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Artichoke for biochemistry, histology, and gene expression in obstructive jaundice

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SUMMARY

OBJECTIVE: This study aimed to evaluate the hepatoprotective effect of artichoke leaf extract (*Cynara scolymus*) in experimental obstructive jaundice.

METHODS: Rats were separated into three groups, namely, sham, control, and artichoke leaf extract. Ischemia was created for 60 min, and then liver tissue and blood samples were taken at the 90th minute of reperfusion. Artichoke leaf extract was given at a 300 mg/kg dose 2 h before the operation. Antioxidant enzyme activities and biochemical parameters were examined from the tissue and serum. Histopathological findings of the liver were scored semiquantitatively.

RESULTS: Antioxidant enzyme activities in the artichoke leaf extract group were statistically significantly higher than that in the other two groups. Biochemical parameters, which show hepatocellular damage, were found to be similar in both sham and artichoke leaf extract groups. Although the values in the sham group were higher than the artichoke group in terms of protein and gene expressions, no statistically significant difference was found between these two groups. Regarding the hepatocellular effects of obstructive jaundice, the artichoke leaf extract group showed lower scores than the control group in all histopathological scores.

CONCLUSION: The results of this study showed that artichoke leaf extract had a hepatoprotective effect that was associated with the antioxidant and anti-inflammatory effects of artichoke leaf extract.

KEYWORDS: Liver. Obstructive jaundice. *Cynara scolymus*. Protective agent. Antioxidant.

INTRODUCTION

Obstructive jaundice (OJ) is the obstruction of the common bile duct due to complications associated with surgery, idiopathic diseases, and metabolic diseases[1]. It has been shown that bile acids and harmful substances eliminated by bile accumulate in hepatocytes and blood in OJ. As a result, chronic liver damage and fibrosis develop due to cell death[2]. If OJ is not treated appropriately, disturbances in reticuloendothelial system (RES) functions, suppression of the immune system, inhibition of the detergent and antibacterial effects of bile salts, disruption of the intestinal mucosal barrier, bacteremia, and endotoxemia develop[3]. Numerous experimental and clinical studies have been conducted to prevent or reverse oxidative stress and inflammation, which have a significant effect on the pathogenesis of OJ. However, there are still no drugs that are widely used in clinical practice[4].

Oxidative stress is characterized by a disruption in the pro-oxidant-antioxidant balance, in which the formation of reactive oxygen species (ROS) exceeds the capacity of the enzymatic and nonenzymatic antioxidant defense systems[5]. ROS in high concentrations damage all major cell structures, including proteins, lipids, and DNA. Phenolic substances in the antioxidant defense system act as a protective shield against oxidative damage in biological molecules such as proteins, lipids, and DNA[6].

The main phenolic compounds in artichoke (*Cynara scolymus*) leaf extract (ALE) are caffeic acid derivatives, including caffeoylquinic acid derivatives[7]. The antimicrobial, hepatoprotective, choleretic, hypocholesterolemic, hypoglycemic, and

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anticancer effects of ALE have been shown in previous studies[8-10]. It has also been reported that ALE significantly prevented oxidative damage in hepatocyte membranes[11].

This study aimed to evaluate the hepatoprotective effect of ALE in experimental OJ, a subject that has not been previously studied.

METHODS

The study was carried out following the principles of the National Laboratory Animal Use and Care Directive, with the approval of the XXX University Experimental Medicine Research Center Animal Ethics Committee. A total of 30 adult male Wistar albino rats included in the study were cared for under the conditions determined by XXX University Experimental Medicine Research and Application Center. The groups were organized as follows:

Group 1 (sham group, n=10): After laparotomy, the common bile duct was released from the surrounding tissues in rats in this group, no other procedure was performed, and no treatment was given.

Group 2 (control group, n=10): After the common bile duct was freed from the surrounding tissues in rats in this group, it was double tied with 5-0 silk and cut between the sutures, no other procedure was performed, and no treatment was given.

Group 3 (ALE group, n=10): After the common bile duct was freed from the surrounding tissues in rats in this group, it was double tied with 5-0 silk and cut between the sutures, then 300 mg/kg/day artichoke extract was given via orogastric tube for 10 days.

After the surgical procedures were completed, the rats were sacrificed on postoperative day 10, and liver tissue and blood samples were taken. The obstructive jaundice model in the study is a model that has been widely used by both our team members and other researchers[12-14].

The ALE used in this study was prepared in XXX University, Faculty of Engineering and Natural Sciences, Department of Chemical Engineering. The dose of ALE to be used in the study was determined as 300 mg/kg, which is preferred in studies in the light of various articles scanned in the literature. Furthermore, in the literature, it was seen that the extract is mainly applied as oral gavage[15,16].

Supply of tissues

Liver tissue and blood samples were taken on postoperative day 10. The tissues were placed on ice and, to remove blood, were washed with cold distilled water and physiological saline, then packed sterile and frozen in liquid nitrogen. In the Science Faculty Biochemistry Department Research Laboratory, the tissues were maintained at -80°C, blood was centrifuged at 10,000 rpm for 5 min at 4°C, and sera were obtained and stored at -80°C until assay.

Biochemical studies

Total protein from the tissues was extracted, the protein concentration of protein lysate was determined, and Western blot analyses were performed. For evaluating antioxidant enzyme activities, catalase, superoxide dismutase, and glutathione peroxidase activities were determined. Serum aminotransferases (AST and ALT), serum lactate dehydrogenase (LDH) activity, serum alkaline phosphatase (ALP), creatine kinase (CK), total protein (TPROT) and albumin (ALB), total bilirubin (TBIL), direct bilirubin (DBIL), and C-reactive protein (CRP) levels were also measured.

Microsomal proteins were separated with the SDS-polyacrylamide gel electrophoresis (SDS-PAGE) method by applying the vertical electrophoresis technique for determination of expression at protein level. RNA isolation from the tissues obtained to determine the effects of the study plant components on the gene expression levels of the enzymes was performed using the RNA TRIZOL method. cDNA from total RNAs was synthesized using 18 nucleotide long oligo d(T) primer and Moloney-Murine Leukemia Virus Reverse Transcriptase. The expression of the mRNAs of the relevant genes was determined in the Bio-rad qRT-PCR device using iTaq Universal SYBR Green Supermix (Cat. No.: 172-5124).

Histopathological examination

The samples were evaluated by the same pathologist blinded to the groups. Histopathological examination of the samples was made using an OLYMPUS brand BX51TF model light microscope. Bile duct proliferation, focal ("spotty") necrosis, inflammation, and granuloma formation were evaluated semi-quantitatively in the liver section.

Statistical analysis

Biochemical data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows software, version 25.0 (SPSS Inc., Chicago, IL, USA). Statistical analysis of pathological scores was performed using R 3.6.0 (https://www.r-poject.org).

RESULTS

General

One rat from Group 2 and Group 3 died during the study, and no new rats were added in their place.

Biochemical results

Total phenolic/flavonoid antioxidant amounts of artichoke extract

The total phenolic content of the artichoke methanol extract was 38.03±0.95 μg GAE/mg, and the total flavonoid amount was 18.11±0.26 μg QE/mg.

Antioxidant enzyme activities

SOD, catalase, and GPx enzyme activities in the artichoke group were statistically significantly higher than that in the other two groups (p<0.05) (Table 1).

Other biochemical parameters

According to these results, all parameters were statistically significantly higher in the control group compared to the other groups (p<0.05). Although the CRP levels were higher in the artichoke group than the sham group, no statistically significant difference was found between these two groups (p>0.05). However, all other parameters were statistically significantly higher in the artichoke group compared to the sham group (p<0.05).

Protein and gene expression study results

When albumin and globulin protein expressions and albumin, globulin, and prothrombin gene expressions were evaluated, it was observed that the values for each parameter in the control group were statistically significantly lower than that in the other two groups (p<0.05). In terms of protein and gene expressions, although the values in the sham group were higher than in the artichoke group, no statistically significant difference was found between these two groups (p>0.05) (Figure 1).

Histopathological results

There was a statistically significant association between biliary duct proliferation/spotty necrosis and the study groups (p<0.001 for both).

There was a statistically significant association between inflammation and the study groups (p<0.001). The proportion

Table 1. Antioxidant enzyme activities of the study groups.

Groups	SOD	Catalase	GPx
Sham (Group 1)	15.43±1.97[a]	260.58±28.98[a, g]	34.53±2.80[a, g]
Control (Group 2)	19.52±4.09[b]	315.06±28.98[b]	58.87±3.25[b]
Artichoke (Group 3)	25.35±2.41	377.51±13.67	94.37±4.30

[a] Significantly different, Group 1 vs. Group 3, p<0.05; [b] Significantly different, Group 2 vs. Group 3, p<0.05; [g] Significantly different, Group 1 vs. Group 2, p<0.05.

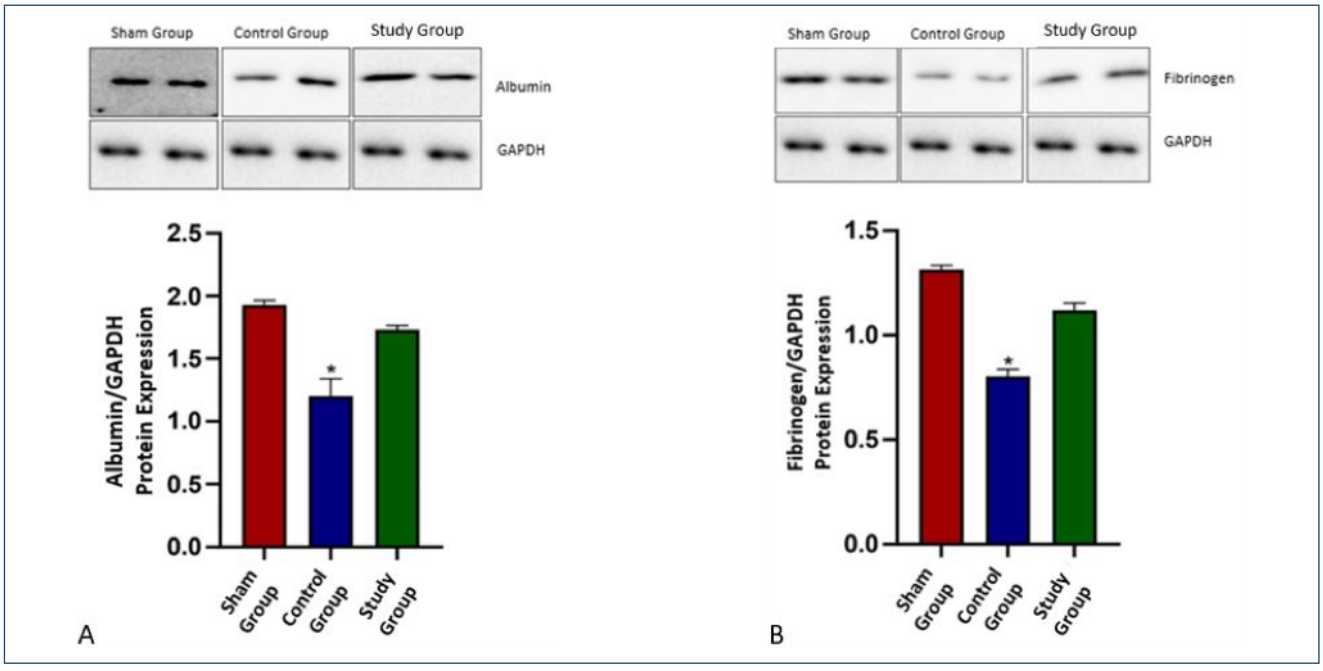

Figure 1. **(A)** Albumin protein expression (*p<0.05). **(B)** Fibrinogen protein expression (*p<0.05).

of no inflammation was higher in Group 1 (n=8, 80%) compared to Groups 2 and 3.

There was a statistically significant association between granuloma and the study groups (p<0.001). The proportion of no granuloma was higher in Groups 1 and 3 compared to Group 2. Conversely, the proportion of existing granuloma was higher in Group 2 (n=7, 87.5%) compared to Group 1 (n=0, 0%) and Group 3 (n=1, 12.5%).

The histopathological differences between Groups 2 and 3 are shown in Figure 2.

DISCUSSION

In OJ, increased bile acids promote the expression of free oxygen radicals from neutrophils and macrophages and induce oxidative damage by stimulating free oxygen radical formation from mitochondria[17]. Quantitative analysis in animal studies has shown that supplementation with ALE can increase SOD, CAT, GSH, and GPx in the liver[18]. In this study, SOD, CAT, and GPx enzyme activities were measured and found that all three antioxidant activities in the ALE group were significantly higher than in the other two groups (p<0.05). These data suggested that ALE is effective in preventing damage by increasing the antioxidative effect against oxidative damage (Table 1).

Elevated circulating levels of ALT, AST, ALP, LDH, and OCT enzymes suggest hepatocellular damage[19]. It was demonstrated that ALE reduced serum ALT and AST levels in a carbon tetrachloride (CCl4)-induced hepatotoxicity model in mice[20]. In this study, the values of liver function tests were significantly higher in the control group than that in the other groups (p<0.05). CRP values indicating inflammation were highest in the control group, followed by the ALE group, and were lowest in the sham group. The higher CRP values in the control group compared to the other two groups are significant in showing inflammation caused by hepatocellular damage due to OJ.

Low protein and albumin levels or high prothrombin time or INR indicate decreased synthetic function and hepatic decompensation[21]. When the albumin and globulin protein expressions and albumin, globulin, and prothrombin gene expressions were evaluated in this study, it was observed that the values of each parameter in the control group were lower than in the other two groups, showing a statistically significant difference (p<0.05). In terms of protein and gene expressions, although the values in the sham group were higher than in the artichoke group, no statistically significant difference was found between these two groups (p>0.05) (Figure 1). These data suggested that ALE protects the synthetic function of liver and helps in compensation.

Mehmetcik et al.[22] reported that administration of ALE to rats significantly reduced transaminase activity and alleviated histopathological change. In this study, there was a statistically significant correlation between the formation of bile duct proliferation and the study groups (p<0.001). While bile duct proliferation was not seen in the sham group, it was evident in the other two groups, and significantly more severe bile duct proliferation was observed in the control group than in the ALE group. There was a statistically significant correlation between spotty necrosis and the study groups (p<0.001). Spotty necrosis was not seen in the sham group but was mild in the ALE group and moderate-severe in the control group. Inflammation was seen in all groups. While mild inflammation was observed in very few samples in the sham group, mild-to-moderate inflammation was more common in the control group than in the ALE group. Although the ALE group showed lower scores than the

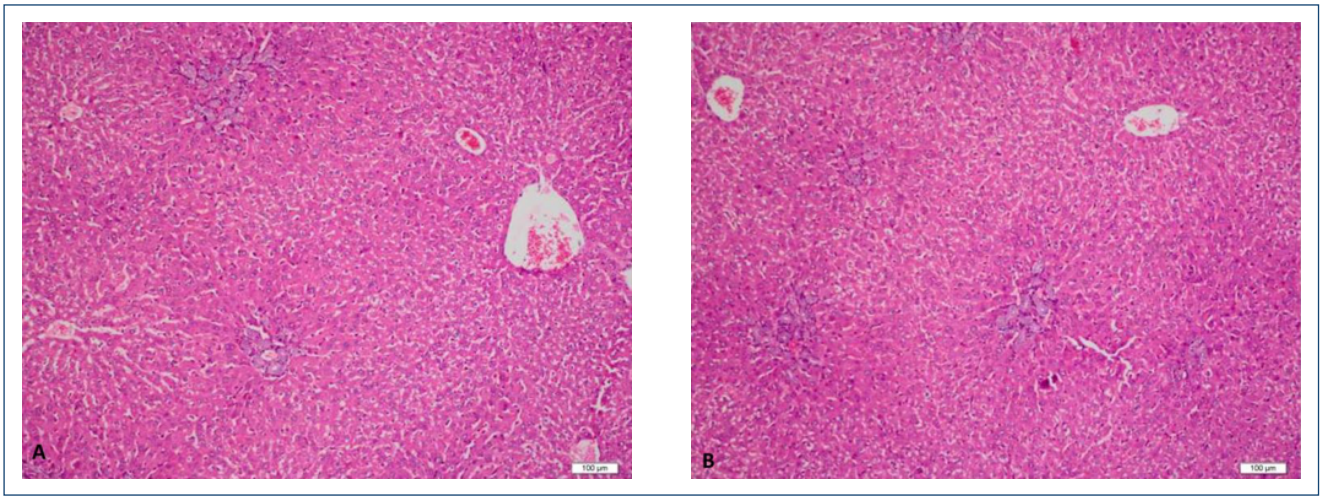

Figure 2. (A and B) Milder bile duct proliferation, spotty necrosis, and inflammation were observed in Group 3 compared to Group 2 (H&E, ×100).

control group in all histopathological scores in determination of hepatocellular effects of OJ, the most significant difference was seen in granuloma formation. Granuloma showing chronic inflammation was not seen in the sham group but was more intense in the control group than in the ALE group.

CONCLUSION

In the light of these results, it was concluded that artichoke extract has a positive effect on OJ and this effect is related to the antioxidant and anti-inflammatory effects of artichoke.

ETHICAL COMMITTEE APPROVAL

In accordance with Research and Publication Ethics, Ethics Committee approval was received from Selçuk University Experimental Medicine Application and Research Center on June 28, 2019, with the decision number 2019-25.

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AUTHORS' CONTRIBUTIONS

SC: Conceptualization, Investigation, Methodology, Writing – original draft. **BÇ:** Investigation, Resources. **PC:** Methodology, Resources. **İB:** Data curation, Investigation, Visualization, Writing – original draft. **HGB:** Investigation, Resources. **FS:** Resources, Validation. **SK:** Supervision, Validation. **SH:** Supervision, Validation. **KK:** Investigation, Methodology, Supervision, Visualization. **MŞ:** Writing – review & editing.

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