Journal of Anatolian Environmental and Animal Sciences
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
DOI: https://doi.org/10.35229/jaes.1801041

JAES
Year: 11, 2026 (1-9)
AÇEH
Yıl: 11, 2026 (1-9)

ARAŞTIRMA MAKALESİ RESEARCH PAPER

Effects of Letrozole Administration on Metabolic, Inflammatory, and Oxidative Responses in Rat Serum and Brain Tissues

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Received: *13.10.2025* ***Accepted:*** *12.12.2025*

How to cite: **Ermiş, M., Karakaş, E., Erol, H., Acı, R., Ümit, F., & Çiftci, G. (2026).** Effects of letrozole administration on metabolic, inflammatory, and oxidative responses in rat serum and brain tissues. *J. Anatolian Environ. Anim. Sci.*, ***11***, 1-9. https://doi.org/10.35229/jaes.1801041

Atıf yapmak için: **Ermiş, M., Karakaş, E., Erol, H., Acı, R., Ümit, F., & Çiftci, G. (2026).** Letrozol uygulamasının rat serum ve beyin dokusunda metabolik, inflamatuar ve oksidatif yanıtlar üzerine etkileri. *Anadolu Çev. Hay. Bil. Derg.*, ***11***, 1-9. https://doi.org/10.35229/jaes.1801041

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Abstract: Letrozole is a drug that reduces estrogen synthesis and is used in the treatment of breast cancer. Its long-term use may cause systemic and neurological effects. In this study, biochemical, inflammatory, and neurological parameters were examined in Letrozole administered female rats. The study included a control group and a Letrozole-treated group (1 mg/kg for 21 days), each comprising six rats. On day 21, blood and brain tissue samples were collected from the rats. Proinflammatory cytokines and $A\beta_{1-40}$ levels were measured by ELISA, Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) levels by Rel Assay® Diagnostics kits, and biochemical parameters and minerals by an autoanalyzer. In the Letrozole group, compared to the control group, increases were observed in serum total protein, albumin, glucose and sodium levels, while urea levels decreased (P<0.05). In the serum lipid profile, increases in TG and LDL levels and a decrease in HDL levels were detected, although these changes were not statistically significant (P>0.05). Serum IL-1α, IL-1β, TNF-α, NF-κB, $A\beta_{1-40}$, and TOS levels increased significantly (P<0.01), whereas TAS levels decreased (P<0.05). In whole brain tissue, Na, Cl, P, Mg, TC, TG, $A\beta_{1-40}$, and NF-κB levels were significantly elevated in the Letrozole group compared to controls (P<0.05). In conclusion, Letrozole treatment was found to induce marked metabolic, inflammatory, and oxidative changes in serum and brain tissue, which may contribute to the disruption of systemic homeostasis and the activation of neuroinflammatory responses.

Keywords: $A\beta_{1-40}$, brain, inflammation, letrozole, oxidative stress, rat.

Letrozol Uygulamasının Rat Serum ve Beyin Dokusunda Metabolik, İnflamatuar ve Oksidatif Yanıtlar Üzerine Etkileri

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Öz: Letrozol, östrojen sentezini azaltan ve meme kanseri tedavisinde kullanılan bir ilaçtır. Uzun süreli kullanımı sistemik ve nörolojik etkiler oluşturabilir. Bu çalışmada, Letrozol uygulanan dişi ratlarda biyokimyasal, inflamatuar ve nörolojik parametreler incelendi. Çalışma, her biri altı rat içeren kontrol grubu ve 21 gün boyunca 1 mg/kg Letrozole uygulanan deney grubundan oluştu. 21. gün sonunda ratlardan kan ve beyin dokusu örnekleri alındı. Proinflamatuar sitokinler ve $A\beta_{1-40}$ düzeyleri ELISA ile Toplam Antioksidan (TAS) ve Toplam Oksidan (TOS) Durumu Rel Assay® Diagnostics kitleriyle, biyokimyasal parametreler ve mineraller ise otoanalizörle ölçüldü. Letrozol grubunda, kontrol grubuna kıyasla serum total protein, albümin, glukoz ve sodyum düzeylerinde artış, üre düzeyinde ise azalma gözlendi (P<0,05). Serum lipid profilinde TG ve LDL düzeylerinde artış, HDL düzeyinde ise azalma tespit edildi, ancak bu değişiklikler anlamlı bulunmadı (P>0,05). Serum IL-1α, IL-1β, TNF-α, NF-κB, $A\beta_{1-40}$ ve TOS düzeyleri anlamlı olarak artarken (P<0,01), TAS düzeyinde azalma saptandı (P<0,05). Tüm beyin dokusunda, letrozol grubunda kontrol grubuna kıyasla Na, Cl, P, Mg, TC, TG, $A\beta_{1-40}$ ve NF-κB düzeylerinin anlamlı şekilde arttığı belirlendi (P<0,05). Sonuç olarak, letrozol tedavisinin serum ve beyin dokusunda belirgin metabolik, inflamatuvar ve oksidatif değişikliklere yol açtığı ve bunun sistemik homeostazın bozulmasına ve nöroinflamatuvar yolların aktivasyonuna katkıda bulunabileceği belirlendi.

Anahtar sözcükler: $A\beta_{1-40}$, beyin, inflamasyon, letrozole, oksidatif stres, rat.

INTRODUCTION

Letrozole, a potent aromatase inhibitor, suppresses the conversion of androgens to estrogens and markedly reduces circulating estrogen levels (Pant, 2007). Although widely used in the treatment of estrogen receptor-positive breast cancer, Letrozole has recently become a preferred agent for inducing a polycystic ovary syndrome (PCOS) model in rats. This model successfully reproduces key features of human PCOS, including hyperandrogenism, anovulation, and cystic ovarian morphology (Kafali et al., 2004; Manneras et al., 2007). Hyperandrogenism promotes the development of insulin resistance and hyperglycemia, leading to increased oxidative stress, which in turn exacerbates inflammatory responses and abdominal fat accumulation (Siddiqui et al., 2022).

Aromatase, an enzyme belonging to the CYP450 superfamily, is expressed primarily in the ovaries as well as in the adrenal glands, adipose tissue, and brain (Grodin et al., 1973). Estrogen is known to possess antioxidant and anti-inflammatory properties. A decrease in circulating estrogen levels leads to increased systemic ROS and elevated expression of inflammatory cytokines (Straub, 2007). ROS can increase intracellular calcium (Ca^{2+}) levels through ion channels, disrupting mitochondrial membrane permeability; additionally, they activate NF-κB pathways, leading to elevated expression of proinflammatory cytokines such as TNF-α and IL-1β (Agarwal et al., 2012; Biswas, 2016; Vaziri and Rodríguez-Iturbe, 2006). It has been reported that inflammatory markers are increased and dyslipidemia develops in rat models treated with Letrozole (Al-Harbi et al., 2025; Ibrahim et al., 2022). The effects of letrozole on protein synthesis, renal and hepatic function, electrolyte homeostasis, as well as glucose and insulin metabolism (assessed via C-peptide levels), remain insufficiently elucidated. Investigating changes in serum parameters such as sodium (Na^+), potassium (K^+), chloride (Cl^-), urea, creatinine, albumin, total protein, glucose, and C-peptide may contribute to a more comprehensive understanding of letrozole's systemic effects. Additionally, measurements TAS and TOS, which are commonly used to assess oxidative stress levels, could provide clearer insights into the drug's cellular effects (Erel, 2004). On the other hand, the expression of aromatase enzyme not only in peripheral tissues but also in brain tissue, along with the well-known neuroprotective properties of estrogen, necessitates the evaluation of Letrozole's effects on the central nervous system (CNS) (Grodin et al., 1973).

Estrogen is a crucial neurosteroid that supports synaptic plasticity, suppresses oxidative stress, and modulates inflammatory responses. Indeed, in Alzheimer's patients, a negative correlation has been observed between brain aromatase mRNA levels and amyloid plaque accumulation, suggesting that estrogen deficiency may play a role in neurodegenerative processes (Yue et al., 2005). In this regard, measuring the levels of $A\beta_{1-40}$ in animal models treated with letrozole will help us better understand both the neurodegenerative alterations associated with estrogen shortage and possible neuroinflammatory mechanisms. Accordingly, our study aimed to comprehensively assess biochemical parameters, lipid profiles, electrolyte balance, inflammatory cytokines, oxidative stress markers, neuroinflammatory indicators, and $A\beta_{1-40}$ levels in female rats treated with letrozole, in order to elucidate the systemic and central effects of the drug.

MATERIAL AND METHOD

Research Site and Animal Material: In the study, a total of 12 female Wistar albino rats aged 8–10 weeks and weighing 150–200 grams were used. The rats were obtained from the Experimental Research Application and Research Center (DEKAM) of Erciyes University. The study was conducted with the permission of Erciyes University Animal Experiments Local Ethics Committee (numbered 25/241). The rats included in the experiment were housed in pre-disinfected cages, with 4-5 rats per cage, and treatments were administered simultaneously. They had *ad libitum* access to food and water. Throughout the study, environmental conditions were maintained at a temperature of 22±2 °C, 60 % humidity, and a 12-hour light/dark cycle.

Animal study design: Letrozole (Novartis) was prepared fresh daily by dissolving the required amount of the drug in 0.9 % physiological saline (NaCl). The stock solution was vortexed until fully homogenized and protected from light to maintain stability. The solution was adjusted to deliver a final dose of 1 mg/kg body weight, with the volume individually calibrated according to the daily body weights of the rats. The prepared suspension was administered via oral gavage once daily for 21 consecutive days, ensuring consistent dosing throughout the experimental period (Baravalle et al., 2006). The control group received an equivalent volume of 0.9 % physiological saline via oral gavage for the same duration.

Collecting Blood Samples and Obtaining Serum: Rats were anesthetized with intraperitoneal xylazine (10 mg/kg) and ketamine hydrochloride (60 mg/kg), after which thoracoabdominal cavities were opened and cardiac blood samples were obtained. At the end of the 21st day, the study was concluded, and brain tissue as well as blood samples were collected for biochemical analysis. Following a 20 min incubation period and coagulation, the blood samples were centrifuged at +4 °C for 10 min at 1 550 x g. The serum was then extracted and separated into aliquots. The serum samples were kept cold until analysis and were stored at -20 °C.

Preparation of whole brain tissue homogenate: The brain tissue was first washed with ice-cold PBS (0.01 mol/L, pH 7.0-7.2) and weighed. PBS was then added at a 1:9 ratios and homogenized with a homogenizer. Samples were ultrasonicated once or twice on ice to disrupt cell membranes, and then two freeze-thaw cycles were applied for further cell lysis. Half of the resulting homogenate was centrifuged at 15 000 × g for biochemical analyses, while the other half was centrifuged at 3 000 × g for 20 min at +4 °C for ELISA analyses (De Boeck et al., 2003; Ermiş and Çiftci, 2024). The supernatant was aliquoted and stored at −20 °C until analysis.

Determination of some biochemical parameters in serum and brain: Glucose (BioSystems, lot: 11803), creatinine (BioSystems, lot: 11802), total cholesterol (BioSystems, lot: 11805), HDL (BioSystems, lot: 11557), LDL (BioSystems, lot: 11585), triglycerides (BioSystems, lot: 11828), total protein (BioSystems, lot: 11800), albumin (BioSystems, lot: 11573), urea (BioSystems, lot: 12516) and uric acid (BioSystems, lot: 11821) levels in serum and brain were measured by the spectrophotometric method using specific Biosystem kits on an autoanalyzer device (Biosistem A25, Spain). Globulin level was calculated by subtracting albumin from total protein

Determination of the levels of certain minerals in serum and brain tissue: The levels of Na (BioSystems, lot: 5201), Mg (BioSystems, lot: 11797), K (BioSystems, lot: 5202), Ca (BioSystems, lot: 11570), P (BioSystems, lot: 11508), Cl (BioSystems, lot: 5207), and Fe (BioSystems, lot: 11509 in serum and brain tissue supernatants were measured by the spectrophotometric method using an autoanalyzer device (A25 Random Access Analyzer, Biosystems, Spain). In this device, briefly, the sample and reagents were taken and mixed in appropriate amounts by the device, followed by optical reading at a specific time and temperature, and the relevant analyzes results were calculated and retrieved from the device.

Determination of IL-1α, IL-1β, TNF-α, NF-κB, C-peptide and $A\beta_{1-40}$ levels in serum and brain tissue: The levels of IL-1α (Catalogue number: 201-11-0119), IL-1β (Catalogue number: 201-11-0120), TNF-α (Catalogue number: 201-11-0765), C-peptide (Nepenthe Bioscience LLC, Sunnyvale, CA, US; katalog no: NE01A000603), $A\beta_{1-40}$ protein (Nepenthe Bioscience LLC, Sunnyvale, CA, US; katalog no: NE010009203) and NF-κB (Catalogue number: 201-11-5141) in serum and brain tissue supernatants were determined using rat-specific ELISA kits (Sunred Biological Technology; Shanghai, China). The procedure of the kit was followed, and OD_{450} values were calculated using Magellan Standard Tracker (V7–2) software, taking into account the OD_{450} values of the standards.

Determination of Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) amounts in serum; Serum TAS and TOS levels were measured using commercial kits (Rel Assay® Diagnostics, Mega Tip, Gaziantep, Turkey). Spectrophotometric techniques were used to measure the change in the amount of absorption. TAS levels were measured using the method developed by Erel (2005). This assay depends on the sample's antioxidants' capacity to prevent a peroxidase metmyoglobin from oxidizing ABTS (2, 2′-azino-di-3-ethylbenz-thiazoline sulfonate) into $ABTS^{+}$. The TAS level was expressed as mmol Trolox equivalent/L. The tissue TOS level was measured using the method developed by Erel (Erel 2004). TOS method relied on the oxidation of ferrous ions into ferric ions in the presence of various oxidative species in an acidic medium. Xylenol orange was used to test the amounts of ferric ions. The results showed that µmol H_2O_2 equivalent/L.

Statistical Analyses: All statistical analyses for comparisons between two groups were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Data distribution was evaluated with Kolmogorov-Smirnov and Shapiro-Wilk tests. For variables with normal distribution, comparisons were conducted using the Independent Samples t-test, while for non-normally distributed variables, the Mann-Whitney U test was applied. Data are presented as mean ± standard deviation (SD), and a P-value less than 0.05 was considered statistically significant.

RESULTS

The serum lipid profile (TC, TG, HDL, and LDL) is presented in Table 1. In the study, TC and HDL levels were found to decrease in Group 2 compared to Group 1, while TG and LDL levels increased. However, these differences were not statistically significant (P>0.05).

Table 1. Serum levels of total cholesterol (TC) (mg/dL), triglycerides (TG) (mg/dL), high-density lipoprotein (HDL) (mg/dL), and low-density lipoprotein (LDL) (mg/dL).

	Group 1	Group 2	P
TC	82,1±2,7	79,56±1,7	0,463
TG	99±7,3	104,86±5,6	0,543
LDL	11,7±0,8	12,33±0,2	0,526
HDL	48,5±2,1	45,16±1,8	0,263

Serum total protein (TP), albumin (Alb), globulin (Glo), glucose (Glu), C-peptide, urea (URE), creatinine (Cre), and uric acid (UA) levels are presented in Table 2.

Table 2. Serum levels of total protein (TP) (g/L), albumin (Alb) (g/L), globulin (Glo) (g/L), glucose (Glu) (mg/dL), C-peptide (ng/mL), urea (URE) (mg/dL), creatinine (Cre) (mg/dL), and uric acid (UA) (mg/dL).

	Group 1	Group 2	P
TP	63,83±1,5	68,83±1,4	0,04
Alb	34,11±0,2	39,3±1	0,04
Glo	29,71±1,4	29,53±0,9	0,92
Glu	146,01±14,9	241,78±8,3	0,01
C-peptide	7,11±0,6	8,64±0,3	0,06
URE	43,93±1,1	38,58±1,3	0,01
Cre	0,29±0,01	0,33±0,01	0,12
UA	1,1±0,09	1,36±0,3	0,54

Comparison between the two groups demonstrated a significant increase in TP, Alb, and Glu levels in Group 2, alongside a significant decrease in URE levels (P<0.05). Although decreases in GLO levels and increases in Cre, UA, and C-peptide levels were observed, these changes did not reach statistical significance (P>0.05). These findings indicate potential differences between the groups in specific metabolic and renal function parameters.

Comparing serum mineral levels between the Letrozole-treated Group 2 and the control Group 1 revealed a statistically significant increase in Na levels ($P<0.05$). However, no significant differences were observed between the groups for other minerals, including K, Cl, P, Fe, Mg, and Ca ($P>0.05$) (Table 3).

Table 3. Serum mineral levels in the groups.

	Group 1	Group 2	P
Na (mmol/L)	139,16±0,4	141±0,5	0,026
K (mmol/L)	5,56±0,2	4,94±0,2	0,125
Cl (mmol/L)	102±0,5	103,33±0,9	0,235
P (mg/dL)	5,95±0,3	6,48±0,3	0,3
Fe (µg/dL)	126,96±6,6	153,8±13,4	0,105
Mg (mg/dL)	2,33±0,07	2,48±0,1	0,2
Ca (mg/dL)	10,35±0,1	10,53±0,1	0,3

In this study, levels of Na, K, Cl, P, Ca, Fe, and Mg were analyzed in brain tissues from both groups. Selected electrolyte and mineral levels measured in the brain tissues of the two groups are presented in Table 4.

Table 4. Mineral levels in brain tissues of the groups.

	Group 1	Group 2	P
Na (mmol/g)	140,33±1,1	143,5±0,3	0,026
K (mmol/g)	12,37±0,8	13,42±0,1	0,31
Cl (mmol/g)	123,5±1	127,33±0,2	0,002
P (mg/g)	35,28±0,6	38,61±0,1	0,002
Fe (mg/g)	15,5±2,6	20,33±1,7	0,18
Mg (mg/g)	1,1±0,1	1,38±0,03	0,06
Ca (µg/g)	0,68±0,1	0,61±0,04	0,5

Na, Cl, and P levels in brain tissue were found to be significantly higher in Group 2 ($P<0.05$). However, no statistically significant differences were observed between the groups in K, Fe, Mg, and Ca levels ($P>0.05$). These results indicate that while certain electrolytes in brain tissue differed between the groups, not all minerals were affected by these changes.

Table 5. Total cholesterol (TC), triglycerides (TG), total protein (TP), and glucose (Glu) levels in whole brain tissue.

	Group 1	Group 2	P
TC (mg/g)	4±0,6	9,81±0,4	0,002
TG (mg/g)	31,21±2,7	48,28±1,3	0,002
TP (g/g)	2,66±0,3	3,33±0,2	0,18
Glu (mg/g)	0,78±0,2	0,66±0,2	0,8

TC and TG levels in brain tissue were significantly elevated in Group 2 ($P<0.05$). No significant differences were observed between the groups in TP and Glu levels ($P>0.05$). These findings indicate that changes related to lipid metabolism are pronounced in brain tissue, while protein and glucose levels remain comparable (Table 5).

In this study, the levels of IL-1β, IL-1α, TNF-α, NF-κB, and $A\beta_{1-40}$ were evaluated in both serum and whole brain tissue across different groups. Serum levels were analyzed as S-IL-1β, S-IL-1α, S-TNF-α, S-NF-κB, and S-$A\beta_{1-40}$, while brain tissue levels were designated as B-IL-1β, B-IL-1α, B-TNF-α, B-NF-κB, and B-$A\beta_{1-40}$. Additionally, Total Antioxidant Status (S-TAS) and Total Oxidant Status (S-TOS) were measured in serum. These parameters were assessed to determine the levels of inflammation and oxidative stress (Table 6).

The levels of inflammatory markers IL-1β, IL-1α, TNF-α, NF-κB, and $A\beta_{1-40}$ measured in serum were significantly elevated in Group 2 ($P<0.01$). Additionally, Group 2 exhibited an increased serum TOS level and a significant decrease in TAS level ($P<0.05$). These findings indicate that both systemic inflammation and oxidative stress were elevated in Group 2.

Table 6. Levels of IL-1β, IL-1α, TNF-α, NF-κB, and $A\beta_{1-40}$ in serum (S-IL-1β, S-IL-1α, S-TNF-α, S-NF-κB, and S-$A\beta_{1-40}$) and whole brain tissue (B-IL-1β, B-IL-1α, B-TNF-α, B-NF-κB, and B-$A\beta_{1-40}$) of the groups, along with serum Total Antioxidant Status (S-TAS) and Total Oxidant Status (S-TOS) levels.

	Group 1	Group 2	P
S-IL1β (pg/mL)	12,61±0,4	19,59±1	0,002
S-IL1α (pg/mL)	43,04±1,1	77,14±3,6	0,002
S-TNFα (pg/mL)	18,79±0,5	22,7±1,1	0,009
S-$A\beta_{1-40}$ (pg/mL)	27,03±1,4	31,43±2,8	0,002
S-NF-κB (pg/mL)	113,6±15,1	213,6±52,4	0,002
B-IL1β (pg/g tissue)	16,06±0,1	16,1±0,9	0,883
B-IL1α (pg/g tissue)	48,2±1,1	49,3±1,9	0,857
B-TNFα (pg/g tissue)	25,18±1,1	28,62±1,1	0,69
B- $A\beta_{1-40}$ (pg/g tissue)	40,4±3	57,1±4,2	0,3
B-NF-κB (pg/g tissue)	1,44±0,05	1,66±0,04	0,02
S-TOS (µmol/L)	2,4±0,1	3,6±0,1	0,004
S-TAS (mmol/L)	2,84±0,4	1,94±0,5	0,026

In brain tissue, only the levels of NF-κB were significantly elevated in Group 2. No statistically significant differences were observed between the groups for other brain tissue parameters (IL-1α, IL-1β, TNF-α, $A\beta_{1-40}$) ($P>0.05$).

In conclusion, a pronounced increase in inflammation and oxidative stress was observed particularly in the serum of Group 2, whereas the increase in brain tissue was more limited and mainly confined to elevated levels of IL-1β and NF-κB. These findings indicate that systemic inflammation is partially reflected in brain tissue as well.

In the graph, the total mineral levels (Na, K, Cl, P, Fe, Mg, and Ca) in the serum and brain tissues of Group 1 and Group 2 were compared using a stacked bar chart. Each mineral was represented by a different color, and the total mineral levels of both groups were presented in a way that allows direct comparison (Figure 1).

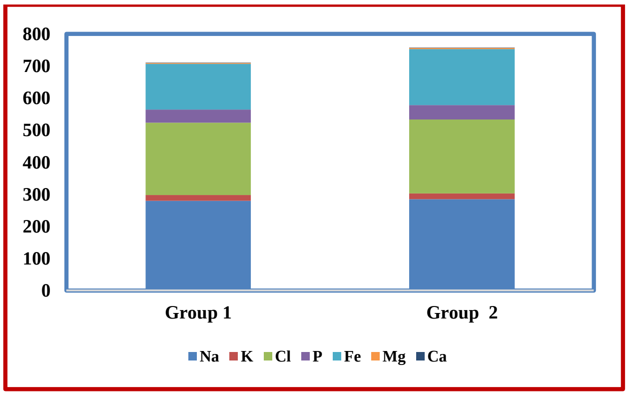

Figure 1. Total mineral levels in the brain and serum.

It was determined that the total mineral level in Group 2 was higher compared to Group 1. This increase was mainly attributed to elevated levels of Na, Cl, and P, while the levels of other minerals K, Fe, Mg, and Ca remained similar between the two groups.

In Group 2, elevated levels of inflammatory markers and amyloid beta were observed in both the brain and serum. These findings suggest that Group 2 exhibited heightened inflammatory and oxidative stress responses, potentially indicating an increased risk of neurodegeneration. Compared to Group 1, both systemic and central nervous system inflammation appeared more pronounced in Group 2 (Figure 2).

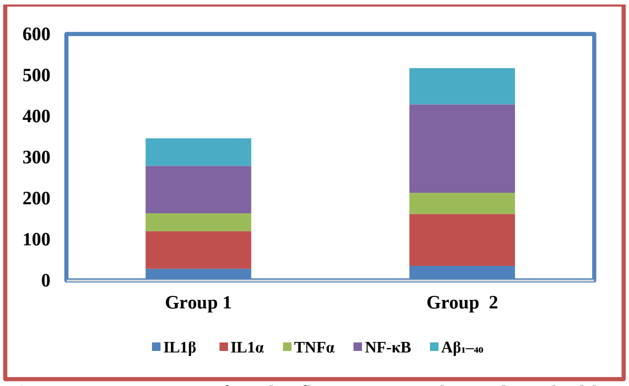

Figure 2. Comparison of total inflammatory marker and amyloid beta levels in brain tissue and serum.

DISCUSSION AND CONCLUSION

Letrozole is a non-steroidal aromatase inhibitor that effectively reduces estrogen synthesis by selectively inhibiting the aromatase enzyme, which catalyzes the conversion of androgens to estrogens in various tissues, including peripheral tissues and the central nervous system. Letrozole has a major therapeutic impact by lowering systemic estrogen levels, especially in the treatment of hormone receptor-positive breast cancer in postmenopausal women. Many clinical investigations have demonstrated its effectiveness in lowering tumor recurrence and enhancing disease-free survival (Goss et al., 2003). Long-term suppression of circulating estrogen levels through aromatase inhibition can lead to significant alterations in various physiological systems beyond tumor cells. Estrogen has been reported to have neuroprotective, anti-inflammatory, and antioxidant effects on the central nervous system (Arevalo et al., 2015; Brinton, 2008). Animal studies have demonstrated that estrogen reduces neuronal cell death, gliosis, and microglial activation, and also suppresses oxidative stress and the production of proinflammatory cytokines (Zhao and Brinton, 2007; Yao et al., 2010). In this context, aromatase inhibitors such as letrozole are thought to affect not only hormonal balance but also nervous system health.

In our study, serum TG and LDL levels were increased, and HDL and TC levels were decreased in Group 2 ($P>0.05$). Estrogen deficiency is known to affect lipid metabolism, but the magnitude of these effects depends on treatment duration, dose, and experimental model. Prolonged estrogen deficiency leads to adverse lipid changes, while short-term or low-dose treatments cause more limited changes, and high doses can cause metabolic side effects (Mauvais-Jarvis, 2011; Xu et al., 2017). Although the literature reports varying findings regarding the effects of aromatase inhibitors on lipid metabolism, some studies suggest that these effects are limited. Love et al. (2005) reported that letrozole may increase LDL and decrease HDL levels, while Wasan et al. (2005) indicated that letrozole may have a more adverse impact on the lipid profiles compared with other aromatase inhibitors. It is believed that the decrease in estrogen levels brought on by aromatase inhibition may affect lipoprotein production and catabolism in the liver, given the regulatory role of estrogen in lipid metabolism (Walsh et al., 1991). In our study, the acute effects of letrozole on the lipid profiles were examined; however, the changes observed in serum were not statistically significant ($P>0.05$).

In our study, a significant increase in serum TP, Alb, and Glu levels and a significant decrease in URE levels were observed in Letrozole-treated Group 2 ($P<0.05$). These findings indicate that letrozole affects metabolic processes. The literature suggests that aromatase inhibitors can induce changes, particularly in glucose metabolism and protein synthesis Letrozole is an aromatase inhibitor that suppresses estrogen production. It has been shown that letrozole treatment reduces estrogen levels by up to 95%, which adversely affects insulin sensitivity and leads to an increase in glucose levels (Mauvais-Jarvis, 2011). In addition, letrozole treatment has been reported to enhance glycolysis in cardiomyocytes, thereby accelerating metabolic remodeling, and this mechanism is noted to be independent of hormonal effects (Lee et al., 2022). The decrease in urea levels may indicate potential effects of letrozole on nitrogen balance, warranting further investigation in this area. The absence of significant changes in kidney function markers such as Cre and UA suggests that letrozole administration generally preserves renal function. On the other hand, a increase in C-peptide levels was observed in Group 2 ($P>0.05$). C-peptide is co-secreted during the cleavage of proinsulin and serves as an indirect marker of endogenous insulin production (Maddaloni and Ceriello, 2022). This increase suggests that letrozole may enhance insulin synthesis in pancreatic beta cells or stimulate insulin secretion. The literature reports that aromatase inhibitors, by lowering estrogen levels, can lead to impairments in glucose metabolism and the development of insulin resistance (Gibb et al., 2016). This situation may lead the pancreas to produce more insulin in response to the increased insulin demand. Therefore, the rise in C-peptide levels can be considered an adaptive response of beta cells to this increased demand (Kim et al., 2016). However, the persistence of this adaptation in the long term may cause beta cell fatigue and functional decline. Additionally, compared to insulin's short half-life, C-peptide levels are noted to be a more reliable biomarker for assessing metabolic status (Wahren et al., 2000). Our study concluded that Letrozole administration affects pancreatic insulin production and secretion, leading to an increase in C-peptide levels; this increase can be regarded as an important adaptive mechanism in regulating metabolic responses and glucose homeostasis. Although the increases observed in creatinine, uric acid, and C-peptide levels were not statistically significant, these changes might become more pronounced with longer treatment durations or higher doses.

In our study, letrozole administration was found to cause a significant increase in serum Na levels ($P<0.05$). The literature indicates that estrogen plays a crucial role in regulating kidney function and electrolyte balance. Estrogen deficiency or agents that reduce estrogen levels, such as aromatase inhibitors, have been reported to cause increased renal sodium retention (Xue and Zhang, 2022). This mechanism suggests that Letrozole may induce changes in sodium homeostasis, leading to elevated serum Na levels. The absence of significant changes in other serum mineral levels (K, Cl, Fe, Mg, Ca) indicates that the homeostasis of these minerals remains relatively stable despite Letrozole treatment. Similarly, previous animal models and clinical studies have reported limited effects of aromatase inhibitors on serum electrolytes (Krysiak et al., 2020; Tomaszewska et al., 2020). These findings are consistent with the mineral parameter results observed in our study.

The significant increase in serum levels of IL-1β, IL-1α, TNF-α, NF-κB, and $A\beta_{1-40}$ in Letrozole-treated Group 2 indicates a marked elevation in systemic inflammation. Literature highlights these proinflammatory cytokines and the NF-κB pathway as key regulators of inflammatory responses (Lawrence, 2009). In particular, IL-1β and TNF-α play central roles in chronic inflammation and are known to trigger pathology in neurodegenerative diseases (Glass and Saijo, 2010). Additionally, elevated amyloid beta ($A\beta_{1-40}$) levels have been associated with neurodegenerative processes such as Alzheimer's disease and tend to increase in parallel with inflammation and oxidative stress (Heneka et al., 2015). The decrease in Serum TAS suggests heightened oxidative stress, supporting the notion that Letrozole reduces estrogen's antioxidant effects (Behl, 2002). Therefore, our findings align with existing literature, demonstrating Letrozole's potential side effects mediated through systemic inflammation and oxidative stress pathways.

The increase in TC and TG levels in brain tissue indicates a direct effect of Letrozole on lipid metabolism. The literature highlights the critical role of estrogen in maintaining brain lipid homeostasis and suggests that agents inhibiting estrogen synthesis, such as aromatase inhibitors, may disrupt this balance (Moser and Pike, 2016). Estrogen has been reported to regulate lipid metabolism in neural cells, providing neuroprotective effects, whereas estrogen deficiency may lead to increased lipid accumulation in the brain, triggering neurodegenerative processes (Vegeto et al., 2020). Conversely, the absence of changes in protein and glucose levels suggests that Letrozole's short-term impact on these metabolic parameters is limited. These findings align with previous studies supporting the specific effects of aromatase inhibitors on brain lipid metabolism, as observed in our study.

In this study, Letrozole administration was found to significantly affect metabolic, inflammatory, and oxidative responses at both central and peripheral levels. The marked increase observed only in NF-κB levels in the brain tissue of Group 2 indicates that Letrozole induces a limited yet notable inflammatory response within the central nervous system. NF-κB is well known as a key regulator of neuroinflammation, controlling microglial activation and the expression of pro-inflammatory cytokines (Glass and Saijo, 2010; Kaltschmidt and Kaltschmidt, 2009). However, the absence of significant alterations in IL-1α, IL-1β, TNF-α, and $A\beta_{1-40}$ levels suggests that Letrozole's effects proceed through specific signaling pathways rather than causing a widespread neuroinflammatory profiles. As estrogen reduction can increase local cytokine production and contribute to neuronal stress and damage (Vegeto et al., 2020), our findings support the notion that selective inflammatory responses may emerge due to the partial loss of estrogen's neuroprotective functions (Miller and Raison, 2016). Considering the central role of IL-1β in microglial activation and in initiating neuroinflammatory processes (Allan et al., 2005), the lack of significant increases in IL-1β further indicates that Letrozole may elicit a more controlled and region-specific pattern of inflammation in brain tissue. This result aligns with reports by Shaftel et al. (2008), emphasizing that central inflammatory responses are typically regulated and show regional variability. On the other hand, the elevated inflammatory markers and $A\beta_{1-40}$ levels detected in both brain tissue and serum in Group 2 demonstrate that Letrozole strongly stimulates systemic inflammation, which parallels central inflammatory processes. The critical roles of proinflammatory cytokines and amyloid beta accumulation in the pathogenesis of neurodegenerative diseases have been extensively documented (Heneka et al., 2015). Systemic inflammation is also known to exacerbate neuroinflammation by activating CNS inflammatory pathways (Perry and Holmes, 2014). Increased oxidative stress and amyloid beta levels contribute to neuronal structural and functional impairment, accelerating neurodegenerative mechanisms (Butterfield and Halliwell, 2019). In this context, Letrozole appears to enhance inflammation and oxidative stress at both peripheral and central levels, with the subsequent elevation of $A\beta_{1-40}$ and pro-inflammatory cytokines potentially facilitating the development of neuroinflammation. Our study demonstrates that Letrozole disrupts systemic homeostasis and promotes inflammatory and oxidative processes, and that these effects are also reflected in brain tissue.

Letrozole treatment induced significant metabolic, inflammatory, and oxidative alterations in both serum and brain tissue, indicating its potential to disrupt systemic homeostasis and to promote neuroinflammatory activity. These findings highlight the need for further comprehensive research on the neurobiological effects of aromatase inhibition.

Conflict of interest statement: None of the authors have any conflicts of interest to disclose.

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