The effects of letrozole on liver function and some biochemical parameters in rats

ABSTRACT

Letrozole (LTZ), is an aromatase inhibitor, that has been widely used in a variety of diseases such as polycystic ovary syndrome, endometriosis, and breast cancer. LTZ is received via the oral route and metabolized in the liver. Therefore, LTZ may have toxic effects like other drugs metabolized in the liver. Based on this, our study aimed to investigate the effect of LTZ on liver function and biochemical parameters. For this purpose, 16 Wistar albino female rats were divided into two groups (n=8): Control and LTZ respectively. The rats in the letrozole group were administered with 2 mL/kg LTZ by oral gavage once a day for 21 days. The Control group received the vehicle once a day for 21 days. Blood samples were collected on the 22nd day of the experiment. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), albumin (ALB), alkaline phosphatase (ALP), direct bilirubin and total bilirubin were measured. Biochemical analysis indicated that ALT, AST, LDH, ALP, and total bilirubin levels were significantly higher in the LTZ administrated group compared to the Control ($p>0.05$). ALB levels decreased in the LTZ group ($p>0.05$). In conclusion, it was determined that LTZ has toxic and detrimental effects on the liver. We suggested that long-term LTZ-administered patients should be under control against liver damage and may have liver-supporting adjuvant therapies for robust liver functions.

Keywords: Albumin, hepatotoxicity, letrozole, liver function tests

INTRODUCTION

Aromatase belongs to the cytochrome P450 system and is expressed by various tissues such as ovaries, adipose tissue, muscle, liver, and breast (Sun et al., 2007). Androgen precursors such as testosterone are involved in the converting reactions of enone rings to phenols for estrogen synthesis. The aromatase enzyme catalyzes above mentioned indispensable steps. Therefore, in pathologies including breast cancer, aromatase inhibitors (AI) are preferred for the inhibition of estrogen production and estrogen receptors. Recently, various steroidal and nonsteroidal AI treatments have been reported in most of the studies. Those in the first, second, and third-generation drugs among the several AI drugs that limit both genomic and non-genomic effects of estrogen, have been approved by the US Food and Drug Administration (FDA). Drugs in the third group are generally used as standard treatment for postmenopausal breast cancer (Kharb et al., 2020; Ratre et al., 2020). Additionally, they are effective as adjuvant therapy and generally well tolerated. Recently, these drugs have been commonly preferred in pathologies such as polycystic ovary syndrome and endometriosis in premenopausal non-fertile women (Barnhart et al., 2003; Bulun and Simpson, 1994; Makav et al., 2023; Mukherjee et al., 2022; Sun et al., 2007).

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Research Article

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Letrozole, as the marketing name Fermara, is a third-generation non-steroidal aromatase inhibitor. The chemical formula of Fermara is (4,4′-[(1H-1,2,4-triazol-1-yl) methylene] bis-benzonitrile) (Mukherjee et al., 2022). LTZ, a triazole derivative, inhibits the conversion of testosterone to estradiol and androstenedione to estrone. Thus, LTZ has been reported to inhibit aromatase activity by 99% and endogenous estrogen synthesis by 97-99% (Wit et al., 2012). LTZ is one of the most potent AIs and is widely used. LTZ has been found to have various positive effects on breast cancer, anovulatory infertility, and spermatogenesis. It is approved for the treatment of hormone receptor-positive, metastatic breast cancer in postmenopausal women. It has a high potential for use in both prevention of the androgenic steroids converting to estrogen and to prevent or reduce the side effects of androgenic steroids (Aydin et al., 2011; Mukherjee et al., 2022; Sun et al., 2007).

In addition to the positive effects of LTZ, many studies have claimed that it may have side effects (Čustović et al., 2019; Perez et al., 2006; Sharma et al., 2014;). The prolonged administration of LTZ has different side effects. The short-term use of LTZ has lower serious side effects. The most common side effects are headache (7%), nausea (6%), fatigue (5%), hot flushes (5%), peripheral edema (6%), rash (2.7%), drowsiness (3.2%) and vomiting (3.8%). Long-term use has been shown to cause more serious side effects. Generally, in breast cancer patients, bone pain, hot flushes, back pain, nausea, and dyspnea (Barnhart et al., 2003; Bulun and Simpson, 1994; Makav et al., 2023; Mukherjee et al., 2022; Sun et al., 2007). Many studies have indicated that LTZ administration leads to toxicity in several tissues and organs. The liver is one of the toxicity targets of LTZ. LTZ use has been shown to increase liver enzymes and cause hepatoxicity. In addition, it has been determined that LTZ may have detrimental effects on the endothelial layer of the central vein of the liver. LTZ has been reported to imbalance the serum lipid profile by an unknown mechanism (Aydin et al., 2011; Gharia et al., 2017; Mukherjee et al., 2022; Moy et al., 2014). The most crucial liver enzymes are considered to be aspartate aminotransferase (AST) and alkaline phosphatase (ALT) (Deveci et al., 2021; Karapehlivan et al., 2023; Kuru et al., 2022)

Based on the mentioned information, our study aimed to investigate the effects of LTZ on liver function and biochemical parameters in rats.

MATERIALS AND METHODS

Animals and ethical procedures

The ethical approval (KAÜ-HADYEK/2024-023) was obtained from Kafkas University Experimental Research Application and Research Center. Considering the principle of reduction from the 4R rule, serum samples of the Control and LTZ-treated groups were obtained from the previous study approved by Kafkas University Experimental Research Application and Research Center with the number 2021/156 for the present study. All procedures were in line with the TR Law 6343/2; 6.7.26 Veterinary Deontology and Helsinki World Medicine Organisation Declaration.

For his study, Wistar Albino female rats were purchased from Kafkas University Experimental Research Application and Research Center. All stages of the study were performed at the same center and under the same conditions. Rats were housed at 22±2 ˚C temperature and 12 h/12 h light/dark cycle. During the experiment, rats were fed with standard food pellets and water ad libitum. Before the experiment procedure rats were fasted for 12 h, allowed for only water.

Groups

In this study, 16 Wistar Albino female rats (200-250 grams and 4 months old) were used. The rats were divided into two groups randomly as given below.

- Control (n=8): Group given vehicle at a dose of 2 ml/kg for 21 days

- Letrozole (n=8): Group given LTZ at a dose of 2 ml/kg for 21 days

LTZ Administration

LTZ (Femara®, Novartis, Istanbul, Turkey) was dissolved in a 1% carboxymethylcellulose (CMC) solution as previously described. The solution was administered to the rats in the Letrozole group by oral gavage at a dose of 2 mL/kg once a day for 21 days (Kafali et al., 2004). The Control group received 1% CMC solution as a vehicle.

Tissue harvesting

The euthanasia procedure was performed on the 22nd day of the experiment, under general anesthesia (ketamine hydrochloride (75 mg/kg) and xylazine (15 mg/kg) intramuscular) by cervical dislocation. Serum samples were obtained and stored at -80ºC for biochemical analysis.

Biochemical analyses

ALT, AST, GGT, LDH, ALB, ALP, direct bilirubin, and total bilirubin were analyzed spectrophotometrically with a Beckman-Coulter AU5800 autoanalyzer (Beckman Coulter®, U.S.).

Statistical analysis

GraphPad 8.1 (San Diego, CA, USA) was used for statistical analyses. The difference between the two groups of biochemical parameters was analyzed by independent samples t-test. The significance was accepted as $p<0.05$.

RESULTS

In this study, ALT, AST, GGT, LDH, ALB, ALP, direct bilirubin, and total bilirubin levels were measured. There was a statistically significant increase in serum ALT levels of the Letrozole group compared to the Control ($p<0.05$). Similarly, AST values showed a statistically significant increase in the Letrozole group compared to the Control ($p<0.05$). There was a statistically significant difference in LDH levels between the Control and Letrozole groups ($p<0.05$). On the other hand, there was no significant difference in GGT levels between the Control and Letrozole groups ($p<0.05$; Figure 1).

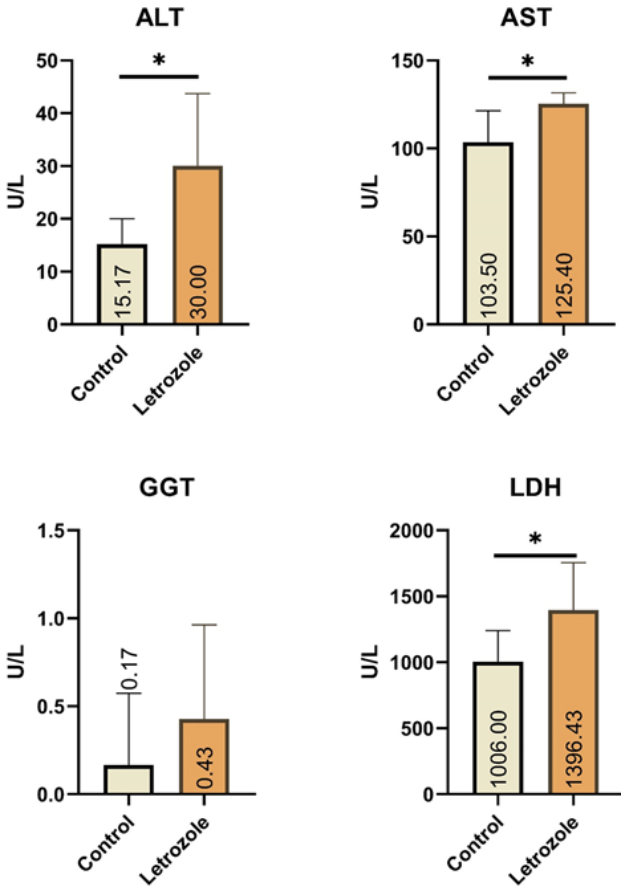

Figure 1. Comparison of serum ALT, AST, GGT, and LDH levels of control and Letrozole groups (*: $p<0.05$). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, LDH: Lactate dehydrogenase.

A statistically significant decrease was observed in serum ALB levels in the Letrozole group compared to the Control ($p<0.01$). When serum ALP levels were analyzed, there was a statistical increase in the Letrozole group compared to the Control ($p<0.05$). Similarly, the total bilirubin level was increased in the Letrozole group compared to the Control ($p<0.01$). However, there was no statistically

significant difference between the Control and Letrozole groups in direct bilirubin levels ($p<0.05$; Figure 2).

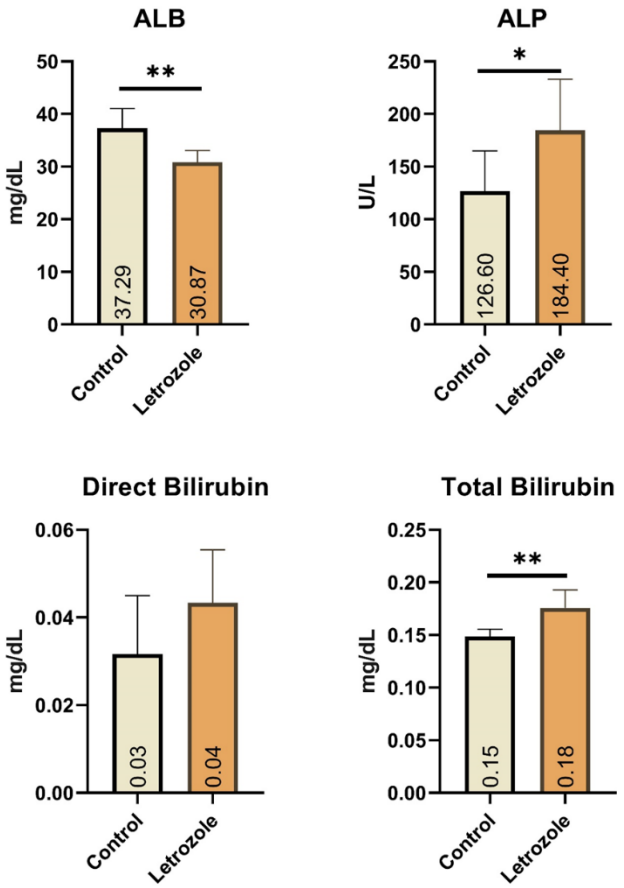

Figure 2. Graphical representation of ALB, ALP, direct, and total bilirubin values of control and Letrozol groups (*: $p<0.05$; **: $p<0.01$). ALB: Albumin, ALP: Alkaline phosphatase.

DISCUSSION

LTZ is a third-generation aromatase inhibitor, generally preferred for its reversibility, selectivity, and efficacy. It is widely used in many conditions including polycystic ovary syndrome, intrauterine insemination, endometriosis, and breast cancer (Jin et al., 2012; Requena et al., 2008; Taniguchi et al., 2011). LTZ is used via the oral route and metabolized in the liver after absorption from the gastrointestinal tract (Mukherjee et al., 2022; Taniguchi et al., 2011). Depending on the metabolism of most drugs in the liver may cause liver toxicity (Akşit et al., 2015; Kutlubay et al., 2009). LTZ is one of the above-mentioned drugs and has the potential to lead to liver toxicity (Aydin et al., 2011). Therefore, in our study, the effects of experimental LTZ-induced liver toxicity in rats were investigated through serum ALT, AST, GGT, LDH, ALB, ALP, direct bilirubin, and total bilirubin levels measurement. Interestingly, there was a significant increase in ALT, AST, LDH, ALB, ALP, and total bilirubin levels in the Letrozole administrated group.

Aminotransferases are enzymes involved in the interconverting of amino and keto acids in carbohydrates and nitrogen. Aminotransferases may be beneficial for the diagnosis of liver diseases such as hepatitis and are a sensitive indicator of liver cell damage. The increase in serum aminotransferase level may be due to the passage of the enzyme in the cell into the serum as a result of hepatocellular necrosis, or it may be due to increased membrane permeability in a level of cell damage that does not end with necrosis. Aminotransferase elevation can generally be assessed at three different levels: severely elevated (usually more than 15 times the normal value), moderately elevated (5-15 times the normal value), and mild elevated (less than 5 times the normal value). ALT and AST are enzymes from the aminotransferase group. ALT which is secreted from hepatocytes is a cytosolic enzyme and relatively more specific to the liver. AST is both cytosolic and mitochondrial sourced. It is also found in striated muscles, the brain, the pancreas, and blood cells as well as the liver (Ersoy, 2012; Green and Flamm, 2002). In our study, we evaluated the effects of LTZ on serum ALT and AST levels. Both enzyme levels were increased. This is a clear indication that LTZ causes liver damage. However, we found that ALT levels increased approximately 2-fold at the end of the 21 days of the experiment. The increase in ALT levels, which is more specific to the liver, caused by LTZ suggested that long-term use of LTZ may cause more serious damage.

ALP is an enzyme synthesized in bone and liver. ALP, which plays an important role in the hydrolysis of phosphate groups of nucleic acids, proteins, and other substrates, is found in the

canalicular membranes of hepatocytes and on the luminal surface of biliary epithelial cells. Serum ALP activity is primarily used as an indicator of hepatic diseases (Fernandez and Kidney, 2007; Wang et al., 2021). Dramatic ALP increases are commonly seen following obstructive biliary disorders, tumor infiltration, and metastasis to the liver (Limdi and Hyde, 2003). GGT is a key enzyme in response to the transpeptidation of functional gamma-glutamyl groups. All mammalian tissues contain GGT but the liver has the greater levels of GGT. GGT is a key marker for most liver pathologies but does not directly specify liver damage. However, increased levels of GGT together with other liver enzymes indicate that the source of damage is the liver (Brennan et al., 2022; Limdi and Hyde, 2003). The fact that serum ALT, AST, ALP, and GGT levels were increased together concluded that LTZ causes liver damage. In addition, GGT and ALP enzymes synthesized in bile duct epithelial cells suggest that LTZ causes severe liver and biliary tract damage.

LDH, an oxidoreductase enzyme, can be synthesized in different tissues. In particular, LDH is the main activator of the pyruvate-to-lactate converting enzymatic reaction. LDH is typically released from necrotic cells (Chaudhary and Chauhan, 2015; Faloppi et al., 2016). In our study, it was determined that the amount of LDH increased with liver-specific enzymes in the LTZ toxicity group. This result indicated that LTZ may create necrotic regions during liver toxicity.

ALB is the most abundant protein in plasma with a concentration of 30-50 g/L, corresponding to 50% of all plasma proteins. ALB is synthesized predominantly in the liver, with a synthesis rate of 150 mg/kg/day at about 10-15 grams per day and in response to the synthesis of 10% of hepatic proteins. ALB enters the bloodstream and helps transport vitamins, enzymes, and other important substances. The final concentration of plasma ALB is balanced through albumin synthesis, intravascular and interstitial influx, catabolism, and loss via renal or intestinal routes (Carvalho and Machado, 2018; Yılmaz et al., 2020). During liver damage, albumin production decreases. In the clinic, ALB is the most frequently used marker to measure the functionality of the liver (Eren et al., 2007; Yılmaz et al., 2020). In our study, serum ALB levels were significantly lower in the LTZ-induced toxicity group. This suggests that ALB levels were decreased secondary to LTZ-induced hepatotoxicity.

Bilirubin is an orange-yellow bile pigment sourced from the catabolism of various heme-containing proteins, particularly hemoglobin. Heme turns into biliverdin, which is converted to unconjugated or indirect bilirubin (UCB). UCB is an insoluble structure and is bound to albumin and circulate. In the liver, glucuronic acid is added to UCB to enhance water-solubility and direct bilirubin. As a result of this conjugation, it is excreted in bile or urine (Guerra Ruiz et al., 2021). Liver lesions cause a decrease in the number of hepatocytes and conclude the uridine diphosphate glucuronic transferase (UDPGT) enzyme, which is involved in conjugation, is not to be produced in sufficient amounts. Therefore, the amount of UCB increases (Işık et al., 2020; Kıncı et al., 2021). In our study, direct and total bilirubin levels were evaluated, and it was revealed that total bilirubin levels increased significantly in the LTZ group. According to our findings, serum total bilirubin levels were increased, and direct bilirubin was not increased indicating that it caused an increase in the amount of unconjugated bilirubin. In conclusion, we suggest that LTZ leads a widespread damage in the liver tissue through deficiency in the UDPGT enzyme.

Taken together, all of our findings indicate that even short-term LTZ administration causes an increase in both liver-specific and other tissue

damage markers. Our findings suggested that damage to the bile ducts, hepatocytes, and necrotic areas in the liver may be present. Aydın et al. (2013) reported that LTZ affected AST, LDH, ALP, and bilirubin values in rats. In addition, hematoxylin and eosin staining of liver tissue revealed congestion, thrombosis, and detached endothelial layer of the central vein (Aydin et al., 2011). These outcomes are in line with the results of our study and indicate that LTZ may result in severe liver damage in long-term use. Gharia et al. (2017) reported that an LTZ-administrated patient was admitted to a hospital with severe liver failure. Li et al. (2023) evaluated the efficacy of AI use, including LTZ, on liver function in cancer patients. AI users have been shown to have worsening liver function after 6 months compared to baseline (Yuechong et al., 2023). In addition, researchers who have studied the pharmacokinetics of LTZ believe that liver failure may significantly increase the half-life of LTZ. Therefore, it is also stated that LTZ-administered patients should be warned about liver damage (Bhatnagar, 2007).

CONCLUSION

In conclusion, LTZ affects the liver at therapeutic doses and may cause severe liver damage at toxic doses. Therefore, we believe that in patients with indications for long-term use of LTZ, an AI, physicians should check the liver function of patients before drug use and these tests should be repeated at regular intervals and adjuvant treatments should be initiated to support the liver when necessary.

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Availability of data and materials: All data and materials of the study are available in contact with the corresponsible author.

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