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Journal of the Institute of Natural & Applied Sciences

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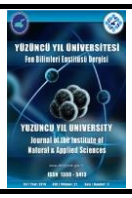

Research Article

Histopathological andAntioxidant Effects of Bromelain on Kidney Tissue of Tyloxapol-Induced Hyperlipidemic Rats

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Article Info

Received: 26.10.2023
Accepted: 19.03.2024
Online August 2024

DOI:10.53433/yyufbed.1381717

Keywords
Bromelain,
Histopathology,
Hyperlipidemia,
Kidney,
Oxidative stress,
Tyloxapol

Abstract: This study was designed to investigate the nephroprotective effect of Bromelain against oxidative stress induced by Tyloxopol-induced hyperlipidemia in rats. Rats (n=6) were divided into three groups. I: Control (C) Group: No treatment was given and only distilled water was given; II: Tyloxapol (TX) Group: Group receiving Tyloxapol (400 mg/kg, i.p); III: Tyloxapol + Bromelain (TX+BR) Group: Group receiving Tyloxapol (400 mg/kg, i.p.) and group receiving Bromelain (250 mg/kg, o.d.) for 18 days. As a result of Tyloxapol administration, oxidative stress parameters SOD and CAT levels decreased, while MDA levels increased. In addition, histopathologic variations were detected in kidney sections. On the other hand, lipid peroxidation decreased and most of the studied parameters improved with combined administration (TX+BR group). According to the results obtained, bromelain may be a potent and alternative agent with protective effect for further studies.

Tyloxapol ile İndüklenmiş Hiperlipidemik Sıçanların Böbrek Dokusu Üzerinde Bromelainin Histopatolojik ve Antioksidan Etkileri

Makale Bilgileri

Geliş: 26.10.2023
Kabul: 19.03.2024
Online Ağustos 2024

DOI:10.53433/yyufbed.1381717

Anahtar Kelimeler
Böbrek,
Bromelain,
Hiperlipidemi,
Histopatoloji,
Oksidatif stres,
Tyloxapol

Öz: Bu çalışma, ratlarda Tyloxopol ile indüklenen hiperlipidemi neticesinde oluşan oksidatif strese karşı Bromelain'in nefroprotektif etkisinin incelenmesi için tasarlandı. Ratlar (n=6) üç grup altında sınıflandırıldı. I: Kontrol (C) Grubu: Herhangi bir uygulama yapılmayan ve sadece distile su verilen grup; II: Tyloxapol (TX) Grup: Tylaxopol (400 mg/kg, i.p) alan grup; III: Tyloxapol + Bromelain (TX+BR) Grup: Tylaxopol (400 mg/kg, i.p) alan ve 18 gün Bromelain (250 mg/kg, o.d.) alan grup. Tyloxapol uygulaması neticesinde oksidatif stres parametrelerinden SOD ve CAT seviyelerinde azalma meydana gelirken MDA seviyelerinde artış görüldü. Buna ek olarak böbrek kesitlerinde histopatolojik varyasyonlar tespit edildi. Bunun aksine çalışmada, kombine uygulama ile (TX+BR grubu) lipid peroksidasyonu azaldı ve çalışılan parametrelerin çoğunda iyileşme görüldü. Elde edilen sonuçlara göre bromelain'in daha sonraki çalışmalar için koruyucu etkiye sahip güçlü ve alternatif bir ajan olabileceği düşünülmektedir.

1. Introduction

Hyperlipidemia due to genetic or environmental causes is defined as serum cholesterol and triglyceride levels above normal. Hyperlipidemia, which includes lipoprotein disorders such as high total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides and low HDL cholesterol, is a risk factor for cardiovascular diseases and a leading cause of morbidity and mortality worldwide (Husain et al., 2022). Coronary arteries and atherosclerosis can be counted as cardiovascular diseases that pose the most important health problems. However, hyperlipidemia not only causes these diseases, but can also cause damage in many organs, including kidney damage (Manzoni et al., 2020). Lipid accumulation in the renal parenchyma is thought to be detrimental to kidney function and therefore hyperlipidemia is known to be one of the most common independent risk factors for chronic kidney disease (Gai et al., 2019).

It is well known that hyperlipidemia is associated with inflammation and oxidative stress, which are generally recognized as the most important risk factors for major cardiovascular diseases such as atherosclerosis (Fidèle et al., 2017). Studies have shown that a diet rich in antioxidants plays an important role in the prevention of cardiovascular disease and cancer and may be preferable to drug therapies for conditions such as hyperlipidemia and hypertension (Nwozo et al., 2017). Stress alters the lipid profile and leads to increased production of free oxygen radicals. A number of studies have reported that stress-induced changes in antioxidant status increase lipid peroxidation, reduce physical activity, and lead to decreased activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) (Chukwuebuka et al., 2021).

Keeping triglyceride and cholesterol levels within limits is crucial for cardiovascular health and having low LDL-C and high HDL-C levels is associated with a reduced risk of atherosclerotic disease (Palabiyik et al., 2023). In order to achieve more effective results in lowering cholesterol levels, drug therapies are applied and statins are one of these therapies. Statins can be produced by microbial or chemical synthesis and are used as inhibitors of cholesterol biosynthesis (Toth & Banach, 2019). While the drugs used are therapeutic, they also have inherent side effects such as hepatotoxicity and nephrotoxicity, which have been demonstrated in various clinical settings. Therefore, researchers have focused on natural compounds with therapeutic properties.

Evidence supporting the effect of increasing fruit and vegetable intake on hyperlipidemia is limited because studies either combined an increase in fruit and vegetable intake with another dietary intervention, such as reducing fat intake, or many studies were not specifically designed to test the effect on hyperlipidemia (Alissa & Ferns, 2017). Ananas comosus (pineapple), which grows in equatorial regions worldwide, has attracted much attention as a phytotherapy and is believed to have anti-cancer, anti-inflammatory and anti-platelet effects, particularly due to its antioxidant potential (Hikisz & Bernasinska-Slomczewska, 2021). Bromelain, an inexpensive by-product waste of pineapple extract, is rich in complex enzymes that play a vital role in various clinical applications and has a highly effective antioxidant effect in the treatment of dichlorvos-induced nephrotoxicity (Colletti et al., 2021; Gürel & Kaya, 2022). Bromelain has extremely low toxicity, is safe for long-term oral administration and is readily absorbed from the intestinal tract without any loss or impairment of activity.

Tyloxapol is a nonionic surface-active oligomer that is a highly valuable substance in the study of triglyceride and cholesterol metabolism and is widely used in in vivo/in vitro biomedical applications. It has many pharmacological effects such as antioxidant, anti-inflammatory and anticancer activities and is used to screen lipid-lowering natural or chemical drugs to study cholesterol and triglyceride metabolism in animal studies (de Sousa et al., 2017).

Our study aimed to evaluate the antioxidant and histopathological effects of bromelain in kidney tissue to alleviate thyroxapol-induced hyperlipidemia in rats.

2. Material and Methods

2.1. Chemicals and materials

Bromelain from pineapple stems (Holland and Barrett) was purchased from the UK and Tyloxapol (Santa Cruz Biotechnology) was purchased from the USA. MDA, SOD and CAT Elisa kits (SunRed Biotechnology) were used for oxidative stress measurement.

2.2. Procurement and preparation of experimental animals

The study was conducted with 18 rats (Rattus norvegicus Domestica, male, 210-300 g) with the approval of Atatürk University Medical Experimental Application and Research Center Ethics Committee dated 15.11.2021 and numbered E-55885869-900-2100312890. Rats were housed in groups of 6 in polycarbonate cages at room temperature (22 ± 2°C), with free access to food and water, on a 12 h light/dark cycle, until the beginning of the experiment. After three groups (I-III) were randomly assigned (n=6), all treatments were performed on rats that were fasted for 12 hours. Anesthesia was administered 24 hours later and kidney tissues were then removed.

2.3. Preparation and application of materials

0.5% (w/v) carboxymethylcellulose was used to prepare an aqueous solution of bromelain and administered to animals by gavage (250 mg/kg per day). A hyperlipidemia model was established by administering tyloxapol (400 mg/kg i.p.) to animals fasted for 12 h after bromelain administration (El-Demerdash et al., 2020; Sulumer et al., 2023). After 24 hours, animals were considered hypercholesterolemic (Sulumer et al., 2023).

2.4. Creation of application group

The 18 Wistar(Rattus norvegicus Domestica) albino rats used in the study were grouped as follows; **I: Control (C) Group:** No treatment was given and only physiologic water [2.5 mL/kg, (i.p)] was given intraperitoneally; **II: Tyloxapol (TX) Group:** Group given Tylaxopol (400 mg/kg, i.p) 30 minutes after physiologic water (2.5 mL/kg, i.p) administration; **III: Tyloxapol + Bromelain (TX+BR) Group**: Group receiving tyloxapol (400 mg/kg, i.p) 24 hours after Bromelain (250 mg/kg, o.d) administration for 18 days.
Rats were considered hyperlipidemic, anesthetized with sevoflurane and sacrificed 24 hours after the end of the treatment. The abdomen of the animals was carefully opened with a vertical incision, kidney tissues were removed and stored at -20 °C and -80 °C until the period of analysis.

2.5. Oxidative stress (MDA, SOD, and CAT) analyze

Kidney tissue was placed in a tube, homogenized at 4000 rpm with the addition of buffer and placed in a homogenizer apparatus (5 x 1 min shaking and 10 sec holding). The lysed tissue was then centrifuged at 13,000 rpm (4°C, 30 min) and the supernatant fraction (homogenate) was removed. For oxidative stress analysis, standards and homogenates prepared as dilute solutions were placed in well plates and reacted according to the procedure documented in the kit. After the reaction, optical density (OD) was measured with a spectrophotometer at a wavelength of 450 nm and the standard curve was calculated.

2.6. Histopathology analysis

Kidney tissues were cut and cassettes were placed in a tissue processing device. The tissues were first kept in formaldehyde for 48 hours, 70%, 80%, 90%, 96%, 100%, 30%, 30 minutes in each alcohol series, 30 minutes in Xylene I and Xylene II separately and 40 minutes in 2 separate paraffin baths and processed in autotechnique (Çakmak, 2020). After this process, tissue embedding (blocking) was performed. The blocks were placed on a microtome to obtain 4 micron thick sections and placed in a water bath at 30-45 °C to be kept on slides. The slides were incubated at 80°C for 30 minutes and then stained with Hemotoxylene-Eosin (H&E) to observe the overall histologic structure. Microscopic examination was performed using a Leica CV5030 Fully Automatic Glass Coverage (Chicago, United States of America). High resolution images of the specimens were taken at ×200 magnification using an Olympus B×60 microscope.

2.7. Statistical analysis

One-way ANOVA test (normal distribution) and GraphPad program (Prism 3.0) were used to compare the groups. All values were expressed as mean ± SEM to emphasize differences between groups. The non-parametric Kruskal-Wallis test was used to analyze the differences between the data obtained using the semi-quantitative method in histopathological examination. Analyses between two groups were performed using the Mann-Whitney U-test. $p < 0.05$ was considered statistically significant

3. Results

3.1. Antioxidant effect of bromelain

In this study, we examined MDA, which is an indicator of lipid peroxidation among the enzymes that contribute to the antioxidant defense mechanism in kidney tissue, as well as SOD, which plays a role in defense against superoxide radicals, and CAT, which detoxifies hydrogen peroxide.

When the MDA data obtained were analyzed, an increase was observed in the kidney tissue in the Tyloxapol treated group. However, this increase is thought to be statistically insignificant. In the group in which Tyloxapol and Bromelain were administered together, Bromelain decreased the observed increase and brought it closer to the data of the control group. However, this decrease is also considered to be statistically insignificant.

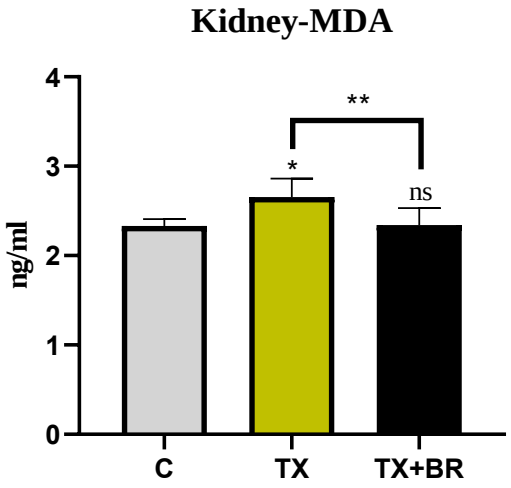

Figure 1. Graphical representation of MDA levels in kidney tissues.
$^{ns}p > 0.05$, $*p < 0.05$ and $**p < 0.01$ vs control group. The data are shown as mean ± SEM (n = 5).

When SOD data, which is one of the enzymatic oxidants, was analyzed, it showed a decrease in the kidney in the tyloxapol treated group compared to the control group and this decrease was found to be statistically significant. In both Tyloxapol and Bromelain treated groups, it was observed that the effect of tyloxapol causing hyperlipidemia was reversed with bromelain and approached the control values. This situation is considered statistically significant.

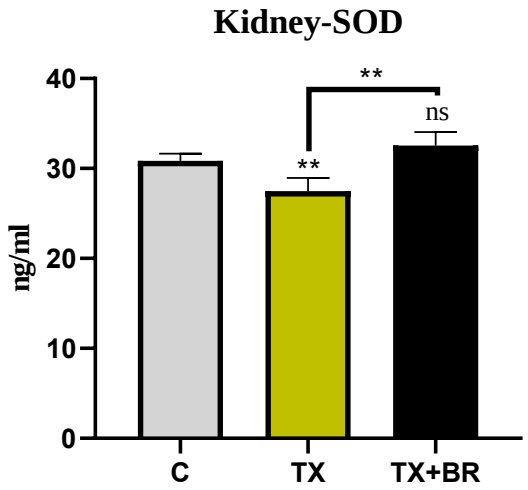

Figure 2. Graphical representation of SOD levels in kidney tissues.
$^{ns}p > 0.05$, $^{*}p < 0.05$ and $^{**}p < 0.01$ vs control group. The data are shown as mean ± SEM (n = 5).

In the analysis of catalase data, the difference between the control group and the group administered Tyloxapol alone was not considered statistically significant, while the difference with Group III was considered significant.

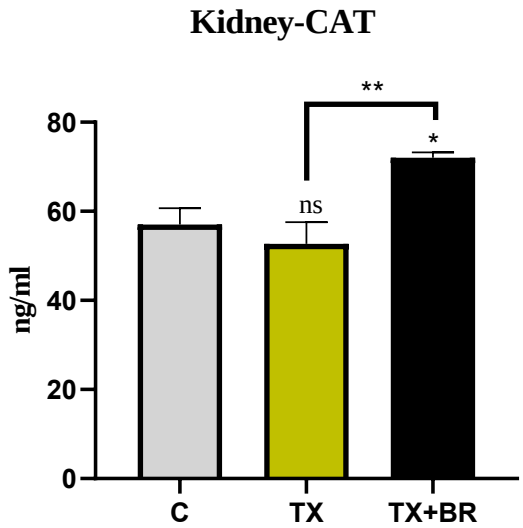

Figure 3. Graphical representation of CAT levels in kidney tissues.
$^{ns}p > 0.05$, $^{*}p < 0.05$ and $^{**}p < 0.01$ vs control group. The data are shown as mean ± SEM (n = 5).

3.2. Effect of bromelain on histopathology

The kidney tissues used in our study were sectioned and examined for histopathology. In the histologic evaluation of the renal cortex of Group C, the microscopy images obtained after H&E staining showed that the renal tubules, glomeruli and renal cavity (Bowman's capsule) had normal structures and no pathologic findings were found (Figure 4, Table 1).

Table 1. Histopathologic findings and scores in kidney tissue

Groups	**C**	**TX**	**TX+BR**
Congestion	− − −	+++	+
Hemorrhage	− − −	++	+
Apoptosis	− − −	+	− − −
Necrosis	− − −	+	− − −
Infiltration	− − −	+++	− − −
Dilatation	− − −	++	− − −

*According to histopathologic results: none (− − −), mild (+), moderate (++), and severe (+++)

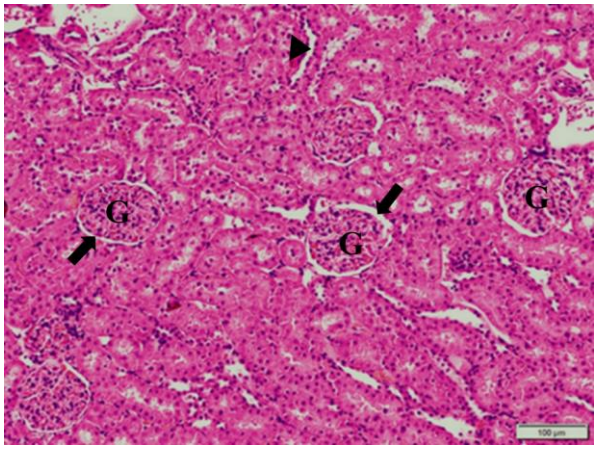

Figure 4. Kidney control (C) group histology images. **G:** glomeruli; **thick arrow:** Bowman's capsule; **arrowhead:** indicates normal histological structures of proximal convoluted tubules (H&E, Bar: 100 μm.).

Significant histopathologic findings including tubular dilatation, congestion, hemorrhage, mononuclear cell infiltration, cell apoptosis and necrosis were observed in the evaluation of TX group (Figure 5).

When bromelain was administered to the rats (TX group) for protection before hyperlipidemia occurred, it was observed that pathologic findings in the kidney tissues decreased due to the effect of bromelain. When TX group was compared with TX+BR group, it was shown in Figure 6 that hemorrhage decreased, congestion, mononuclear cell infiltration, tubular dilatation, cell apoptosis and necrosis were not observed.

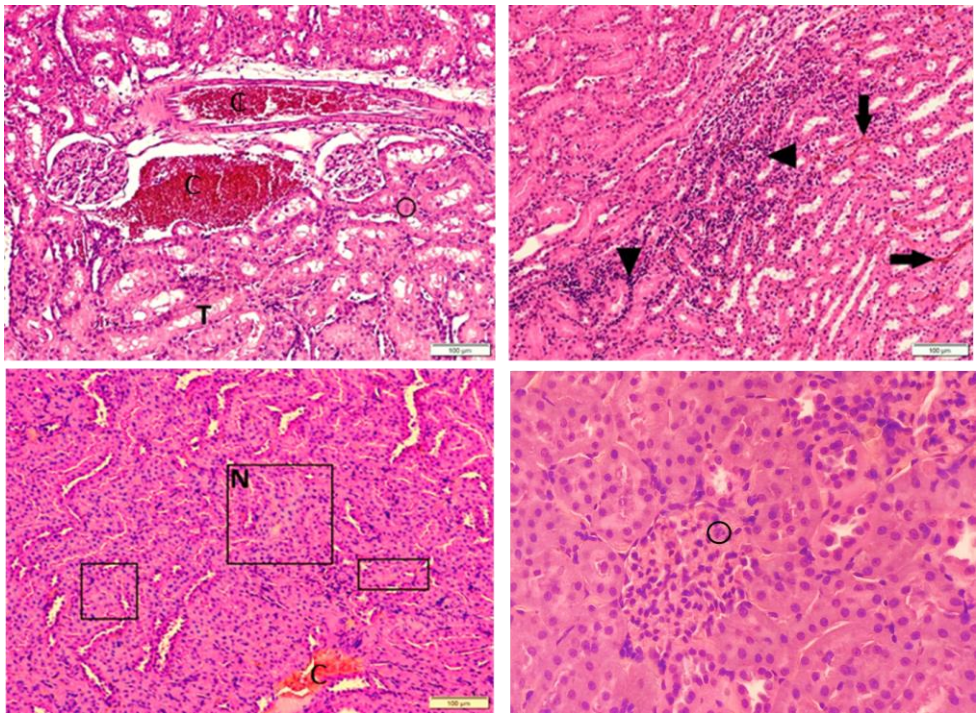

Figure 5. Histological effects of Tyloxapol on kidney tissue in the hypercholesterol group (TX) **circle:** apoptosis, **T:** tubular dilatation; **arrowhead:** mononuclear cell infiltration, **thick arrow:** bleeding (hemorrhage); **N:** necrosis, **C:** congestion (blood supply).

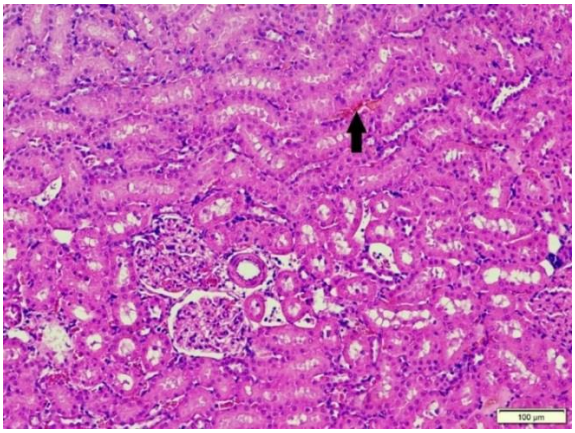

Figure 6. Kidney TX+BR group histology images (H&E, Bar: 100 µm). **Thick arrow:** Hemorrhage.

4. Discussion

Correction of elevated plasma total cholesterol, triglyceride and LDL levels, as well as increased HDL lipid ratios, is paramount in the treatment of hyperlipidemia. Lipoprotein and lipid abnormalities are recognized as risk factors for cardiovascular diseases, which have increased significantly globally in recent years (Iyer & Patil, 2019). Tyloxapol is a non-ionic surfactant that induces hyperlipidemia by inhibiting peripheral lipoprotein lipase responsible for clearance of lipid particles from the body and increasing cholesterol synthesis in the liver and lipid absorption in the intestine (Dudhipala et al., 2021). In our study, an acute hyperlipidemia model was established in animals 24 hours after tyloxapol induction and our model was supported by the study of Parwin and colleagues (Parwin et al., 2019). The accumulation of lipids and other substances in the arterial walls can block the vessel lumen and affect heart tissue, while restricting blood flow to the kidneys and other vital organs, leading to coronary heart disease or kidney failure (Akinmoladun et al., 2021).

In hyperlipidemia, increased production of reactive oxygen species (ROS) by endothelial cells challenges the cell's antioxidant defenses, and increased ROS production from accumulated fat can lead to increased oxidative stress in the blood, which can severely affect other organs (Feng et al., 2022). MDA is an indicator of lipid peroxidation, which triggers protein oxidation and disrupts cell membrane structure and function (Gaschler & Stockwell, 2017). Superoxide dismutase (SOD), a bioactive substance known to have antioxidant properties (oxygen radical scavenger), causes a decrease in antioxidant-SOD levels in liver and kidney tissues of rats under hypercholesterolemic conditions. Under conditions of increased oxidative stress, catalase may represent the most effective antioxidant defense in the body and, like SOD, is an essential enzyme to prevent ROS production (Baldissera et al., 2017). Therefore, in our study, the levels of MDA, SOD and CAT, which are antioxidant parameters in kidney tissue, were investigated in tyloxapol, which causes hyperlipidemia, and bromelain, which is given for preventive purposes. When the results were analyzed, an increase in MDA level was observed as the first parameter in the hyperlipidemic group (TX), while this increase was close to the control level in the combined group (TX+BR). For the antioxidant defense enzyme SOD, a decrease was observed in the TX group compared to the control group, but Bromelain was able to reverse this decrease by providing strong protection before hyperlipidemia. Although catalase, one of the last antioxidant parameters we studied, decreased in the hyperlipidemic group, bromelain increased CAT activity in the combined group and showed that it has a strong anti-oxidation activity. In a study in rats, bromelain was shown to significantly eliminate oxidative stress during isoproterenol-induced myocardial infarction (Allawadhi et al., 2018). Kalaiselvi et al. (2013) reported that bromelain can quench and detoxify free radicals and positively modulate antioxidant activity in rats with 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast cancer (Kalaiselvi et al., 2013).

In another stage of the study, sections of kidney tissue were taken for pathological examination to observe the effects of hyperlipidemia on kidney tissue. In previous studies, glomerular atrophy, hemorrhage and abundant necrosis were detected in kidney tissues after hyperlipidemia (Liu et al., 2021). In another study, foot process fusion, mild damage to glomeruli, and accumulation of lipid droplets in the renal tubule interstitium were observed in the kidney tissues of rats fed a high-fat diet (Li et al., 2022). Singh et al. (2024) reported that tyloxapol-induced mice exhibited many damages, especially inflammation in kidney, liver, pancreas and arterial tissues. In another study on rats, it was reported that hyperlipidemia caused glomerular atrophy and increased Bowman's capsule size in kidney tissue (Gara et al., 2017). In addition, triton-induced inflammation, glomerular fibrosis and tissue necrosis were observed in the kidney tissues of rats (Karale et al., 2022). In a study with bromelain, it was observed that paracetamol caused glomerular atrophy, tubular dilatation, inflammatory cell infiltration and necrotic changes in renal tissue, but treatment with bromelain significantly reduced these damages (Akaras et al., 2023). El-Demerdash et al. (2020) proved that aluminum causes atrophic and fragmented glomeruli in renal tissue and bromelain treatment treats these findings and shows normal glomerular structure. In our study, pathological signs of mononuclear cell infiltration, tubular dilatation, hemorrhagic cell apoptosis, congestion and necrosis were found in the TX group. These findings are significantly different from the control group and demonstrate the success of our model. When the histopathological changes of the kidney tissue were examined, our combined group (TX+BR) showed a significant decrease in the pathological findings observed in the TX group and had a histoprotective effect.

5. Conclusion

In conclusion, our study addressed the effects of Tyloxopol-induced hyperlipidemia. Accordingly, Tyloxopol induced dysfunction in renal tissue, leading to disruption of oxidative-antioxidative balance and tissue damage. The bromelain used in this study is known as a potent agent with many broad spectrum health benefits. In this context, bromelain, which is used as an untested agent, is seen as a promising protective and alternative with the potential to reduce the risk of death and disease as a result of free radical reactions in kidney tissue.

Acknowledgements

We would like to thank the Atatürk University Faculty of Science Molecular Biology/Genetics Departments for their support of our experimental study.

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