Investigation of the effects of antidepressant treatment on hippocampus and hypothalamus endoplasmic reticulum stress in chronic mild stress ınduced depression ın rats

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SUMMARY

Objective: This study aimed to examine the role of endoplasmic reticulum (ER) stress in the pathophysiology of depression in female rats exposed to the chronic mild stress model.

Method: Chronic mild stress model was constituted in 48 female adult rats were and divided into 4 groups as control, depression, depression+1 mg/kg sertraline, and depression+10 mg/kg sertraline. Subcutaneous drug infusions were performed for 14 days using osmotic minipumps. Expression levels of genes in ER stress pathway were evalulated in hypothalamus and hippocampus tissues of rats.

Results: It was found that ATF4 gene expression increased in depression in the hippocampus and decreased with treatment. Hippocampal GRP78 gene expression was downregulated after treatment. Hypothalamic and hippocampal CALR gene expression decreased with treatment, hypothalamic HSP47 gene expression decreased in both treatment groups. Hypothalamic and hippocampal XPB1 gene expression decreased with treatment, hippocampal XPB1 gene expression was further downregulated in the depression +10 mg/kg sertraline group compared to the depression+1 mg/kg sertraline group.

Discussion: These findings show that the ER stress mechanism may have a role in the pathophysiology of depression and that this mechanism can be reversed with treatment. The results of our study have been encouraging for human studies and open the way for new projects to understand and accelerate the antidepressant effect.

Key Words: Major Depression, Endoplasmic Reticulum, Chronic Mild Stress Model, Sertraline, Gene Expression

INTRODUCTION

Depression is a syndrome characterized by profound sadness, sometimes both sad and anxious mood, slowness and stagnation in thought, speech, psychomotor and psychophysiological processes, feeling worthless and powerless, loss of desire, and thinking of hopelessness (1). In the international comorbidity study, the lifetime prevalence of major depressive disorder was 12.7% in men, 21.3% in women, and 17.1% in total (2). Antidepressants are thought to act by modulating the serotonergic and noradrenergic systems.

The endoplasmic reticulum (ER) is a crucial organelle required for processes such as regulation of protein synthesis, modification and folding of proteins, synthesis and distribution of phospholipids and steroids, and calcium balance in the cell endomembrane system of eukaryotic cells. When stress occurs inside the cell (oxidative stress, imbalance in calcium level, etc.), a dysfunction occurs in the endoplasmic reticulum. This situation causes the accumulation of misfolded proteins in the

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extracellular space, causing ER stress (3).

Sertraline, a selective serotonin reuptake inhibitor (SSRI) class antidepressant, is one of the most prescribed psychiatric drugs. Studies in liver tissue have shown that sertraline causes changes in mitochondria and ER functions. Sertraline is thought to have an antidepressant effect by replicating these changes in some brain parts (4).

Recent data suggest that endoplasmic reticulum (ER) stress is involved in the pathophysiology of depression. This increased stress is thought to be reduced by antidepressant treatment, and there is evidence that the underlying mechanism is related to ER stress (5). Evalulation of mRNA level ER stress gene expressions including CALR (calreticulin), GRP78 (HSPA5; heat shock protein family A member 5), CHOP (DDIT3; DNA Damage Inducible Transcript 3), ATF4 (activating transcription factor 4), ATF6, PERK (PKR-like Endoplasmic Reticulum Kinase), eIF2α (Eukaryotic Translation Initiating factor 2α), IRE1 (inositol-requiring enzyme 1), HSP47 (Heat shock protein 47)and XBP-1(x Box Binding Protein) will help to understand this pathway.

This study hypothesizes that the expression levels of ER stress genes increased in rat chronic mild stress (CMS) model and sertralin treatment reduces this elevated ER stress. This study aimed to illuminate the effect of ER stress on the pathophysiology of depression and the development of drugs that act directly on ER stress to overcome the inadequate or delayed response to treatment observed in current treatments. Rat CMS model was constituted and effects of antidepresan treatment was tested. CMS model is accepted as golden standart and widely used to study pathophysiology of human depression.

METHOD

Animal material

This study was carried out with the approval of the Ethics Committee of Necmettin Erbakan University KONUDAM Experimental Medicine Application and Research Center dated 16.01.2020 and numbered 2020-011. Our study was conducted at Necmettin Erbakan University, KONUDAM Experimental Medicine Application and Research Center. A total of 48 female adult rats were and divided into 4 groups as control (n=12), depression (n=12) Depression + 1 mg/kg sertraline group (n=12) and depression + 10 mg/kg sertraline (n=12) groups.

Chronic Mild Stress (CMS) model

Depression was induced by applying the Chronic Mild Stress (CMS) model to adult female rats. Our work consisted of 2 stages. In the first stage, the CMS protocol applied to the animals in the depression groups is summarized in Table 1. Wet cage application was carried out by wetting 333 g of sawdust in a cage with 1.5 liters of water. For the tilted cage procedure, the cages are tilted 60 degrees so that the food portion of the cage is up. Noise stress was developed (approximately 60 dB) with the help of a bell ringing once every 10 seconds for 1 second. Swimming stress was performed by swimming the rats for 10 minutes in the cylinders. Restraint stress was accomplished by physical restraint in restraint apparatuses. For starvation stress, the foods were removed from 16:00 to 09:00 the next day (thus establishing a 17-hour fasting period). Restraint and swimming were not applied for one week after the placement of the osmotic pumps to allow the back area of the animals to recover. Forced swim test (5 min) was applied on the 8th day of the study. (Table-1) .

Forced Swim Test (FST)

The transparent plexiglass cylinder on which the test was carried out is 49 cm high and 19 cm in diameter. For the experiment, the cylinder was filled up to 30 cm with water at approximately 25 ± 1°C. When the animals were relocated, the water was also replenished. The rats were allowed to swim for 15 minutes on the first day, dried with a towel in the drying cage, and then placed back in their cages. The next day, the rats were placed into the water for the forced swimming test for 300 seconds (5 minutes) and recorded with a video camera. Recordings were scored as swimming, clim-

Table 1 Chronic mild stress (cms) protocol applied to adult female rats

	Morn ng			Afternoon			N ght		
Day	T me	Stressor	Durat on	T me	Stressor	Durat on	T me	Stressor	Durat on
0	Open f eld test basal evaluat on								
1	09.00	Restra nt	45 min	12.00	No se	4 hour	-	-	-
2	09.00	Wet cage				7 hour	-	-	-
3	09.00	Restra nt	45 min	12.00	Sw m	10 min	16.00	Starvat on	All n ght
4	09.00	No se	4 hour	14.00	Restra nt	45 min	-	-	-
5	09.00	Sw m	10 min	-	-	-	16.00	T lted cage	All n ght
6	09.00	No se	4 hour	-	-	-	16.00	L ght on	All n ght
7	09.00	Restra nt	45 min	13.00	Fst tra n ng	15 min	-	-	-
8	09.00	Open f eld test	-	12.00	Fst	Placement of osmotic mini-pumps			
9	09.00	No se	4 hour	16.00	L ght on	All n ght	-	-	-
10	09.00	Starvat on			T lted cage		-	-	-
11	09.00	Wet cage	7 hour		-	-	-	-	-
12	10.00	No se	4 hour		T lted cage	All n ght			
13	09.00	Wet cage	7 hour				-	-	-
14	09.00	Restra nt	45 min				16.00	Starvat on	All n ght
15	09.00	No se	4 hour	16.00	L ght on	All n ght	-	-	-
16	09.00	Restra nt	45 min	12.00	No se	4 hour	-	-	-
17	09.00	Wet cage	7 hour	16.00	-	-	-	-	-
18	09.00	Restra nt	45 min	12.00	Sw m	10 min	16.00	Starvat on	All n ght
19	09.00	No se	4 hour	14.00	Restra nt	45 min	-	-	-
20	09.00	Sw m	10 min	16.00	T lted cage	All n ght	-	-	-
21	09.00	Restra nt	45 min	13.00	Fst tra n ng	-	-	-	-
22	Term nat on of exper mental and behav oral tests								

bing, and immobilization at 5-second intervals with the program's use.

Implantation of Osmotic Mini-Pumps and Drug Infussion

Adult female rats were given two different doses of sertraline (1 mg/kg or 10 mg/kg) or DMSO as a carrier for 14 days. After administration of FST, unique osmotic pumps (Alzet 2ML2) that can infuse 5 μl/hour were implanted in all animals. Sertraline was dissolved with DMSO to a total volume of 2 ml. Anesthesia was induced by administering xylazine/ketamine (8/75 mg/kg, intramuscularly). Under mild anesthesia, the upper side of the two scapula bones of the rats was shaved and cleaned with iodine solution. Then, with a longitudinal incision, osmotic pumps kept in saline were carefully implanted under the skin, and the incision area was sutured. After cleaning the area with the iodized solution, 2 ml of saline was injected subcutaneously into the animals for postoperative care, minimizing the fluid loss during anesthesia. Afterward, the dryness of the cornea was prevented by dripping saline into the eyes of the rats placed in the resting cages. After two weeks of sertraline and DMSO infusion, behavioral changes in rats were determined by FST.

Depressive behaviors were evaluated with the parameters of immobilization frequency (number of immobilization in 5 minutes), immobilization time, and percentage of movement.

In the second stage, all animals were decapitated under light sedation after FST was administered on the 22nd day, and plasma and serum samples were collected. After rapid removal of the brain tissues of the animals, the hippocampus and hypothalamus regions were dissected and immediately frozen in liquid nitrogen and stored at -80 ˚C for gene expression analysis.

Total RNA Isolation and Quality Control

Total RNA isolation was performed by homogenization of tissue samples was carried out in 1000 μl of TRIzol. Homogenates were incubated for 5 minutes at room temperature (RT), then 200 μl of chloroform was added, and after a short vortexing process, incubated again at RT for 15 minutes. The samples were centrifuged at 12000 g for 15 minutes at +4 ºC. The supernatant containing the RNA was transferred to new eppendorf tubes and 500 µl of isopropanol was added and inverted several times. After incubation for 10 min at RT, the samples were centrifuged at 12000 g for 10 minutes at +4 ºC. After removing the supernatant, the pellet was ethanol washed and centrifugated at 12000 g for 10 minutes at +4 ºC. After drying at RT for 5-10 min, the pellet was dissolved by adding 50 µl of nuclease-free water. The quality and quantity of total RNA samples were evaluated using a Nanodrop device and measuring at the A260/A280 and A260/230 ratios. The quality of the RNA samples was also evaluated by 1% agarose gel electrophoresis.

Table 2. Primer sequences of genes used in RT-PCR analyzes

Gene	Primer sequence (5 -3)	PCR product (bp)
ATF4	TTCGACCAGTCGGGTTTG GGAGAACCCATGAGGTTTGA	93
ATF6	GAAGGGATCACCTGCTGTTAC GTCCATCACCTGACAGTCAATC	152
CALR	CGGCTACGTGAAGCTGTT ACGTTCTTGCCCTTGTAGTT	144
CHOP	AACGGAAACAGAGTGGTCAG GGTCAGGCGCTCGATTT	137
EIF2A	GGTTTCTTGGCAGCCATTT TGCAACTTTAGGCTCCTCAC	100
GRP78	TGGTATTCTTCGAGTGACAGC GACCATCCTTTCAATTTCTTCAGG	109
HSP47	AGATGCAGAAGAAGGCTGTT GTTCTTGTCGATGGCCTCA	113
IRE-1	GCGCATCACAAAGTGGAAGTA ACATACAGAGTGGGCGTCA	75
PERK	CAAAGTAGATGACTGCAATTACGC TCCAGCCACGCATTGAAATA	144
XBP1	CCAGAACATCTTCCCATGGAT GGGTCCAACTTGTCCAGAAT	89
PGK1	ATGCAAAGACTGGCCAAGCTAC AGCCACAGCCTCAGCATATTTC	104
CycA	TATCTGCACTGCCAAGACTGAGTG CTTCTTGCTGGTCTTGCCATTCC	126

Cleanup of gDNA Contamination of Total RNA Samples

DNAse-I enzyme reaction was performed according to the manufacturer's instructions to eliminate possible gDNA contamination. 10 µg total RNA was made up to 100 µl total volume with DNAse-I reaction mix. RNA samples were incubated at 37 ˚C for 10 minutes after adding 2 U of DNAse-I enzyme. Then, 1 µl of 0.5 M EDTA was added, and the reaction was stopped by incubating at 75 ˚C for 10 minutes.

Reverse Transcriptase (RT) Reaction

cDNA was synthesized from the quality-controlled RNA samples using the manufacturer's protocol. To obtain single-strand cDNA from 2 µg/20 µl total RNA; 1 µl Oligo dT and 1 µl Random hexamer were added to 2 µg/20 µl total RNA and incubated at +70 ˚C for 5 minutes. Then, 2 µl of RNAse inhibitor and 4 µl of dNTP were added to the resulting reaction mixture and incubated at +25˚C for 5 minutes. Reverse transcriptase enzyme (2 µl) was added and incubated at +25˚C for 10 minutes and then at +37 ˚C for 60 minutes. The reaction was stopped by incubating at +70˚C water bath for 10 minutes. The cDNA samples obtained were stored at -20 ºC until use.

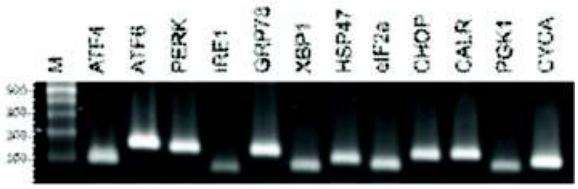

Figure 1. Agorose gel electrophores image of RT-PCR products of the genes used in the study. (M: 100 size standard)

Primer Design

The primer design of the target genes to be used in the study was carried out using the IDT PrimerQuest (http://eu.idtdna.com/home/home.aspx) program. The primer sequences of the genes used in the analysis are summarized in Table 2.

Real-Time Quantitative Polymerase Chain Reaction (qPCR)

Expression levels of target and reference genes was performed using a real-time PCR device (Bio-Rad CFX Connect Real-Time PCR System). SyberGreen, a dye that binds to double-stranded DNA, was used for the reaction. A total volume of 20 µl polymerase chain reaction was prepared including 10 µl of 2X SyberGreen master mix, 2 µl of cDNA, 5 pMol of forward and reverse primer. The PCR protocol was set at +95 ˚C for 10 min denaturation and 40 cycles (30 sec at 95 ˚C, 30 sec at 60 ˚C, 30 sec at 72 ˚C). In addition, the melting curve analysis was performed. Temperature was brought to 95 ˚C for 1 minute, then reduced to 55 ˚C and gradually increased to 95 ˚C again. Ct (threshold cycle) values target and reference genes were obtained. The resulting PCR products electrophorosed on a 2% agarose gel.

Statistical analysis

The "Resource Equation" method was used to determine the sample size (6). Ct values PGK1 and CYCA reference genes were used for internal control and normalisation of target genes and 2(-ΔCt) values were determined. Differences observed in gene expression levels between groups were compared using a one-way analysis of variance (ANOVA) using the SPSS package program. The least significant difference (LSD) test was used to determine possible differences between means for

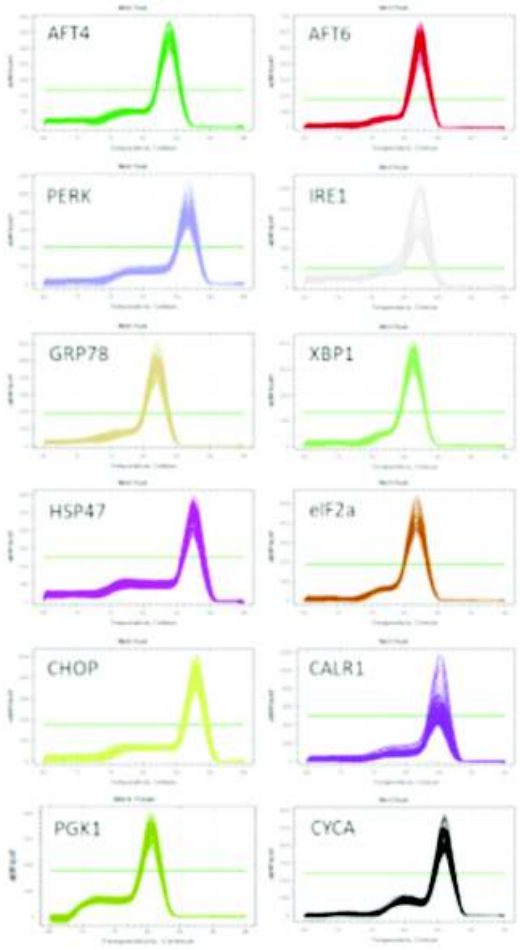

Figure 2. Melting curve analysis of the genes used in the study

groups. Findings with a p-value below 0.05 were considered statistically significant.

RESULTS

Chronic mild stress model was constituted in adult female rats and effects of two different sertraline doses were evaluated. Behavioral test indicated that CMS model was effective to generate depression like behaviorous. Sertraline infussion (10 mg/kg) is effective to prevent depression like behaviours.

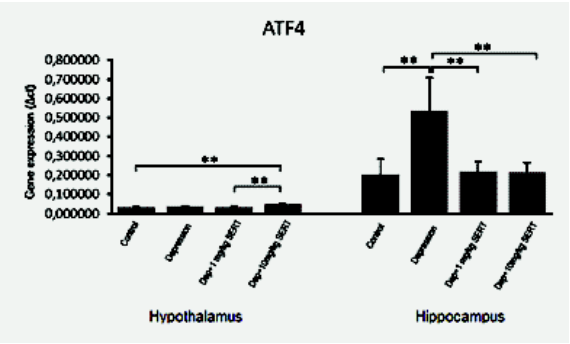

Figure 3: ATF4 gene expression levels in control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline groups. 2(-ΔCt) values were presented as mean means ± SEM. * p<0.05, ** p<0.01, *** p<0.001.

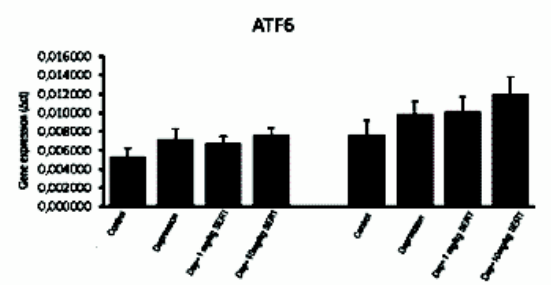

Figure 4: ATF6 gene expression levels in control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline groups. 2(-ΔCt) values were presented as mean means ± SEM. * p<0.05, ** p<0.01, *** p<0.001

Total RNA isolation was performed from hippocampus and hipotalamus tissue samples of control, depression, depression + 1 mg/kg sertraline, and depression + 10 mg/kg sertraline group rats. Quality/control was checked with spectrophotometric and agarose gel electrophoresis. All total RNA samples were of good quality to be used in the qPCR analysis.

mRNA level expression of target genes in the total ER stress pathway and two reference genes (PGK1 and CycA) used for normalization were determined by qPCR. PCR products of all genes were observed in agarose gel (2%) electrophoresis (Figure 1). In addition, melting curve analyzes indicated that all PCR products were specificly amplified target genomic regions (Figure 2).

Figures 3-12 describes expression levels of target genes in ER stress pathway. ATF6 (Figure 4), PERK (Figure 5) and IRE1 (Figure 7) gene expression levels were not significant different between the experimental groups. ATF4 gene expression in the hippocampus was significantly higher in the depression group than in the control group ($p<0.05$). A statistically significant decrease in hippocampus ATF4 gene expression was detected in the depression+1 mg/kg sertraline group and the depression+10 mg/kg sertraline group compared to the depression group ($p<0.05$; Figure 3). It was observed that hypothalamic ATF4 gene expression increased significantly in the depression+10 mg/kg sertraline group compared to the depression+1

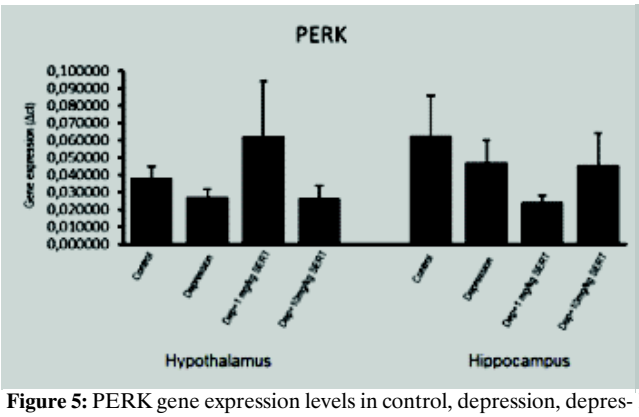

Figure 5: PERK gene expression levels in control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline groups. 2(-ΔCt) values were presented as mean means ± SEM. * p<0.05, ** p<0.01, *** p<0.001

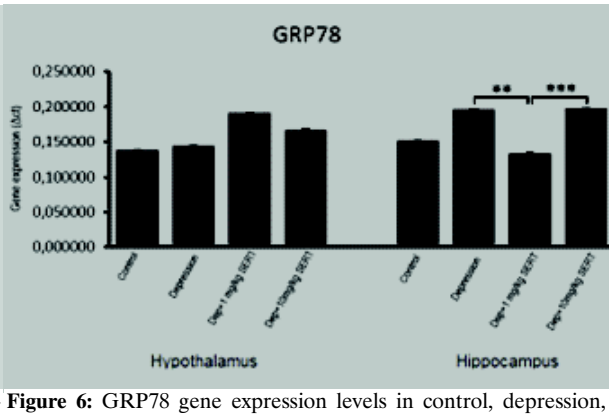

Figure 6: GRP78 gene expression levels in control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline groups. 2(-ΔCt) values were presented as mean means ± SEM. * p<0.05, ** p<0.01, *** p<0.001

mg/kg sertraline and control group. (p<0.05; Figure 3).

Hippocampus GRP78 gene expression was significantly downregulated in the depression+1 mg/kg sertraline group than in the depression group. (p<0.05) Hippocampal GRP78 gene expression was statistically significantly higher in the depression+10 mg/kg sertraline group than in the depression+1 mg/kg sertraline group. (p<0.05; Figure 6)

There was a statistically significant upregulation in hippocampal CHOP gene expression in the depression+10 mg/kg sertraline group compared to the control group. (p<0.05) Hypothalamic CHOP gene expression was significantly increased in the depression+1/kg sertraline group compared to the depression and control groups. (p<0.05; Figure 8)

A significant decrease in CALR gene expression was detected in the hypothalamus of the depression+1 mg/kg group compared to the depression group (p<0.05). There was a significant downregulated CALR gene expression in the hippocampus of the depression+10 mg/kg sertraline group compared to the depression group.(p<0.05; Figure 9).

Hypothalamic eIF2α gene expression was significantly higher in the depression+10 mg/kg sertraline group than in the other three groups. (p<0.05; Figure 10). A statistically significant decrease was found in hypothalamus HSP47 gene expression in the depression+1 mg/kg sertraline and depression+10 mg/kg sertraline group compared to the depression group (p<0.05; Figure-11).

Hypothalamic XPB1 gene expression was found to be significantly decreased in the depression+1 mg/kg sertraline group compared to the depression group (p<0.05) In addition, XPB1 gene expression in the hypothalamus was significantly lower in the depression+1 mg/kg sertraline group compared to the control group (p<0.05). Hippocampal XPB1 gene expression was statistically significantly down-

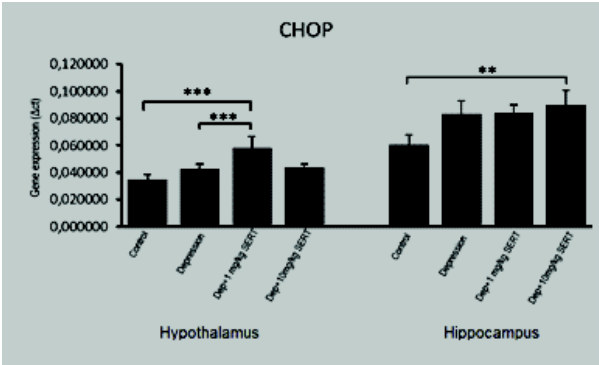

Figure 8: CHOP gene expression levels in control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline groups. 2(-ΔCt) values were presented as mean means ± SEM. * p<0.05, ** p<0.01, *** p<0.001

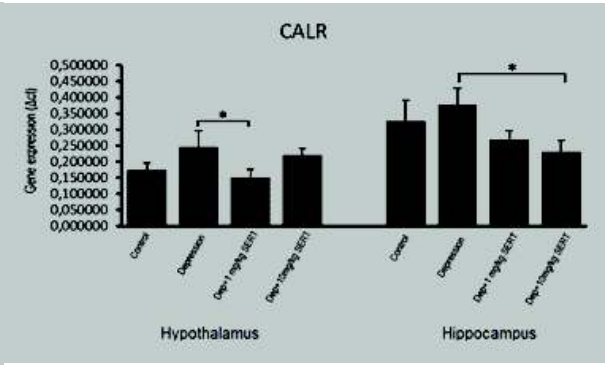

Figure 9: CALR gene expression levels in control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline groups. 2(-ΔCt) values were presented as mean means ± SEM. * p<0.05, ** p<0.01, *** p<0.001

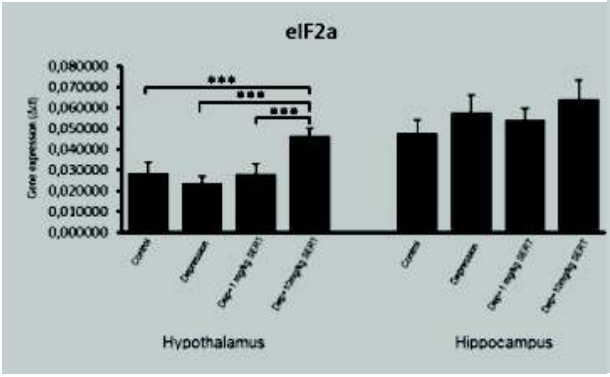

Figure 10: eIF2a gene expression levels in control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline groups. 2(-ΔCt) values were presented as mean means ± SEM. * p<0.05, ** p<0.01, *** p<0.001

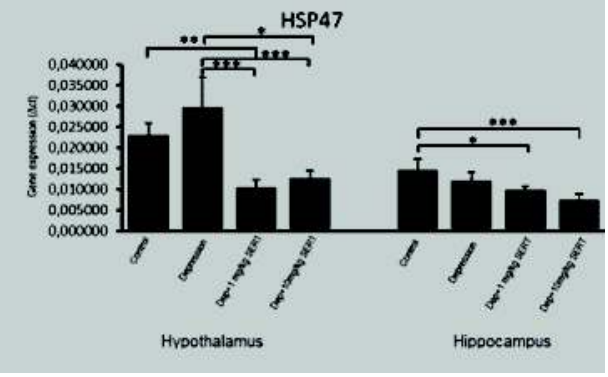

Figure 11: HSP47 gene expression levels in control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline groups. 2(-ΔCt) values were presented as mean means ± SEM. * p<0.05, ** p<0.01, *** p<0.001

regulated in the depression+10 mg/kg sertraline group compared to the depression group. Hippocampal XBP1 gene expression was lower in the depression+10 mg/kg sertraline group than in the depression+1 mg/kg sertraline group. (p<0.05; Figure-12).

DISCUSSION

Depression is a prevalent mood disorder accompanied by depressed mood, loss of interest, changes in appetite, sleep, energy levels, and autonomic changes (7). There are many effective treatments for depression, but it is estimated that one-third of patients with depression do not respond adequately to antidepressants (8,9). Therefore, the pharmacological mechanism of action of antidepressant drugs needs to be clarified. Sertraline is widely used in depression treatment. In this study, a Chronic mild stress model was constituted female rats and effects of sertraline was evaluated using two different doses. During experimental procedures, others also reported that 10 mg/kg sertraline is effective can be used as a golden standart experimental rat depression model.

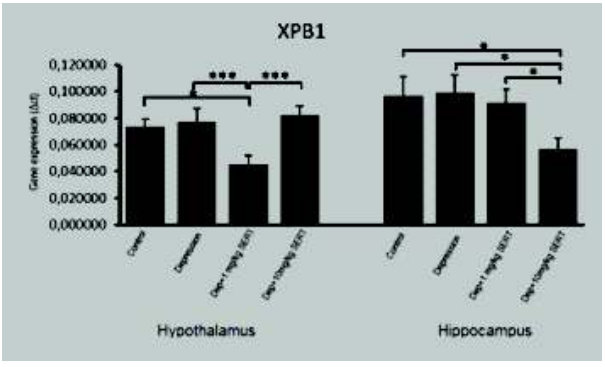

Figure 12: XBP1 gene expression levels in control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline groups. 2 (-ΔCt) values were presented as mean means ± SEM. * p<0.05, ** p<0.01, *** p<0.001

The endoplasmic reticulum (ER) is an essential intracellular organelle involved in the post-translational modification, smooth folding and synthesis of secreted proteins, and calcium homeostasis (10). Various physiological conditions, such as hypoxia, stress, hypoglycemia, decreased calcium, oxidative stress, and a fat-rich diet can impair the protein folding process, resulting in the accumulation of unfolded and misfolded proteins in the ER (11). Several studies have shown that people with depression have structural abnormalities in brain tissue. Among the mechanisms involved in the pathophysiology of this condition, disorders in the endoplasmic reticulum have also been mentioned.

Although it is stated that ER stress may play a role in the pathophysiology of depression, there are not enough studies describing how ER stress changes with treatment in depression and the mechanism of this effect.

The finding of increased expression of hippocampal ATF4 in depression in our study coincides with the information that ATF4 gene expression is increased in the rat hippocampus with learned helplessness, as mentioned in the study of Timberlake et al. (12), and supports our hypothesis that ER stress causes depression. In addition, Omi et al. (13) reported that fluvoxamine, a Sig-1R stimulant SSRI, provided ATF4 modulation. Similarly in our study, hippocampal ATF4 gene

expression levels were downregulated after sertraline treatment. This finding indicates that Sig-1R stimulation may be necessary to treat ER stress-induced depression effectively.

Although it was reported in the study of Pavlovsky et al. (14) that ATF6 gene expression increased in the striatum of rats after chronic restraint stress, no significant change was found in ATF6 gene expression in our study. In addition, this result does not support the previous literature (15) where fluoxetine induces apoptosis by increasing ATF6 levels. These conflicting results reflect the influence of different pathophysiological mechanisms.

In a study by Sharma et al. (16), inhibition of hippocampal PERK expression could improve cognitive functions. In a study by Ma et al. (17), desipramine provided antitumor activity by inducing autophagy through PERK / eIF2α and ATF6 signaling pathways in glioma. Another study (17) stated that activation of the PERK-eIF2α signaling pathway after chronic defeat stress causes depression and memory impairment. There was no significant change in PERK gene expression between the groups in our study. It is impossible to explain this situation for a single reason; different mechanisms may cause this inconsistency.

GRP78 gene expression increased in the temporal cortices of patients with depression who died due to suicide (18). In a study by Nevell et al. (19), GRP78 levels increased significantly compared to patients with MDD. In a study by Tan et al. (20), there was an increase in GRP78 gene expression after chronic unpredictable stress in rats. In our study, no change was observed in GRP78 expression in depression. However, Jangra et al. (21) reported that a decrease in hippocampal GRP78 gene expression was observed with honokiol treatment. Similarly, in this study, hippocampal GRP78 gene expression was decreased in the depression+1 mg/kg sertraline group compared to the depression. This finding supports our hypothesis that antidepressant treatment reduces ER stress levels.

Previous literature indicated that CHOP gene expression was increased in depression(19) and in rats with learned helplessness (12). Pavlovsky et al. (14) reported elavated CHOP gene expression in the striatum of rats exposed to chronic restraint stress. Also, Huang et al. (22) stated that CHOP gene expression was upregulated after social defeat stress in rat's amygdala. Lastly, Jangra et al. (21) reported that CHOP gene expression increased after chronic restraint stress in the rat's prefrontal cortex. In our study, an increased but not significant CHOP expression was observed. However, hippocampal and hypothalamic CHOP expression was not decreased in either treatment groups. This finding suggested that sertraline treatment may not affect CHOP driven inhibition of protein synthesis and somehow supports this mechanism in lower doses. Although these differences are difficult to explain, they may be due to methodological differences between studies.

Behnke et al. (18) reported that calreticulin (CALR) expression was increased in the temporal cortices of depressed patients who died due to suicide. In contrast, no significant increase in hippocampal and hypothalamic CALR gene expression was observed in our study. However, sampling strategy of these studies are quite different may cause this variation. Consistent with this research data, Bown et al. (23) reported no significant change in CALR gene expression levels in some patients who died after suicide attempts. This result suggests that further research is needed to reach consistent data on this subject. In addition, hypothalamic CALR gene expression was lower in the sertraline+1 mg/kg group than in the depression group. The hippocampal CALR gene expression was significantly decreased in the depression+10 mg/kg sertraline group compared to the depression group. These findings supports our hypothesis that antidepressant treatment reduces ER stress.

No study has been found for HSP47 with any mental illness or antidepressant treatment in the current literature. The decrease in hypothalamic HSP47 gene expression in the depression+1 mg/kg sertraline and depression+10 mg/kg sertraline group compared to the depression group confirms our hypothesis that a decrease in ER stress biomarkers is expected with effective antidepressant treatment in depression.

It was reported an increased hippocampal XBP1 gene expression in rats with learned helplessness (12) and social defeat stress (24). Pavlovsky et al. (14) also suggested that increased XBP1 gene expression might be responsible for depression. Our study indicated an unsignificantly upregulated XBP1 expression in depression groups of hypotalamus and hipocampus. Spesifically, hippocampal XBP1 gene expression was decreased in the depression+10 mg/kg sertraline group compared to the depression group. In addition, the results in the hippocampal XBP1 gene expression in the study show that ER stress is reduced with a higher treatment dose.

Although we obtained striking results regarding the effect of ER stress on depression, the findings should be interpreted with caution. First, it should not be overlooked that there are different measurement techniques for the ER stress level. Secondly, although we used sertraline, an effective treatment for depression, studies involving antidepressants that act through different mechanisms of action are needed.

Our study illustrates that ER stress might play a crucial role in the pathophysiology of depression and that this situation can be reversed with sertraline antidepressant therapy. In future studies, there is a need for human studies that can confirm the data obtained in our research and elucidate the mechanisms by which the change in ER stress occurs.

Conflict of Interest

The authors declare no conflict of interest.

Authors' contributions

All authors contributed to the study's conception and design. Data collection was performed by Mustafa KARAAĞAÇ, Mehmet AK and Ercan KURAR. Mustafa Karaağaç wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

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