Bromelain ameliorates inflammation and hyperlipidemia by modulating oxidative stress and lipid metabolism in hyperlipidemic rats

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Abstract

Hyperlipidemia is the major cause of cardiovascular diseases (CVDs) and responsible for major deaths worldwide since it contains abnormal levels of circulating plasma lipids. Bromelain (BRO) is a bioactive compound obtained from the pineapple stem belonging to the Bromeliaceae family. Through the modulation of the inflammation pathway, BRO can be considered a promising natural therapeutic agent for improving human health problems. Therefore, the present study aims to evaluate the effect of BRO hypolipidemic, biochemical, histopathologically, and molecularly in hyperlipidemic rats. Total cholesterol (TC), triglyceride (TG), and LDL cholesterol (LDL-C), AST, and ALT values were measured from blood samples. Oxidative stress markers and histopathological examination were assessed in the heart and liver tissues. Finally, to determine Srebp-1c, Lxr-α, matrix metalloproteinases (MMP), and inflammation, the gene expressions of Il-1β, Il-6, and Tnf-α in the same tissues were examined. BRO treatment prevented the increase in hyperlipidemic levels caused by tyloxapol administration. It reduced the rise in LDL cholesterol and triglyceride levels. In addition; lipid peroxidation levels induced by tyloxapol in rats showed that Bromelain protected the change in SOD and CAT activities by acting on oxidative stress parameters. BRO was also found to have a histopathologically protective effect against liver and heart tissue damage caused by hyperlipidemia. Inhibition of expression of Srebp-1c, Lxr-α, Mmp-2, Mmp-9 and proinflammatory cytokines Il-1β, Il-6, and Tnf-α genes also appeared. It was concluded that bromelain, an untested agent for hyperlipidemia, may be a promising new agent to reduce mortality and morbidity associated with free radical reactions, and inflammation in the liver and heart tissue.

INTRODUCTION

Cholesterol and triglycerides are steroid alcohols found in both human and animal cells and are important clinically identified plasma lipids. In addition to being a cell component of cholesterol, it acts as a precursor for steroid hormones, bile acids oxysterols, and vitamin D (Bahulikar et al. 2018) It is also an important molecule for cell growth and function and is responsible for meeting signaling, biophysical and endocrine requirements (Nazih and Bard, 2020). In addition to playing an important role in mammalian membrane homeostasis, excess cellular cholesterol is toxic and therefore cholesterol biosynthesis requires strict regulation (Reboldi and Dang, 2018). Both deficiency and excess cholesterol cause disease (Schade et al. 2020).

High cholesterol level is associated with many diseases, especially coronary heart diseases (CHD). These include stroke (ischemic stroke), including atherosclerosis, blood pressure, diabetes, myocardial infarction, and coronary artery diseases. Uncontrolled cholesterol intake is an important factor leading to foam cell formation and atherogenesis. LDL (Low-Density Lipoprotein) levels increase individual susceptibility to atherosclerosis and its complications, as well as being associated with cardiovascular risk in human populations (Saxena and Chandra, 2020). The relationship between high HDL-C (High-Density Lipoprotein Cholesterol) concentration or low LDL-C concentration and reduced risk of atherosclerotic cardiovascular disease is widely accepted (Palabıyık et al. 2022). Various studies have been conducted to find ways to decrease LDL-C concentrations and increase HDL concentrations. It may
be necessary to initiate drug therapy in addition to lifestyle changes to reduce the risk of cardiovascular disease as well as high LDL-C (Saini et al. 2004).

Cholesterol homeostasis is also known to be controlled by internal factors such as genetics, body weight, endocrine factors and circadian rhythm, and other external therapeutic and nutritional factors such as statins, ezetimibe, plant sterol therapy and weight loss. Dietary recommendations have long been the primary method for efforts to lower cholesterol levels. However, it has been observed that this method alone does not work, and only minor changes in cholesterol levels can be obtained. In general, diet and exercise cause a 10–15% decrease in LDL-C levels (Scirica and Cannon 2005). For this reason, drug treatments have been used to obtain more effective results, and these treatments include statin treatments and non-statin treatments. However, undesirable side effects were observed during these treatments as well (Gross and Figueredo 1973; Pedersen and Tobert 2004).

Tyloxapol is a very valuable substance in studies for triglyceride and cholesterol metabolism. It is used in animal models to screen for lipid-lowering natural or chemical drugs and to examine cholesterol and triglyceride metabolism (Takahashi et al. 2003). This substance promotes cholesterol synthesis through increased 3-hydroxyl-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA) activity and increases plasma cholesterol levels (Iqbal et al. 2015). It is known that diets rich in antioxidants play an important role in the prevention of cardiovascular disease and cancer. It has been shown to be preferred over drug treatments in diseases such as hyperlipidemia and hypertension. Due to their antioxidant potential, many plants belonging to the Bromeliaceae family, especially Ananas comosus (Ananas), are of great interest as phytotherapeutic and are also considered to have anticancer, anti-inflammatory and anti-platelet effects. At the same time, A. comosus stem extract is an inexpensive by-product waste rich in complex enzymes identified by bromelain, which is very important in some clinical applications, especially in tumor growth modulation, wound healing, anti-inflammatory effect, anti-diarrhea and aiding digestion. Bromelain also presents a new perspective in terms of the treatment of metabolic disorders and metal toxicity (Al-Otaibi et al. 2015).

Therefore, the present study was prepared to evaluate the protective efficacy of bromelain in alleviating tyloxapol-induced hyperlipidemia in rats.

**MATERIAL AND METHOD**

**Supply of substances**

0.5% (w/v) CMC (Carboxymethylcelulose) was used to prepare the aqueous solution of Bromelain (Holland and Barrett, England) used in the study. It was then sonicated at 40°C for 30 minutes. Tyloxapol (Santa Cruz Biotechnology, USA) was dissolved in normal saline (pH 7.4) with 1–2 drops of Twin 80 (Baldissera et al. 2017).

**Animals and experiment design**
This study was carried out with the approval of Atatürk University Medical Experimental Application and Research Center ethics committee, dated 15.11.2021 and numbered E-55885869-900-2100312890. A total of 18 male Wistar rats (210–300 g) obtained from the Institution's Experimental Animals unit were used. Animals were randomly assigned to three groups (A-C) (n = 6). Rats were kept in polycarbonate cages and on a 12-hour light/dark cycle until the start of the experiment at room temperature (22 ± 2 ºC) with free access to food and water.

Group A: The group that received physiological water [2.5 mL/kg, intraperitoneal (i.p)]

Group B: The group that received tylaxopol (400 mg/kg, i.p) 30 minutes after physiological water (2.5 mL/kg, i.p) administration

Group C: The group that received bromelain (250 mg/kg, o.d) orally for 18 days before 24 hours tyloxapol (400 mg/kg, i.p) administration.

Tyloxapol (400 mg/kg, i.p) and Bromelain (250 mg/kg, o.d) concentrations were determined by literature review and preliminary trials (Zarzecki et al. 2014; El-Demerdash et al. 2020). All applications were performed on rats that were fasted for 12 hours and after 24 hours the animals were anesthetized with sevoflurane. Blood samples from rats and heart and liver tissues were removed for further studies. The excised tissues were first treated with liquid nitrogen for molecular analysis and then brought to -80°C. Tissues were placed in 10% buffered formalin phosphate solution for histopathology. At the end of the procedure, the animals were sacrificed.

**Anti-hyperlipidemic Study**

During slaughter, blood samples were taken to determine total cholesterol (TC), triglyceride (TG), LDL-C, HDL-C, ALT and AST values. TC, TG and HDL-C concentrations were determined by enzymatic color testing using purchased commercial kits. LDL-C concentration is:

\[
LDL = TC - HDL - \frac{5}{4}TG
\]

was calculated with the formula (Friedewald et al. 1972; Sami Khaza 2013).

ALT and AST concentrations were also determined using standard kits.

**Oxidative stress parameters**

In this section, the enzymatic antioxidants Malondialdehyde (MDA) and Superoxide dismutase (SOD) and Catalase (CAT), which are indicators of unsaturated fatty acid peroxidation for liver and heart tissues used in our study, were determined. Elisa Kits (SunRed Biotechnology) were used to determine the MDA, SOD and CAT levels of the samples taken from the tissues. After the procedure was completed, the optical density (OD) was measured in a spectrophotometer at a wavelength of 450 nm. The OD values of the sample were applied to the regression equation for concentration calculation.

**Histopathological Study**
The heart and liver tissues were cut and cassetted and transferred to the tissue tracking device. Then, blockage was performed and 4 micron thick sections were taken from the blocked tissues. Sections were stained with hematoxylin-eosin. High resolution pictures (x 200 magnification) of the samples were taken using an Olympus (B x 60, Japan) microscope.

**Tissue homogenization, RNA Isolation and cDNA Extraction**

0.03 g of liver and heart tissue were weighed and then the EcoPURE RNA Isolation Kit (EcoTech) procedure was used for homogenization and RNA isolation. The concentration and purity of the obtained RNAs were measured with the Nanodrop device. RNA samples were kept at -80°C until further processing. VitaScript cDNA Isolation Kit (Procomcure) was used for cDNA extraction. Concentration and purity of each group were measured with the Nanodrop instrument.

**Primary Design of Genes and Real-Time PCR**

In order to observe the anti-inflammatory effects of the Bromelain substance used in the study on the heart and liver tissues, the *Gapdh* gene as well as the *Il-1β*, *Il-6*, *Tnf-a*, *Srebp-1c*, *Lxr-a*, *Mmp-2* and *Mmp-9* genes were used as a cleaning agent. Before the Real-Time PCR procedure, lyophilized gene primers (Sentebiolab Biokim) were prepared for processing as written in the protocol. The primers of the genes used are shown in Table 1.
Table 1
Primer Sequences and Accession Numbers for the Genes Used in the Study

<table>
<thead>
<tr>
<th>GENE SYMBOL (RAT)</th>
<th>PRIMER SEQUENCE</th>
<th>ACCESS NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Il-1β</td>
<td>F: 5'-AAA AAT GCC TCG TGC TGT CT-3’</td>
<td>NM_031512</td>
</tr>
<tr>
<td></td>
<td>R: 5’-TCG TTG CTT GTC TCT CCT TG-3’</td>
<td></td>
</tr>
<tr>
<td>Il-6</td>
<td>F: 5'-AGT TGC CTT CTT GGG ACT GA-3’</td>
<td>NM_012589.2</td>
</tr>
<tr>
<td></td>
<td>R: 5’-ACT GGT CTG TTG TGG GTG GT-3’</td>
<td></td>
</tr>
<tr>
<td>Tnf-α</td>
<td>F: 5'CGG GGT GAT CGG TCC CAA CAA-3’</td>
<td>NM_012675.3</td>
</tr>
<tr>
<td></td>
<td>R: 5’-GTG GTT TGC TAC GAC GTG GGC-3’</td>
<td></td>
</tr>
<tr>
<td>Srebp-1c</td>
<td>F: 5'-GGA GCC ATG GAT TGC ACA TTT GA-3’</td>
<td>Vijayakumar &amp; Nachiappan 2017</td>
</tr>
<tr>
<td></td>
<td>R: 5’-CTG TGT CTC CTG TCT CAC CCC-3’</td>
<td></td>
</tr>
<tr>
<td>Lxr-α</td>
<td>F: 5'-GTA CAA CCC TGG GAG TGA GA-3’</td>
<td>Zhao et al. 2015</td>
</tr>
<tr>
<td></td>
<td>R: 5’-GGT TGA TGG AGA CAT AGG CA-3’</td>
<td></td>
</tr>
<tr>
<td>Mmp-2</td>
<td>F: 5’-ACC ACG GAT CTG AGC AAT-3’</td>
<td>NC_051354.1</td>
</tr>
<tr>
<td></td>
<td>R: 5’-TAC TGG ACC CAC GCC TAC-3’</td>
<td></td>
</tr>
<tr>
<td>Mmp-9</td>
<td>F: 5’-TTG GCT TCC TCC GTG ATT-3’</td>
<td>NC_051338.1</td>
</tr>
<tr>
<td></td>
<td>R: 5’-CCC TAC TGC TGG TCC TTC-3’</td>
<td></td>
</tr>
<tr>
<td>Gapdh</td>
<td>F: 5’-AAA CCC ATC ACC ATC TTC CA-3’</td>
<td>NM_017008.3</td>
</tr>
<tr>
<td></td>
<td>R: 5’-ATA CTC AGC ACC AGC ATC ACC-3’</td>
<td></td>
</tr>
</tbody>
</table>

Real-Time PCR was performed with the VitaScript kit (Procomcure). Gene expression profiling was performed on the Rotor-Gene PCR instrument using SYBER Green (Bio-Rad, Hercules, CA) based qPCR method. Results were analyzed after repeating the same procedure for each tissue and gene.

Performing Statistical Analysis

Measurements were performed in 3 replicates for each animal and group. One-way ANOVA test was used to compare groups. Tukey Test, one of the multiple comparison tests, was used to determine the difference between groups found to be different. q-RT PCR results were statistically evaluated using Prism (GraphPad Software, San Diego, CA) as follows: ns p > 0.05 (not significant); *p < 0.05 (significant); **p < 0.01 (very significant); *** or ****p < 0.001 (highly significant).

RESULTS
**Effects of BRO on Biochemical and Antioxidant Parameters**

TC, TG, LDL-C, HDL-C, ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) values in the serum of the control and administered groups are shown in Figure 1.

When the healthy control (HC) and TYX group were compared in the study, TC, TG, LDL-C and HDL-C levels increased significantly in the TYX group. However, BRO treatment at a dose of 250 mg/kg administered significantly reduced these increased levels. Similar to these results, ALT and AST levels, which are liver function enzymes, increased significantly in the TYX group, while 250 mg/kg BRO administration significantly reduced these increased levels. MDA, SOD and CAT values for the groups used in the study are shown in Figure 2.

In the MDA results, the values in the TYX group increased when compared to the HC group. This increase was considered significant (p<0.01) for liver tissue. This increase observed in the BRO-treated groups decreased and approached the HC group data. When the data obtained for SOD is examined, the data in the liver tissue is at a lower level compared to the heart tissue. While the SOD data showed a decrease in the TYX group, the values approached the HC values in the 250 mg/kg BRO-administered group. However, these values were considered to be statistically insignificant for heart and liver tissue. When the CAT data are examined, there is no statistically significant difference between the groups for liver tissue. For heart tissue, when HC and treatment groups were compared separately, a statistically significant difference was observed (p<0.01), while the difference between TYX and TYX+BRO was statistically insignificant.

**Histopathological Evaluations**

The visuals of the histopathological evaluations made with H&E staining are given in Figure 3. Histopathological examination with H&E staining in Figures 3a and 4a showed normal histology of the liver and heart in the HC group. While the liver tissues were normal histology in the HC group, apoptosis, and necrosis, sinusoidal dilatation, bleeding, congestion and mononuclear cell infiltration were observed in the cells in the hyperlipidemia (TYX) group. On the contrary, it was observed that pathological findings decreased in the TYX group when BRO was given for preventive purposes before hyperlipidemia occurred.

It was observed that congestion decreased, bleeding, mononuclear cell infiltration, sinusoidal dilatation, cell apoptosis, and necrosis were not observed in the treatment with BRO. Histopathological images of the heart tissue are given in Figure 4 and, similar to the liver tissue, normal histology was observed in the heart tissues in the HC group.

In the BRO group, it was observed that the pathological findings in the heart tissues decreased due to the effect of bromelain. When the TYX group was compared with the TYX+BRO group, congestion decreased, and hemorrhage, mononuclear cell infiltration, cell apoptosis, and necrosis were not observed.

**Il-1β, Il-6 and Tnf-α Gene Expressions**
Gene expression graphs of the genes used in the study for both liver and heart tissue are given in Figure 5.

When the $I{I}-1\beta$ gene expression data were examined; BRO application brought the gene levels activated by TYX closer to the HC group for both liver and heart tissue. This inhibitory effect of BRO was more pronounced in liver tissue. Inhibition in liver tissue was statistically significant, whereas inhibition in heart tissue was not statistically significant.

When the $I{I}-6$ gene expression data were examined; when the $I{I}-6$ gene expression data of the TYX-treated groups were compared with the HC group, an increase was observed in both liver and heart tissue. While this increase is statistically insignificant for liver tissue, it is significant for heart tissue. As a result of BRO application, the level of $I{I}-6$ gene expression in liver tissue was found even lower than the HC group data. In heart tissue, although there was an inhibition at this gene level, it remained above the HC group. This decrease was considered statistically insignificant.

When the $Tnf-a$ gene expression data are examined; while similar results were obtained for the other two gene levels in liver tissue, no similarity was observed for heart tissue. In the liver tissue, there was an increase in the $Tnf-a$ gene level in the TYX group and an inhibition as a result of BRO administration. However, both the increase and decrease are statistically insignificant. In heart tissue, BRO application did not cause an inhibition on the increase in gene expression caused by TYX. On the contrary, it caused a small increase in gene expression. However, these increases in both TYX and TYX+BRO application groups were not statistically significant.

When we looked at the $Srebp-1c$ data, tyloxapol caused an increase in liver tissue, but this increase was not statistically significant. Again, the combination group brought this increase closer to that of the control group, and the reduction was not statistically significant. However, when comparing the combination group and the hyperlipidemia group, a statistically significant difference was found ($p<0.05$). When the heart tissue data were examined, a significant increase was observed in the hyperlipidemia group ($p<0.001$), and bromelain protection brought this increase closer to the control group data ($p<0.01$).

In $Lxr-a$ data, the same results were observed for both tissues, but the increase caused by tyloxapol was more pronounced in liver tissue ($p<0.001$). These increases again approached the control group data with bromelain applied for preventive purposes.

When $Mmp-2$ and $Mmp-9$ data are taken together, statistically significant increases were seen in both liver and heart tissue in the TYX group. These increases were in liver and heart ($p<0.05$) for $Mmp-2$. For $Mmp-9$, it was found in the liver ($p<0.01$) and in the heart ($p<0.0001$). In the TYX+BRO group, the increases caused by tyloxapol were reversed by the effect of bromelain. These decreases are statistically significant for both gene expression.

**DISCUSSION**
In general, a high-fat diet is a risk factor for overall body fat deposition, particularly visceral fat. There are many synthetic hypolipidemic drugs that are useful in lowering blood cholesterol levels in patients with cardiovascular diseases and at risk for such diseases. However, these drugs have natural side effects such as hepatotoxicity and nephrotoxicity that have been proven in various clinical settings. Natural treatments are required to keep the side effects of these drugs minimal and to prevent harmful secondary effects (Kumar et al. 2011; Björnsson 2017). As a result, herbal remedies for hyperlipidemia are gaining popularity as they have fewer side effects, are cheaper, are more readily available, and are safe (Serrano et al. 2009).

The aims of this study are to determine whether bromelain will have a hypolipidemic effect on tyloxapol-induced hyperlipidemia in male Wistar rats, show whether bromelain can positively affect antioxidant mechanisms against oxidative damage caused by hyperlipidemia, show whether bromelain can have a positive effect on tyloxapol-induced hyperlipidemia, mainly liver and heart disease caused by hyperlipidemia, determine whether bromelain has a protective effect against tissue damage, and finally to evaluate the effect of bromelain on increased pro-inflammatory cytokine levels as a result of hyperlipidemia.

In this experimental protocol, tyloxapol-induced hyperlipidemic levels were inhibited by oral single dose (250 mg/kg) bromelain protection for 18 days, the increase in total cholesterol levels was blocked and the increase in LDL cholesterol and triglyceride levels was significantly reduced. In addition; we observed the ability of bromelain to act on oxidative stress parameters while preserving SOD and CAT activities, as well as the change in lipid peroxidation levels induced by tyloxapol in rats. It was also determined that bromelain had a histopathologically protective effect against liver tissue damage caused by hyperlipidemia. Finally, the anti-inflammatory properties of bromelain were determined by determining the expression levels of proinflammatory cytokines Il-1β, Il-6 and Tnf-a genes, metalloproteinases (Mmp-2 and Mmp-9), and Srebp-1c and Lxr-a genes, which play a major role in hyperlipidemia, which can be used as an inflammation marker.

Widely used to inhibit the enzymatic activity of lipoprotein lipase, tyloxapol causes accumulation of TC, TG, and LDL-C in plasma (Janicki and Aron 1962). This model has been particularly used to screen for natural or chemical hypolipidemic drugs (Schurr et al. 1972). Rony et al. (2014) showed that maximum plasma triglycerides and total cholesterol were reached 20 hours after tyloxapol administration. In the current study, the plasma lipid profile of rats in the control group did not change during the experimental period (Rony et al. 2014). However, intraperitoneal injection of tyloxapol resulted in high lipid concentrations in plasma. After 24 hours of tyloxapol injection, a statistically significant increase in TC, TG, and LDL-C concentrations was noted compared to the control group. High plasma TC and LDL-C levels cause lipid atherogenesis and are considered a major risk factor for the development of insulin-resistant, atherogenic risk and metabolic syndrome.

It is believed that TYX combines with triglycerides in plasma in a way that reduces their rate of hydrolysis by enzymes (clearing factor lipase or lipoprotein lipase) to interfere with their removal from the circulation
by extrahepatic tissues (Hall et al. 2000). In addition, injection of this compound in rats has been shown to increase hepatic secretion of VLDL-K, resulting in elevated blood cholesterol and triglyceride levels (Khanna et al. 2002).

Oral administration of bromelain (250 mg/kg) before being hyperlipidemic with TYX caused a significant decrease in serum TC, TG and LDL-C levels. The cholesterol-lowering activity of bromelain was probably mediated by the improvement of LDL-C catabolism (Mbikay et al. 2014).

In addition, bromelain-like compounds are involved in cholesterol metabolism such as 3-hydroxyl-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA), acyl CoA cholesterol acyl transferase (ACAT) and lecithin-cholesterol acyl transferase (LCAT) to reduce cholesterol biosynthesis. It is known to reduce serum lipids through other mechanisms including the modulation of enzyme activities (Hosomi et al. 2010).

Decreased liver functions due to various reasons can be recognized by enzyme parameters such as LDH, AST, and ALT synthesized and metabolized by the liver. Thus, especially AST and ALT are the most sensitive markers showing hepatic injury (Dancygier 2010), and previous studies have shown that these markers increase after renal dysfunction after hyperlipidemia (Kathak et al. 2022). High levels of AST and ALT in serum indicate necrotization of the liver. Therefore, these parameters are preferred when investigating agents that may have adverse effects on the liver. Reliable results were found regarding the parameters of liver injury from hyperlipidemia in rats. In our study, a significant increase was observed in AST and ALT levels after tyloxapol treatment, which supports the literature.

These liver function enzymes were significantly reduced in rats pre-fed with bromelain at a dose of 250 mg/kg. This indicates the positive effect of bromelain on serum liver function levels. In the current study, the antagonistic role of bromelain from A. comosus stem against tyloxapol-induced oxidative damage and biochemical degradation was also examined. There is little evidence of the efficacy of bromelain as a natural product to overcome the toxicity of this nonionic oligomer. It is known that hyperlipidemia disrupts the oxidant-antioxidant balance in tissues and causes various biochemical and physiological dysfunctions (Yang et al. 2008). Oxidative stress is the best known among the mechanisms that cause cell death and tissue damage caused by almost all agents, not just hyperlipidemia (Haushalter et al. 2019). The generation of reactive oxygen species during hyperlipidemia is a pathological factor that plays a key role in causing hepatic, cardiac, and renal dysfunction. In addition, as a result of the production of free radicals (such as trichloromethyl and peroxy), which initiate some strong lipid peroxidation, antioxidant defenses are overcome in the liver, oxidative destruction and serious tissue damage to cell membranes occur (Askin et al. 2022). Recently, many studies have focused on alleviating organ damage caused by hyperlipidemia.

In our study, the ameliorated effect of bromelain showed itself in the reduction of oxidative stress. Therefore, in this study, antioxidant parameters were used as markers for bromelain's efficacy against tyloxapol-induced liver and heart injury.
When the results of MDA, the first parameter used for this purpose, were examined, it was determined that tyloxapol increased MDA levels in all three tissues and thus caused oxidative stress. It was observed that bromelain brought the increased MDA levels closer to the healthy control group and brought the damage under control. Therefore, targeting MDA represents a promising approach to prevent the impact of oxidative damage on distant organs, particularly the heart and kidney (Sherif and Al-Shaalan 2018).

The protective effects of bromelain have been reported in various experimental clinical studies over the years in chemical-induced oxidative and apoptotic damage to various tissues and organs (Dave et al. 2012). However, there is no evidence from well-designed experimental studies to indicate the effects of bromelain on the liver and heart after hyperlipidemia. Our study showed that bromelain can exert strong protection following hyperlipidemia and reduce myocardial damage through an antioxidant-mediated pathway in the second window of protection. This protective effect was also mediated by the activity of the SOD enzyme, particularly in heart tissue, as the first line of defense against oxidative damage. Studies have shown increased activation of antioxidant enzymes in distant organs after the initial acute injury of the heart and kidney (Wang et al. 2017).

The increase in catalase enzyme as a result of tyloxapol application, which is another finding we obtained as a result of our study, is also compatible with the literature data (Mantha et al. 1993). However, bromelain did not cause a significant decrease in catalase enzyme levels increased as a result of tyloxapol administration. The level of catalase in the heart and liver tissue was preserved the same.

In previous studies, histopathologically increased cytoplasmic vacuolation, vascular permeability and leukocyte infiltration in liver, kidney and heart tissue after hyperlipidemia, as well as degenerative changes and increased secondary congestion have been reported (Abdou and ElMazoudy 2010). In our study, histopathologically, in the tyloxapol group; hemorrhage, congestion, mononuclear cell infiltration, sinusoidal dilatation, cell apoptosis, and necrosis were detected. Significant differences were found in TYX scoring values compared to the healthy control group. The occurrence of liver and heart damage after hyperlipidemia showed that our modeling was successful. Also in liver and heart tissues; The significant reduction in histopathological changes observed in the TYX + BRO groups compared to the TYX group demonstrated the histoprotective efficacy of BRO use. Especially when apoptosis decreases after hyperlipidemia, renal inflammation also decreases. It was thought that this could potentially be demonstrated in other organs affected by hyperlipidemia (Daemen et al. 1999). Our study also shows that the use of BRO partially reduces apoptosis and inflammation. Our findings prove that this idea is correct with histopathological findings.

Inflammation is the response to infection and injury in conditions such as swelling, redness, and pain in a particular area of the body. It is a very important process in pathology. It is a necessary response in cell homeostasis due to its important role in restoring tissue structure and function (Agarwal et al. 2019). Inflammation involves interactions of resident cells, soluble mediators, and extracellular matrix molecules. With a successful inflammatory response, harmful stimuli are cleared and physiological function returns to normal. Faulty inflammatory response may cause morbidity and may have adverse
effects on longevity (Tasneem et al. 2019). The duration of inflammation varies with damage, and too much inflammation leads to some deadly and dangerous diseases such as osteoarthritis, rheumatoid arthritis, Crohn's syndrome, intestinal diseases and cancer. In this case, cytokines are activated. Especially Il-1β, Il-6 and Tnf-a are the most important of these cytokines. These are the most frequently used parameters in the evaluation of inflammation in experimental studies (Agarwal et al. 2019).

In our study, findings have been obtained that trigger a systemic inflammatory response in liver and heart tissue as a result of hyperlipidemia application, collection of inflammatory cells, and increasing the level of these three pro-inflammatory cytokines resulting in distant organ damage. However, bromelain pretreatment reversed the significant increase in cytokine (especially Il-1β and Il-6) levels triggered by hyperlipidemia. When Tnf-a gene expression data is examined; while similar results were obtained for the other two gene levels in liver tissue, no similarity was observed for heart tissue. There is an increase in Tnf-a gene level in the tyloxapol group in the liver, and inhibition as a result of bromelain administration. However, both the increase and decrease are statistically insignificant. In heart tissue, bromelain application did not cause an inhibition on the increase in gene expression caused by tyloxapol. On the contrary, it caused a small increase in gene expression. However, these increases in both tyloxapol and tyloxapol + bromelain administration groups were not statistically significant.

Literature reviews related to the anti-inflammatory effects of bromelain support our findings. Bromelain application decreased oxidant and inflammatory parameters (Il-1β and Tnf-a) in NaOH-induced corrosive burns and in turn increased antioxidant levels (Şehirli et al. 2021). Another study on the anti-inflammatory effects of bromelain shows that bromelain treatment can effectively reduce neutrophil migration to areas of acute inflammation and promote specific removal of the CD128 chemokine receptor as a potential mechanism of action (Fitzhugh et al. 2008). Another study anecdotally reported that bromelain taken orally reduced inflammation in ulcerative colitis in humans. Proteolytically active bromelain is known to reduce the expression of mRNAs encoding proinflammatory cytokines by human leukocytes in vitro. These proinflammatory cytokines include granulocyte colony-stimulating factor (G-CSF), interferon (IFN)-γ, Il-6, Il-1β and TNF (Onken et al. 2008). Similarly, bromelain reduced the development of allergic airway disease by exerting an anti-inflammatory effect in a murine model. Bromelain achieved this effect by altering CD4 + to CD8 + T lymphocyte populations. The same investigators suggested that bromelain may have similar effects in the treatment of human asthma and hypersensitivity disorders as a result of the reduction in allergic airway disease outcomes (Secor et al. 2005). Bromelain may have shown an anti-inflammatory effect by showing similar effects in the present study.

Srebp-1c is a membrane-bound transcription factor involved in lipid metabolism by regulating triglyceride synthesis and storage in hepatocytes. Lxr-a is the main upstream regulatory gene of Srebp-1c expression, and the activation of Lxr-a induces the overexpression of Srebp-1c and downregulates the expression of ApoA5 protein, ultimately increasing triglyceride levels. Furthermore, inhibition of Lxr-a expression resulted in decreased Srebp-1c expression and triglyceride synthesis (Serrano et al. 2009). In one study, Srebp-1c and Lxr-a gene expression was investigated in hyperlipidemia model rats. Although the expression levels of both genes were increased in the hyperlipidemic group, the ethanol extract of Usnea
used for treatment reversed these increases (Zhu et al. 2017). A study by Zhu et al. found that the plant *Valeriana jatamansi* has a lipid-lowering mechanism of action and down-regulates the increased *Srebp-1c* and *Lxr-a* protein expression caused by hyperlipidemia (Zhu et al. 2016). In our study, *Srebp-1c* and *Lxr-a* gene levels were upregulated in the tyloxapol-induced hyperlipidemia group in both liver and heart tissue, while this increase was downregulated in the combined group. Therefore, our results are compatible with the data in the literature.

Studies have been conducted to demonstrate the effect of statin lipid-lowering therapy on other factors affecting cardiovascular disease risk. Administration of this therapy has been shown to affect cholesterol concentrations and lower LDL concentrations, further reducing the risk of CVD. MMPs are potential indicators of arterial inflammation. In addition, other studies have shown that statin therapy significantly reduces *Mmp-2* and *Mmp-9* levels in human and animal models, thereby stabilizing atherosclerotic plaques and reducing the risk of CVD (Kosowski et al. 2022). In previous studies, serum *Mmp-2* activity was found to be positively correlated with total cholesterol and LDL-C levels. While no correlation was found with HDL cholesterol and triglyceride levels (Derosa et al. 2007), a negative correlation was found between plasma MMP-2 protein level and HDL cholesterol in patients with coronary artery disease (Bencsik, et al. 2015). As far as we know, there are few studies examining *Mmp-2* and *Mmp-9* gene levels based on hyperlipidemia. Considering our findings, it was observed that *Mmp-2* and *Mmp-9* levels were significantly increased in both tissues in the hyperlipidemic group. On the other hand, bromelain, which is used for preservative purposes, managed to prevent this increase by having the opposite effect. Based on the studies conducted, it is thought that with this decrease provided by bromelain, atherosclerotic plaque progression and fragility can be stopped and the rupture of the plaque can be prevented.

**CONCLUSION**

In summary, as a result of our findings, bromelain has a hypolipidemic effect because it reduces the increase in TC, TG, and LDL-C in rats with hyperlipidemia induced by tyloxapol, it also has an antioxidant effect by positively affecting the parameters related to oxidative stress, as well as against liver and heart damage histopathologically in hyperlipidemic rats. concluded that it may have a protective effect. Finally, it was determined that it has anti-inflammatory effects at the gene level. In conclusion, our study was after hyperlipidemia; oxidant and antioxidant balance are disturbed, causing tissue damage and an increase in the levels of inflammatory mediators, leading to dysfunction in liver and heart tissues. Bromelain, an untested agent for hyperlipidemia, may be a promising new agent to reduce mortality and morbidity associated with free radical reactions in liver and heart tissue. For the reasons mentioned above, we believe that this component can be recommended together with drugs to prevent many diseases, especially cardiovascular diseases, which are associated with hyperlipidemia. However, further investigations are necessary to elucidate the mechanism of action of this compound.

For the genes targeted in the current study, a protein-protein interaction network was created using the STRING online database, and according to the results of this network analysis, it was seen that all the genes examined in the study interacted with each other (Fig. 6).
Abbreviations

**ALT** Alanine Aminotransferase  
**ANOVA** Analysis of variance  
**AST** Aspartate Aminotransferase  
**BRO** Bromelain  
**CAT** Catalase  
**cDNA** Complementary DNA  
**HDL** High-Density Lipoprotein  
**LDL** Low-Density Lipoprotein  
**MDA** Malondialdehyde  
**qPCR** Quantitative polymerase chain reaction  
**RNA** Ribonucleic acid  
**SOD** Superoxide dismutase  
**STRING** The search tool for the retrieval of interacting genes  
**TYX** Tyloxapol  

Declarations

**Ethical Approval**

Animal studies were carried out with the approval of the ethics committee of Atatürk University Medical Experiment Application and Research Center numbered E-55885869-900-2100312890.

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**Availability of data and materials**

Data will be provided by the authors upon request.
References


**Figures**
Figure 1

Effects of BRO on serum TC, TG, LDL-C, HDL-C, ALT and AST after the formation of hyperlipidemia. Tukey's multiple range tests were used, * indicates significant differences between other groups studied and control (ns: no significant, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001). Abbreviations used: HC Healthy control; TYX: Tyloxapol (hyperlipidemia) group; BRO: Bromelain.
Figure 2

Effects of BRO on liver and heart tissues MDA, SOD and CAT after TYX-induced hyperlipidemia. Tukey’s multiple range tests were used,* indicates significant differences between other studied groups and control (ns: no significant, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001). Abbreviations used: HC Healthy control; TYX: Tyloxapol (hyperlipidemia) group; BRO: Bromelain
Figure 3

H&E staining of liver tissue. **a:** Liver HC; normal structure of the liver **b:** Liver-TYX1; C: congestion, thick arrow: hemorrhage, N: necrosis; **c:** Liver-TYX2; circle: apoptosis, C: Congestion, arrowhead: mononuclear cell infiltration; **d:** Liver-TYX3; thin arrow: sinusoidal dilatation; **e:** Liver-TYX+BRO; decreased congestion (bar, 100 µm). TYX: Tyloxapol, BRO: Bromelain
Figure 4

H&E staining of heart tissue. 

- **a**: Heart-HC; normal structure of the heart 
- **b**: Heart-TYX1; C: congestion, thick arrow: hemorrhage, arrowhead: mononuclear cell infiltration; 
- **c**: Heart-TYX2; circle: apoptosis, C: Congestion, arrowhead: mononuclear cell infiltration; 
- **d**: Heart-TYX+BRO; decreased congestion (bar, 100 µm). 

TYX: Tyloxapol, BRO: Bromelain.
Figure 5

Relative mRNA expression levels of *Il-1β*, *Il-6*, *Tnf-α*, *Srebp-1c*, *Lxr-α*, *Mmmp-2* and *Mmmp-9* in the liver and heart tissues of the groups used in the study. HC: Healthy control, TYX: Tyloxapol, BRO: Bromelain.
Figure 6

Screening for hyperlipidemia and inflammation target genes. Based on different types of evidence, the interaction networks of the targets examined in the study were visualized using the STRING web resource (http://string-db.org). mRNA expression affected by TYX and BRO aggregated in network analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- floatimage1.png