Section A-Research paper

Effect of estradiol on synaptic proteins and neuroinflammatory markers in unilateral and bilateral ovariectomized rat model

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Abstract

Estradiol, the primary estrogen hormone in females, plays a crucial role in the regulation of synaptic function and neuroinflammation within the central nervous system. The present study is aimed to investigate the effect of estradiol on mRNA expression of synaptic proteins and neuroinflammatory markers in unilateral and bilateral ovariectomized rat models, a commonly used method to induce estrogen deficiency and study the consequences of hormonal changes. Two months old Wistar albino female rats were divided into five experimental groups, each consisting of 6 animals. Unilateral and bilateral ovariectomized rats were treated with estradiol (10 mg/Kg/b.wt) subcutaneously for 30 days. A significant decrease was observed in the mRNA levels of Synuclein, SNAP25, PSD-95, Neuroligin, and MMP-9 along with a significant increase in the expression levels of IL-6 both in unilateral and bilateral ovariectomized rat models, indicating potential disruptions in synaptic function and neuronal plasticity. Furthermore, the bilateral ovariectomy group exhibited a more pronounced decrease when compared to the unilateral ovariectomy group. Moreover, when comparing the expression levels of these genes between the unilateral and bilateral ovariectomy groups after estradiol treatment, we observed a significant increase in Synuclein, SNAP25, PSD-95, Neuroligin, MMP-9 expression along with a significant decrease in the mRNA levels of IL-6 in the unilateral ovariectomy group compared to the bilateral ovariectomy group. The findings suggest that estradiol plays a crucial role in regulating synaptic function, neuroprotection, inflammation, and cognitive processes. Understanding the molecular mechanisms underlying estradiol's effects on gene expression in these genes may contribute to the development of therapeutic interventions for conditions associated with estrogen deficiency.

Keywords: ovariectomy; unilateral; bilateral; Eatradiol; synaptic proteins; neuroinflammatory markers

Introduction

Estradiol, the primary estrogen hormone in females, plays a crucial role in the regulation of synaptic function and neuroinflammation within the central nervous system. It exerts its effects through interactions with various synaptic proteins and influences the expression of neuroinflammatory markers. And also influence the expression of genes involved in synaptic function, neuroprotection, inflammation, and cognitive processes (Villa et al., 2016).

Unilateral and bilateral ovariectomy models are widely used to study the effects of estradiol deficiency and hormone replacement therapy on brain function. Unilateral ovariectomy involves the removal of one ovary, while bilateral ovariectomy involves the removal of both ovaries. These models mimic the hormonal changes that occur during menopause and provide a means to investigate the effects of estradiol on synaptic proteins and neuroinflammatory markers in the absence of endogenous hormone production. Recent studies using unilateral and bilateral ovariectomy models have shed light on the effects of estradiol on synaptic proteins and neuroinflammatory markers (Rocca et al., 2011).

Synaptic proteins are essential for maintaining synaptic structure and function, regulating neurotransmission, and facilitating synaptic plasticity. Estradiol has been shown to modulate the expression of synaptic proteins in the brain. For example, studies have demonstrated that estradiol increases the expression of synaptic proteins such as synaptophysin, PSD-95 (postsynaptic density protein 95), and SNAP-25 (synaptosome-associated protein 25). These proteins are involved in neurotransmitter release, synaptic vesicle trafficking, and synaptic connectivity. The modulation of synaptic proteins by estradiol suggests its role in influencing synaptic plasticity and cognitive function (Tunc-Ozcan et al., 2018; Lite et al., 2019; Li et al., 2019).

Neuroinflammation is characterized by the activation of glial cells and the release of pro-inflammatory cytokines, which can disrupt synaptic function and contribute to the progression of neurological disorders. Estradiol has been shown to modulate the expression of neuroinflammatory markers, including cytokines such as interleukin-6 (IL-6), tumor necrosis

factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β). Studies have demonstrated that estradiol treatment reduces the expression of these pro-inflammatory cytokines in the brain, indicating its anti-inflammatory properties. The modulation of neuroinflammatory markers by estradiol suggests its potential role in protecting against neuroinflammation and associated neurodegenerative diseases (Sharma et al., 2018; Singh et al., 2020).

Understanding the impact of estradiol on synaptic proteins and neuroinflammatory markers in unilateral and bilateral ovariectomized rat models can provide valuable insights into the mechanisms underlying hormonal regulation of brain function and its relevance to menopause-related neurological disorders. Hence, the present study is aimed to study the effect of estradiol on the gene expression of synaptic proteins (Synuclein, SNAP25, PSD-95, and Neuroligin) and neuroinflammatory markers (MMP-9, and IL-6) in unilateral and bilateral ovariectomized rat models.

Materials and Methods

Animals

In accordance with the NIH Guide for Care and Use of Animals, two-month-old Wistar albino female rats were housed under a 12/12 h reversed light/dark cycle. At 8:00 h, the lights went out. They were kept at 80-85% of their regular body weight during the maze testing by consuming fewer calories. 5g of weight gain per week were allowed for growth.

Ovariectomy procedure

Rats were given general anaesthesia, 60 mg/kg ketamine hydrochloride intramuscularly, and 50 mg/kg sodium pentobarbital intraperitoneally before the ovaries were extracted unilaterally and bilaterally through midline abdominal incisions. The uterine horns and arteries were severed 0.5-1 cm before the ovary. Adipose tissue was cut and ligated before being returned to the abdominal cavity. A monofilament suture was used to close the wounds. After the procedure, a preventive dose of 4000 IU benzathine penicillin G was administered intramuscularly, and the animal was allowed to awaken from anaesthesia (Huether, McCance, 2019). The animal was immediately returned to its cage after waking up from anaesthesia.

Following surgery, the animal was kept in a heated cage (25-27 ℃) for at least two hours. Following surgery, the animal was kept in a heated cage (25-27 ℃) for at least two hours. The animals were separated for the first few days following surgery. While the animal was recovering, the cage was cleaned on a regular basis. For postoperative pain relief, the animals received a subcutaneous injection of 5 mg of Rimadyl per kg of body weight in saline twenty-four hours after surgery (Jakob et al., 2012).

Hormonal treatment

After ovariectomy, rats were split into 5 study groups, each with a total of 6 animals. Two experimental groups (unilateral OVX and bilateral OVX) received 100 millilitres of sesame oil diluted with 10 mg of 17b-estradiol-3-benzoate. 100 ml of sesame oil was given to the control groups twice, separated by a 24-hour period (Dulce, 2012). After 30 days, the experimental animals were killed and organs were dissected and utilized for the molecular analysis.

Experimental design

Animals were grouped into five and each containing of 6 animals. Group I- Normal control rats; Group II- Unilateral ovariectomized rats; Group III- Unilateral ovariectomized rats treated with estrdiol subcutaneously (10 mg/Kg/b.wt) for 30 days; Group IV- Bilateral ovariectomized rats. Group V: Bilateral ovariectomized rats treated with estrdiol subcutaneously (10 mg/Kg/b.wt) for 30 days.

5.2.2 Gene expression analysis

Isolation of total RNA, cDNA conversion and real-time PCR: Using the method described by Fourney et al. (1988), total RNA was extracted using a TRIR kit from the brain tissue of control and diabetic rats with and without estradiol treatment. (Total reagent for RNA isolation.One millilitre of TRIR was used to homogenise 100 mg of fresh tissue. This mixture received 0.2 mL of chloroform, which was then added, vortexed, and kept at 4 C for several minutes. It was then centrifuged for 15 minutes at 12,000 x g (at 4 ℃). The aqueous phase (top layer) was carefully transferred to a micro centrifuge tube after centrifugation. Equal parts of isopropanol were then added, mixed by vortexing for 15 seconds, and then the tube was kept at 4° C for 10 minutes. The mixture was centrifuged once more for 10 minutes at 4 degrees Celsius and 12000 g, with the supernatant being discarded. After vortexing 1mL of 75 percent ethanol over the RNA pellet,

the concentration of RNA was determined through spectrophotometry. The results are given in micrograms (μg).

Following the directions provided by the manufacturer (Seraing, Belgium), the complementary DNA was produced from 2 mg of total RNA using the RT (reverse transcriptase) kit that was purchased from Takara. To carry out Real-Time PCR, a total of 45 μL of water, 2x reaction buffer (Takar sybr green mastermix), forward and reverse primers (primer sequences are listed in Table: 5.1), and primers for the target and house-keeping gene (β-actin) were all thoroughly mixed and spun down. 5 μl of template complementary DNA for the samples, 5 μl of control DNA (positive control), and 5 μl of water (negative control) were placed in separate PCR vials. 45 μl of the reaction mixture was added to the reaction bottle, and the reaction was set up for 40 cycles (95 °C for 5 min, 95 °C for 5 s, 60 °C for 20 s, and 72 °C for 40 s). Using a PCR machine (Stratagene MX 3000P, Agilent Technologies, 530l, Stevens Creek Blvd, Santa Clara CA, 95051), the collected data were plotted on a graph, and relative quantification was calculated using the amplification and melt curves. The qualifying amount of mRNA was determined using the comparison method.

5.2.3 Statistical analysis

To determine the significance of specific differences between the control and experimental groups, the obtained data were statistically analysed using one-way analysis of variance (ANOVA) and student Newman Keul's comparison tests with Graph Pad Prism (version 5). At $p<0.05$, the significance was taken into account.

5.3 Results

5.3.1 Estradiol promotes synnuclein mRNA expression in ovariectomized rats

Figure 1 represents the mRNA expressions of synnuclein in ovariectomized rats. The results suggest that ovariectomy leads to a decrease in Neuroligin expression, indicating potential disruptions in synaptic adhesion and neuronal connectivity. However, estradiol treatment appears to counteract this effect by increasing Neuroligin expression, particularly in the unilateral ovariectomy group.

5.3.2 Estradiol raises the mRNA expression of SNAP25 in ovariectomized rats

Figure 2 depicts the mRNA expressions of SNAP25 in ovariectomized rats. A significant decrease in SNAP-25 expression was observed in ovariectomized rats, indicating potential disruptions in synaptic function. However, estradiol treatment appears to attenuate this effect by increasing SNAP-25 expression, particularly in the unilateral ovariectomy group which indicates a potential role of estradiol in modulating SNAP-25 expression and potentially restoring synaptic function in the context of ovariectomy.

5.3.3 Estradiol increases the mRNA expression of PSD-95 in ovariectomized rats

Figure 3 shows the mRNA expressions of PSD-95 in ovariectomized rats. Our findings revealed that when compared to the control group, the expression of PSD-95 was significantly decreased in both the unilateral ovariectomy and bilateral ovariectomy groups. Furthermore, the bilateral ovariectomy group exhibited a more pronounced decrease in PSD-95 expression compared to the unilateral ovariectomy group. These results indicate that bilateral ovariectomy has a greater impact on reducing PSD-95 expression compared to unilateral ovariectomy. However, estradiol treatment showed a significant increase in the expression of PSD-95 particularly in the unilateral ovariectomy group when compared to the control group.

5.3.4 Estradiol raises the mRNA expression of Neuroligin in ovariectomized rats

Figure 4 represents the mRNA expressions of Neuroligin in ovariectomized rats which were also studied in the present study. It indicates that when compared to the control group, the expression of neuroligin was significantly decreased in both the unilateral and bilateral ovariectomy groups. After estradiol treatment, a significant increase was observed in neuroligin expression in the unilateral ovariectomy group compared to the bilateral ovariectomy group which suggests that estradiol may play a role in synaptic development and plasticity through the regulation of Neuroligin expression.

5.3.5 Estradiol raises the mRNA expression of MMP-9 in ovariectomized rats

Figure 5 shows the mRNA expressions of **MMP-9** in ovariectomized rats. The results showed that ovariectomy led to a decrease in MMP-9 expression, indicating potential disruptions in extracellular matrix remodeling and synaptic plasticity. However, estradiol treatment countered this effect by increasing MMP-9 expression, particularly in the unilateral ovariectomy

group. This suggests that estradiol may play a role in restoring extracellular matrix remodeling and synaptic plasticity following ovariectomy

5.3.6 Estradiol declines the mRNA expression of IL-6 in ovariectomized rats

Figure 6 shows the mRNA expressions of IL-6 in ovariectomized rats. IL-6 expression, a pro-inflammatory cytokine associated with immune responses and neuroinflammation, was found to be upregulated in ovariectomized rats, potentially contributing to memory loss. However, estradiol treatment reduced IL-6 expression in both unilateral and bilateral ovariectomized rats, indicating its potential therapeutic effect in mitigating memory-related deficits associated with ovariectomy.

Discussion:

Estradiol, as the primary estrogen hormone in mammals, plays a crucial role in regulating gene expression within the central nervous system. Its effects are particularly notable in various physiological processes, including synaptic function, neuroprotection, inflammation, and cognitive processes. Consequently, numerous studies have focused on understanding how estradiol influences the expression of specific genes involved in these pathways. In the context of unilateral and bilateral ovariectomized rat models, which simulate the depletion of endogenous ovarian hormones, investigating the impact of estradiol on gene expression becomes even more significant.

Synuclein is a family of proteins involved in synaptic function and implicated in neurodegenerative diseases. The present result suggests that, ovariectomy leads to a decrease in synnuclein expression, indicating potential disruptions in synaptic function and neuronal plasticity (Fig.1). However, estradiol treatment appears to counteract this effect by increasing synnuclein expression, particularly in the unilateral ovariectomy group. A study by Srinivasan et al. (2019) demonstrated that estradiol replacement therapy after ovariectomy significantly upregulated the gene expression of Synuclein in the hippocampus of rats. These findings suggest that estradiol may exert a neuroprotective effect by modulating Synuclein expression which coincide with the present results represented in figure.1.

SNAP25 is a crucial component of the SNARE complex, essential for synaptic vesicle fusion and neurotransmitter release. In the present study we observed significant changes in the expression of SNAP-25 between groups (Fig.2). These findings suggest that ovariectomy leads to a decrease in SNAP-25 expression, indicating potential disruptions in synaptic function. However, estradiol treatment appears to attenuate this effect by increasing SNAP-25 expression, particularly in the unilateral ovariectomy group. This indicates a potential role of estradiol in modulating SNAP-25 expression and potentially restoring synaptic function in the context of ovariectomy. A study by Guerra-Araiza et al. (2012) revealed that estradiol replacement in bilateral ovariectomized rat's upregulated SNAP25 gene expression in the prefrontal cortex. These results suggest that estradiol may influence synaptic plasticity and cognitive function by modulating SNAP25 expression.

PSD-95 is a scaffolding protein that plays a key role in organizing and anchoring synaptic proteins. Our findings (Fig.3) revealed that when compared to the control group, the expression of PSD-95 was significantly decreased in both the unilateral ovariectomy and bilateral ovariectomy groups. Furthermore, the bilateral ovariectomy group exhibited a more pronounced decrease in PSD-95 expression compared to the unilateral ovariectomy group. These results indicate that bilateral ovariectomy has a greater impact on reducing PSD-95 expression compared to unilateral ovariectomy.

To evaluate the effect of estradiol treatment on PSD-95 expression, we administered estradiol to the ovariectomized rats and analyzed PSD-95 levels. Our results demonstrated that after estradiol treatment, the expression of PSD-95 was significantly increased in the unilateral ovariectomy group when compared to the control group. Moreover, when we compared the expression levels of PSD-95 between the unilateral and bilateral ovariectomy groups after estradiol treatment, we observed a significant increase in PSD-95 expression in the unilateral ovariectomy group compared to the bilateral ovariectomy group. It has been reported in a study by Garcia-Segura et al. (2010), estradiol replacement in ovariectomized rats increased the gene expression of PSD-95 in the hippocampus. This finding suggests that estradiol may promote synaptic remodeling and enhance synaptic strength by modulating PSD-95 expression.

Considering genes involved in synapse formation and maintenance, influencing synaptic connectivity and neural circuitry, Neuroligin expression (Fig.4) was also studied in the present

study. Our results indicated that when compared to the control group, the expression of neuroligin was significantly decreased in both the unilateral ovariectomy and bilateral ovariectomy groups. Moreover, the bilateral ovariectomy group exhibited a more pronounced decrease in neuroligin expression compared to the unilateral ovariectomy group. This suggests that bilateral ovariectomy has a greater impact on reducing neuroligin expression compared to unilateral ovariectomy and after estradiol treatment; the expression of neuroligin was significantly increased in the unilateral ovariectomy group when compared to the control group. Furthermore, when we compared the expression levels of neuroligin between the unilateral and bilateral ovariectomy groups and after estradiol treatment, we observed a significant increase in neuroligin expression in the unilateral ovariectomy group compared to the bilateral ovariectomy group. This highlights the potential role of estradiol in modulating neuroligin expression and potentially restoring synaptic function and neuronal connectivity in the context of ovariectomy. A study by Baudry et al. (2013) demonstrated that estradiol replacement in bilateral ovariectomized rats increased Neuroligin gene expression in the amygdala. These findings suggest that estradiol may play a role in synaptic development and plasticity through the regulation of Neuroligin expression.

Additionally, the expression of genes such as MMP-9 & IL-6 (Fig.5&6) involved in tissue remodeling and neuroinflammation were also analyzed in the current study. These findings suggest that ovariectomy leads to a decrease in MMP-9 expression, indicating potential disruptions in extracellular matrix remodeling and synaptic plasticity. However, estradiol treatment appears to counteract this effect by increasing MMP-9 expression, particularly in the unilateral ovariectomy group. This highlights the potential role of estradiol in modulating MMP-9 expression and potentially restoring extracellular matrix remodeling and synaptic plasticity in the context of ovariectomy. In a study by Li et al. (2018), estradiol replacement in unilateral ovariectomized rats suppressed the gene expression of MMP-9 in the hippocampus. These results suggest that estradiol may attenuate neuroinflammation and protect against tissue damage by modulating MMP-9 expression.

Considering IL-6, a pro-inflammatory cytokine involved in immune responses and neuroinflammation, our findings indicate that ovariectomy leads to an upregulation of IL-6 expression, which may contribute to memory loss. However, estradiol treatment appears to

attenuate this effect by reducing IL-6 expression in both unilateral and bilateral ovariectomized rats compared to their respective non-treated counterparts. These results highlight the potential importance of estradiol in regulating IL-6 expression and suggest its therapeutic potential in mitigating memory-related deficits associated with ovariectomy. A study by Gonzalez-Perez et al. (2007) showed that estradiol replacement in bilateral ovariectomized rats decreased the gene expression of IL-6 in the hippocampus. These findings suggest that estradiol may exert anti-inflammatory effects by modulating IL-6 expression.

Conclusion:

Estradiol exerts significant influence on the gene expression of Synuclein, SNAP25, PSD-95, Neuroligin, MMP-9, and IL-6 in unilateral and bilateral ovariectomized rat models. The findings suggest that estradiol plays a crucial role in regulating synaptic function, neuroprotection, inflammation, and cognitive processes. Understanding the molecular mechanisms underlying estradiol's effects on gene expression in these genes may contribute to the development of therapeutic interventions for conditions associated with estrogen deficiency.

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Figures with legends:

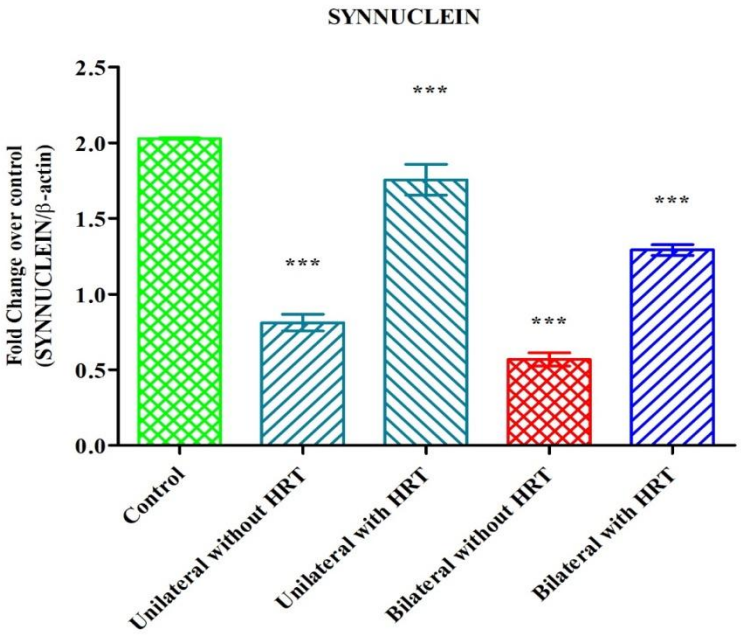

Figure 1.Effect of Estradiol on mRNA expression of synnuclein in ovariectomized rats. Each bar represents mean ± SEM of 6 animals. The significance was considered at p< 0.05.

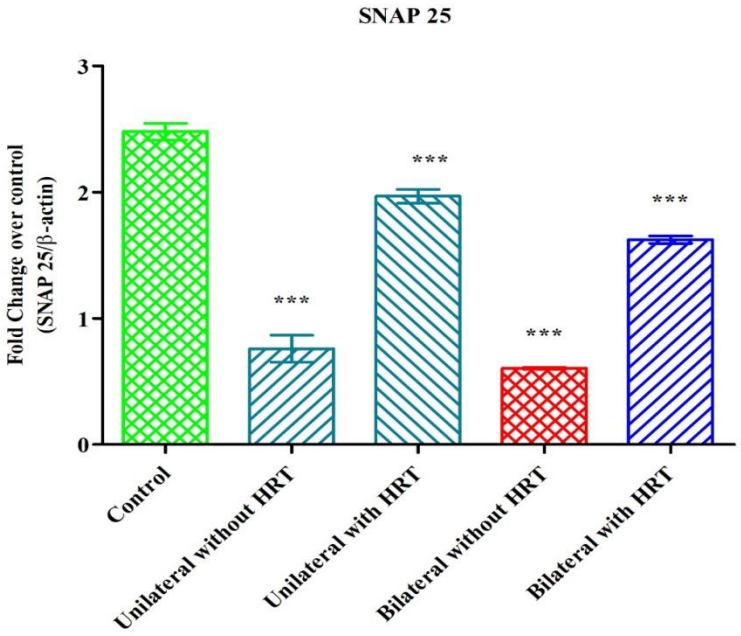

Figure 2.Effect of Estradiol on mRNA expression of SNAP-25 in ovariectomized rats. Each bar represents mean ± SEM of 6 animals. The significance was considered at p< 0.05.

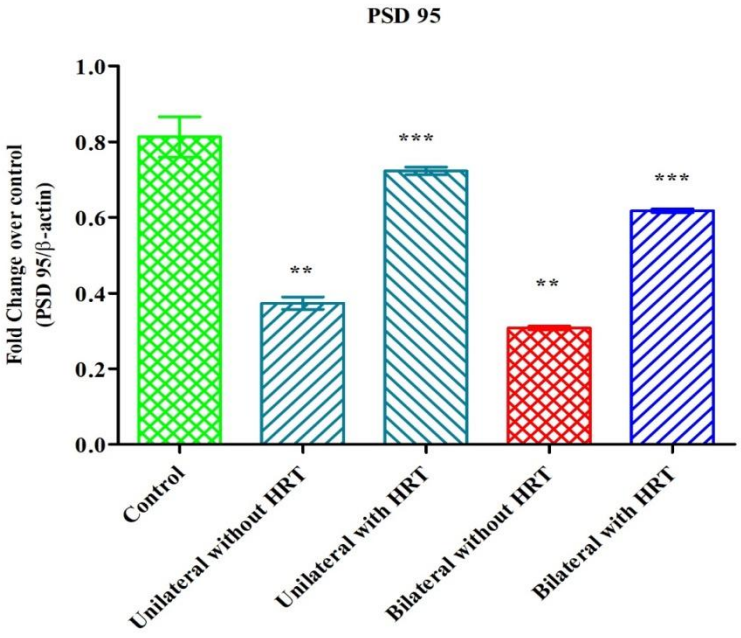

Figure 3.Effect of Estradiol on mRNA expression of PSD-95 in ovariectomized rats. Each bar represents mean ± SEM of 6 animals. The significance was considered at p< 0.05.

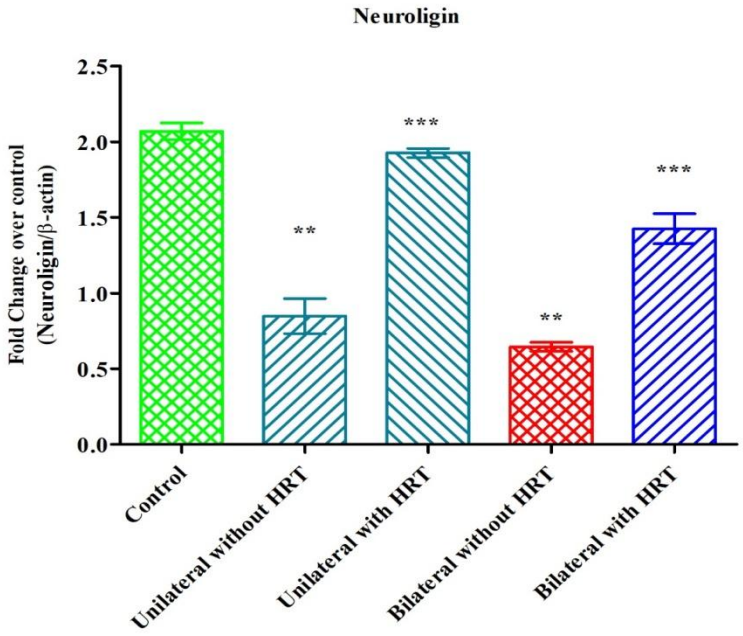

Figure 4.Effect of Estradiol on mRNA expression of Neuroligin in ovariectomized rats. Each bar represents mean ± SEM of 6 animals. The significance was considered at p< 0.05.

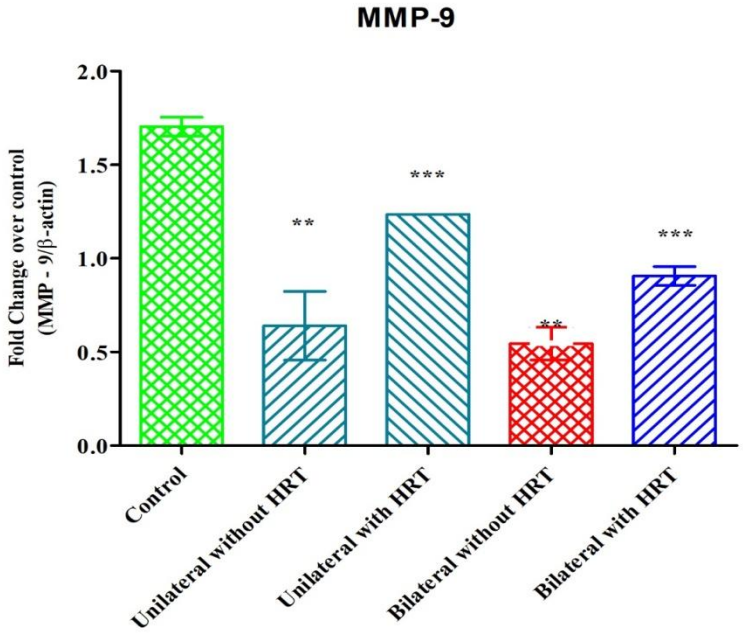

Figure 5.Effect of Estradiol on mRNA expression of MMP-9 in ovariectomized rats. Each bar represents mean ± SEM of 6 animals. The significance was considered at p< 0.05.

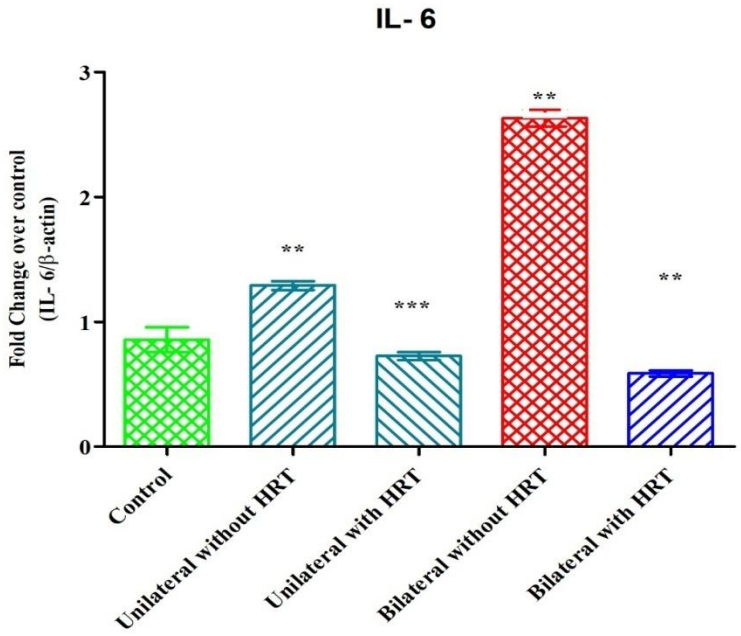

Figure 6.Effect of Estradiol on mRNA expression of IL-6 in ovariectomized rats. Each bar represents mean ± SEM of 6 animals. The significance was considered at p< 0.05.