



## Evaluating the effect of genistein on the amount of BDNF protein in the spinal cord of diabetic ovariectomized rats

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### ABSTRACT

*BDNF (Brain derived neurotrophic factor) is a secretion protein and a member of the neurotrophin family of growth factors. Studies have shown that in ovariectomized animals, the expression of BDNF decreased. However, recommending more estrogen increases its expression. Genistein is a phytoestrogen found in soy and soy products in large amount. The compound has attracted medical science researchers and public individuals, owing to the fact that it helps in the prevention and treatment of several diseases such as diabetes, menopause symptoms, osteoporosis, heart and blood vessel diseases, renal disease, and different cancers. In this study, the effect of genistein was studied as an alternative to estrogen in the expression of BDNF in ovariectomized rats of type 2 diabetes. Sixty (60) female rats were randomly divided into 4 groups. The SHAM group received laparotomy and drug solution (DMSO+PEG). In the ovariectomy group (OVX), the ovary on both sides of the abdomen were removed. The diabetic ovariectomy group (OVX. DI) had type 2 diabetes after ovariectomy. In the diabetic ovariectomy group treated with genistein (OVX. DI. G), the rats received genistein 1 mg / kg / day after ovariectomy and induction of diabetes. Thereafter, sampling of spinal cord was carried out. The amount of BDNF was measured using RTPCR. The results showed that the injection of genistein significantly increased the amount of BDNF expression in the spinal cord when compared with the diabetic ovariectomy group (OVX.DI) and ovariectomy group (OVX) ( $p < 0/05$ ). Since BDNF is a member of the neurotrophic factor family, its expression is required for the regulation of growth, survival, and preservation of neurons and ovarian steroid. Therefore, genistein can be said to increase BDNF in the spinal cord.*

**Keywords:** Genistein, BDNF, Diabetes, Spinal Cord.

### INTRODUCTION

Every year, most women undergo ovariectomized bilateral surgical operation, due to tumors, benign ovary, or for the prevention of malignancies, and as such, they experience early menopause [1]. One of the risks faced by women who have ovariectomized bilateral operation before getting to the natural age of menopause is the occurrence of neurological diseases[2]. Nowadays, there are evidences that steroid hormones, such as estrogen, are neuroprotective and therefore play the role of a sexual hormone. They can protect the environment and central nervous system against risks such as lack of oxygen, glucose etc[3]. Diabetes is one of the other related risk factors of age, which has become a threat to menopausal women [4]. This disease is one of the most common endocrine disorders and more than 100 million people are known to suffer from the disease yearly. It is the seventh leading cause of death worldwide[5]. The most important disorder is neuropathy, which is as a result of diabetes. It includes environmental nerves disorder [6]. It is associated with structural changes in environmental nerves that include axonal atrophy, demyelinating, neurofibrillary decrease and slowing of nerve fibers reconstruction [7]. Neuropathies are the common complications of long term diabetes and more than 50% of diabetic patients are known to suffer from this disorder [8]. As an important and significant disease, they provide the ground for foot wound and cutting of end parts [9]. Brain derived neurotrophic factor (BDNF), relative to factors of nerve growth, plays a regulatory role in neural differentiation, synaptic plasticity, and cell death [10,11]. It should be mentioned that estrogen has interference performance with BDNF. As studies have shown that estrogen receptors are in the cells of the brain, which include BDNF receptors or expression of BDNF. Estrogen regulates the activity of neurotrophin system. However, studies have also shown that in ovariectomized animals, the expression of BDNF decreased and therefore more estrogens are required to increase its expression [12]. But treatment with estrogen increases the incidence of

breast cancer and cervical adenocarcinoma. Some researchers emphasize that some plants like phytoestrogen with natural formula have fewer side effects. It is especially the case in breast and cervical cancer of humans [13]. It seems that they can serve as an alternative to estrogen. Genistein acts similar to estrogens as one of the most important effective product of phytoestrogens like soy. Its structural similarities to estrogen is the reason for its high affinity towards estrogen receptors, making it capable of performing estrogenic effects in the body [14]. Phytoestrogens are weak agonists of estrogen. They can provide strong effects with a small amount of estrogen in the environment. Perhaps the correct prediction is that they provide more estrogenic properties in menopausal women [15]. Some studies have shown that phytoestrogen adjusts some biochemical processes in the central nervous system. BDNF expression in the brains of ovariectomized rats increased when fed with soybeans or diet that contained steroid for 8 weeks [16]. Also, there are evidences that soy phytoestrogens in the frontal cortex of female rats with the race of Retired, increased BDNF [17]. Therefore, this study was carried out to investigate the effects of neuroprotective genistein on BDNF expression in the spinal cord of ovariectomized diabetic rats.

## MATERIALS AND METHODS

### *Animal Care*

Forty female Wistar rats (weighing 200-250 g) were purchased from the Experimental Animal Research Center, Faculty of Medicine, Tabriz University, Tabriz, Iran. All rats were kept under controlled conditions (temperature 22-24°C with 12:12 h light and dark cycle) and received standard chow diet and water *ad libitum* for 1 week. This study was approved by the University Ethics Committee. After 1 week, the rats were divided randomly into 4 groups (n=10) as follows; 1. Sham: This group underwent only surgery without ovariectomy, 2. OVX: (bilateral ovariectomy), 3. OVX.D (OVX + Diabetes), 4. OVX.D.G: (OVX + Diabetes + Genistein), before the experiment begins, all animals, except sham, underwent a bilateral ovariectomy. The ovaries were excised and oviducts replaced, with minimum disruption to surrounding soft tissues.

Induction of type 2 diabetes: After 10 days of recovery, the rats in the diabetic groups were fed with HFD (58% fat, 17% carbohydrate and 25% protein) *ad libitum* for the initial period of 4 weeks, and then a low dose of STZ (35 mg/kg) was injected intraperitoneally (IP) in a 0.1 M citrate buffer (pH 4.5), the blood glucose level was measured with a glucometer in all rats after the induction of diabetes. Animals with blood glucose levels of more than 200 mg/dl were selected as diabetic rats.

Genistein (sigma Chemical Inc. St. Louis, MO, USA) dose (1 mg/kg/day. SC) administration for 4 weeks was performed for related groups.

### **Molecular analysis**

#### *Total RNA extraction and real time PCR*

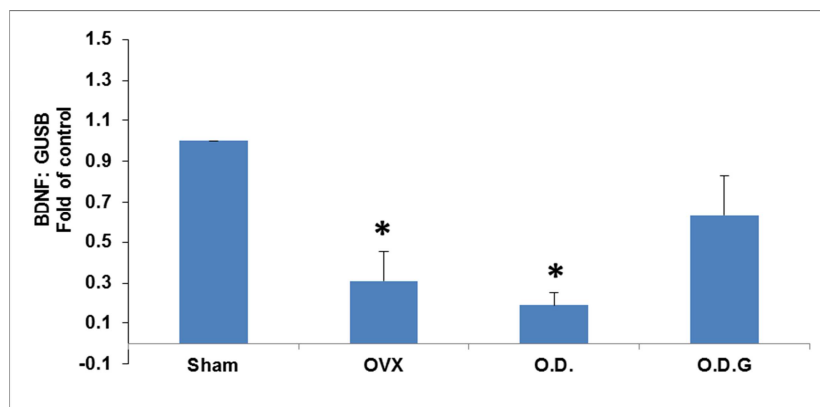
At the end of 8 weeks, animals were anaesthetized with ketamine (50 mg/kg) and xylazine (8 mg/kg) and their hearts were isolated. qPCR studies were conducted as described in previous studies. The comparative gene expression of BDNF analyzed by real time polymerase chain reaction (realtime PCR) is described as follows: For extraction procedure, frozen spinal cord samples were homogenized in Trizol reagent (Gibco/BRL) according to the manufacturer's recommendations (using chloroform layer separation followed by treatment with isopropanol and ethanol). The RNA content and purity of samples were measured using UV spectrophotometry and tested for integrity by gel electrophoresis with ethidium bromide. Reverse transcription (RT) was accomplished using Taqman MicroRNA reverse Transcriptase kit (Applied Biosystems) according the manufacturer's instructions. Real time quantification for the expression of microRNA and mRNA was performed with a SYBRgreen PCR Master Mix, and Real-time PCR reactions were performed on a Bio-Rad iQ5 detection System (Richmond, CA, USA). The *gap* expression was evaluated as a housekeeping gene for sample variation in RT reaction. To accurately detect mature BDNF, a realtime quantification using Ambion primers was performed for BDNF (Ambion, Austin, TX) with a primer sequence of CUGCCAAUCCAUGGUCACAG (www.mirbase.org). The 2-(DDCt) method was used to determine the comparative quantitative levels of BDNF. The results were expressed as fold-difference to relevant controls.

### **Statistical analysis:**

Results are expressed as mean  $\pm$ SEM. All data were analyzed by one-way ANOVA with Tukey's Multiple Comparison post hoc test, and Student's t-test was used to evaluate significant differences ( $p < 0.05$ ) between the groups.

## RESULTS

Expression of BDNF gene in the spinal cord of ovariectomized diabetic rat: The expression level of BDNF gene in the spinal cord of OVX and OVX+DI was significantly lower than that of the control ( $p < 0.05$ , Figure 1). On the other hand, treatment with genistein for 2 months resulted in a significant increase in the expression of BDNF ( $p < 0.05$ , Figure 1). Figure 1 shows that genistein increased the expression of BDNF. In comparison with the OVX and OVX+DI group, genistein increased the tissue BDNF level, 2 months after treatment ( $p < 0.05$ ).



**Figure 1. The effects of OVX,DI and genistein treatment on BDNF gene expression.**

*Sham operation or bilateral OVX and DII was performed, and then OVX and OVX,DI rats were subcutaneously administered with genistein (1mg/kg/day) or placebo (DMSO+PEG) for 8 weeks. BDNF expression was determined using real-time quantitative PCR. The values were expressed as the fold of change ( $\times$  Basal) against the control (no treatment), and in Mean  $\pm$  SEM,  $n = 6$ , each one with triplicate samples. \* where  $P < 0.05$  compared to the control.*

## DISCUSSION

The results of this study demonstrated that OVX and specially OVX+DII significantly reduced BDNF gene expression in the spinal cord. Treatment with genistein increased BDNF gene expression in the spinal cord in OVX and especially OVX+DII rats. BDNF is a member of the neurotrophic factor family, and plays a key role in regulating the growth, survival, and preservation of neurons [18]. In addition, BDNF plays a key function in learning and memory [19]. It has been suggested that decreased production of BDNF is a pathogenetic factor common to major depression and Alzheimer's disease, which might clarify the relationship between both disorders [20]. Singh (1995), in his evaluation of the effect of OVX and E2 replacement on BDNF mRNA expression using *in situ* hybridization, extended the hypothesis that 17- $\beta$  estradiol (E2) may exert a neurotrophomodulatory role. It was shown that E2 deprivation leads to a reduction in BDNF mRNA in ovariectomized rat, and that E2 replacement therapy was more effective in maintaining BDNF mRNA in the hippocampus, suggesting the need for ovarian steroid in the expression of BDNF [21]. However, estrogen use is associated with increased incidence of breast cancer [22]. In a study, it was shown that phytoestrogens occupied the ER- $\beta$  binding site and regulates the expression of BDNF mRNA [23]. In this study, it seems that genistein increased BDNF expression probably via these estrogen receptors and exerted its neuroprotective effect on the spinal cord. In this study, the expression of BDNF reduced in OVX compared to the sham group, and replacement of estrogen with genistein led to increased BDNF expression in the spinal cord. In addition, 17 $\beta$ -estradiol, daidzein and genistein notably enhanced hippocampus neuronal cell proliferation and viability. Similar to the effect of 17 $\beta$ -estradiol, daidzein and genistein induced an increase in the percentage of cells in S phase. Daidzein and genistein significantly increased the expression of BDNF mRNA and protein levels [24]. Krabbe et al. (2007) demonstrated that Plasma levels of BDNF decreased in humans with type 2 diabetes independently of obesity. They showed that the Plasma BDNF is inversely associated with fasting plasma glucose, but not with insulin [25]. In the present study, the expression of BDNF significantly decreased in ovariectomized diabetic rats in comparison to the control group, in which genistein reversed these effects and increased BDNF expression in the spinal cord. In animals, BDNF is concerned with the improvement of insulin resistance. BDNF decreases food intake and lowers the blood glucose level in obese diabetic mice [26–28]. The role of BDNF in metabolism is supported by investigations on BDNF-deficient mice, as it extends obesity and hyperphagia in early adulthood [29]. However, when BDNF was administered to normal rats or mice, it had no effect on blood glucose level, demonstrating that BDNF exerts its effects by promoting insulin sensitivity [26]. In addition, BDNF activates numerous signaling pathways such as phosphatidylinositol-3 kinase/Akt among others [30].

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