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Investigating the Protective Effect of Ellagic Acid on Cholemic Nephropathy in Cholestatic Rats

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Abstract

Background: Cholemic nephropathy (CN), a renal dysfunction caused by bile acids, is a severe complication of chronic liver damage and bile duct ligation (BDL), which may lead to complete kidney failure.
Objectives: This study investigated the protective effect of ellagic acid on CN in cholestatic rats.
Methods: Sixty male Wistar rats weighing about 180 - 200 g were randomly divided into 6 groups for in vivo investigation. The rats were randomly divided into 5 groups of 10. Cholestasis was induced in rats by closing the bile ducts; then, the animals were treated with different doses of ellagic acid ([illegible] and 50 mg/kg). Then, the induction effect of cholestasis and the protective effects of ellagic acid on serum and urinary factors, oxidative stress indices, and histopathological changes in liver and kidney tissue were investigated.
Results: Bile duct ligation in rats led to an [illegible]um and urinary factors. It was also associated with increased reactive oxygen species (ROS), lipid peroxidation, glutathione (GSH) oxide form, decrease in antioxidant systems, GSH and severe histopathological changes, and fibrosis of the liver and kidney tissues. Another finding of this research was the beneficial effect of ellagic acid in improving serum and urinary factors, oxidative stress indices, and histopathological changes.
Conclusions: Due to its antioxidant properties, ellagic acid can potentially serve as a novel therapeutic approach for treating kidney damage caused by increased serum levels of bile acids.

Keywords: Ellagic Acid, Oxidative Stress Indicators, Rats, Bile Duct Ligation

Background

Cholestasis is a disorder that affects the secretion of bile and is commonly observed in various liver diseases (1, 2). Cholestasis could develop in response to xenobiotics (e.g., alcohol or drugs) or diseases (3). It has been found that kidneys are the most affected extrahepatic organs during cholestasis (4). The accumulation of potentially cytotoxic chemicals (e.g., hydrophobic bile acids), which are routinely excreted through the bile flow, seems to play a fundamental role in developing cholestasis-induced renal injury or cholemic nephropathy (CN) (5).

Cholestasis can be categorized as extra-hepatic or intra-hepatic. Extra-hepatic cholestasis occurs due to bile duct obstruction caused by stones or tumors, whereas intra-hepatic cholestasis results from genetic defects or the adverse effects of certain drugs on hepatocytes and bile duct cells (1, 6-8). Cholestasis induces the production of prostaglandins, elevates the level of bile salts, causes endotoxemia, increases nitric oxide production and the level of opioids, and triggers vascular changes (8-11). Other effects of cholestasis include the deposition of bilirubin, bile acids, and cholesterol, which are typically secreted into bile (12, 13). In addition to the complications that arise due to the failure of bile excretion during the obstruction of the bile duct, liver, and kidney tissue damage can also be

observed.

Cholestatic liver diseases lead to damage and disruption of kidney function. This was first reported by Quincke (1899) in jaundice patients who subsequently developed kidney damage (14). Renal changes in obstructive jaundice are known as CN, resulting in kidney dysfunction in jaundice patients with histomorphological evidence in kidney tissue. The histological changes caused by CN include a wide range of kidney diseases, predominantly affecting the distal part of the nephron with the formation of intraluminal cysts (15). The relationship between obstructive jaundice and kidney damage is a well-known clinical phenomenon and an unresolved problem (14).

Polyphenol compounds, specifically ellagic acid, have been demonstrated to possess potent antioxidant activity (16). Ellagic acid is available in natural sources, such as green tea, pomegranate, strawberry, raspberry, walnut, and eucalyptus tree bark (17). Besides its antioxidant properties, ellagic acid has also exhibited a range of pharmacological activities, including anticancer, anti-allergic, antimalarial, and anti-inflammatory effects (17-19). Furthermore, the antioxidant properties of ellagic acid are effective both in vivo and in laboratory conditions (20).

2. Objectives

The present study investigated the protective effects of ellagic acid on CN in cholestatic rats.

3. Methods

The study was conducted on male Wistar rats weighing between 220 and 280 g, which were obtained from the Research Institute of Khorramabad University. Five groups of rats were evaluated and housed in specialized cages under controlled environmental conditions, with a constant temperature of 22°C and a 12-hour light-dark cycle. Water and compressed food (pellets) were available ad libitum except during surgery and experiments. The 5 test groups consisted of a sham group, a bile duct ligation (BDL) group, a BDL group receiving ellagic acid at a rate of 10 mg/kg/day, a BDL group receiving ellagic acid at a rate of 25 mg/kg/day, and a BDL group receiving ellagic acid at a rate of 50 mg/kg/day, with 10 rats assigned to each group. All experimental procedures were conducted according to ethical standards for animal treatment approved by the Ethics Committee of Lorestan University of Medical Sciences.

3.1. Cholestasis Surgery

The present study aimed to investigate the effects of BDL on rats. A sham group was included, in which surgery was conducted without BDL, while the cholestasis group underwent surgery with BDL (21). Prior to surgery, the rats were anesthetized using an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (10 mg/kg). The abdominal region was shaved thoroughly, and 70% alcohol was used to disinfect the skin. A 3-cm longitudinal incision was made in the midline of the abdomen using a surgical knife, and the skin and muscles of the abdominal wall were opened in 2 stages. Using identifying the duct, forceps was placed under it and tied with 4 - 0 silk thread at 2 separate points after which the duct was cut. Thereafter, the abdominal wall was sutured in 2 layers of muscle and skin with silk thread. Following the procedure, 1 mL of normal saline was administered intraperitoneally. The surgical site was disinfected with alcohol or Betadine after surgery. Two days after the surgery, the color of the animals' urine changed, and their ears turned yellow, indicating successful cholestasis surgery.

3.2. Analysis of Serum and Urine Biochemical Factors

To assess serum and urine biochemical factors, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), and lactate dehydrogenase (LDH), a fasting blood sample was obtained from the chest area of the rat. Subsequently, the serum of the blood samples was separated by centrifugation at a speed of 2500 rpm for 15 min at a temperature of 30°C. Urine samples were prepared and underwent similar centrifugation at the aforementioned settings, and the supernatant was used for subsequent analysis.

3.3. Preparation of Liver Homogenate for Measuring Oxidative Stress Markers

The liver of the rat was immediately removed and washed with normal saline that had been chilled. Following this, a 10% homogenate was prepared in 1.15% (w/v) potassium chloride. The homogenate was then centrifuged at a speed of 7000 rpm for 10 min at 4°C.

3.4. Measurement of Oxidative Stress Markers in Liver and Kidney Tissue Samples

The supernatant solution obtained from the homogenized liver and kidney samples was used to measure lipid peroxidation by quantifying the malondialdehyde content, as well as to assess reactive oxygen species (ROS), antioxidant capacity, and glutathione (GSH) regeneration levels.

3.5. Lipid Peroxidation Analysis in Liver Tissue

Lipid peroxidation in the liver was evaluated by a colorimetric method involving the measurement of thiobarbituric acid reactive substances (TBARS) based on the method described by Fraga et al. (22). Briefly, 0.1 mL of tissue homogenate was mixed with 2 mL of TCA-HCL-TBA reagent (consisting of 37% TBA, HCL [0.25 mol], and 15% TCA at a ratio of 1:1:1) and subsequently cooled after 15 min in a boiling bain-marie. This was followed by centrifugation at 3500 rpm for 10 min at room temperature, and the absorbance of the resulting clear supernatant was measured at 535 nm against the blank.

3.6. Measurement of Reactive Oxygen Species Production

The production rate of ROS was determined as follows:

In this study, 500 mg of liver tissue was added to 5 mL of Tris-hydrochloride buffer (40 mM, pH = 7.4, and temperature of 4°C) and homogenized using a homogenizer. Thereafter, 100 μL of the resultant homogenate mixture was mixed with 1 mL of cold Tris-hydrochloride buffer (40 mM and pH = 7.4) and 2′,7′-dichlorofluorescein diacetate (final concentration of 1 μM) was added. The samples were then incubated for 15 min at a temperature of 37°C in darkness, after which the fluorescence intensity was measured at an excitation wavelength of 485 nm and an emission wavelength of 525 nm using a fluorimeter.

For the measurement of GSH in liver tissue, the tissue was first homogenized at a ratio of 1: 10 (w/v) with chilled 0.02 M EDTA solution. Next, 5 mL of the homogenized liver mixture was mixed with 4 mL of water and 1 mL of 50% TCA. The resultant mixture was centrifuged at 3000 rpm for 15 min, following which 2 mL of the supernatant was mixed with 4 mL of 0.4 M Tris buffer and 0.1mL of 0.01 DTNB molar. The mixture was shaken well, and its absorbance was read at 412 nm after 5 min.

Finally, for the measurement of total antioxidant capacity (ferric reducing antioxidant power [FRAP]), 100 μL of liver tissue homogenate was added to 3 mL of FRAP solution containing 2.5 mL of acetate buffer (300 mM and pH = 3), 0.25 mL of ferric chloride solution (20 mM), and 0.25 mL of TPTZ solution. The mixture was then incubated at room temperature for 5 min.

After centrifugation (1 min, 10 000 g), the absorbance of the samples was measured with a spectrophotometer set to a wavelength of 593 nm. The resulting measurements were incorporated into the standard curve formula to calculate the total antioxidant capacity in terms of micromolar vitamin C.

3.7. Examining Liver and Kidney Tissue Sections to Investigate the Tissue Changes Caused by Cholestasis

To examine the tissue changes induced by cholestasis, liver, and kidney, tissue sections were prepared from both the sham and cholestatic rats. In the experimental group, the animals were killed using chloroform before their livers were removed; the same procedure was carried out in the sham group. Following washing with a 10% formalin solution as a stabilizer, the samples were dehydrated using alcohol (ethanol) and clarified using toluene. Thereafter, the samples were embedded in paraffin wax, and 5-micron thick slices were obtained using a microtome (Cambridge Medical Instruments United Kingdom). Subsequently, the samples were placed on slides and stained using hematoxylin and eosin (H & E) staining. Once the staining process was complete, the samples were fixed into the slices using Entellan glue for long-term preservation. Finally, the liver and kidney tissue sections were examined under an optical photomicroscope (Zeiss, Germany) to compare the differences between the normal and cholestatic groups. The Needle scoring system method was used to assess the tissue changes observed (23).

3.8. Data Collection Methods

Data collection involved performing calculations using GraphPad Prism version 6, employing a 1-way analysis of variance to compare groups. After each significant F, the analysis was continued with Tukey's post hoc test. Statistically, P values less than 0.05 were assumed to be significant.

4. Results

4.1. The Results of Examining the Weight of Rats with Cholestatic and Cholemic Nephropathy

The present study aimed to evaluate the weight status of rats afflicted with cholestatic and CN. Figure 1 reveals that after 14 days following BDL, there were no significant differences in the body weight of the experimental groups. However, there was a statistically significant increase in the average weights of the liver and kidneys among the cholestatic group compared to the control group ($P < 0.001$). Notably, a substantial weight reduction was observed among those receiving ellagic acid at various doses compared to the BDL group ($P > 0.05$).

4.2. The Results of the Analysis of Biochemical Indicators of Liver Damage and Cholestasis in the BDL Animal Model

We analyzed biochemical indicators of liver damage and cholestasis in the BDL animal model. As shown

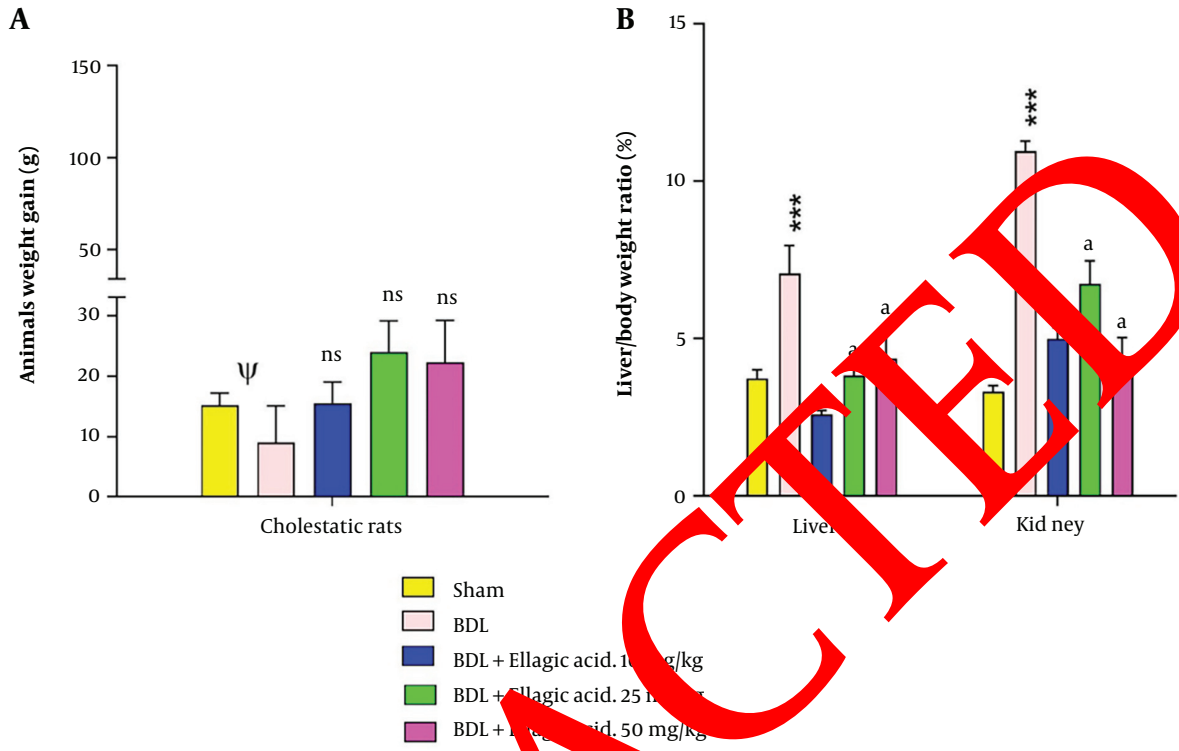

Figure 1. Changes in liver weight and animal weight in cholestasis (values are expressed as mean ± SD for 6 animals per group. The statistical significance of these changes was assessed using a significance level of $P < 0.001$ for the control group, compared to which the results of the experimental groups were evaluated. In this regard, the asterisks (***) represent a significant difference between the control and test groups, while a indicates a significant difference between the BDL group and test groups. Additionally, ns was used to indicate no statistically significant difference between the BDL group and test groups ($P > 0.05$), whereas the Ψ was used to indicate no significant difference between the control group and the test groups ($P > 0.05$). Abbreviation: BDL, bile duct ligation.

in Figure 2, a significant increase in the average blood concentration of ALT, AST, alkaline phosphatase (ALP), LDH, gamma-glutamyl transferase (γ-GT), and bilirubin were observed in cholestatic animals compared to the control group ($P < 0.001$). Moreover, our findings suggest that the administration of 10, 25, and 50 mg/kg of ellagic acid can significantly reduce the serum level of tissue damage markers compared to the BDL group (Figure 2).

Analysis of Urinary Indicators

A significant increase in the amount of creatinine, bilirubin, blood urea nitrogen (BUN), ALP, and γ-GT in cholestatic animals compared to the control group ($P < 0.001$). Moreover, the administration of 10, 25, and 50 mg/kg of ellagic acid can significantly reduce the serum and urinary levels of tissue damage markers compared to the BDL group ($P > 0.05$).

4.4. Analysis of Oxidative Stress Indices in Liver and Kidney

According to Table 1, an investigation of oxidative stress indices in liver and kidney tissue samples indicated a notable elevation in the production of ROS and lipid peroxidation, a decrease in GSH levels, and a decline in antioxidant capacity across all cholestatic animals ($P < 0.05$). However, the administration of ellagic acid as a therapeutic intervention in BDL animals exhibited a significant reduction in oxidative stress markers and associated complications in cholestatic animals ($P < 0.05$). These results suggest that ellagic acid may exhibit a protective effect against oxidative stress-induced damage in the liver and kidney tissues of cholestatic animals.

4.5. Histological Changes of the Liver in Cholestatic Animals

Figure 4 and Table 2 demonstrate significant histological changes in the liver tissue samples of cholestatic animals. Specifically, we observed tissue necrosis, widespread inflammation, and an increase in collagen deposition (i.e., tissue fibrosis) in the BDL group. However, the administration of ellagic acid in cholestatic animals appeared to prevent tissue damage caused by cholestasis in rats. Our results suggest that ellagic acid may hold promise as a therapeutic agent for

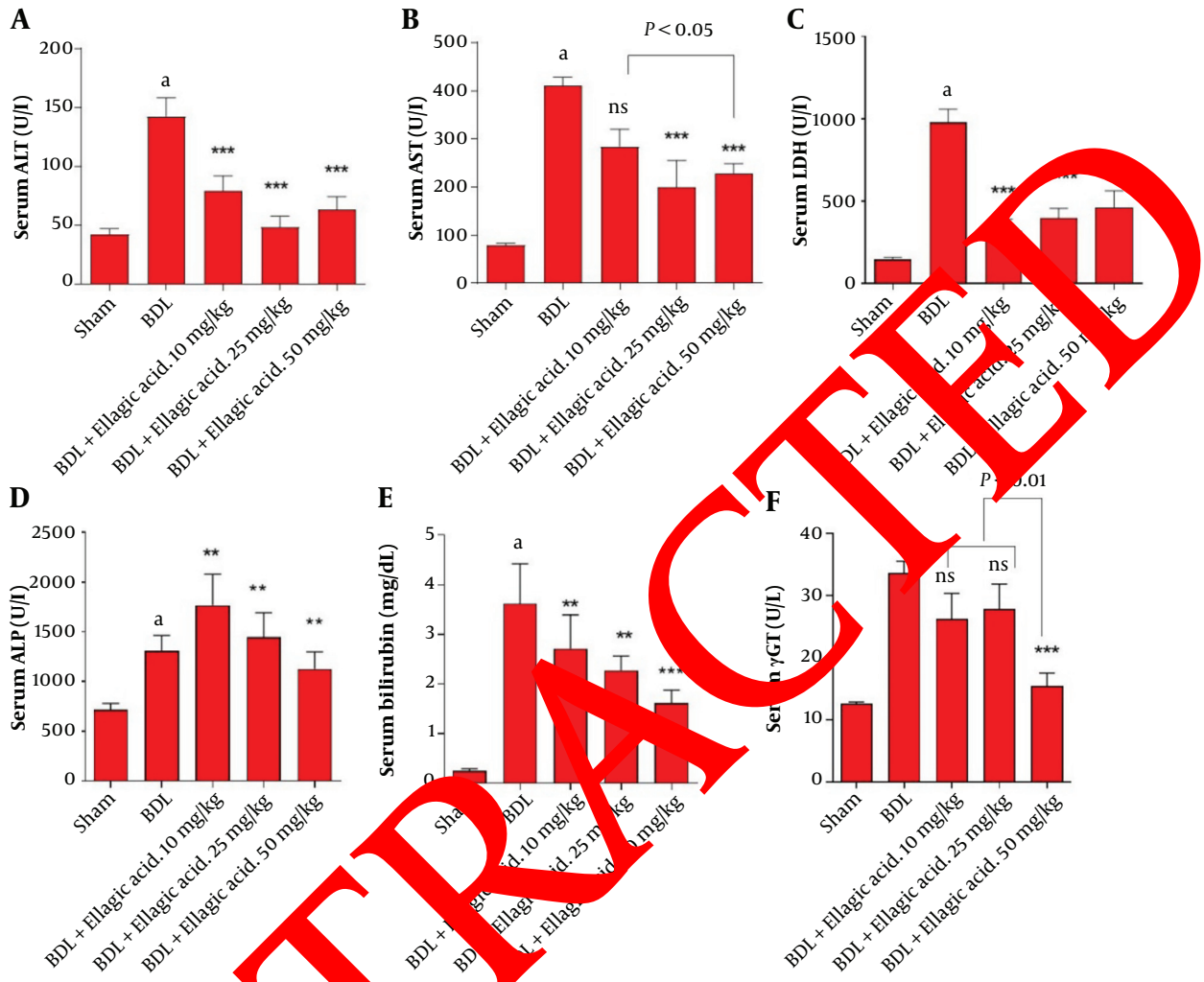

Figure 2. Serum biochemical changes in cholestatic animals (14 days after BDL surgery) and the effect of ellagic acid administration. Values are expressed as mean ± SD for 6 animals per group. a indicates a significant difference compared to the control group ($P < 0.001$), *** indicates a significant difference compared to the control group ($P < 0.001$), ** $P < 0.01$, and ns indicates no significant difference compared to the BDL group ($P > 0.05$). Abbreviations: BDL, bile duct ligation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; γ-GT, gamma-glutamyl transferase.

the prevention and treatment of liver damage associated with cholestasis.

Table 3 shows the degree of liver tissue changes in cholestatic animals.

4.6. The Investigation of the Impact of Ellagic Acid on the Histological Changes in Kidney Tissue of Cholestatic Animals

As indicated by Figure 5 and Table 3, the occlusion of the bile duct in rats resulted in severe histopathological alterations in the kidney tissue, including interstitial inflammation, atrophy, vascular congestion, and tubular necrosis, when compared to the sham group.

Table 3 shows the degree of kidney tissue changes in cholestatic animals.

Furthermore, animals that received 10, 25, and 50 mg/kg of ellagic acid demonstrated an ameliorating effect on the histopathological changes in kidney tissue as compared to the BDL group. The degree of kidney tissue modifications in cholestatic animals can be observed in Table 3.

It can be concluded that ellagic acid has a protective effect on the kidney tissue of cholestatic animals, which is evident from the mitigation of histopathological alterations observed in this study. These findings suggest that ellagic acid supplementation may serve

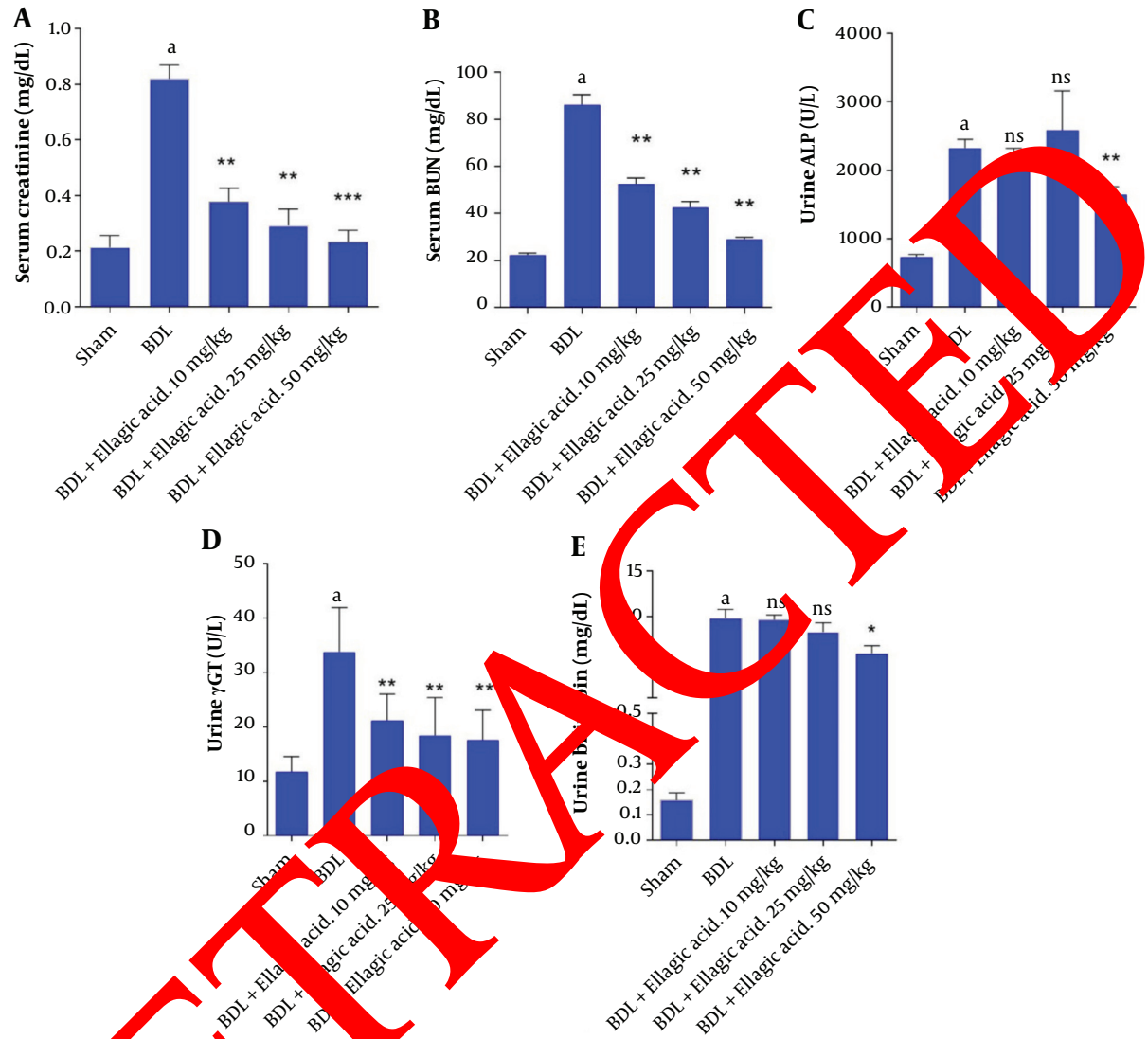

Figure 3. Urinary and se[rum] indicators of kidney damage and the effect of ellagic acid administration in 6 animals per group. Values are expressed as mean ± SD, indicating a significant difference co[mpared] to the control group ($P < 0.001$). Asterisks denote a significant difference compared to the control group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0$[.] represent n[o] significant d[ifference] compared to the BDL group ($P > 0.05$). Abbreviations: BDL, bile duct ligation; BUN, blood urea nitrogen; ALP, alkaline p[hos]phatase; [GGT], gamma-glut[amyl] [trans]ferase.

as a [pro]mising strategy for managing kidney damage caused b[y] cholestasis.

5. Discussion

Cholestasis is caused by various disorders and diseases in humans. Alcohol consumption, viral liver infections, xenobiotics, liver cancer, and cirrhosis are associated with cholestatic liver disease (24, 25). Although the liver is the first vulnerable organ affected by cholestasis, other extrahepatic organs, especially the kidneys, are affected by damage and dysfunction in cholestasis and cirrhosis (26). Cholemic nephropathy is a complication that occurs under the influence of cholestatic liver disease and cirrhosis in the kidney. The lack of effective treatment strategies to prevent or reduce possible damage highlights the need for a comprehensive understanding of the exact mechanism underlying these abnormalities to inform corresponding treatment strategies. In this study, we investigated the protective effects of ellagic acid

Table 1. Oxidative Stress Indicators in Liver and Kidney Tissue of Cholestatic Animals [a]

Treatment	ROS Formation (Fluorescent Intensity)	Lipid Peroxidation (nmol of TBARS/mg Protein)	Glutathione (μmol of GSH/mg Protein)	Total Antioxidant Capacity (μM of Vitamin C Equivalent)
Oxidative Stress Index Liver				
Sham	83889 ± 16040	1.77 ± 0.06	19.86 ± 3.64	3.60 ± [illegible]
BDL	177433 ± 17806 [b]	3.15 ± 0.40 [b]	7.9 ± 0.76 [b]	[illegible] ± 0.03
BDL+ellagic acid (10 mg/kg)	151845 ± 8149 [c]	2.14 ± 0.21 [c]	8.10 ± 0.97	2.82 ± 0.13
BDL+ellagic acid (25 mg/kg)	166952 ± 8267 [c]	2.13 ± 0.19 [c]	9.19 ± 0.50 [c]	[illegible] ± 0.17 [c]
BDL+ellagic acid (50 mg/kg)	131253 ± 4373 [c, d]	2.27 ± 0.18 [c]	12.45 ± 2.62 [c]	3.49 [illegible] [c]
Oxidative Stress Index Kidney				
Sham	95641 ± 10500	1.60 ± 0.52	18.56 ± [illegible]	3.77 ± 0.12
BDL	162720 ± 7875 [b]	5.02 ± 0.54 [b]	5.[illegible] ± 0.82 [b]	2.38 [illegible]
BDL+ellagic acid (10 mg/kg)	148697 ± 5568 [c]	2.54 ± 0.46 [c]	[illegible] 1.16	[illegible] ± 0.12 [c]
BDL+ellagic acid (25 mg/kg)	154678 ± 7937 [c]	3.20 ± 0.55 [c]	9.84 ± [illegible]	3.28 ± 0.20 [c]
BDL+ellagic acid (50 mg/kg)	148484 ± 11803 [c]	3.26 ± 0.49 [c]	9.55 ± 0.82	3.20 ± 0.12 [c]

Abbreviations: ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; GS[illegible] glutathione; BDL, bile duct ligati[illegible]
[a] Values are expressed as mean ± SD in 6 animals per group.
[b] Significant differences compared to the control group ($P < 0.05$).
[c] Significant differences compared to the BDL group ($P < 0.05$).
[d] Significant difference compared to the groups receiving ellagic acid at 10 and 25 mg/kg ($P <$ [illegible]

Table 2. Grading of Pathological Liver Tissue Damage in Cholestatic Animals and [illegible] of Ellagic Acid [illegible]nistration

Treatment	Confluent Necrosis	Focal Necr[illegible]is	P[illegible]lammation	Bile Duct Proliferation	Total Grade
Sham (vehicle+treated rats)	-	-	-	-	Normal
BDL rats	-	++	+++	++	4
BDL+ellagic acid (10 mg/kg)		+	+	-	3
BDL+ellagic acid (20 mg/kg)			+	+	2
BDL+ellagic acid (50 mg/kg)	-	-	+	+	2

Abbreviation: BDL, bile duct ligati[illegible]

Table 3. Grading of Patholo[illegible]cal Damages of Kidney Tiss[illegible] Cholestatic Animals and Impacts of Ellagic Acid Administration

Treatment	Focal Necrosis	Tubular Atrophy	Interstitial Inflammation	Vascular Congestion	Total Grade
Sham (vehicle+treated ra[illegible]		-	-	-	Normal
BDL rats		++	+++	++	4
BDL[illegible]llagic acid [illegible]mg/kg)	-	+	+	-	3
BDL+[illegible]ic acid ([illegible]0 mg/kg)	-	-	+	+	2
BDL+ellag[illegible]d (50 mg/kg)	-	-	+	+	2

Abbreviation: Bi[illegible]le duct ligation.

on cholemic nephropathy in cholestatic rats. Although the precise mechanisms involved in the pathogenesis of cholestatic nephropathy have yet to be clearly defined, multiple studies suggest a central role of oxidative stress and related events in the development of this condition (27).

Biochemical indicators (serum-urinary) were analyzed in this study to evaluate liver and kidney abnormalities. The results confirmed significant changes in biochemical indices (serum-urinary) in animals with BDL, inducing cholestasis and kidney abnormalities. Specifically, we observed a significant increase in oxidative stress

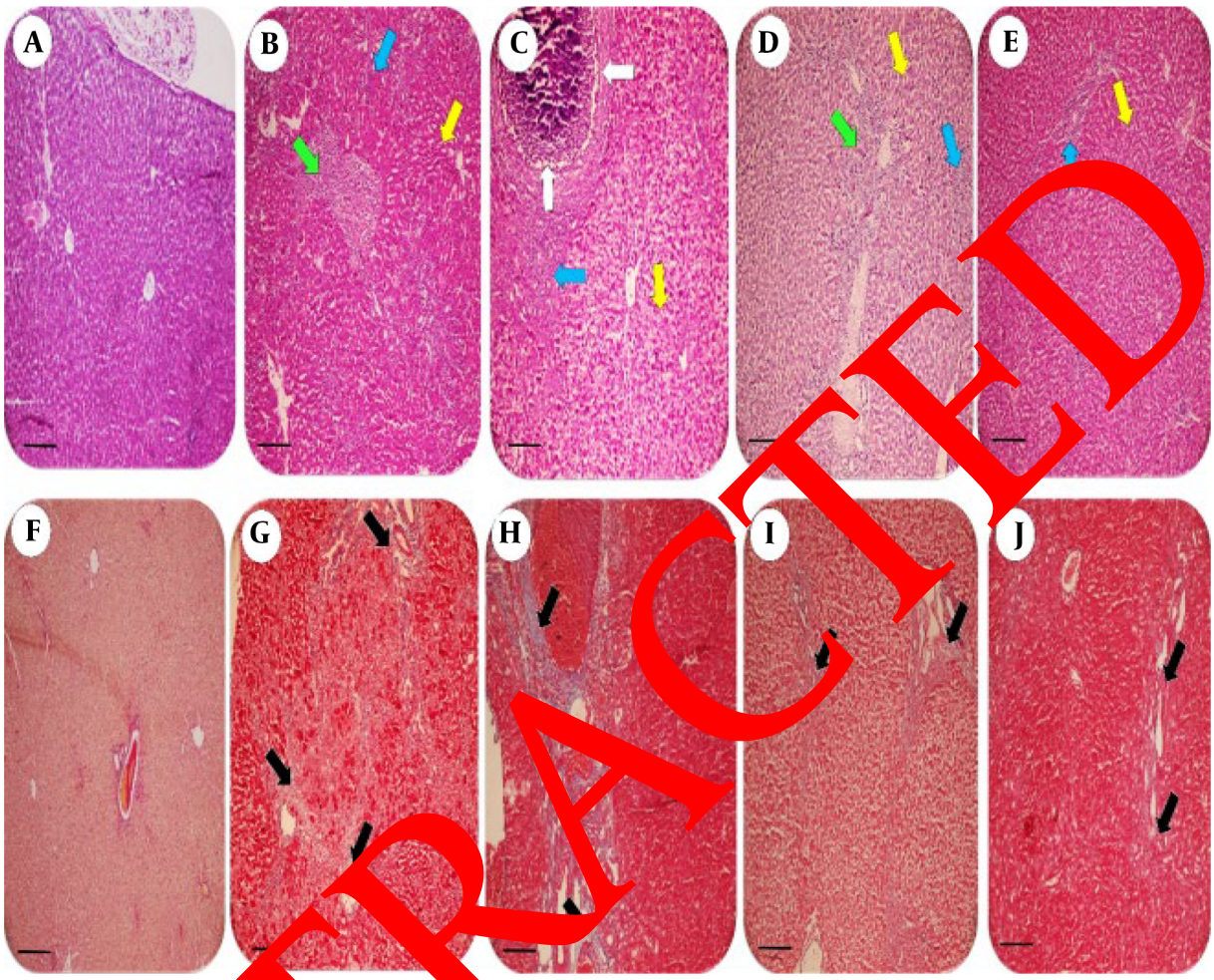

Figure 4. Histopathological changes of liver tissue samples in cholestatic animals (14 days after bile duct ligation surgery) and the effect of ellagic acid administration. The top row shows hematoxylin and eosin staining and the bottom row shows Masson's trichrome staining (to reveal the degree of tissue fibrosis). A and F represent the control group, where no specific changes are observed in liver tissue. B and G represent the bile duct ligation group, where histopathological changes appeared as tissue necrosis (white arrow). Bile duct proliferation (blue arrow), tissue inflammation (green arrow), and congestion of sinusoids (yellow arrow) also appeared. The black arrow in the bottom row represents the fibrotic changes in the liver tissue. C and H represent the group receiving ellagic acid at the rate of 10 mg/kg. D and I represent the group receiving ellagic acid at the rate of 25 mg/kg, and J represent the group receiving ellagic acid at the rate of 50 mg/kg.

biomarkers in the kidney and liver tissue samples of the animals subjected to BDL. Disturbances in cellular redox balance and destruction of macromolecules and critical cellular targets, such as DNA, lipids, and proteins, were evident in the tissue of all cholestatic animals.

Previous investigations have demonstrated the role of hydrophobic bile acids in the pathogenesis of cholestasis-related CN (28, 29). Orellana et al. assessed the effect of BDL on oxidative stress production and compared its effects in the liver and all tested animals (30). They concluded that disruption of the oxidant-antioxidant balance may contribute to cholestatic liver damage in the BDL rat model (27). Although no significant change was observed in the number of antioxidant enzymes in kidney tissue following BDL, lipid peroxidation was found to increase. As noted by Orellana et al., further research is required to fully evaluate the role of oxidative stress in renal dysfunction in cholestasis (30). The results obtained here are consistent with this suggestion.

Kaler et al. conducted a study to investigate the relationship between the concentration of bile acids in serum and urine, nephrotoxicity, and kidney function in rats subjected to a BDL operation for 15 days (31). Changes in the concentrations of bile acids, functional markers, and kidney damage were measured in serum and urine samples and compared with those of control animals that

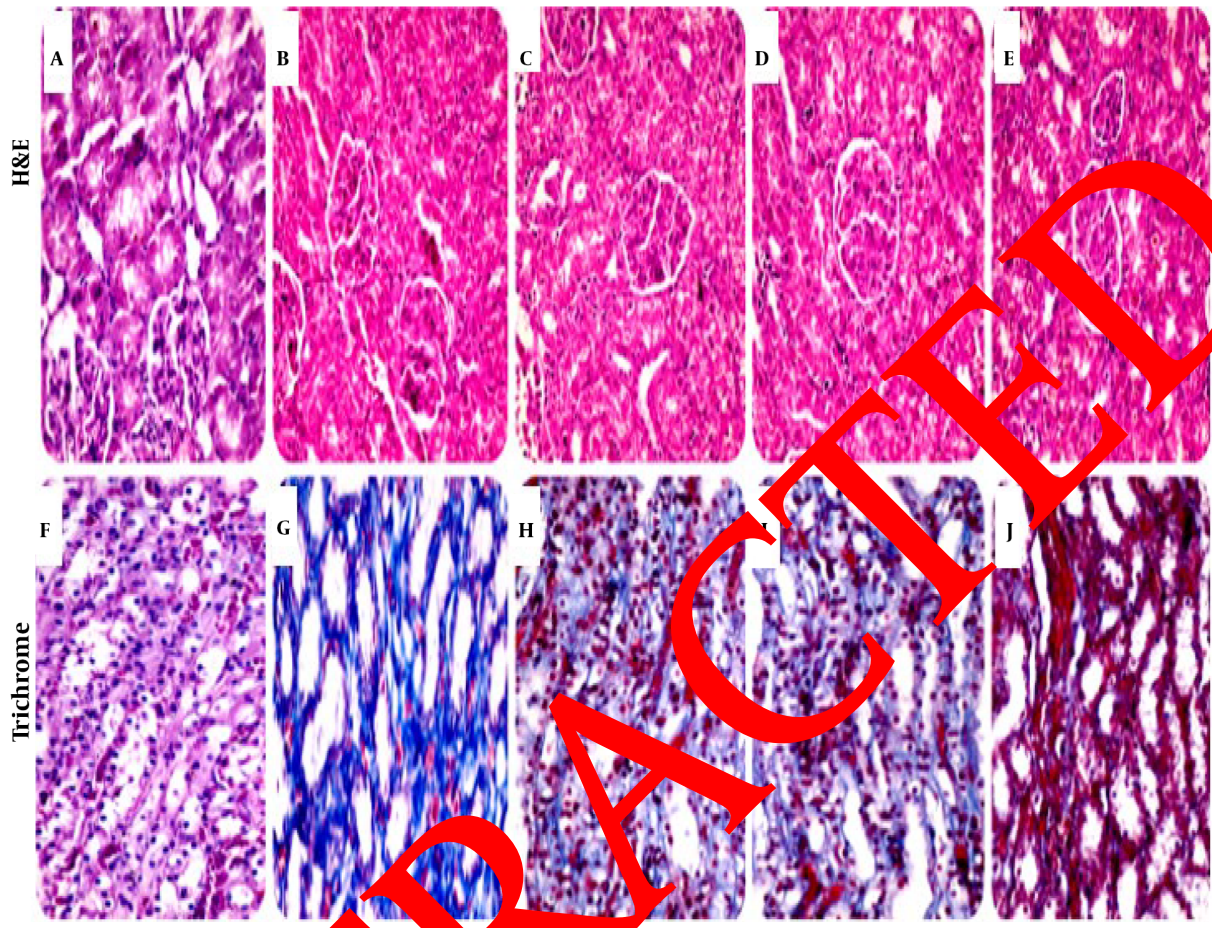

Figure 5. Histopathological changes of kidney tissue in cholestatic animals (14 days after bile duct ligation surgery [BDL]) and the effect of ellagic acid administration. The top row shows hematoxylin and eosin staining and the bottom row Masson's trichrome staining (to reveal the degree of tissue fibrosis). A and F represent the control group, where no specific changes are observed in their kidney tissue samples. B and G represent the BDL group in which histopathological changes appeared in the form of tubule atrophy, necrosis, inflammation, and congestion. In the bottom row, the image shows the fibrotic changes in the kidney tissue. C and H represent the group receiving ellagic acid at the rate of 10 mg/kg. D and I represent the group receiving ellagic acid at the rate of 25 mg/kg. E and J represent the group receiving ellagic acid at the rate of 50 mg/kg.

did not undergo BDL surgery (31). The findings of their study indicated that temporary renal dysfunction, water transfer, and ion-specific histological changes in proximal tubules occurred 3 - 4 days after the BDL operation, particularly at [illegible] and urinary concentrations of bile acids [illegible]. These observations are consistent with our results.

Besides examining the role of oxidative stress in cholestatic renal dysfunction, this study also evaluated histopathological and fibro-textural changes in liver and kidney tissues. Collagen deposition was identified as a prominent histopathological finding in all cholestatic animals, especially in the later stages of CN (days 14 and 28 after the BDL operation). Although tissue fibrosis is a complex process, several studies have established a close association between oxidative stress and fibrosis (27).

Ellagic acid, by virtue of the hydroxyl and methoxy groups present in its structure, is capable of inhibiting and trapping free radicals, thereby mitigating oxidative stress. Ellagic acid exhibits potent antioxidant properties in various pharmacological activities (32, 33). The results of recent research confirm the beneficial effects of ellagic acid on serum and urinary biomarkers, oxidative stress indices, and histopathological changes. For instance, Hwang et al. (34) investigated the antioxidant potential of ellagic acid and demonstrated its protective effect against oxidative stress-induced hepatocyte damage by preventing ROS production, cell damage, apoptosis and necrosis, and mitochondrial depolarization (a major cause of ROS). Furthermore, ellagic acid administration

was found to protect against liver injury caused by lightning-induced oxidative stress by preventing cell death and increasing GSH, ALT, and AST levels in rats (34). In another study, Pari and Sivasankari investigated the protective and antioxidant properties of ellagic acid against oxidative damage induced by cyclosporin A. These researchers reported that oral administration of different concentrations (12.5, 25, and 50) of ellagic acid in rats for 21 consecutive days significantly reduced oxidative stress markers and improved liver damage markers (ALT, AST, ALP, and LDH), as well as histopathological changes in liver tissue (35). Therefore, the aforementioned research results are consistent with our findings.

5.1. Conclusions

Oxidative stress has been widely recognized as a significant factor in the development and progression of various diseases, particularly kidney disorders. As a result of its substantial antioxidant properties, ellagic acid has emerged as a potential therapeutic strategy for addressing complications associated with kidney damage. However, further extensive studies in this field are required to gain a better understanding of its precise mechanisms.

Acknowledgments

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Footnotes

Authors' Contri[illegible]tion: AK conceived and designed the evalua[illegible]n and dra[illegible]d the ma[illegible]script. FM participated in desig[illegible]g the eva[illegible]tio[illegible], performed parts of the [illegible]tistical a[illegible]alysis, and h[illegible]ed to draft the manuscript. MA re[illegible]luate[illegible] data, revised the manuscript, performed the s[illegible]tical analysis, and revised the manuscript. NH and FK [illegible]e responsible for working with animals and performing [illegible]oratory tests. HM and AK conceived and designed the evaluation and drafted the manuscript. All authors read and approved the final manuscript.

Conflict of Interests: The first and third authors are cousins.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: IR.LUMS.REC.1401.011.

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