Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Protective Effect of *Lycium barbarum* on Renal Injury Induced by Acute Pancreatitis in Rats

Tugba Dogan, Kadir Batcioglu, Elif Kayhan Kustepe[1,2], Ayse Burcin Uyumlu, Yakup Yilmaztekin

Department of Biochemistry, Faculty of Pharmacy, İnonu University, [1]Department of Histology and Embryology, Faculty of Medicine, İnonu University, [2]Department of Basic Medical Sciences, Faculty of Medicine, İnonu University, Malatya, Turkey

Submitted: 05-Nov-2021 Revised: 25-Nov-2021 Accepted: 23-Dec-2021 Published: 28-Mar-2022

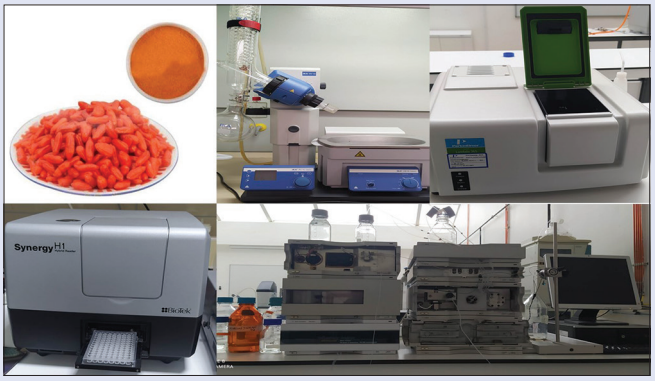
ABSTRACT

Background: *Lycium barbarum* (LB) is a plant species that is well known in Chinese traditional medicine and is also considered a nutrient, belonging to the Solanaceae family, also called goji berry or wolfberry. **Objectives:** The aim of this study was to investigate the protective efficacy of LB, in kidney damage caused by acute pancreatitis (AP). **Materials and Methods:** In the study, we used 36 female Wistar albino rats (12 in each group) which were divided into three groups: Control, cerulein (100 μg/kg b. wt. intraperitonally) and Cerulein + LB (6 mg/ml/day gastric gavage) group. Serum lipase, Interleukin (IL)-1, and IL-6 levels were measured. Superoxide dismutase, catalase (CAT), glutathione peroxidase enzyme activity assays and 8-hydroxy deoxyguanosine, malondialdehyde (MDA), and protein levels were measured in kidney tissue samples. In addition, histopathological analysis was performed in kidney tissue samples. **Results:** According to the findings, in the AP model created with Cerulein, administration of LB plant extract decreased oxidative stress and damage caused by AP in the kidney tissue and partially suppressed the inflammatory reactions in the tissue. **Conclusion:** According to the findings, in the AP model created with Cerulein, administration of LB extract decreased oxidative stress and in kidney damage caused by AP.

Key words: Acute pancreatitis, antioxidant enzymes, kidney Injury, *Lycium barbarum*, oxidative stress

SUMMARY

- In the cerulein-induced acute pancreatitis model, administration of *Lycium barbarum* plant extract showed a partial suppressive effect on inflammatory reactions in the tissue by reducing the oxidative stress and damage caused by acute pancreatitis.



Abbreviations used: LB: *Lycium barbarum*, AP: Acute pancreatitis, IL: Interleukin, TNF α: Tumor necrosis factor alpha, MDA: Malondialdehyde, CAT: Catalase, GSHPx: Glutathione peroxidase, SOD: Superoxide Dismutase, 8-OHdG: 8-hydroxydeoxyguanosine, ROS: Reactive oxygen species.

Correspondence:

Prof. Kadir Batcioglu,
Department of Biochemistry, Faculty of Pharmacy,
İnonu University, Battalgazi, Malatya, Turkey.
E-mail: kadir.batcioglu@inonu.edu.tr
DOI: 10.4103/pm.pm_516_21

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Acute pancreatitis (AP) occurs as a result of the development of a widespread inflammation as a result of digestive enzymes, which are normally inactive in the pancreas, digest pancreatic tissues as an early activity with an etiological factor whose cause is not yet known.[1] Due to severe inflammation in the pancreas, adjacent and distant organs of the pancreas also occurs damage in different intensities. The kidney, liver, spleen, and lungs are firstly among these organs.[2] Although the etiological factors that cause AP show a lot of variety, the clinical picture that occurs whatever happens of the etiological factor is characterized by the self-digestion of the tissue (autodigestion) with the activation of enzymes in the pancreas, and the inflammation, edema, hemorrhage and necrosis that develop accordingly.[3]

Cytokines such as interleukin (IL)-1 and IL-6 are released from activated cells. AP is transformed from a local inflammatory process to a disease showing systemic effects through these inflammation mediators.[4] Pancreatic necrosis, multiple organ failure, and mortality are seen commonly in this disease, which can cause cardiovascular, pulmonary, and renal failure in the early period and septic problems in the late period.[5-7]

Renal dysfunction is among the serious complications of AP. The picture, which is seen in 80% of the patients and named "Nephropathia Pancreatica" by Otto, is characterized by proteinuria and hematuria. It has been reported that 23% of patients who develop acute renal failure depend on AP, and the mortality rate in these patients is about 80%. Acute tubular necrosis has been associated with the severity of pancreatic inflammation. If the hemodynamic changes occurring in AP are not corrected with appropriate treatment in a short time, renal dysfunctions develop. The development of septic complications also plays a role in the etiology of renal dysfunction.[8]

Cite this article as: Dogan T, Batcioglu K, Kustepe EK, Uyumlu AB, Yilmaztekin Y. Protective effect of *Lycium barbarum* on renal injury induced by acute pancreatitis in rats. Phcog Mag 2022;18:152-9.

In many studies in the literature, it has been reported that the degenerative effects of cytokines such as IL-1, IL-6, and tumor necrosis factor-alpha (TNF-α), whose concentrations increase in the systemic circulation as a result of inflammation developing in the etiology of renal damage. In addition, early activated pancreatic-derived proteases and lipases and oxidative damage caused by free oxygen radicals play a role in this process. Due to these interrelated factors, the process becomes extremely complicated.[9-11]

Oxidative stress plays a critical role in the initial and progression stages of AP, as in many diseases. Many experimental models have been put forward to support the accuracy of this approach.[12-15] Free oxygen radicals (reactive oxygen species [ROS]) and cytokines are take place in the center of the molecular mechanism in the development of progressive hemorrhagic necrosis that occurs in the early stages of AP.[16-18] There is a dynamic balance between ROS, which is continuously produced in the organism under physiological conditions, and antioxidant defense systems, and therefore harmful effects do not occur. However, in AP, reactive oxygen radicals, whose concentrations increase with severe inflammation that develops suddenly and rapidly, disrupting the balance in favor of oxidants, create severe oxidative stress.[15,19,20]

Lycium barbarum (LB) is a plant species that is well known in Chinese traditional medicine and is also considered a nutrient, belonging to the *Solanaceae* family, also called goji berry or wolfberry. This plant is up to 3 meters tall, its leaves are gray-green, its petiole is very short and there are 1–3 flowers on each branch. Fruits are 3–8 mm in diameter, 6–20 mm in length, and have a dark red color. Its role in preventing various diseases has been demonstrated many times in experimental, clinical, and epidemiological studies.[21,22] The important bioactive ingredients of LB are complex glycoconjugates with glycoprotein structure. It contains a peptide structure consisting of 6 monosaccharides (galactose, glucose, rhamnose, arabinose, mannose, and xylose) and 18 amino acids. Oligosaccharides, which are one of the main components in its structure, show efficiency in cell–cell interaction and adhesion, cell migration, blood coagulation, immune response, and wound healing, and these activities are some of their many known functions.[23-25] LB contains many bioactive vitamins (Vitamin A, B_1, B_2, C), carotenoids (zeaxanthin, lutein, lycopene, cryptoxanthin), cerebroside, β-sitosterol, proline, scoleptin, and betaine, which are known to have strong antioxidant effects.

It has been reported that LB shows anti-inflammatory, antineoplastic, antipyretic, antidiabetic, antisenyl, immunomodulatory, and antiaging effects due to this rich content.[26-30]

Although it is known that barbarum suppresses inflammation and increases antioxidant capacity in AP,[31-34] no study investigating its effectiveness against nonpancreatic organ damage has been found. In this study, we investigated whether LB extract has a protective effect against renal injury in rats with experimentally induced AP by cerulein.

MATERIALS AND METHODS

Reagents and chemicals

Sodium Carbonate (≥99.9%), Nitro Blue tetrazolium Chloride (≥90.0%), 2-(2-Thiobarbituric acid) (≥98%), Acetic Acid (≥99%), Sodium azide (≥99.5%), n-Butanol (99.8%), and Sodium Carbonate (≥99.9%) were acquired from Merck Chemicals, USA. Sodium Potassium Tartrate (99%), Sodium Hydroxide (98%–100.5%), Copper Sulphate pentahyrate (98.0%), Folin-Ciocalteu-Phenol Reagent, Xanthine (≥99%), Ethylenediaminetetraacetic acid (99%), Bovine serum albumin (BSA) (≥98%), Hydrogen peroxide (30%), Sodium Dodecyl Sulfate (≥99.0%), NADPH-(=97%), Tris hydrochloride (>99%), Ethanol (≥99.8%), Proteinase K (≥90%), RNase T1, RNase A (≥95%), Nuclease P1, Alkaline phosphatase, Acetonitrile (99.8%), Sodium Acetate (≥99%), Glutathione (>98%), and Cerulein (≥95%) were acquired from Sigma-Aldrich Chemicals, USA.

Rat IL-1 β (BMS630TEN) and Rat IL-6 (BMS625TEN) Elisa Kits were obtained from eBioscience ELISA Kits, Thermo Fisher Scientific, USA. Quanti chrome lipase kit was obtained from BioAssay Systems, USA.

Design of study

This study was carried out in Inonu University Turgut Ozal Medical Center, in the Experimental Research Unit, by the rules stipulated by the Inonu University animal ethics committee (Research protocol number: 2011/A-16). 36 female Wistar albino rats, 3–4-month old, with an average weight of 250–300 g were used. During the study, all animals were kept in polypropylene cages (4 animals were kept at each cage) and fed *ad libitum* standard feed and tap water. 12 h light/12 h dark period was applied and the room temperature was hold of 22°C–24°C.

A total of 36 female rats were divided into 3 groups randomly selected with 12 animals in each group.

- Group 1: The control group was made only saline physiological injection was used as a solvent during the study and fed with *ad libitum* standard feed and water for 10 days
- Group 2: Induction of AP was provided with 50 μg/kg b. wt cerulein applied twice intraperitoneally with a total dose of 100 μg/kg b. wt with 2 h intervals, after giving *ad libitum* standard feed and water. Saline was used as a solvent[35]
- Group 3: 10 days before cerulein injection LB extract was given by i.g., gavage method at a dose of 6 mg/ml/day for 10 days. In this time, it continued to be fed with standard feed and water.[36]

Extraction processes of *Lycium barbarum* samples

Sun-dried goji berry berries (LB) harvested in Manisa-Turkey and sold in the local market were purchased. Sun-dried LB fruits were pounded in a mortar and pulverized. After taking 10 g of the powdered material and adding 200 mL of methanol-water (v/v, 80:20%) at 60°C, it was mixed with a magnetic stirrer for 1 h. At the end of the process, the mixture was centrifuged at 5000 rpm for 25 min. After the upper liquid part was taken into another tube, 20 mL (80°C) of pure water was added to the tube with the residue and it was centrifuged again at 5000 rpm for 25 min. This process was carried out in 3 repetitions. The extracts collected at the end of each centrifugation were combined. These extracts were evaporated in the evaporator until 25 mL remain. Methanol: water (v/v, 80:20%) was added to this extract until it was completed to 100 mL. Then, the obtained extract was vortexed and kept at 4°C for 10 h, all solvent in the extract was evaporated in the evaporator. The obtained *L. barbarum* extract powder was dissolved in distilled water to a final concentration of 6 mg/ml and used completely. The yield of LB extract is 5%. The all steps for this extraction procedure are shown in Figure 1.[37] Dosage adjustment was modified according to the study of Zhao *et al*. to be administered at 6 mg/kg/day for 10 days.[36]

Biochemical analysis

At the end of 10-day feeding period, cerulein injections were made and after 24 h rats were sacrificed by cervical dislocation. The rats whose ribcages were opened were blood withdrawn by entering the heart with an injector and kept in closed tubes for 10 min at room temperature and centrifuged at 3500 rpm for 5 min to obtain serum. The separated serum was stored at −40°C for measurement of lipase, IL-1, and IL-6. Both kidneys were taken after sacrification. The right kidney was saved

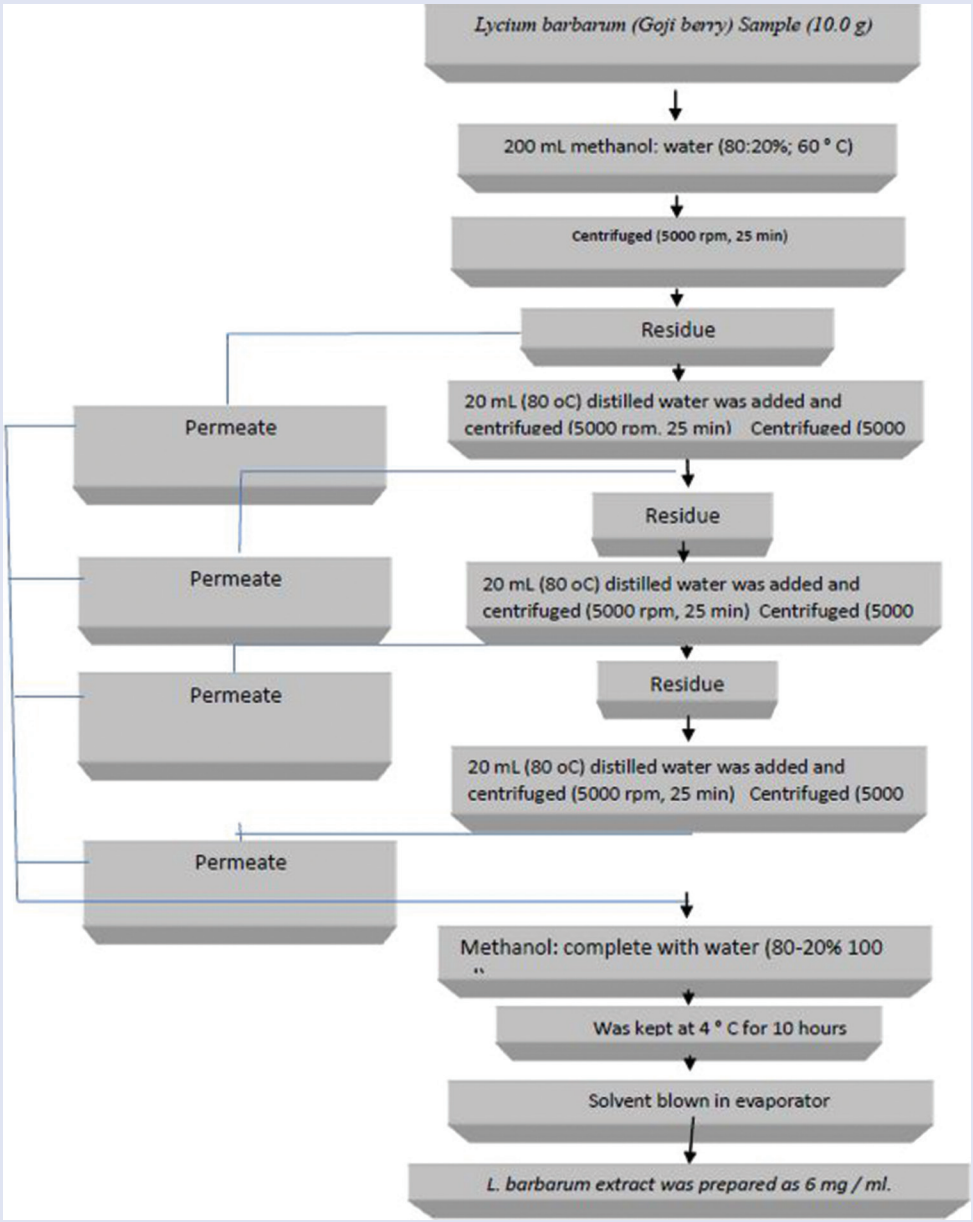


Figure 1: Extraction process steps for *Lycium barbarum* (Goji berry) samples

in 10% formaldehyde solution for histopathological examination. The left kidney was used for biochemical measurements.

Preparation of kidney tissues for biochemical analysis

Kidney tissues, which were delivered to the laboratory by cold chain, were transferred to glass tubes and cold 50 mM phosphate buffer (6.059 g of K_2HPO_4, 2.07 g of KH_2PO_4 were taken and dissolved in 800 ml of distilled water and the pH was adjusted to 1000 ml) (pH 7.4) was added by creating a 1:10 w/v ratio. It was then homogenized for 5 min at 6000 rpm in the ultra-Turrax T25 (IKA Werke GmbH, Staufen, Germany) homogenizer. The obtained homogenates were sonicated 3 times for 10 s at 20 s intervals using VWR Bronson scientific sonicator (VWR Int. Ltd. Merck House Pool, UK). The supernatant was obtained by centrifuging homogenates at 4°C at 13,500 g for 15 min (Centrifuge 5415R, Eppendorf AG, Hamburg, Germany). The supernatants were stored at −80°C for protein quantity determination, Superoxide dismutase (SOD), CAT, GPx activity determination (SANYO Biomedical Co., Co., MDF-U537, Osaka, Japan). 500 µl of homogenate was used for the measurement of malondialdehyde (MDA) levels.

Measurment of biochemical parameters

Protein levels of samples were measured according to Lowry method and BSA was used as protein standard.[38] MDA levels were measured using thiobarbituric acid reactive substances assay method developed by Mihara and Uchiyama, and the results were expressed in nmol/mg protein.[39]

SOD activity was determined according to the method of McCord and Fridovich. This method is based on the inhibition by SOD of the reduction of cytochrome C through superoxide radicals produced in the xanthine-xanthine oxidase system.[40] Enzyme activities were calculated

assuming that 50% inhibition of the reduction of cyt c is equivalent to 1 U of SOD enzyme activity and the results were given as U/mg protein. Pure SOD enzyme was used to draw the standard graph.

Determination of Catalase enzyme activity was made according to the method of Luck H.[41] The decomposition of H_2O_2 to water and molecular oxygen by the catalytic activity of CAT was monitored spectrophotometrically at 240 nm. Specific activity was expressed in terms of U/mg protein.

GPx activity measurement was made according to the method of Lawrence and Burk.[42] The activity was assayed by following the oxidation of NADPH at 340 nm for 3 min, 25°C, in the presence of Glutathione reductase (GR) and GSH. One unit of activity was defined as oxidation of 1 μmol NADPH per minute. The final GPx activity levels were expressed as U/mg protein.

8-hydroxy deoxyguanosine quantification

DNA isolation

One gram of kidney tissue taken into the test tube was homogenized with a solution containing 10 mL of 1% SDS and 1 mM EDTA in an Ultra-Turrax T25 (IKA Werke GmbH, Staufen, Germany) homogenizer at 6000 rpm for 5 min. Homogenates were incubated with Proteinase K for 30 min at 37°C. After adding 0.5 mL pH 7.4 1 M Tris-HCl, it was extracted with 1 volume of Chloroform: Isoamyl alcohol (24:1, v: v). After the phases were separated by centrifuge, the aqueous phases were collected. DNA was precipitated with 0.1 volume of absolute ethanol kept at −20C. After centrifugation, DNA washed with 70% ethanol was solubilized with 2 ml 1.5 mM NaCl, 150 μM Na-Citrate, and 1 mM EDTA solution. The solution was incubated at 37°C for 30 min with RNase T1 and RNase A. DNA solution was extracted again with chloroform: isoamyl alcohol solution and precipitated with 5 m NaCl.[43]

After dissolving precipitated DNA pellets with 0.5 mL 20 mM CH_3COONa, 30 min at 37°C. 60 min at 37°C by incubating with 63 mg nuclease P1 and adding 50 μL 1 m Tris-HCl. 6.3 U was incubated by alkaline phosphatase.[44]

High-performance liquid chromatography analysis

High-performance liquid chromatography (HPLC) analyzes were made with the manual injection Agilent 1100 (Agilent Technologies, Inc., Headquarters, Santa Clara, United States) device. Parts of 20 μL of the isolated DNA samples were loaded into the C18 HPLC column (ACE, Aberdeen, Scotland) with an internal diameter of 4.6 mm with a particle size of 3 μm. The analysis was done isocratically with 0.1% (v: v) acetic acid mobile phase containing 3% (v: v) acetonitrile at a flow rate of 2 ml/min and was carried out at UV 297 nm.[45] It was graphed with the concentration corresponding to the peak areas obtained as a result of analysis of solutions of different concentrations prepared by the 8-hydroxy deoxyguanosine (8-OHdG) standard under the same chromatographic conditions. Using the correct equation of the generated graph, 8-OHdG content of the samples was determined and expressed as nmol/mg DNA.

Measurement of pancreatic lipase level in serum

Pancreatic lipase measurement was performed by the procedure specified in the supplied pancreatic lipase Bioassay Systems' Quanti chrome lipase kit.

Cytokine determination in serum

At the end of the study, IL-1 β and IL-6 levels of the samples taken from all groups were measured using the ELISA method (eBioscience Rat-IL-1 β Platinum ELISA Kit) and the results were calculated in pg/ml with the help of the standard graph.

Histopathological examination of kidney tissue

Kidney tissue samples taken for histological examination were detected for 48 h with 10% formaldehyde. After fixation, kidney tissue samples were embedded in paraffin blocks after a routine histological tissue follow-up procedure. Cross-sections 6 μm thick were prepared with the help of microtomes from paraffin blocks. After sections taken on slides were stained with H and E (H and E), and photographs were taken by examining with Nikon Optichot-2 light microscope and Nikon DS-L3 Camera control-Image analysis system (Nikon Corporation, Tokyo, Japan).[46]

Statistical analyze

One-sample Kolmogorov–Smirnov test was applied first to test whether the data conformed to the normal distribution, and it was observed that the data conformed to the normal distribution. Analysis of variance test was used to compare between groups of data showing compliance with normal distribution and the LSD *post hoc* test was performed to analyze which parameters belonged to the observed differences between the groups. In all these tests, the statistical significance level was considered to be $P < 0.05$. All statistical analyses were performed using SPSS (SPSS 15.0 Inc., Chicago, IL, USA).

RESULTS

Biochemical findings

The results of all parameters obtained in this study are summarized in below in graphs [Figure 2].

According to the results of antioxidant parameters in our study; SOD and GPx enzyme activity showed a statistically significant decrease in the AP group compared to the control group. On the other hand, LB application showed a significant increase compared to the AP group ($P < 0.05$). Although there was a certain increase in CAT activity in the AP group compared to the control group, the difference between the groups was not statistically significant but showed a certain increase ($P > 0.05$). Similarly, although lower CAT levels were determined in the LB group compared to AP group, the difference was not statistically significant ($P > 0.05$).

Although there was a certain increase in MDA level in the AP group compared to the control, the difference between the groups was not statistically significant ($P > 0.05$). The LB group decreased in a statistically significant way the MDA level, which was high in both the control and AP groups ($P < 0.05$). The amount of 8-OHdG did not cause a significant change in the AP group compared to the control. LB group decreased in a statistically significant way the amount of 8-OHdG in both control and AP groups ($P < 0.05$).

The lipase level we obtained in the study showed a statistically significant increase in the AP group compared to the control group ($P < 0.05$). In addition, the lipase level in the LB group showed a statistically significant increase compared to both the control and AP groups ($P < 0.05$).

According to our study findings, IL-1 level showed a significant increase in the AP group compared to the control ($P < 0.05$). However, the LB group significantly decreased the IL-1 level, which increased depending on the AP group ($P < 0.05$). There was no statistically significant difference between the 1st and 3rd groups ($P > 0.05$).

According to our study findings, although a certain increase in IL-6 level was observed in the AP group compared to the control group, the difference between the groups was not statistically significant. LB group decreased in a statistically significantly IL-6 level, which was high in both control and AP groups ($P < 0.05$).

If we present all biochemical parameters in a single table collectively;

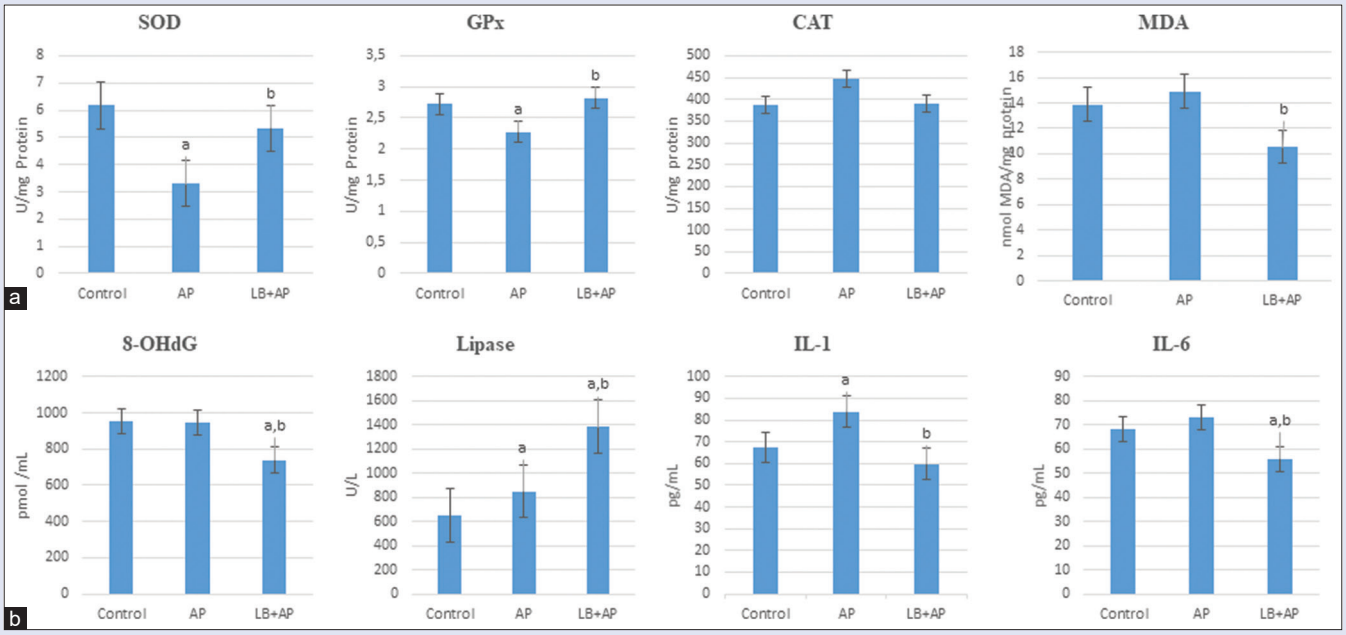


Figure 2: Control group, acute pancreatitis and *Lycium barbarum* + Acute pancreatitis experimental groups (a) $P < 0.05$ compared with the control group (b) $P < 0.05$ compared to Acute pancreatitis group

Histopathological examination

The cortical [Figure 3a and b] and medullary [Figure 3c] structures in the kidney tissue sections in the control group were evaluated as normal histological structure.

In the cross-sections of the kidneys in the AP group, collapse in the glomeruli commonly, dilatation in the Bowman gap, increase in the density of eosinophilic cytoplasm in distal and proximal tubular epithelial cells, and heterochromatic and pycnotic nucleus structure were noted [Figures 3c and 4a].

Place-to-place hemorrhage was detected in the renal cortex [Figure 4b] and medulla [Figure 4c]. Distal and proximal tubular degeneration around the hemorrhagic areas in the cortex [Figure 4b] and degeneration of the medullary tubular structures in the regions close to the hemorrhagic areas in the medulla [Figure 4c] were detected.

When the sections in the treatment group were examined, it was observed that there was a significant decrease in the histological findings of kidney damage observed in the AP group. Glomeruli were evaluated in normal appearance. Glomeruli were evaluated in normal appearance. Hemorrhage was not detected in kidney sections in this group. However, there was minimal eosinophilic cytoplasm concentration in proximal and distal tubular epithelial cells [Figures 4d and 5a] and damage to in place to place tubular epithelial cells [Figure 5b].

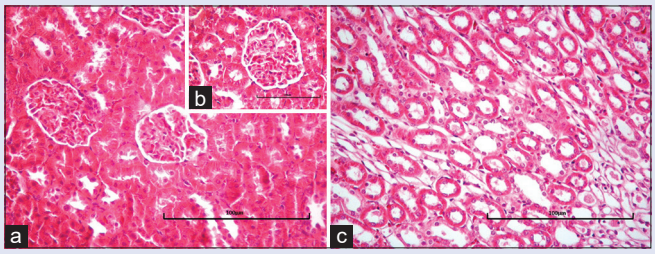


Figure 3: Histological analysis of the control group. (a and b) Cortex had a normal histological structure, H and E, ×20 and × 40. Scale bars = 100 μm (a, b). (c) Medulla had a normal histological structure, H and E, ×20. Scale bar = 100 μm

DISCUSSION

AP is an acute inflammatory disease of the pancreas that can affect surrounding tissues and distant organs at varying levels.[47] Acute kidney injury (AKI) occurs in 70% of patients with severe AP, and this complication significantly increases the risk of AP-related mortality.[48] In this study, we investigated whether there is a protective effect of LB plant extract known to have anti-inflammatory and antioxidant-effective ingredients in preventing developing kidney tissue damage linked to AP, which is experimentally created with cerulein in the rats.

With the onset of AP, increases of serum lipase and amylase enzymes released from acinar cells are biomarkers used in biochemical measurements. In a study conducted in humans by Zhou *et al.*, an increase in serum lipase level was observed in patients with severe AP due to the increase in the severity of the disease.[49] In our study, serum lipase level increased significantly ($P < 0.05$) in the experimental AP group induced with cerulein, but the application of LB extract could not prevent this increase in serum lipase level. This result does not coincide with the study conducted by Xiong *et al.*, which found that in the AP model with cerulein in mice, the administration of LB polysaccharides significantly reduced the level of lipase increased as a result of the formation of AP.[34]

It has been reported in several studies that LB has anti-inflammatory activity alongside its antioxidant effect.[50,51] LB extract has been observed to show both anti-inflammatory and immune-stimulating effects. However, the anticancerogenic effect of LBP is also thought to occur with the immune-stimulatory effect.[52] In our study, the induction of AP with cerulein was observed to cause an increase in levels of inflammation mediators such as IL-6, IL-1 resulting in corresponding

Table 1: İn the experimental groups, kidney tissue, superoxide dismutase, catalase, glutathione peroxidase activities and quantity levels of malondialdehyde, lipase, ınterleukin-6, ınterleukin-1 and 8-hydroxy deoxyguanosine

Parameter	Grup 1 control (*n*=12)	Grup 2 AP (*n*=12)	Grup 3 LB + AP (*n*=12)
SOD (U/mg protein)	6.1820±1.05271	3.3039±0.77575[a]	5.3379±0.76156[a,b]
GPx (U/mg protein)	2.7230±0.42872	2.2766±0.42419a	2.8187±0.36986[b]
CAT (U/mg protein)	387.31±93.64	446.96±111.25	390.81±103.13
MDA (nmol MDA/mg protein)	13.8835±2.82542	14.9041±2.66453	10.5363±1.90565[a, b]
8-OHdG (pmol/mL)	954.1033±324.71434	947.8408±230.46918	738.8942±184.57902[a, b]
Lipase (U/L)	647.9558±139.95630	848.9525±101.05653[a]	1384.2558±264.70683[a,b]
IL-1 (pg/mL)	67.3333±13.59367	83.8333±16.35867[a]	59.6667±17.94098[b]
IL-6 (pg/mL)	68.4508±15.82049	73.0925±10.56462	55.9850±9.09854[a,b]

[a]*P*<0.05 compared with control group, [b]*P*<0.05 compared with AP group, Values are given as mean±SD. SD: Standard deviation; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde; GPx: Glutathione peroxidase; 8-OhdG: 8-hydroxy deoxyguanosine; IL: Interleukin; LB: *Lycium barbarum*; AP: Acute pancreatitis

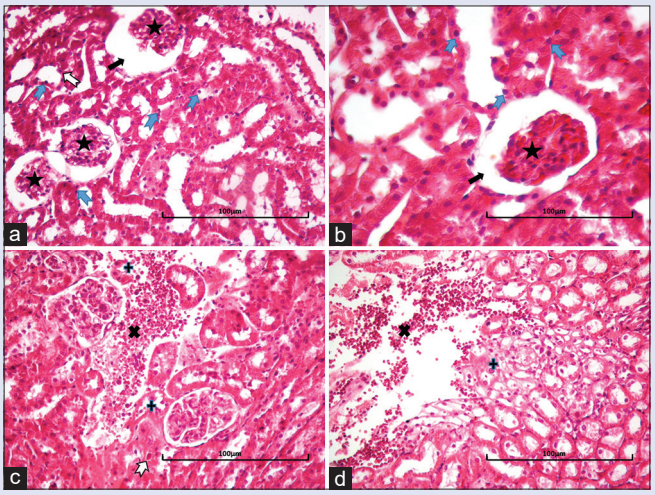


Figure 4: Histological analysis of the acute pancreatitis group. Glomerular collapse (aster), dilatation of Bowman's spaces (black arrow), increased eosinophilic cytoplasm density in the distal and proximal tubule epithelium (white arrow), heterochromatic and pycnotic nuclei (blue arrow) were observed. Hemorrhage areas (x) were detected in the renal cortex (distal and proximal tubule degeneration around the hemorrhagic area [+]) and medulla (degeneration of the medullary tubules around the hemorrhagic area [+]), H and E, Scale bars = 100 µm. (a, c and d) ×20. (b) ×40

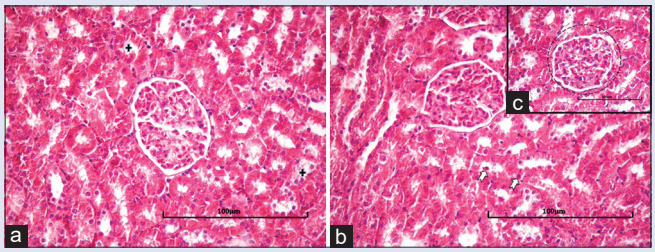


Figure 5: Histological analysis of the *Lycium barbarum* + acute pancreatitis group, H and E, Scale bars = 100 µm. (a) Glomerular had a normal histological structure and there was local damages in tubule epithelial cells (+), ×20. (b) No hemorrhage detected in the glomeruli and minimal eosinophilic cytoplasm condensation was observed in proximal and distal tubular epithelial cells (white arrow), ×20. (c) Glomerular had a normal histological structure (circle), ×40

pancreatic inflammation. According to our findings, IL-1 and IL-6 levels were increased in the AP group compared to the control group, and it was observed that these increased cytokine levels decreased significantly with LB administration. Similar to our findings, in a study conducted by Xiong *et al.*, in the AP model with cerulein in mice, the delivery of LB polysaccharides resulted in a decrease in the level of IL-6 and TNfA, which has increased due to AP.[34] These data show that the LB plant has an anti-inflammatory effect. In the study of Qi *et al.*, the changes observed in IL-1 and IL-6 levels with the administration of LBP against AKI are consistent with the findings of our study.[17]

In addition to the findings we obtained in our study, we examined the kidney tissues histopathologically. In our findings, dilation in the bowman ranges of the kidneys and consequent pressure increase point to intercellular fluid increase and picnotic and heterochromatic nucleus structure in proximal and distal tubule epithelial cells. These findings, which we observed in kidney tissue sections, prove that kidney tissue damage was formed in the AP group. Tissue damage occurring outside of the pancreas is usually a complication that occurs with acute necrotizing pancreatitis, and in line with histological data, we can say that the model developed in our experimental animals is compatible with necrosis of pancreatitis. The underlying pathological findings in the kidney tissue are the congestion and collapse caused by renal circulatory disorder and the oxidative stress triggered by the ischemia shaped accordingly. In many studies in the literature, findings in AKI associated with AP support the findings in our study.[53-55]

We examined antioxidant parameters such as SOD, GPx, CAT, MDA, and 8-OHdG to reveal kidney damage and oxidative stress. In our study, there was a significant reduction in kidney SOD and GPx activities compared with the control group of the AP model, while there was an increase in CAT activity and MDA level. With LB administration, there was an increase in kidney SOD and GPx activities and a decrease in MDA level. These results show that kidney damage and oxidative stress due to AP can be cured by LB. It is thought that this effect of LB is due to the anti-inflammatory and antioxidant-effective components it contains. The changes observed in SOD, GPx and MDA parameters through the use of the LB plant in many studies support the findings we have made in our study. However, CAT showed an increase unlike other antioxidant enzymes SOD and GPx. CAT and GPx are two antioxidant enzymes responsible for the detoxification of hydrogen peroxide and other organic peroxides. During the dismutation of superoxide radicals, which are intensely produced during oxidative stress, a significant amount of hydrogen peroxide is formed by the SOD enzyme. GPx provides the first line of defense in the detoxification of accumulated peroxides and therefore GPX activity begins to decrease faster than the CAT enzyme. In fact, the presence of peroxide at low concentrations can stimulate CAT enzyme production and cause high activity to be measured.[26,35,51,52,56] The changes observed in SOD activity and MDA levels by Xie *et al.*, given different doses of LBP (200, 400, and 600 mg/kg b. wt) against kidney damage caused by lead acetate in mice support the findings of our study.[57] In many studies, the use of LB has shown positive results

in diseases that develop due to oxidative stress in different organs.[58,59] In these studies, the observed changes in antioxidant parameters (SOD, GPx, and MDA) are in agreement with the data we obtained in our study. These results show us that the use of LB in many organ damage with its antioxidative effects is promising in obtaining positive results.

On the other hand, the increase of free radicals in the cell causes significant damage to DNA. 8-OHdG is considered to be the most important indicator of DNA damage.[60-63] The increase in the amount of 8-OHdG probably occurs as a result of oxidative interference of mitochondrial and nuclear DNA by free radicals.[64] It has been shown that LBP has protective effects against DNA damage in the pancreas in diabetic rats.[65] In the findings of our study, no significant difference was observed in tissue 8-OHdG level depending on the development of AP. This may be due to the more conserved localization of nuclear and mitochondrial DNA in the cell. However, administration of LB caused a decrease in 8-OHdG levels. This result suggests that LB extract can reverse the hydroxylations that occur in DNA. There is a need for extensive studies at the molecular level on this subject.

CONCLUSION

Pancreatic tissue is often focused on in AP, and possible damage and loss of function in other organs are not taken into account sufficiently. However, damage to other organs, especially kidney, is as important as pancreatic damage caused by AP. LB extract can be considered as a protective agent against kidney damage and oxidative stress caused by AP. In addition, it can reduce local and systemic effects by limiting the severity of fire, which is the most important factor determining the prognosis of the disease, with its anti-inflammatory effects. Although the protective activity of LB was demonstrated in this study, the bioactive molecules responsible for these effects should be separated and their share in the activity should be determined in future studies.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Yokoi H, Naganuma T, Higashiguchi T, Isaji S, Kawarada Y. Prospective study of a protocol for selection of treatment of acute pancreatitis based on scoring of severity. Digestion 1999;60 Suppl 1:14-8.
2. Khokhar AS, Seidner DL. The pathophysiology of pancreatitis. Nutr Clin Pract 2004;19:5-15.
3. Norman J. The role of cytokines in the pathogenesis of acute pancreatitis. Am J Surg 1998;175:76-83.
4. Bhatia M, Wong FL, Cao Y, Lau HY, Huang J, Puneet P, *et al.* Pathophysiology of acute pancreatitis. Pancreatology 2005;5:132-44.
5. Lee HA, Hills EA. Acute pancreatitis and renal failure. Postgrad Med J 1965;41:471-6.
6. Browne GW, Pitchumoni CS. Pathophysiology of pulmonary complications of acute pancreatitis. World J Gastroenterol 2006;12:7087-96.
7. Raghu MG, Wig JD, Kochhar R, Gupta D, Gupta R, Yadav TD, *et al.* Lung complications in acute pancreatitis. JOP 2007;8:177-85.
8. Koyuncu A, Gökgöz S. Systemic Complications In Acute Pancreatitis. Cumhuriyet University, Faculty of Medicine Journal 2001;23:65-72.
9. Sanfey H, Bulkley GB, Cameron JL. The role of oxygen-derived free radicals in the pathogenesis of acute pancreatitis. Ann Surg 1984;200:405-13.
10. Telek G, Ducroc R, Scoazec JY, Pasquier C, Feldmann G, Rozé C. Differential upregulation of cellular adhesion molecules at the sites of oxidative stress in experimental acute pancreatitis. J Surg Res 2001;96:56-67.
11. Granger J, Remick D. Acute pancreatitis: Models, markers, and mediators. Shock 2005;24 Suppl 1:45-51.
12. Ramudo L, Manso MA, Sevillano S, de Dios I. Kinetic study of TNF-α production and its regulatory mechanisms in acinar cells during acute pancreatitis induced by bile-pancreatic duct obstruction. J Pathol A J Pathol Soc Gt Britain Irel 2005;206:9-16.
13. Pereda J, Sabater L, Aparisi L, Escobar J, Sandoval J, Viña J, *et al.* Interaction between cytokines and oxidative stress in acute pancreatitis. Curr Med Chem 2006;13:2775-87.
14. Escobar J, Pereda J, Arduini A, Sandoval J, Sabater L, Aparisi L, *et al.* Cross-talk between oxidative stress and pro-inflammatory cytokines in acute pancreatitis: A key role for protein phosphatases. Curr Pharm Des 2009;15:3027-42.
15. Leung PS, Chan YC. Role of oxidative stress in pancreatic inflammation. Antioxid Redox Signal 2009;11:135-65.
16. Liu Q, Djuricin G, Nathan C, Gattuso P, Weinstein RA, Prinz RA. The effect of epidermal growth factor on the septic complications of acute pancreatitis. J Surg Res 1997;69:171-7.
17. Qi W, Tan DX, Reiter RJ, Kim SJ, Manchester LC, Cabrera J, *et al.* Melatonin reduces lipid peroxidation and tissue edema in cerulein-induced acute pancreatitis in rats. Dig Dis Sci 1999;44:2257-62.
18. Al Mofleh IA. Severe acute pancreatitis: Pathogenetic aspects and prognostic factors. World J Gastroenterol 2008;14:675-84.
19. Barón V, Muriel P. Role of glutathione, lipid peroxidation and antioxidants on acute bile-duct obstruction in the rat. Biochim Biophys Acta 1999;1472:173-80.
20. Andican G, Gelisgen R, Unal E, Tortum OB, Dervisoglu S, Karahasanoglu T, *et al.* Oxidative stress and nitric oxide in rats with alcohol-induced acute pancreatitis. World J Gastroenterol 2005;11:2340-5.
21. Luo Q, Li Z, Huang X, Yan J, Zhang S, Cai YZ. *Lycium barbarum* polysaccharides: Protective effects against heat-induced damage of rat testes and H_2O_2-induced DNA damage in mouse testicular cells and beneficial effect on sexual behavior and reproductive function of hemicastrated rats. Life Sci 2006;79:613-21.
22. Potterat O. Goji (*Lycium barbarum* and L. Chinense): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Med 2010;76:7-19.
23. Luo Q, Cai Y, Yan J, Sun M, Corke H. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. Life Sci 2004;76:137-49.
24. Chen Z, Soo MY, Srinivasan N, Tan BK, Chan SH. Activation of macrophages by polysaccharide-protein complex from *Lycium barbarum* L. Phytother Res 2009;23:1116-22.
25. Reeve VE, Allanson M, Arun SJ, Domanski D, Painter N. Mice drinking goji berry juice (*Lycium barbarum*) are protected from UV radiation-induced skin damage via antioxidant pathways. Photochem Photobiol Sci 2010;9:601-7.
26. Li XM, Ma YL, Liu XJ. Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. J Ethnopharmacol 2007;111:504-11.
27. Jing L, Cui G, Feng Q, Xiao Y. Evaluation of hypoglycemic activity of the polysaccharides extracted from *Lycium barbarum*. Afr J Tradit Complement Altern Med 2009;6:579-84.
28. Wu HT, He XJ, Hong YK, Ma T, Xu YP, Li HH. Chemical characterization of *Lycium barbarum* polysaccharides and its inhibition against liver oxidative injury of high-fat mice. Int J Biol Macromol 2010;46:540-3.
29. Xin YF, Wan LL, Peng JL, Guo C. Alleviation of the acute doxorubicin-induced cardiotoxicity by *Lycium barbarum* polysaccharides through the suppression of oxidative stress. Food Chem Toxicol 2011;49:259-64.
30. Tang WM, Chan E, Kwok CY, Lee YK, Wu JH, Wan CW, *et al.* A review of the anticancer and immunomodulatory effects of *Lycium barbarum* fruit. Inflammopharmacology 2012;20:307-14.
31. de Souza Zanchet MZ, Nardi GM, de Oliveira Souza Bratti L, Filippin-Monteiro FB, Locatelli C. *Lycium barbarum* reduces abdominal fat and improves lipid profile and antioxidant status in patients with metabolic syndrome. Oxid Med Cell Longev 2017;2017:9763210.
32. Gao Y, Wei Y, Wang Y, Gao F, Chen Z. *Lycium barbarum*: A traditional chinese herb and a promising anti-aging agent. Aging Dis 2017;8:778-91.
33. Chang JS, Lee YJ, Wilkie DA, Lin CT. The neuroprotective and antioxidative effects of submicron and blended *Lycium barbarum* in experimental retinal degeneration in rats. J Vet Med Sci 2018;80:1108-15.
34. Xiong GF, Li DW, Zheng MB, Liu SC. The effects of *Lycium barbarum* polysaccharide (LBP) in a mouse model of cerulein-induced acute pancreatitis. Med Sci Monit 2019;25:3880-6.
35. Warzecha Z, Ceranowicz P, Dembinski A, Cieszkowski J, Kusnierz-Cabala B, Tomaszewska R, *et al.* Therapeutic effect of ghrelin in the course of cerulein-induced acute pancreatitis in rats. J Physiol Pharmacol 2010;61:419-27.
36. Zhao R, Li QW, Li J, Zhang T. Protective effect of *Lycium barbarum* polysaccharide 4 on kidneys in streptozotocin-induced diabetic rats. Can J Physiol Pharmacol 2009;87:711-9.
37. Wang NT, Lin HI, Yeh DY, Chou TY, Chen CF, Leu FC, *et al.* Effects of the antioxidants *lycium*

barbarum and ascorbic acid on reperfusion liver injury in rats. In: Transplantation proceedings. Elsevier 2009. p. 4110-3.

38. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
39. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271-8.
40. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049-55.
41. Luck H. Catalase. In: Methods of enzymatic analysis. Academic Press, New York and London. Elsevier; 1965. p. 885-94.
42. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 1976;71:952-8.
43. Noblitt SD, Huehls AM, Morris DL Jr. The role of metal ion binding in generating 8-hydroxy-2′-deoxyguanosine from the nucleoside 2′-deoxyguanosine and the nucleotide 2′-deoxyguanosine-5′-monophosphate. J Inorg Biochem 2007;101:536-42.
44. Thanusu J, Kanagarajan V, Nagini S, Gopalakrishnan M. Chemopreventive potential of 3-[2,6-bis (4-fluorophenyl)-3-methylpiperidin-4-ylideneamino]-2-thioxoimidazolidin-4-one on 7,12-dimethylbenz[a] anthracene (DMBA) induced hamster buccal pouch carcinogenesis. J Enzyme Inhib Med Chem 2010;25:836-43.
45. Koul A, Arora N, Tanwar L. Lycopene mediated modulation of 7,12 Dimethlybenz (a) anthracene induced hepatic clastogenicity in male Balb/c mice. Nutr Hosp 2010;25:304-10.
46. Brzóska MM, Moniuszko-Jakoniuk J, Piłat-Marcinkiewicz B, Sawicki B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol Alcohol 2003;38:2-10.
47. Bradley EL 3rd. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Arch Surg 1993;128:586-90.
48. Wajda J, Dumnicka P, Maraj M, Ceranowicz P, Kuźniewski M, Kuśnierz-Cabala B. Potential prognostic markers of acute kidney injury in the early phase of acute pancreatitis. Int J Mol Sci 2019;20:E3714.
49. Zhou J, Li Y, Tang Y, Liu F, Yu S, Zhang L, *et al.* Effect of acute kidney injury on mortality and hospital stay in patient with severe acute pancreatitis. Nephrology (Carlton) 2015;20:485-91.
50. Nardi GM, Farias Januario AG, Freire CG, Megiolaro F, Schneider K, Perazzoli MR, *et al.* Anti-inflammatory activity of berry fruits in mice model of inflammation is based on oxidative stress modulation. Pharmacognosy Res 2016;8:S42-9.
51. Xiao J, Liong EC, Ching YP, Chang RC, So KF, Fung ML, *et al. Lycium barbarum* polysaccharides protect mice liver from carbon tetrachloride-induced oxidative stress and necroinflammation. J Ethnopharmacol 2012;139:462-70.
52. Mäkelä A, Sternby B, Kuusi T, Puolakkainen P, Schröder T. Phospholipase A2 activity and concentration in several body fluids in patients with acute pancreatitis. Scand J Gastroenterol 1990;25:944-50.
53. Wu Q, Liu LT, Wang XY, Lang ZF, Meng XH, Guo SF, *et al. Lycium barbarum* polysaccharides attenuate kidney injury in septic rats by regulating Keap1-Nrf2/ARE pathway. Life Sci 2020;242:117240.
54. Zhang XP, Wang L, Zhou YF. The pathogenic mechanism of severe acute pancreatitis complicated with renal injury: a review of current knowledge. Digestive diseases and sciences. 2008;53:297-306.
55. Malmstrøm ML, Hansen MB, Andersen AM, Ersbøll AK, Nielsen OH, Jørgensen LN, *et al.* Cytokines and organ failure in acute pancreatitis: Inflammatory response in acute pancreatitis. Pancreas 2012;41:271-7.
56. Jones DP, Eklöw L, Thor H, Orrenius S. Metabolism of hydrogen peroxide in isolated hepatocytes: Relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H_2O_2. Arch Biochem Biophys 1981;210:505-16.
57. Xie W, Huang YY, Chen HG, Zhou X. Study on the efficacy and mechanism of *Lycium barbarum* polysaccharide against lead-induced renal injury in mice. Nutrients 2021;13:2945.
58. Li J, Shi M, Ma B, Zheng Y, Niu R, Li K. Protective effects of fraction 4a of polysaccharides isolated from *Lycium barbarum* against KBrO 3-induced renal damage in rats. Food Funct 2017;8:2566-72.
59. Amagase H, Sun B, Borek C. *Lycium barbarum* (goji) juice improves *in vivo* antioxidant biomarkers in serum of healthy adults. Nutr Res 2009;29:19-25.
60. Du M, Hu X, Kou L, Zhang B, Zhang C. *Lycium barbarum* polysaccharide mediated the antidiabetic and antinephritic effects in diet-streptozotocin-induced diabetic Sprague Dawley rats via regulation of NF-$κ$B. Biomed Res Int 2016;2016:3140290.
61. Gan F, Liu Q, Liu Y, Huang D, Pan C, Song S, *et al. Lycium barbarum* polysaccharides improve CCl_4-induced liver fibrosis, inflammatory response and TLRs/NF-kB signaling pathway expression in wistar rats. Life Sci 2018;192:205-12.
62. Cadet J, Douki T, Gasparutto D, Ravanat JL. Oxidative damage to DNA: Formation, measurement and biochemical features. Mutat Res 2003;531:5-23.
63. Geyikoglu F, Emir M, Colak S, Koc K, Turkez H, Bakir M, *et al.* Effect of oleuropein against chemotherapy drug-induced histological changes, oxidative stress, and DNA damages in rat kidney injury. J Food Drug Anal 2017;25:447-59.
64. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: Mechanisms, mutation, and disease. FASEB J 2003;17:1195-214.
65. Xu M, Zhang H, Wang Y. The protective effects of *Lycium barbarum* polysaccharide on alloxan-induced isolated islet cells damage in rats. Zhong Yao Cai 2002;25:649-51.