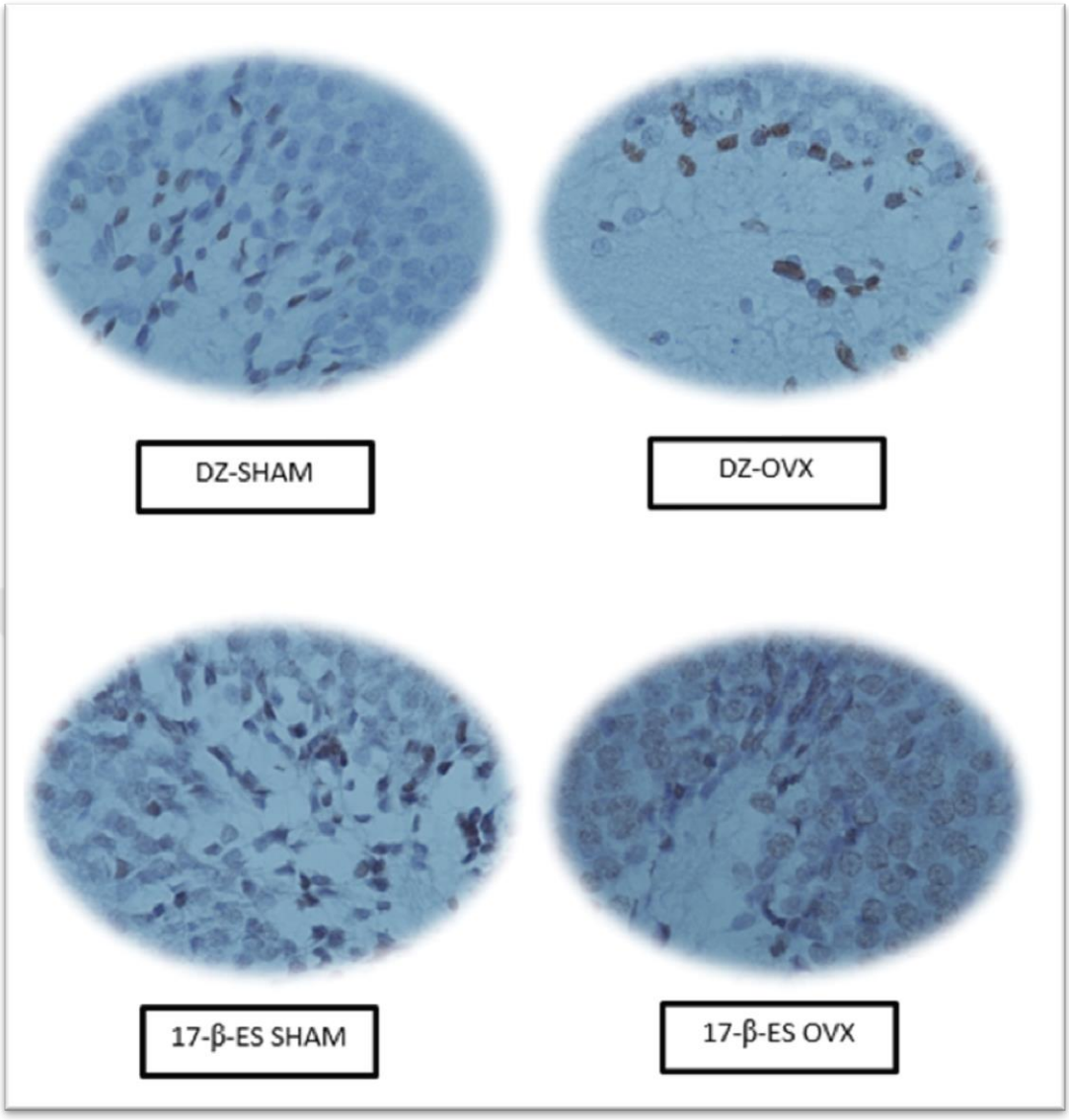

Figure 4.45. Immune staining of hippocampal TUNEL positive cells of 17-β ES and DNP treated rats in anxiety study (40X)

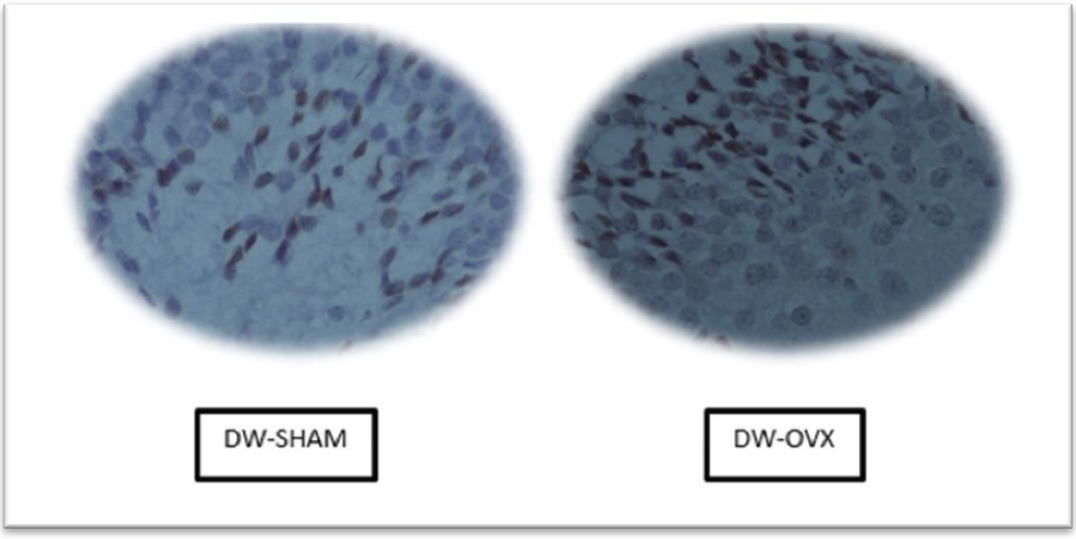

Figure 4.46. Immune staining of hippocampal TUNEL positive cells of DW treated rats in anxiety study (40X)

4.2.3.2 Results of Depression Study

4.2.3.2.1 Result of SER Positive Cells Count

The main effect of treatment was significant on SER positive cell count in the hippocampus region, $F(4, 40) = 113.23$, $p = 0.001$, $\eta^2 = 0.92$. The hippocampus region of subjects in the HD-LBP and IMIP treatment groups had higher count of SER positive cell than those in the other treatment groups. DW treated and 17-β ES treated groups had similar and least number of SER positive cells (Figure 4.47).

The main effect of the operation was significant on SER positive cell count in the hippocampus region, $F(1, 40) = 4.18$, $p = 0.048$, $\eta^2 = 0.09$. SHAM operated groups had more SER positive cells than OVX operated groups.

The interaction effect between treatment and operation was significant, $F (4, 40) = 7.02$, $p = 0.001$ $\eta^2 = 0.41$. HD-LBP and IMP treated groups had more SER positive cells than other groups in SHAM operated condition, whereas DW and 17-β ES treated groups had lower SER positive cells than other treatment groups in OVX condition. In other words, sham operated groups had higher number of SER positive cells than overectomy group in DW treatment condition, however there were no

significant differences between ovariectomy and sham groups in other treatment condition (Figure 4.48-4.50.).

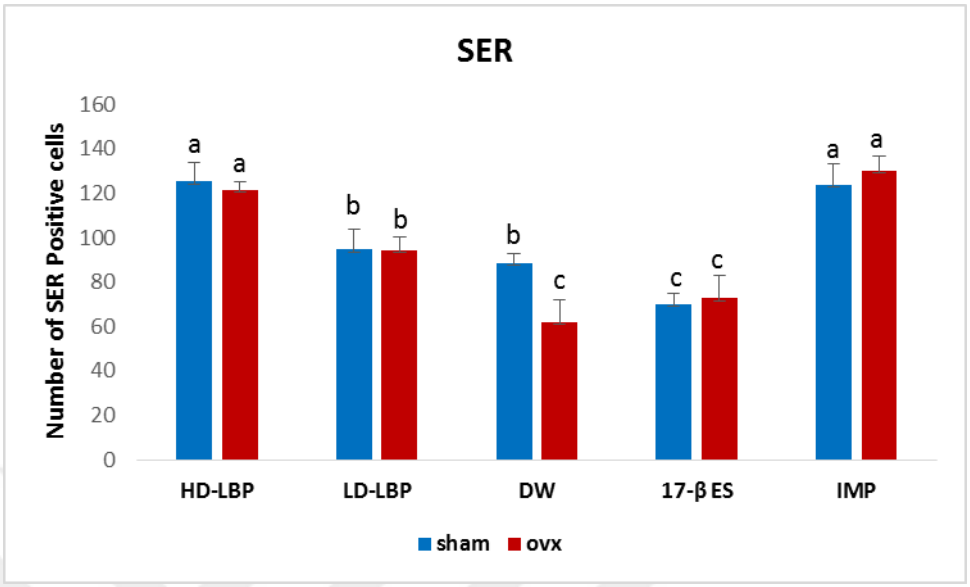

Figure 4.47. Mean (S.E.M.) Number of SER positive cells in depression study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

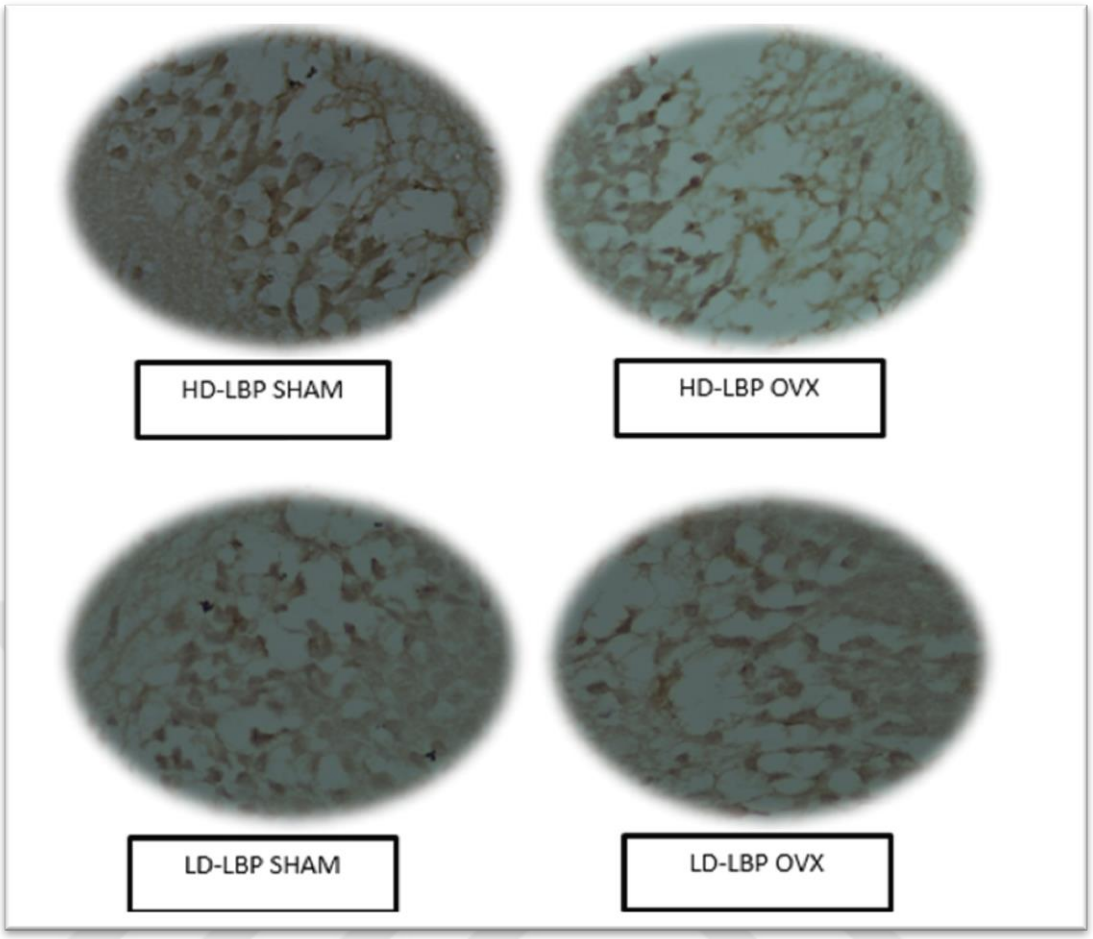

Figure 4.48. Immune staining of hippocampal SER positive cells of HD-LBP and LD-LBP treated rats in depression study (40X)

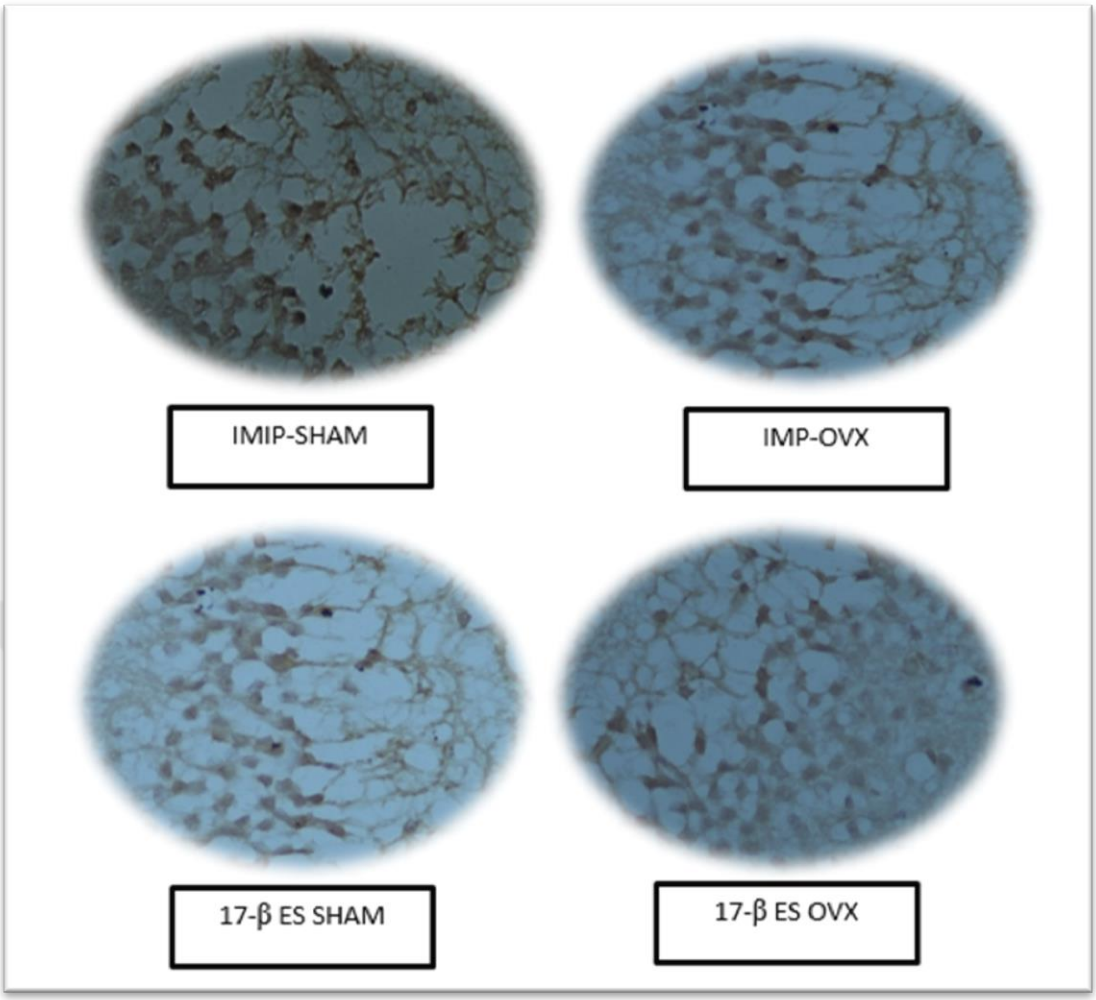

Figure 4.49. Immune staining of hippocampal SER positive cells of 17-β ES and IMP treated rats in depression study (40X)

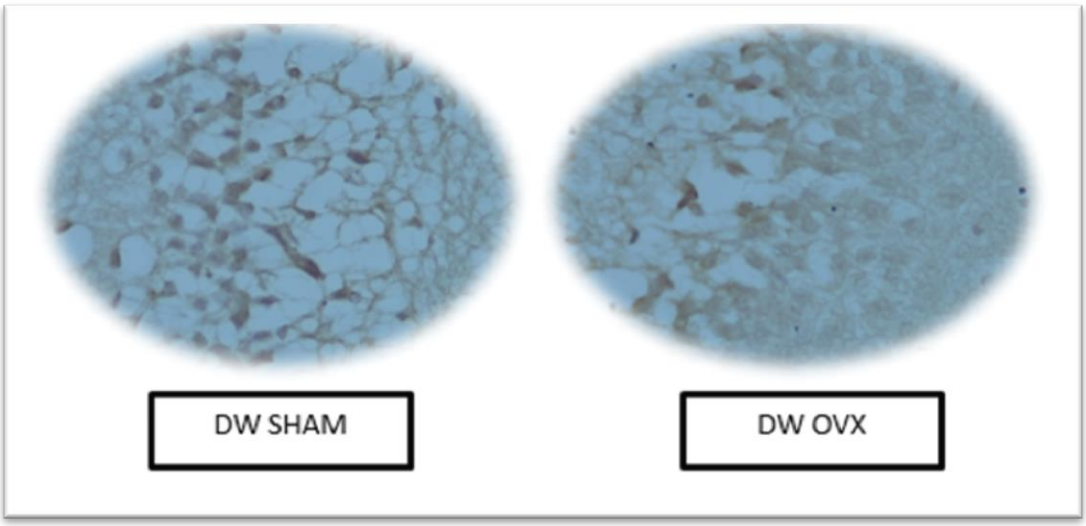

Figure 4.50. Immune staining of hippocampal SER positive cells of DW treated rats in depression study (40X)

4.2.3.2.2 BDNF Receptor Detected Results

The main effect of treatment was significant on BDNF positive cell count in the hippocampus region, $F(4, 40) = 35.63$, $p = 0.001$, $\eta^2 = 0.78$. The hippocampus region of subjects in the HD-LBP treatment groups had most number of BDNF positive cells than those in the other treatments groups. DW treated and 17-β ES treated groups had similar and least number of BDNF positive cells (Figure 4.51.).

The main effect of the operation was significant on BDNF positive cell count in the hippocampus region, $F(1.40) = 4.62$, $p = 0.038$, $\eta^2 = 0.10$. SHAM operated groups had more BDNF positive cells than OVX operated groups.

The interaction effect between treatment and operation was significant, $F (4, 40) = 5.33$, $p = 0.002$ $\eta^2 = 0.35$. HD-LBP and IMP treated groups had more BDNF positive cells than other groups in SHAM operated condition, whereas DW and 17-β ES treated groups had lower BDNF positive cells than other treatment groups in OVX condition (Figure 4.52-4.54).

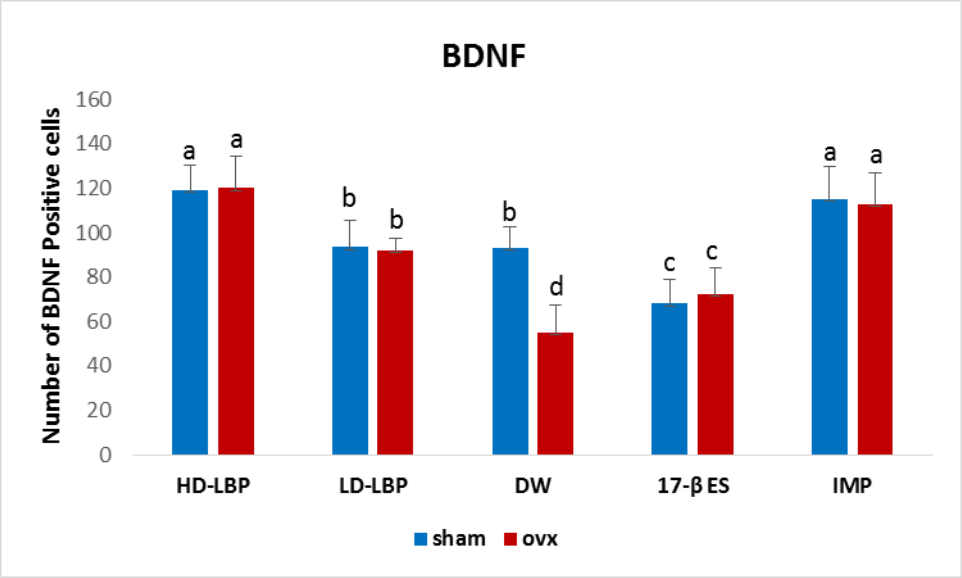

Figure 4.51. Mean (S.E.M.) Number of BDNF positive cells in depression study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

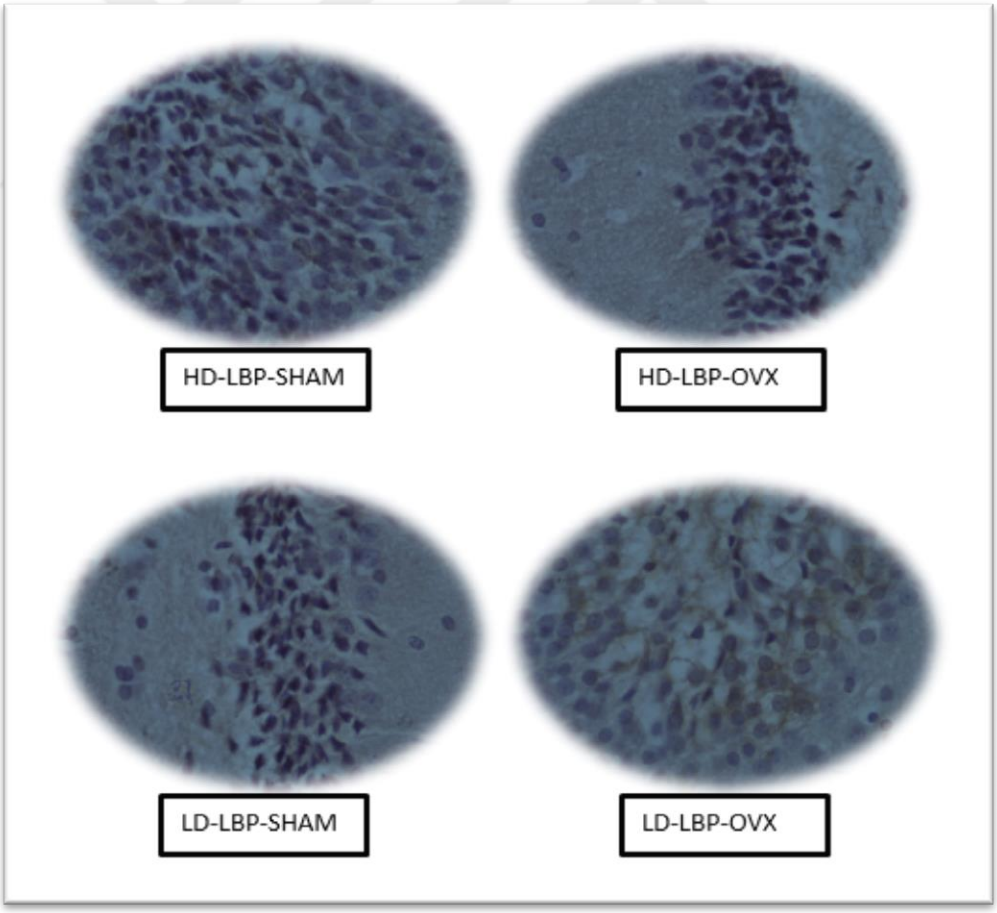

Figure 4.52. Immune staining of hippocampal BDNF positive cells of HD-LBP and LD-LBP treated rats in depression study (40X)

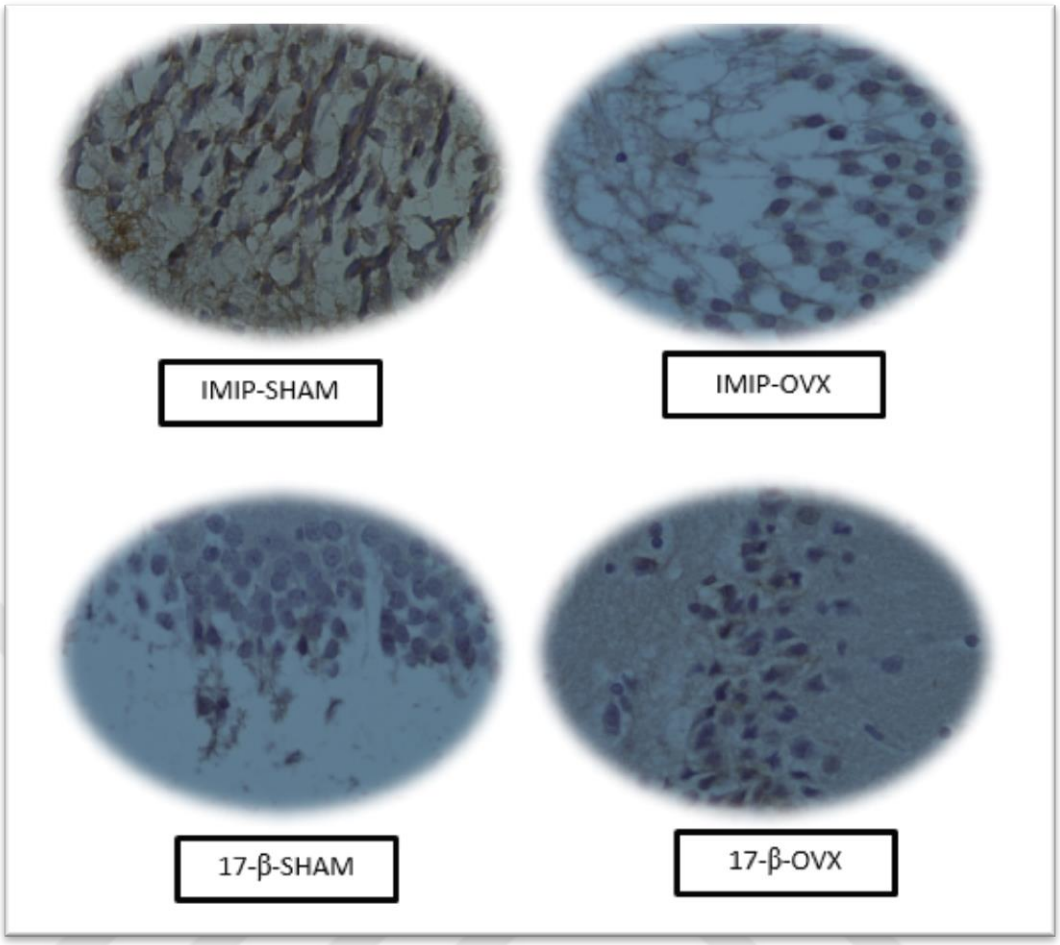

Figure 4.53. Immune staining of hippocampal BDNF positive cells of 17 β ES and IMP treated rats in depression study (40X

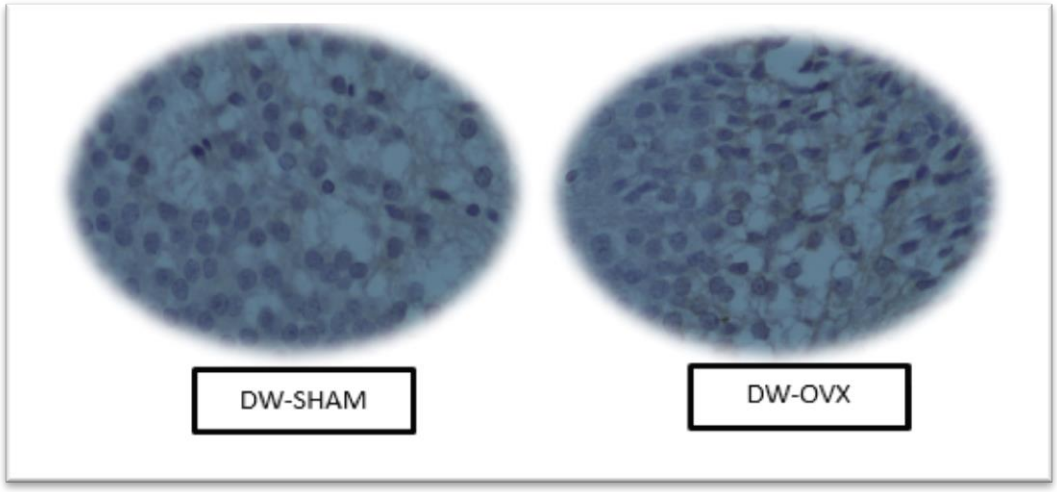

Figure 4.54. Immune staining of hippocampal BDNF positive cells of DW treated rats in depression study(40X)

4.2.3.2.3 Apoptotic Cell Count Results

The main effect of treatment was significant on apoptotic cell count in the hippocampus region, $F(4, 40) = 162.85$, $p = 0.001$, $\eta^2 = 0.94$. Although the hippocampus region of subjects in the HD-LBP treatment groups had lowest TUNEL positive cells, DW treated groups had most TUNEL positive cells than those in the other treatment groups (Figure 4.55.).

The main effect of the operation was significant on TUNEL positive cell count in the hippocampus region, $F(1, 40) = 328.46$, $p = 0.001$, $\eta^2 = 0.89$. SHAM operated groups had fewer number of TUNEL positive cells than OVX operated groups.

The interaction effect between treatment and operation was significant, $F(4, 40) = 162.59$, $p = 0.001$ $\eta^2 = 0.94$. HD-LBP and DW treated groups had lower APOP positive cells than other groups in SHAM operated condition, whereas DW treated groups had higher APOP positive cells than other treatment groups in OVX condition (Figure 4.56-4.59.).

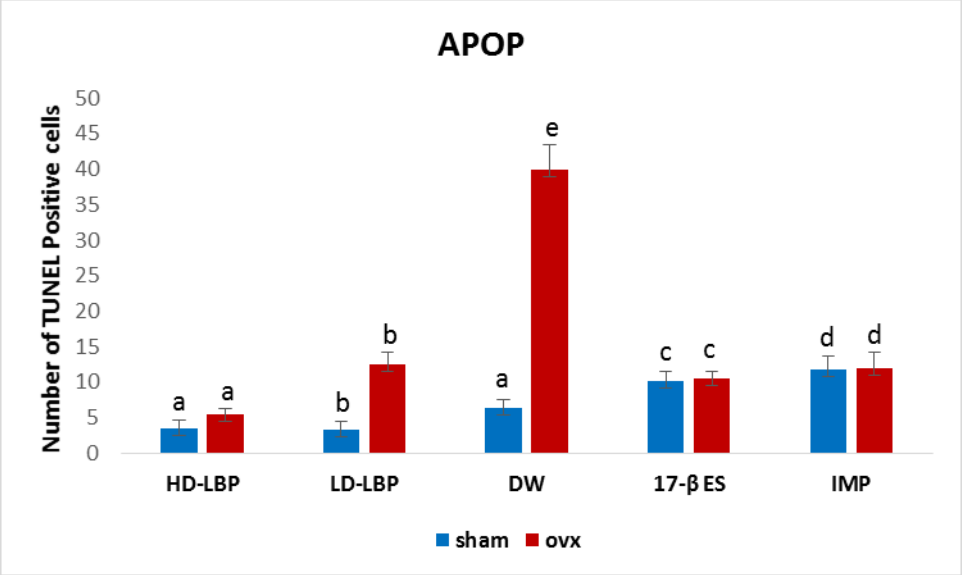

Figure 4.55. Mean (S.E.M.) Number of TUNEL positive cells in depression study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

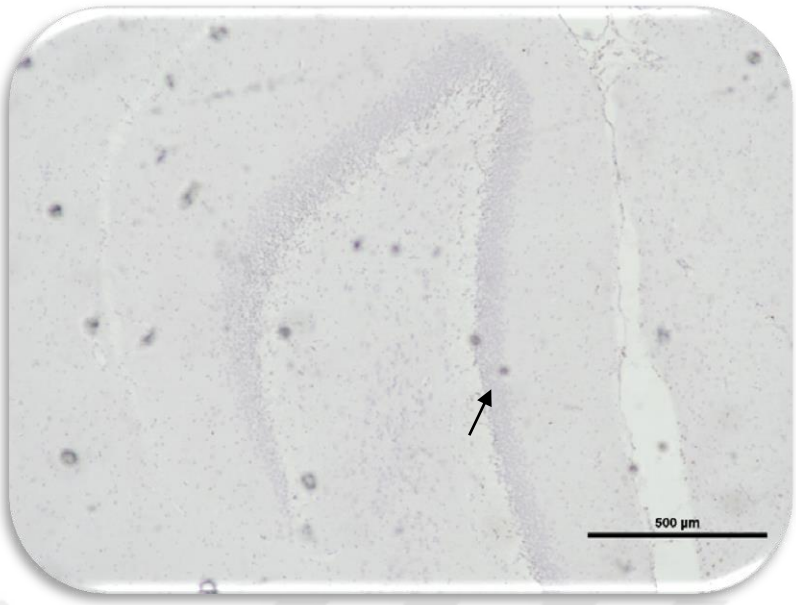

Figure 4.56. TUNEL staining of hippocampus region (4X)

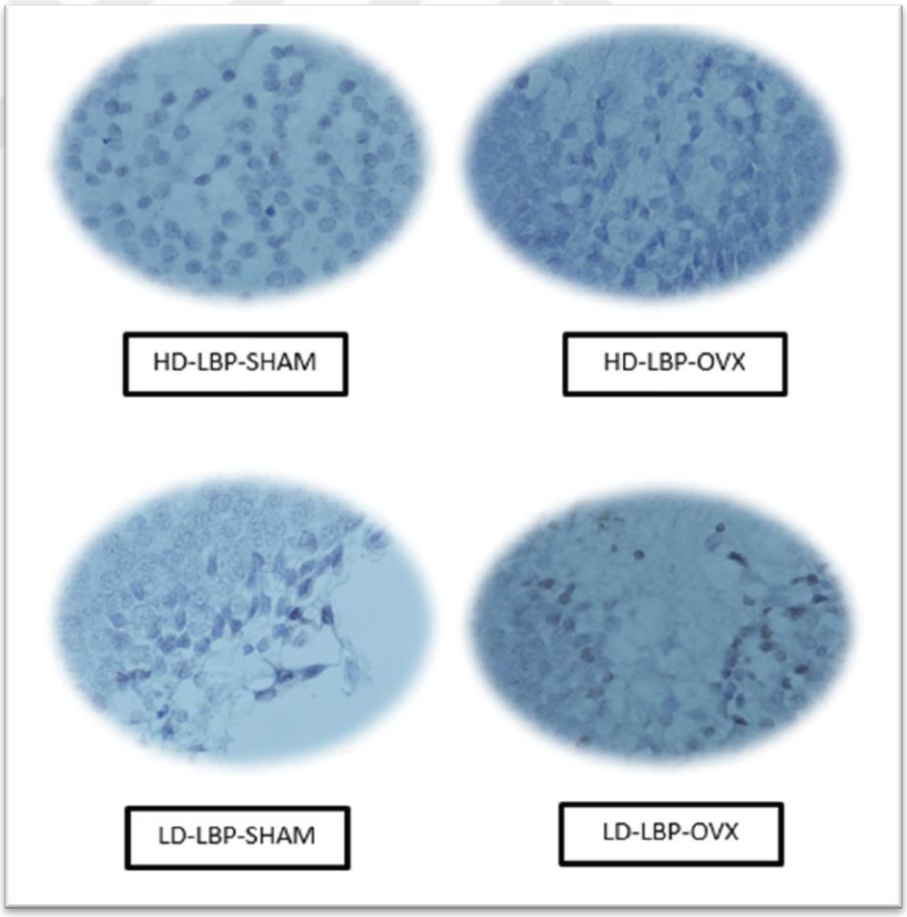

Figure 4.57. Immunstaining of the hippocampal TUNEL positive cells of HD-LBP and LD-LBP treated rats in depression study (40X)

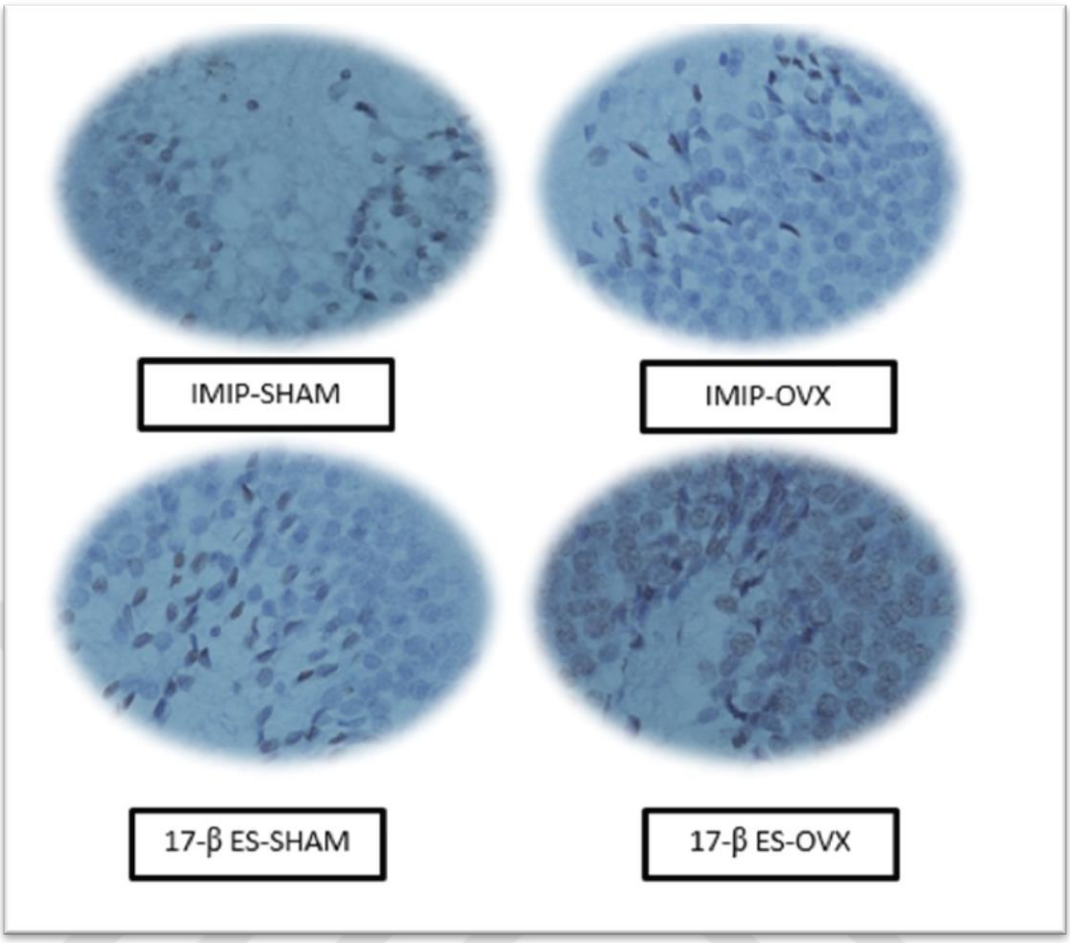

Figure 4.58. Immunstaining of the hippocampal TUNEL positive cells of 17-β ES and IMP treated rats in depression study (40X)

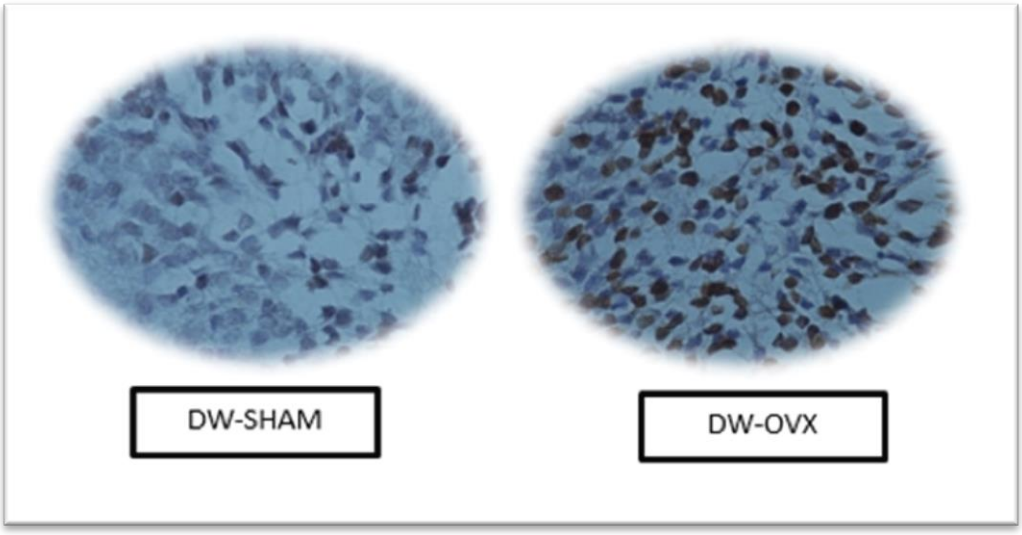

Figure 4.59. Immunstaining of the hippocampal TUNEL positive cells of DW treated rats in depression study (40X)

4.2.4 Result of Learning and Memory Study

4.2.4.1 Serotonin Receptor Detected Results

The main effect of treatment was significant on SER positive cell count in the hippocampus region, $F(4, 40) = 55.80$, $p = 0.001$, $\eta^2 = 0.85$. The hippocampus region of subjects in the HD-LBP and DNP treatment groups had most count of SER positive cell than those in the other treated groups. DW treated groups had least SER positive cells than those in the other treatment groups (Figure 4.60.).

The main effect of the operation was significant on SER positive cell count in the hippocampus region, $F(1, 40) = 10.50$, $p = 0.002$, $\eta^2 = 0.21$. DW treated groups in SHAM operated condition had more SER positive cells than OVX operated condition.

The interaction effect between treatment and operation was significant, $F (4, 40) = 5.25$, $p = 0.002$ $\eta^2 = 0.34$. HD-LBP treated groups had more SER positive cells than other groups in SHAM operated condition, whereas DW and 17-β ES treated groups had lower SER positive cells than other treatment groups in OVX condition (Figure 4.61.-4.63.).

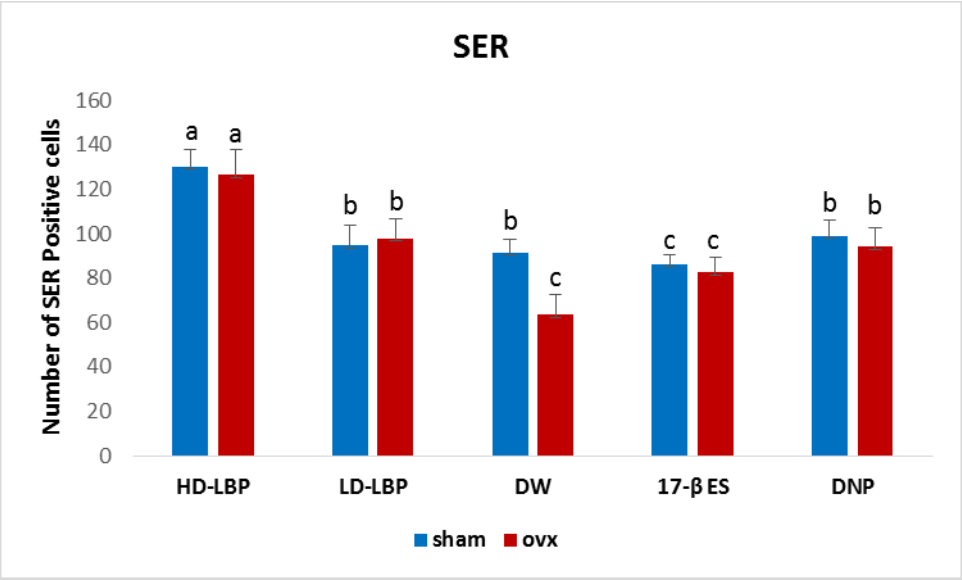

Figure 4.60. Mean (S.E.M.) Number of SER positive cells in learning study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

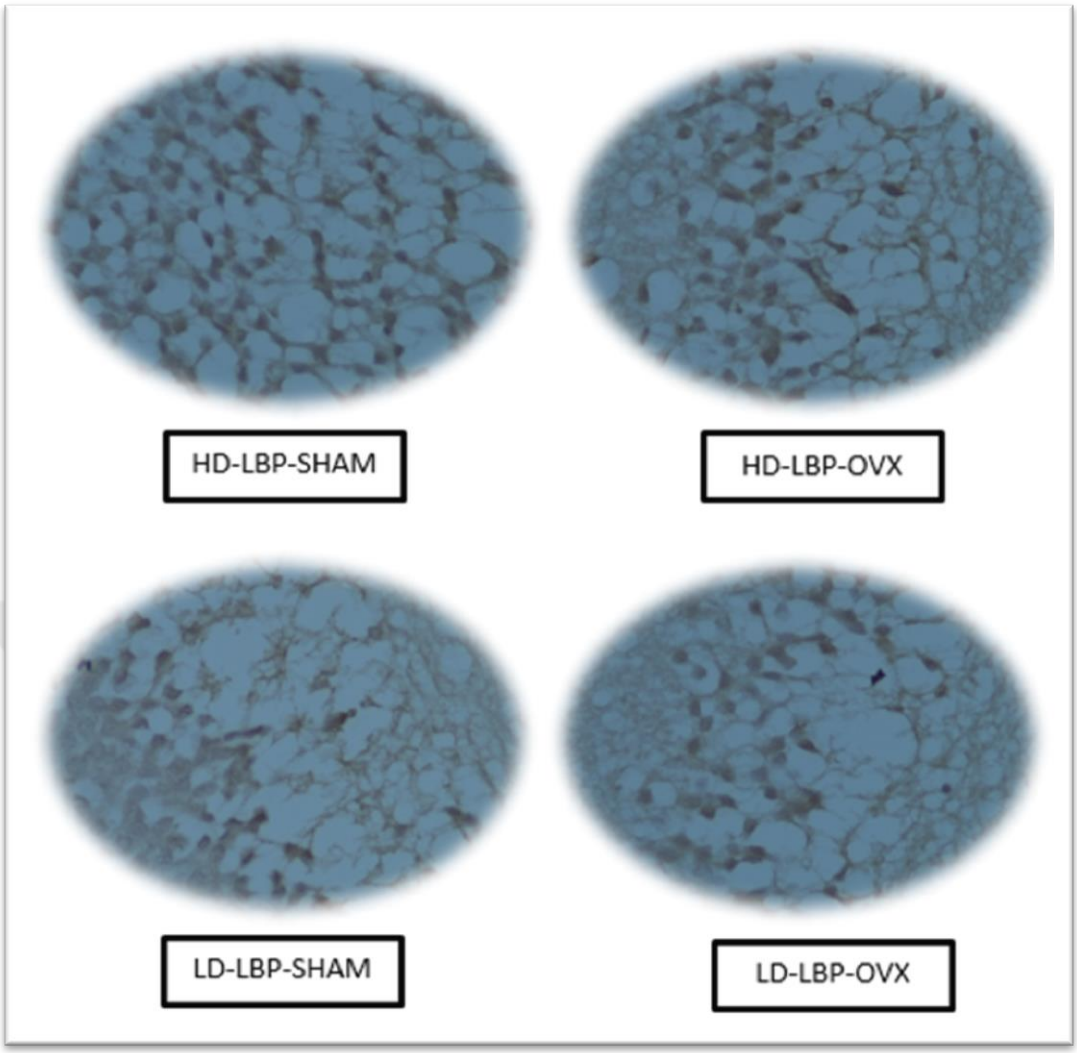

Figure 4.61 Immunstaining of the hippocampal SER positive cells of HD-LBP and LD-LBP treated rats in learning study (40X)

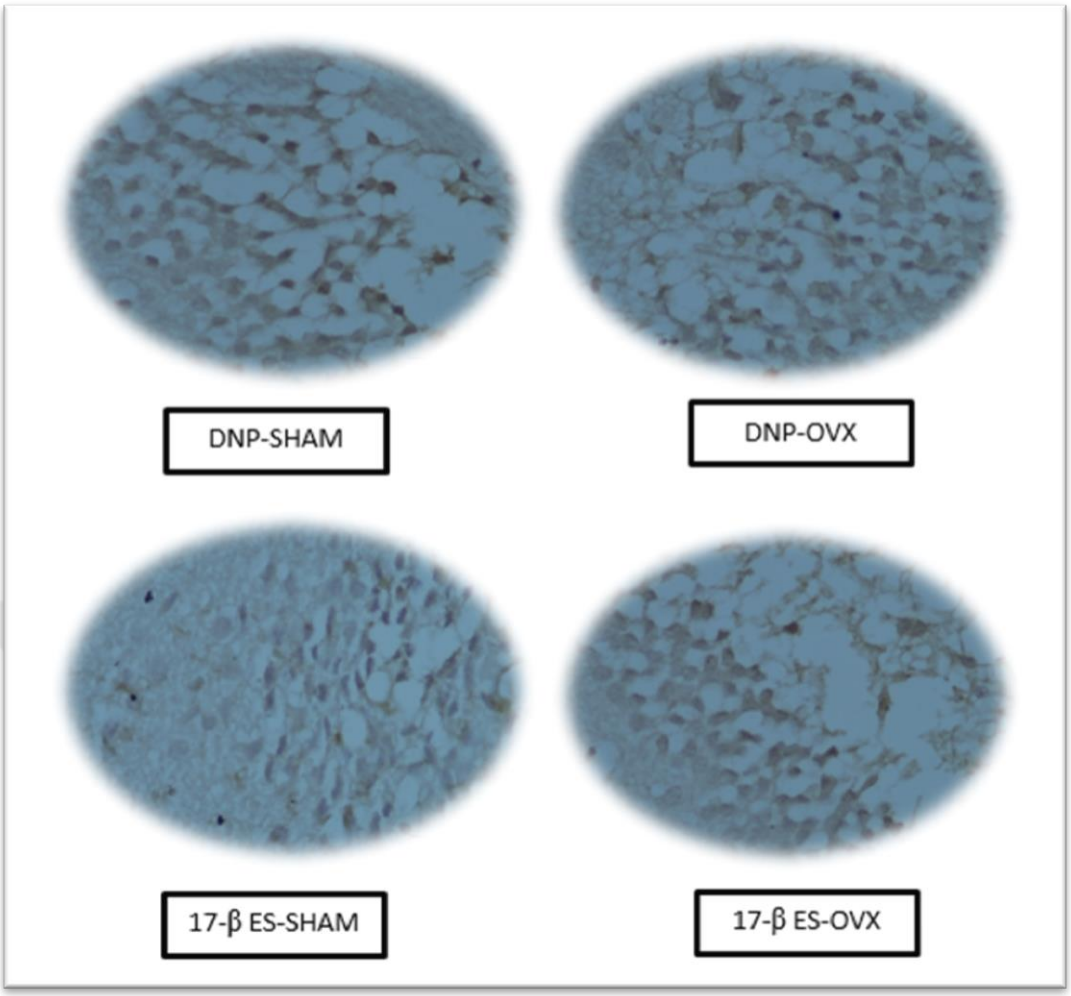

Figure 4.62. Immunstaining of the hippocampal SER positive cells of 17-β ES and DNP treated rats in learning study (40X)

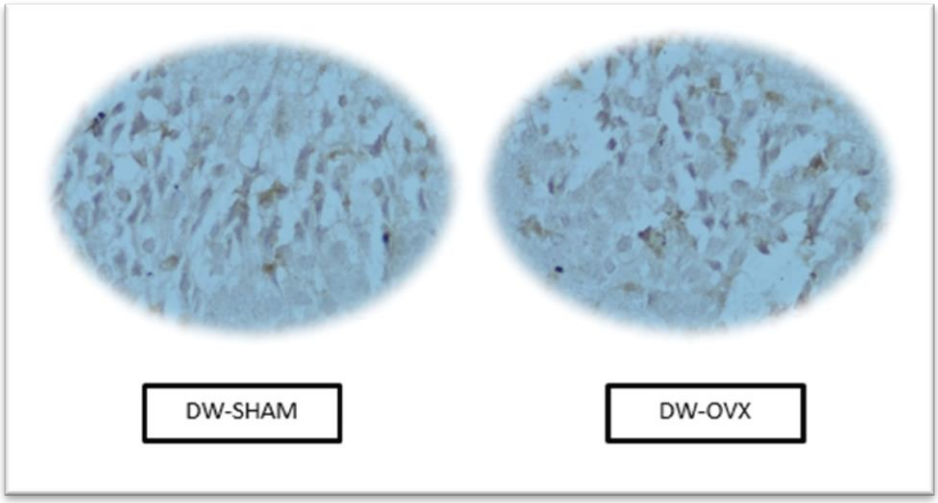

Figure 4.63. Immunstaining of the hippocampal SER positive cells of DW treated rats in learning study (40X)

4.2.4.1.1 Result of BDNF Positive Cells

The main effect of treatment was significant on BDNF positive cell counts in the hippocampus region, $F(4, 40) = 51.39$, $p = 0.001$, $\eta^2 = 0.84$. The hippocampus region of subjects in the HD-LBP treated groups had most BDNF positive cells than those in the other treated groups. LD-LBP treated and 17-β ES treated groups had similar BDNF positive cells count. However, DW treated groups had least BDNF positive cells than those in the other treatment groups (Figure 4.64.).

The main effect of the operation was significant on BDNF positive cell counts in the hippocampus region, $F(1, 40) = 12.85$, $p = 0.001$, $\eta^2 = 0.24$. DW treated groups in SHAM operated condition had more BDNF positive cells than OVX operated condition.

The interaction effect between treatment and operation was significant, $F (4, 40) = 4.71$, $p = 0.003$ $\eta^2 = 0.32$. HD-LBP treated groups had more BDNF positive cells than other groups in SHAM operated condition, whereas DW and treated groups had lower BDNF positive cells than other treatment groups in OVX condition (Figure 4.65-4.67.).

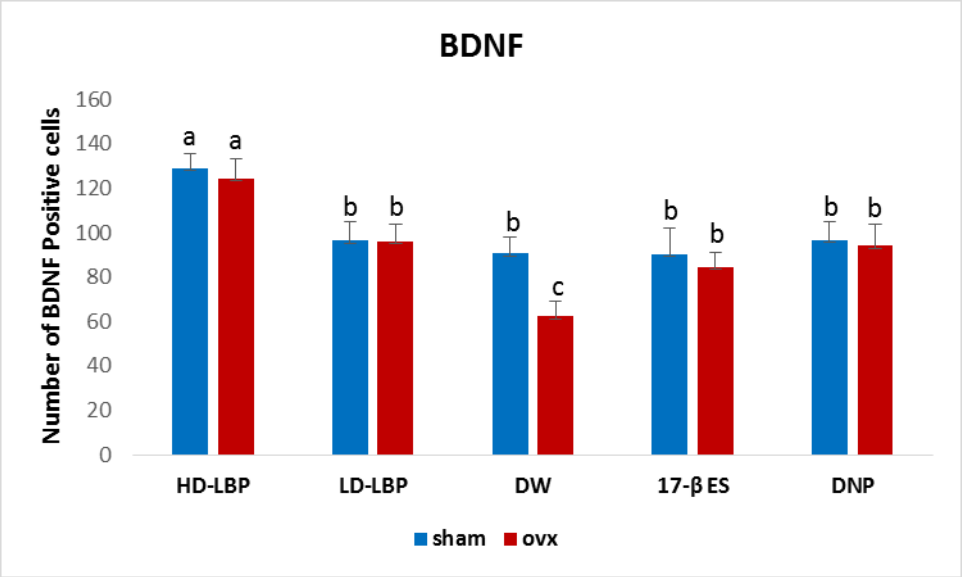

Figure 4.64. Mean (S.E.M.) Number of BDNF positive cells in learning study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

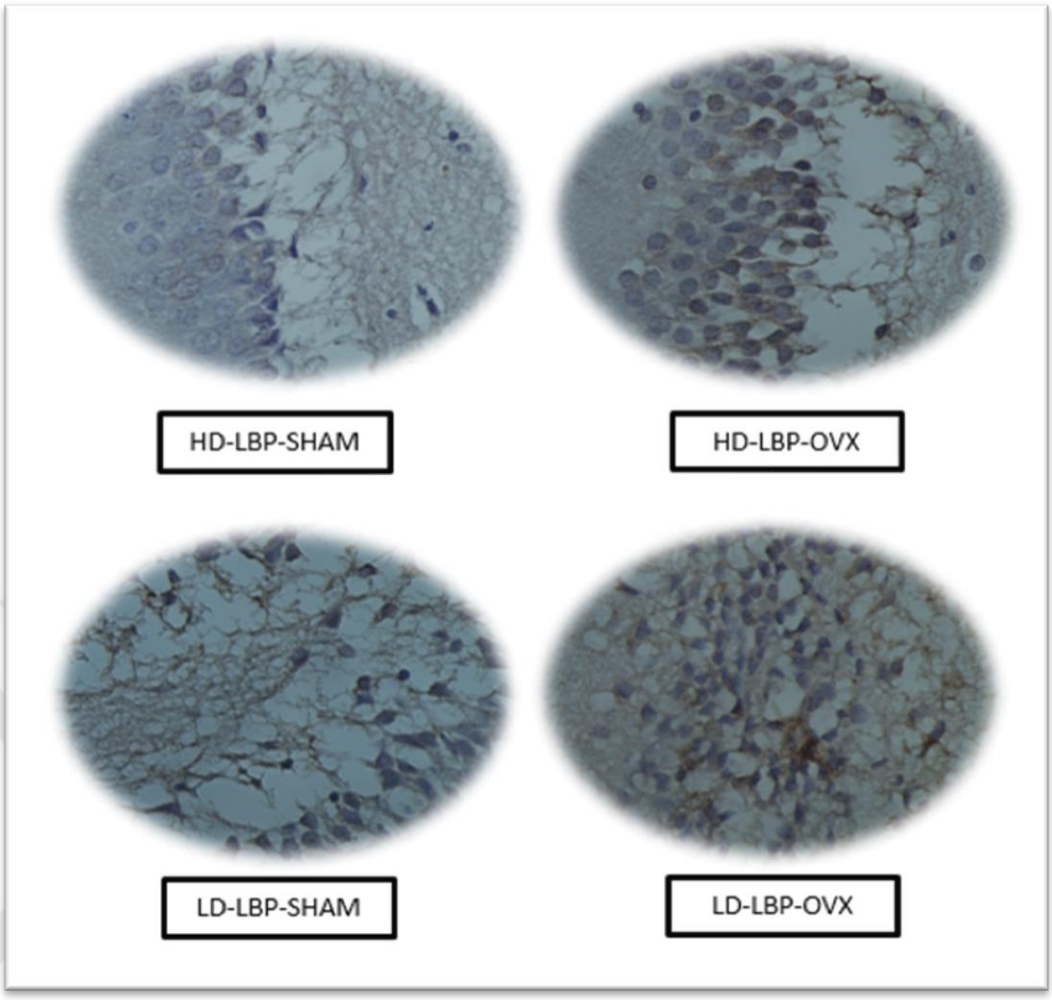

Figure 4.65. Immunstaining of the hippocampal BDNF positive cells of HD-LBP and LD-LBP treated rats in learning study (40X)

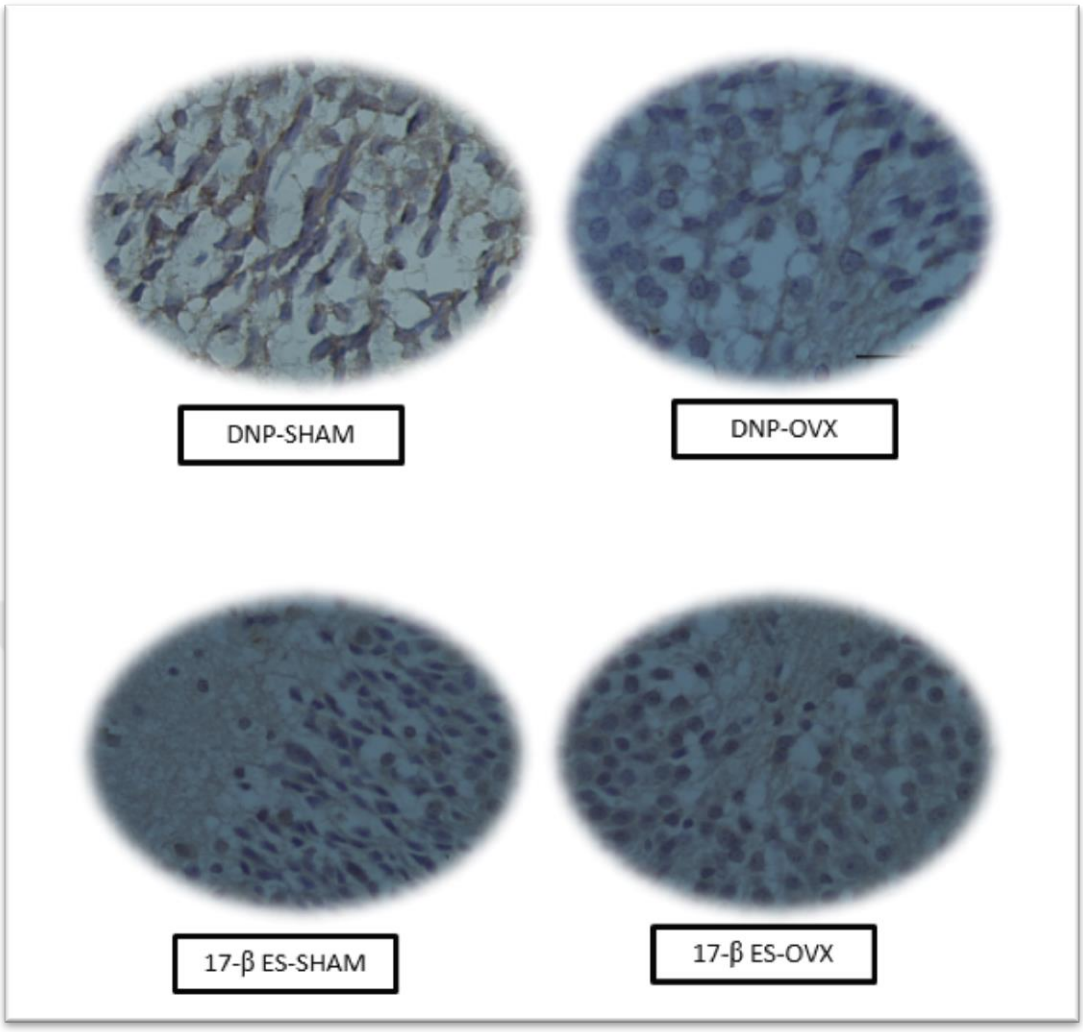

Figure 4.66. Immunstaining of the hippocampal BDNF positive cells of 17-β ES and DNP treated rats in learning study (40X)

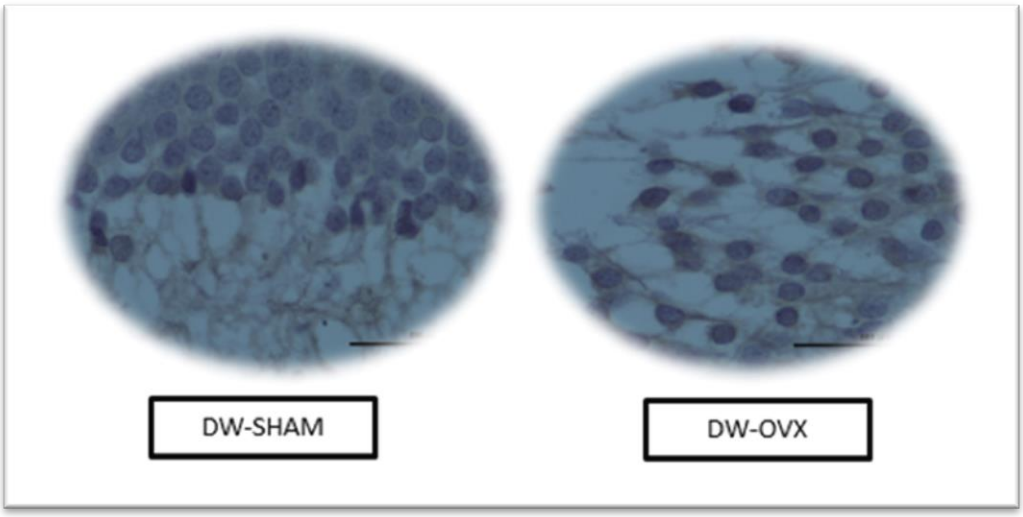

Figure 4.67. Immunstaining of the hippocampal BDNF positive cells of DW treated rats in learning study (40X)

4.2.4.1.2 Apoptotic Cell Count Results

The main effect of treatment was significant on apoptotic cell count in the hippocampus region, $F(4, 40) = 126.14$, $p = 0.001$, $\eta^2 = 0.93$. Although the hippocampus region of subjects in the HD-LBP treatment groups had lowest count of apoptotic cells, DW treated groups had most the cell numbers than those in the other treated groups (Figure 4.68.).

The main effect of the operation was significant on SER positive cell count in the hippocampus region, $F(1, 40) = 262.62$, $p = 0.001$, $\eta^2 = 0.87$. SHAM operated groups had fewer TUNEL positive cells than OVX operated groups.

The interaction effect between treatment and operation was significant, $F (4, 40) = 112.78$, $p = 0.001$ $\eta^2 = 0.92$. HD-LBP and LD-LBP treated groups had lower TUNEL positive cells than other groups in sham operated condition, whereas DW treated groups had higher TUNEL positive cells than other treatment groups in ovx condition (Figure 4.69.-471.).

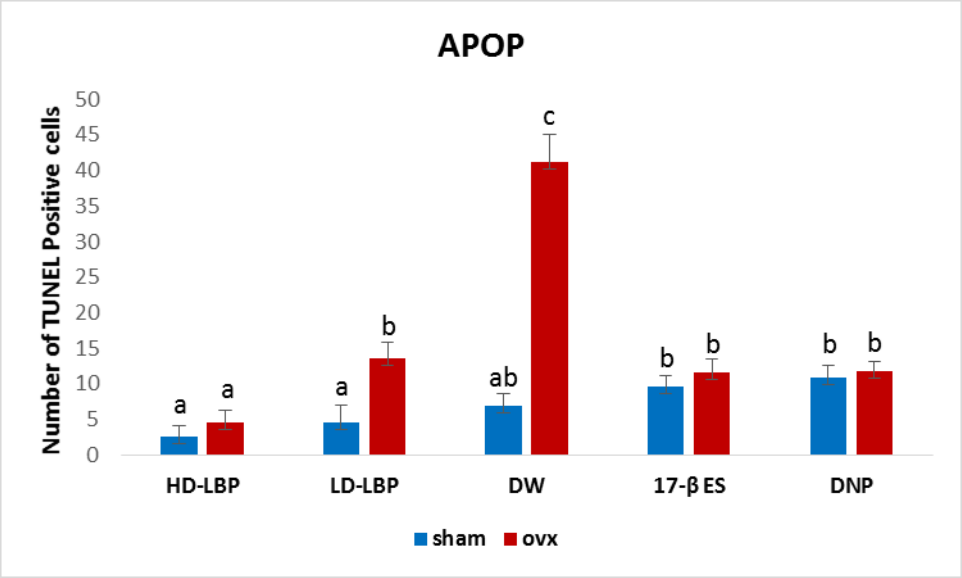

Figure 4.68. Mean (S.E.M.) Number of TUNEL positive cells in learning study. Mean-values with the same letters within vertical columns are significantly different ($p<0.05$).

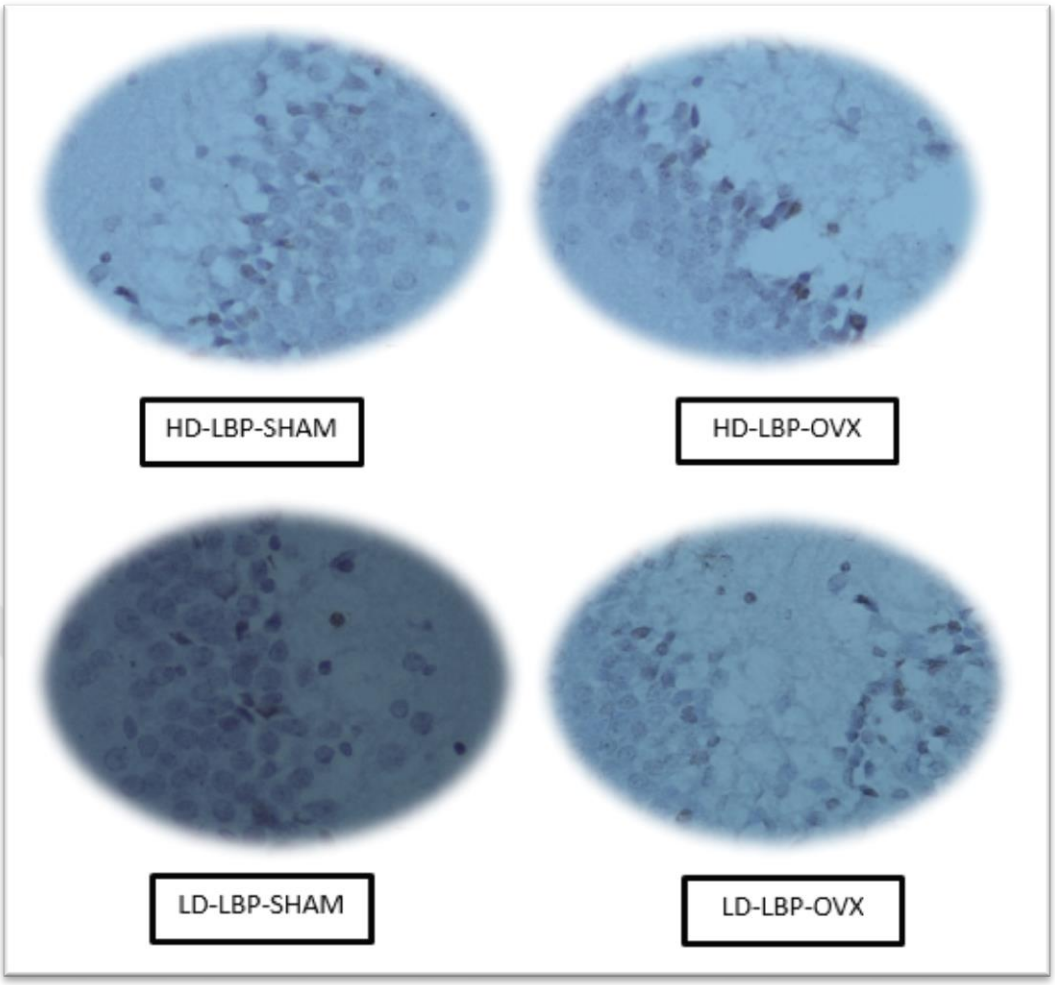

Figure 4.69. Immunstaining of the hippocampal TUNEL positive cells HD-LBP and LD-LBP treated rats in learning study (40X)

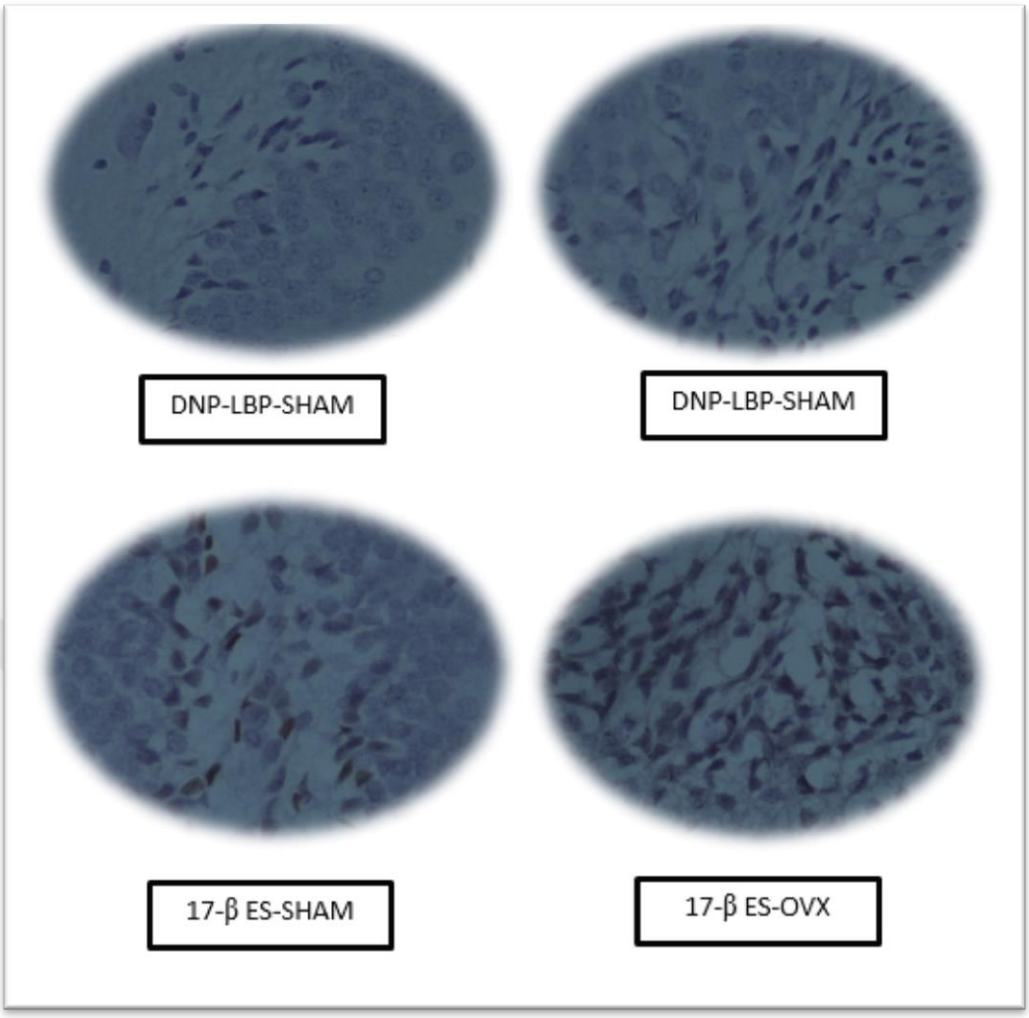

Figure 4.70. Immunstaining of the hippocampal TUNEL positive cells of 17-β ES and DNP treated rats in learning study (40X)

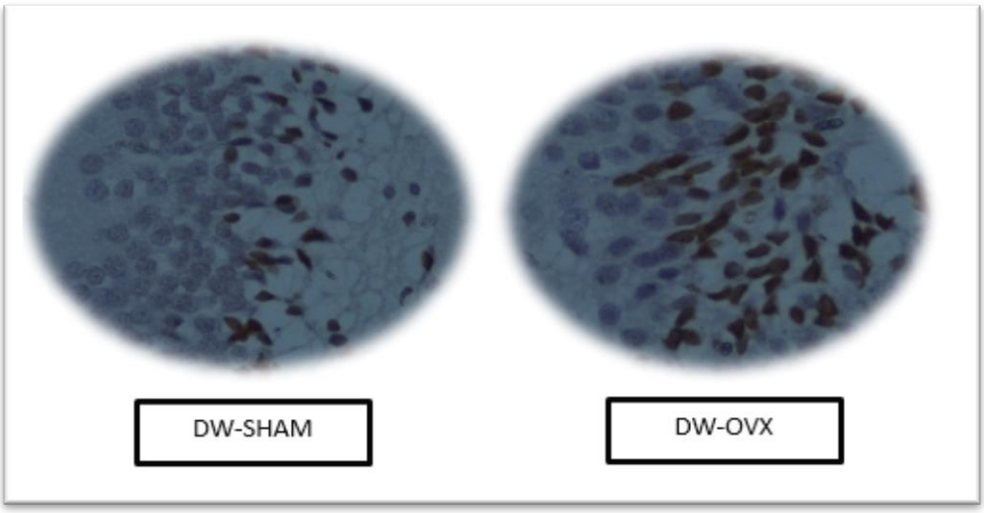

Figure 4.71. Immunstaining of the hippocampal TUNEL positive cells of DW treated rats in learning study (40X)

4.3 Discussion of Anxiety Study

Lycium barbarum fruit grows in China and its have many beneficial effects on human and animal. In our previous study, we have showed that the fruit have anxiolytic effect on rats (Pehlivan Karakaş et al., 2016). It is reported that the fruit enhance anxiety like behavior of human or rodents owing to its polysaccharides (Gao et al., 2015). In the present study, we aimed to explain effects of the fruit polysaccharides on the anxiety behavior of ovariectomized female rats and to explain biochemical and histochemical mechanism of the anxiety behavior.

Until today, *L. barbarum* fruit has been studied in many behavioral experiments but its effect on behavior of ovariectomized rats have been not yet studied. In this present anxiety study, ovariectomized and sham operated female rats were exposed open field test and elevated plus maze test for measurement of anxiety behavior after various treatments applications. In the open field test, decreased time spent in the center, increased time spent at the edge of open field and decreased mobility in the open field were accepted as high anxiety level (Benabid et al., 2008). However, in the elevated plus maze test, decreased total distance travelled, increased time spent at the closed arms, decreased time spent at the open arm, mobility and velocity were accepted as high anxiety level. This results were tried to clarify with mechanism of under these behaviors.

Our previous study demonstrated that methanol extraction of *Lycium barbarum* fruit decrease anxiety level of rats (Pehlivan Karakaş et al., 2016) and similarly the present results showed that LBP treatment decreased anxiety level of ovariectomized female rats. In the open field test, sham operated groups were lower anxiety level than ovx-operated groups but treatments had no effect in these groups behavior. Morover, the elevated plus maze test results showed that rats with sham operated condition travelled more and were more mobile than those in ovx-operated condition and, HD-LBP and LD-LBP treatments groups were more mobile than DW treated groups. These findings showed that HD-LBP and LD-LBP treated groups were less anxious than DW group and similarly, sham operated groups were less anxious than ovx operated groups. In literature, although it was showed that LBP enhance stress induced anxiety like behavior (Gao et al., 2015), but the present study did not show such effects of LBP on anxiety like behavior of ovariectomized rats.

Previous research suggests some mechanisms such as high serotonin level and antioxidant level due to LBP; however, did not provide measures for their claim (Pehlivan Karakaş et al., 2016). Such plausible mechanisms and others were illuminated in this study in the following section.

The behavioral distruption is caused by accumulating oxidative stress in brain. The oxidative stress level was decreased by increasing antioxidant defence system enzymes such as SOD, CAT, GPX and by decreasing MDA level. We hypothesized that ovariectomy induced anxiety like behavior was caused by decreasing antioxidant enzmes activities, so we analyzed antioxidant enzyme levels in serum of ovx/sham operated female rats after the applying behavioral tests. We tested SOD, CAT, GPX, 17-β ES and MDA serum concentration of ovx or sham operated female rats. The present biochemical findings indicated that both HD-LBP and LD-LBP treatments in rats had lower anxiety behaviors than other treatment groups. Moreover, these treatments increased SOD enzyme activities. These results showed that LBP treatments increased antioxidant defence. In the literature, it was showed that the levels of SOD and CAT enzyme activities were high concentration in rats with decreased oxidative stress, but MDA levels was high concentration in these rats serum (Zhao et al., 2015). Moreover, in ovx rats serum had decreased 17-β ES level but sham group had more 17-β ES level than ovx rats group (Han et al., 2015). Similar to the last outcomes of literature, HD-LBP treated rats had more SOD activity level than 17-β ES treated groups and sham operated groups had more SOD enzyme activity level than ovx operated groups. These outcomes showed that HD-LBP treatment had more antioxidant effect on ovariectomized female rats than other treatments. These findings showed similarity with the previous research finding, indicating that LBP application increased SOD activity in rats serum levels (Zhao et al., 2015). However, CAT and GPX enzyme activity results showed that 17-β ES treated groups were less CAT and GPX enzyme activities than other groups. 17-β ES and MDA measures showed that there was no difference in all groups. These results showed that there was a low decreasing the level of 17-β ES hormone of the ovariectomized rats serum because of the fact that these rats might showed resistant to this stress. Another possibility of this result is that it may be caused by less recovery time after the ovariectomy.

Behavioral distruption can be caused by changing of neurotransmitters level in brain such as hippocampus region. Especially, ovariectomy operation caused changing of neurotransmitter level by decreasing estrogen level in serum or some brain regions (Han et al., 2015).

Although it was reported that fruits of *Lycium barbarum* had high level of antioxidant activity and decrease anxiety like behavior, in the literature no experimental research was present about how it affected hippocampal neurotransmitter level such as serotonin and BDNF or hipocampal cell apoptosis level of ovariectomized rats. We aimed to explain effects of LBP on changing of hippocampal neurotransmitters and apoptosis level of ovariectomized rats. The BDNF is one of the nerve growth factors that plays an important role in anxiety behavior of human or rodents (Duman and Monteggia, 2006). Most studies showed that decresed anxiety levels were caused by increased BDNF levels in hippocampus region (Chen et al., 2015). We hypothesized that if ovariectomy operation caused anxiety like behaviors by decreasing BDNF level in hippocampus, LBP treatment might reverse this situation by increasing the BDNF level of the ovariectomized rats. For this purpose, we tested the BDNF levels of ovariectomized rats after the collected brain tissues. Our results showed that HD-LBP treatment goups had more BDNF level than other treatment groups in hippocampus region. In LD-LBP treatment groups were similar BDNF level to DZ treated groups and all treatment groups were more BDNF level than DW treatment (control) groups. The findings of present study suggested that increasing BDNF might be caused by activation of Gamma-amino butyric acid (GABA) receptors because GABA receptors caused both of increasing BDNF and its receptors level in hippocampus region. The receptors are TrkB receptors which play a key role decreasing anxiety like behavior (Zhao et al., 2015). However, ovariectomy caused increasing anxiety like behavior by decreasing BDNF level and thus it might be reversed down regulation of BDNF and TrkB pathway. It has been also reported that the TrkB activation by BDNF stimulates the Akt phosphorylation and decreased BDNF level of ovariectomized rats hippocampus region might be caused by low activation of the Akt phosphorylation (Rosa et al., 2016).

However, serotonin hormones and its receptors plays a important role in anxiety behavior, are responsible for anxiolytic effects. Previous research has indicated that a decrease in serotonin level induces anxiety (Briley et al., 1990). In literature, it is known that LBP decreased anxiety level by changing antioxidant biomarkers (Zhao et al., 2016) but it is unknown that whether or not anxiolytic effects of LBP on ovariectomized rats was caused by increasing hippocampal SERs. Based on this information, we aimed to investigate SER in hipocampus region of ovariectomized rats treated with LBP. The study findings showed that HD-LBP treated-ovariectomized rats had more serotonin receptors level than other treatments groups. This result supported our behavioral results and thus HD-LBP might show its anxiolytic effect by increasing hippocampal SER level.

One of the anxiety reasons is increased apoptosis of hipocampal neurons. It is known that antioxidants decrease apoptosis level of rats brain. LBP is one of the most powerfull antioxidants fruit polysaccharides. Previous study indicated that 20 mg/kg dose of LBP treatment decreased TUNEL positive neurons in hippocampus (Wang et al., 2014). Moreover, another study showed that LBP decreased oxidative stress and apoptosis of hippocampal neurons of rats. (Zhou et al., 2016) In the direction of these given evidences, we aimed to analyze whether or not anxiolytic effect of LBP on ovariectomized rats were performed by its reduced effect of apoptotic cell and evaluated TUNEL positive cell count in hippocampus region of ovariectomy and sham operated rats. In present study, DW treated-ovariectomized rats had high level of TUNEL positive cell counts than those in sham-operated groups. Moreover, HD-LBP and LD LBP had more TUNEL positive neurons than other groups but HD-LBP treatments were more effective than LD-LBP treatments groups. These beneficial effects of LBP might be due to its increasing SOD enzyme activity in these groups.

4.4 Discussion of Depression Study

In menopause or ovariectomy, reduction of ovarian hormones caused gradually decreasing of 17-β ES levels in serum of human or rodents. More recently, there has been emerging evidence that decreasing 17-β ES level resulted in

increasing oxidative stress in body, especially in brain (Park et al., 2016). In addition to this, there is another research finding indicating that depression like behavior is caused by increasing oxidative stress in brain (Patki et al., 2015). Especially, hippocampus region of the brain was more associated with behavioral distruptions (Mao et al., 2017). In last years, *L. barbarum* was mostly used for behavioral distruptions such as depression and the study showed antidepressant effect of *L. barbarum* (Pehlivan Karakaş et al., 2016; Zhang et al., 2012). The effects of LBP on hippocampal serotonin level of rats were not yet known. In present study, we examined the effects of polysaccharides from *L. barbarum* and the effects of the polysaccharides on ovariectomized female rats depression like behavior. In addition, the investigation of antioxidant and some hippocampal neurotransmitters mechanisms under the depression behavior were tried to explain.

Many drugs were used in treatment of depression and the antidepressant was tricyclic antidepresants such as IMP and selective serotonin re-uptake inhibitors (SSRIs) such as fluoxetine (Ostadhadi et al., 2016). We used forced swimming test for measurement of depression behavior of ovariectomized rats. The decrease in the immobility time in the FST indicated the less depressive of the rats (Ostadhadi et al., 2016). The FST results showed that IMP treated groups were less depressive than HD-LBP, LD-LBP and 17-β ES treatment groups and these groups were less depressive than DW (control) groups. Moreover, DW treated-sham operated groups were less depressive than DW treated-ovx operated groups. IMP is a powerfull antidepressant drug (Ostadhadi et al., 2016) and was used as a positive control in the present study. Although results of the study showed that LBP treatments were not as effective as IMP treatment but LBP treatments were more active than other treatment groups. In present study, antidepressant drug decreased immobility time paradigm of the Porsolt test.

There is evidence that depression is accompanied by an increased antioxidant defenses system dysfunction. Oxidative stresses contribute to the pathogenesis of depressive disorders (Mendez-David et al., 2015). Many antidepressant drugs decrease oxidative stress in chronically stressed animals (Mendez-David et al., 2015). It is known that LBP treatment increased SOD and CAT level and decreased MDA level of rats serum (Zhou et al., 2016). Our results was in line with previous

study, and showed that HD-LBP, 17-β ES and IMP treatments groups had higher level of SOD enzyme activity than DW group. Moreover, DW treated-sham operated subjects had higher amount of SOD activity than DW treated-ovx operated condition ones. These findings are consistent with the previous reports, indicating that ovx operated rats had lower amount of SOD activity than sham operated groups (Morrone et al., 2016). In CAT and GPX enzyme activities, 17-β ES treatment group had lower amount of CAT and GPX enzyme activities than other groups. 17-β ES concentration of all groups were not different from each other. Recovery time after OVX aplication is very important for decresing estrogen level in blood, the time might be unsufficient for decreasing estrogen level. In MDA concentration measurement, HD-LBP treatment groups had lower amount of MDA than LD-LBP and IMP treated rats. It is well known that primary antioxidant enzymes include SOD, GPX and CAT. SOD dismutates superoxide radicals to form H_2O_2 and O2. GPX is an enzyme responsible for reducing H_2O_2 or organic hydroperoxides to water and alcohol, respectively. CAT catalyses the breakdown of H_2O_2 to form water and O_2 (Changbo and Zhaojun, 2012).

Gamma-amino butyric acid is the primary inhibitory neurotransmitter. As in anxiety, $GABA_B$ receptors were mainly found in lymbic system in brain, playing an important role in depression distruptions (Ghaderi and Jafari, 2014). Antidepressant drugs showed its effect by this $GABA_B$ system such as baclofen. The BDNF is an important regulatory protein and mainly expressed in nervous system. (Jamal et al., 2015). Moreover, BDNF is implicated in the pathophysiology of depression (Yu and Chen, 2011). Postmortem studies have shown that hippocampal BDNF levels are decreased in depressed patients and increased in patients receiving antidepressant treatment (Hritcu and Gorgan, 2014). In present study, we examined the effects of LBP treatments on hippocampal BDNF level of ovariectomized rats. The results indicated that IMP treatments had highly effective on hippocampal BDNF level of the ovariectomized rats. The effects of HD-LBP treatments were close the effects of IMP treatments. Because the IMP drug regulates BDNF level, LBP treatments may regulate depression behavior by this way. DW-treated-ovx operated rats groups had least hippocampal BDNF level and thus ovariectomy operation might be distruped by BDNF pathway.

Increasing evidences show that hippocampal synaptic plasticity plays a crucial role in the pathogenesis of depression (She et al., 2015). However, in the present research, immunohistochemical analysis showed that results of HD-LBP, LD-LBP, 17-β ES and IMP treatments group were similar each other, but DW treated-sham operated group had higher amount of SER positive cells than DW treated-ovx operated group.

4.5 Discussion of Learning and Memory Study

Ovariectomy operation caused learning and memory impairments because of decreasing estrogen level. In previous study, we showed that *L. barbarum* enhanced learning and memory behavior of rats (Pehlivan Karakaş et al., 2016). However, the effects of the *L. barbarum* or its polysaccharides on ovariectomized rats behavior and under the mechanisms of the behavior have not been studied in literature up to now. Thus, we hypothesed that high and low doses of LBP would enhance learning and memory behavior of ovariectomized rats because of high level of antioxidant contents of LBP. Ovariectomized rats were exposed to Morris water maze test for measurements of learning and memory behavior. The current findings showed that HD-LBP treatments decreased time spent to find platform in Morris water maze test. Moreover, DW treated-sham operated groups spent less time to find platform than those in ovx operated groups. These results showed that HD-LBP treatments enhance memory performance of ovariectomized rats.

Increased antioxidant enzyme activity caused enhancement of learning and memory of rats. *L. barbarum* has high antioxidant content, thus we tested antioxidant enzyme activity of rat serum such as SOD, CAT, GPX and MDA, and 17-β ES. Our results showed that HD-LBP and 17-β ES treatment groups had higher level of SOD enzyme activity. However, CAT activity was similar in all groups. DW treated-sham operated groups had higher level of SOD, GPX, MDA and 17-β ES than those in ovx operated groups. Previous studies indicated that LBP incresed level of antioxidant enzymes and thus it may enhance learning and memory behavior of ovariectomized rats via antioxidant mechanisms.

In many studies, hippocampus region were related to learning and memory functions (Swart, 2016). SER and BDNF hippocampal level played an important role in the memory performance (Pytka et al., 2017). Increased hippocampal SER and BDNF enhances distrupted learning and memory functions of rats (Chan and Ye, 2017). Other important effect was apoptosis level of hippocampal neurons. In present study, we analyzed numbers of receptors of SER and BDNF, and apoptosis level of ovariectomized rats hippocampus. Our results showed that HD-LBP treatment increased the number of SER and BDNF positive neurons and decreased TUNEL positive neurons in hippocampus region of the rats. The behavioral effects of LBP might be based on SER and BDNF payhway in hippocampus region.

Consistently, we observed that ovariectomy operation induced significant cognitive impairments in the MWM tests. The activation of SER receptors by HD-LBP treatments decreased these cognitive impairments. We also found that LD-LBP increased memory function as well as the effect of the DNP treatments, might be associated with the similar increased SER level.

Moreover, learning activity is also responsible for signal transduction between two nerve cells. The most important neurotransmitters for learning: acetylcholine, norepinephrine and glutamic acid (glutamate). If this neurotransmitter is less secreted, memory problems may arise. Apart from acetylcholine, neuropinephrine and glutamate are also important neurotransmitters. These are: neurotransmitters such as dopamine, seratonin, endorphin, stress hormone, histamine, taurine. Neurotrophins are required for the development of nerve cells. Neurotrophins found in vertebrates; Neurotrophin-3, neurotrophin-4/5, neurotrophin-6 and neurotrophin-7. The most common neurotrophins are BDNF and its receptor TrkB (Murer et al., 2001). It plays a role in the formation of BDNF long term potential, synaptic plasticity and long term memory in the adult brain. In the same way, action (Bliss and Collingridge, 1993; Martin et al., 2000) is found in BDNF pre- and postsynaptic nerves. Both retrograde and anterograde are carried. BDNF moves through autocrine and paracrine mechanisms (Murer et al., in 2001). BDNF triggers neuronal activity and consequently learning by three different mechanisms (I) the contribution of ionotropic glutamate receptors and voltage-dependent Ca channels from postsynaptic sites is dependent on the uptake of Ca^{2+} into the cell

(Hartmann et al., 2001) (Ii) Dependent upon the uptake of Ca into the presynaptic region (Balkowiec And Katz, 2002) and (Iii) Ca uptake due to Ca release from intracellular Ca deposits, which is not dependent on intracellular uptake (Griesbeck et al., 1999). Activation of the BDNF and Trkb receptor has been shown to induce intracellular phospholipase Ca^{2+}, Phosphatidylinositol 3-kinase pathway is activated. Activation of the Ca^{2+}-challmodulin-dependent kinase by CaMKII also activates signaling regulators, mitogen-activated protein kinase (Segal, 2003).

BDNF is a neurotrophin acting at several levels in the brain (Kruse et al., 2007). BDNF was shown to be responsible for the survival, maintenance and growth of neurons (Wang et al., 2015). It also plays a critical role in synaptic plasticity and memory processes (Yu et al., 2009). In activity-dependent synaptic plasticity, BDNF enhances long-term potentiation in the hippocampus (Figurov et al., 1996) but blocks the induction of long-term depression in the visual cortex (Kumura et al., 2000). BDNF is a member of the neurotrophin family of growth factors and is concentrated in brain regions involved in learning and memory, such as the hippocampus and amygdala (Scharfman and MacLusky, 2006).

BDNF is a growth factor well known to be related to cognition (Piepmeier and Etnier, 2015). Oxidative stress is manifested by an elevation of the lipid peroxidation product such as MDA, and by a reduction of CAT and SOD levels. So, we investigated the potential effects of treatment with LBP against oxidative damage induced on ovariectomized rats. SOD and CAT are important antioxidant enzymes in the body. The ability for removing free radicals is reflected indirectly by the activity of these enzyme (Schuessel et al., 2005). MDA is a degradation product of lipid peroxidation, and its quantity is indicative of free radical-induced cell damage (Zhao et al., 2015)

SOD is the first and most important line of antioxidant enzyme defence against oxidative stress and particularly oxygen radicals. SOD scavenges superoxide by converting it to peroxide. Peroxide, in turn, is destroyed by catalase, which is widely distributed in all animal tissues. Antioxidants play a profound role in protecting erythrocytes against oxidative stress. Thus, SOD and catalase act in amutually supportive way with antioxidant enzymes to provide a protective defence

against ROS. GHS-Px could particularly catalyze the reductive action of GSH toH_2O_2 to protect the integrity of plasma membrane and functions. (Wu et al., 2010).

Antioxidants play a major role in protecting biological systems against reactive oxygen species and reflect the antioxidant capacity of the system (Wu et al., 2010) In view of the above and, considering the currently noted decline in MDA levels as well as elevated enzyme activities of SOD, GSH-Px, CAT and level of GSH in both lycium barbarum polysaccharides-treated groups, the potential of the lycium barbarum polysaccharides becomes evident as an anti-peroxidative and an antioxidant agent (Wu et al., 2010).

5. CONCLUSIONS

The aim of the present study was to investigate whether or not the *L. barbarum* polysaccharides is effective in reducing anxiety and depression like behaviors and in enhancing spatial learning performance of ovariectomized rats and clarify under the biochemical and immunohistochemical mechanisms of the behavioral effects. The present experiment is important for the first time evidence for the behavioral effect of *L. barbarum* polysaccharides on ovariectomized rats. This study also explored the biochemical and immunohistochemical mechanisms for behavioral effects of LBP treatments. For these purpose, we treated overiectomized rats with high or low doss of LBP and some drugs and 17-β estradiol. In particular, HD-LBP treatment and LD-LBP and various drugs treatment were more effective in reducing anxiety and depression like behaviors and improving spatial learning behaviors of ovariectomized female rats than DW treatment.

However, *L. barbarum* includes antioxidant contents and increased serum SOD enzyme activity of female ovariectomized rats. Also, HD-LBP increased SER receptor and BDNF neurotransmitters and decreased apoptotic cell count in hippocampus region of sham or ovx operated rats. In conclusion, the findings of this study suggest that the *L. barbarum* polysaccharides decreases the level of anxiety and depression like behaviors and increases spatial learning behavior in ovariectomized Wistar Albino female rats via its powerful antioxidant properties and its effects of changing hippocampal neurotransmitters level.

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7. CURRICULUM VITAE

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Publications (SCI):

1. Fatma Pehlivan Karakaş, Hamit Coşkun, Kadir Sağlam, **Bihter Gökçe Bozat**. "*Lycium barbarum* L. (Goji berry) Fruits Improves Anxiety and Depression-Like Behaviors, and Learning Performance: Moderating Role of Sex". *Turkish Journal of Biology*, 40: 762-771, **2016**. DOI: 10.3906/biy-1507-114.
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2. Fatma Pehlivan Karakaş, Hamit Coşkun, Hayriye Orallar, **Bihter Gökçe Bozat**. "The Effects of *Lycium barbarum* (Goji berry) Polysaccharides on Antioxidant Enzymes in Ovariectomized Rats". 1st International Congress on Medicinal and Aromatic Plants, Konya/ Turkey, 9 -12 May, **2017**.

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2. Fatma Pehlivan Karakaş, **Bihter Gökçe Bozat**, Günce Şahin, Arzu Uçar Türker. "Farklı Dozlardaki Besin Stresinin Keçi Sedefi (*Galega officinalis* L.) Bitkisinin Sekonder Metabolit Üretimi ve Antioksidan Aktivitesi Üzerine Olan Etkileri". 23. Ulusal Biyoloji Kongresi, 5-9 Eylül **2016.** Özet kitapçığı, Sayfa 76 (**Oral presentation**). Gaziantep, TÜRKİYE.
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