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Original Article

Trigonelline Attenuates Obstruction-Induced Jaundice in Experimental Rats by Inhibiting A-SMA/TGF-B/Smad-3 Expression

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ABSTRACT

Background and Objectives: Obstructive jaundice is characterized by excessive accumulation of bilirubin due to blockage of bile ducts. Chronic obstruction can result in various complications. Trigonelline, a plant alkaloid derived from fenugreek seeds, has been extensively studied for its hepatoprotective effects. This study aimed to investigate the putative mechanism of action of trigonelline in an experimental model of obstructive jaundice. **Materials and Methods:** Obstructive jaundice was induced in young Sprague-Dawley rats (140-150 g) by ligating their common bile duct. Then they were treated orally with vehicle (1% dimethyl sulfoxide), curcumin (20 mg/kg), or trigonelline (50, 100 and 200 mg/kg) for 28 days. **Results:** Trigonelline (200 mg/kg) exerted protective efficacy against obstructive jaundice, as depicted by marked inhibition (p<0.001) of elevated serum AST, ALT, ALP, GGT, total bilirubin and direct bilirubin. The molecular underpinnings suggested that obstruction-induced alterations in hepatic levels of SOD, GSH, MDA and NO were effectively (p<0.001) ameliorated by trigonelline. Furthermore, RT-PCR quantification of hepatic fibrotic markers showed that trigonelline effectively down-regulated (p<0.001) hepatic α-SMA, Collagen-1, TGF-β and Smad-3 mRNA expression. Furthermore, histopathological analysis of hepatic tissues suggested that trigonelline effectively (p<0.001) reduced obstruction-induced morphological perturbations. **Conclusion:** Trigonelline ameliorated obstructive jaundice via elevated oxido-nitrosative stress and up-regulated the mRNA expression of α-SMA, Collagen-1, TGF-β and Smad-3 in hepatic tissue. Thus, trigonelline can be considered a valuable therapeutic agent for the management of obstructive jaundice.

Keywords: Collagen-1, Obstructive jaundice, Smad-3, TGF-β, Trigonelline, α-SMA.

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INTRODUCTION

Obstructive jaundice is a medical condition characterized by the accumulation of bilirubin in the body, resulting in a yellowish discoloration of the skin and eyes.[1] This condition arises from an obstruction in the biliary tract, which can have various underlying causes, including gallstones, tumors and strictures.[2] Obstructive jaundice is less common in young patients compared to older adults, primarily due to differences in the underlying causes of bile duct obstruction.[2] While it is typically seen in older adults, particularly those with gallstones or malignancies, certain conditions such as biliary atresia (estimated incidence is around 1:10,000-15,000 live births), choledochal cysts (1:100,000-150,000 people), parasitic infections, primary sclerosing cholangitis and autoimmune diseases are more commonly encountered in younger individuals.[3,4] Thus, proper diagnosis and treatment of obstructive jaundice are crucial, as it can have serious consequences if remains unaddressed.

The pathophysiology of obstructive jaundice involves the disruption of the normal flow of bile from the liver to the duodenum, resulting in the accumulation of bilirubin in the bloodstream. This excess bilirubin then seeps out of the blood vessels into the surrounding fatty tissue, leading to the characteristic yellowish discoloration of the skin and mucous membranes.[5] Furthermore, choledocholithiasis, or the presence of gallstones in the bile duct, as well as malignancies such as cholangiocarcinoma, periampullary tumors and pancreatic tumors, are several other causes of obstructive jaundice.[6] In addition, benign strictures, such as those resulting from chronic pancreatitis and iatrogenic causes, such as biliary tract damage from invasive procedures, can also lead to obstructive jaundice.[7] During obstructive jaundice, the accumulated bile acids, inflammation and mitochondrial dysfunction lead to hepatic oxidative stress, which contributes to liver injury.[6] The excessive

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production of Reactive Oxygen Species (ROS) damages lipids, proteins and DNA, which are responsible for producing collagen and Extracellular Matrix (ECM) components and, eventually, fibrosis.[8] Thus, researchers have focused on antioxidant therapies for the timely management of the obstruction by inhibiting oxidative stress and preventing further liver damage.

Accurate diagnosis of obstructive jaundice requires a comprehensive approach, including thorough medical history, physical examination and laboratory tests. The initial steps often involve assessing the patient's symptoms, such as abdominal pain, nausea and weight loss, as well as conducting a physical examination to identify signs of jaundice and potential underlying causes.[2] The liver is the primary target organ for many of the toxicities, as it is the first organ exposed to everything absorbed in the small intestine and the liver is responsible for the metabolism of foreign substances to compounds, which may lead to hepatotoxicity.[9] Thus, liver function tests in combination with bilirubin levels help to differentiate between obstructive and non-obstructive jaundice. Patients with obstructive jaundice typically present with a gradual onset of jaundice, accompanied by other symptoms such as pruritus, dark urine and light-colored stool.[10]

Current treatments for obstructive jaundice primarily aim to relieve bile duct obstruction, restore normal bile flow, prevent further liver damage and improve patient outcomes.[5,11] Surgical interventions, including cholecystectomy and biliary bypass surgery, have been effective but are associated with significant costs. Pharmacological treatments such as cholestyramine, broad-spectrum antibiotics (e.g., ceftriaxone and metronidazole) and antihistamines (e.g., hydroxyzine) have been widely used; however, their unwanted side effects limit their clinical usage.[5] Palliative care, such as biliary stent placement, pain management, nutritional support and malabsorption management, are also important treatment options; however, they focus on symptom relief rather than cure.[5] Thus, there is an urgent need to identify new therapeutic agents with significant efficacy and minimal adverse events.

Trigonelline is a plant alkaloid widely isolated from fenugreek seeds and is also detected in other plant species, including *Coffea arabica* (coffee), *Medicago sativa* (alfalfa) and *Trifolium incarnatum* (Clover).[12] Fenugreek,[13] as well as its active component trigonelline,[14] have been Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (FDA). Over the past decades, extensive research on trigonelline has uncovered its multi-targeted therapeutic effects across various pathological conditions, including neurodegenerative diseases (such as peripheral neuropathy, stroke, Parkinson's and Alzheimer's disease), metabolic syndrome, lipid homeostasis, cancers, inflammation-related disorders and oxidative stress.[15] Trigonelline exhibits hepatoprotective effects in diverse animal models, including diabetes-induced hepatotoxicity, non-alcoholic fatty liver disease[16,17] and carbon tetrachloride-induced hepatotoxicity.[18] These hepatoprotective effects of trigonelline are attributed to its ability to antioxidant, anti-inflammation, antifibrotic and antiapoptotic potential. Although this experimental data is promising, its potential against obstructive jaundice has yet to be evaluated. Therefore, this study aimed to investigate the putative mechanism of action of trigonelline in an experimental model of obstructive jaundice.

MATERIALS AND METHODS

Animals

Young adult male Sprague Dawley rats (5-6 weeks, 140-150 g) were purchased from Xi'an Gaoxin Hospital, Xi'an, China and quarantined for one week in-house at the institute animal house under standard laboratory conditions, that is, a temperature of 24±1°C, relative humidity of 45-55% and normal light/dark cycle. Animals had free access to standard chow-pelleted food and water. The experimental protocol was approved (approval no. 478261SY) by the Institutional Animal Ethics Committee of Kangping Experimental Animal Research Institute, China and was performed in accordance with the guidelines of the National Institute of Health Guide for Care and Use of Laboratory Animals.

Induction of obstructive jaundice

Obstructive jaundice was induced in young Sprague-Dawley rats according to a previously reported method.[19] Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.30 mL/0.1/kg) and midline laparotomy was performed under sterile conditions. The Common Bile Duct (CBD) was identified and freed from the surrounding soft tissue. Then, it ligated with 4-0 silk and the abdominal incisions were closed in 2 layers with continuous 3-0 silk sutures. The animals were allowed access to food postoperatively.

Experimental groups

Animals were randomly divided into various groups (n=15) as follows:

Sham group: Rats were subjected to the surgical procedure but without ligation of the CBD. They were treated with a vehicle (10 g/kg of 1% aqueous DMSO (Dimethyl Sulfoxide [DMSO) solution, p.o.) for 28 days.

Vehicle control group: Rats were subjected to the surgical procedure for CBD ligation. They received treatment with vehicle (10 g/kg of 1% aqueous DMSO solution, p.o.) for 28 days.

Curcumin (20 mg/kg)-treated group: Rats were subjected to the surgical procedure for CBD ligation. They were then treated with curcumin (20 mg/kg, p.o., purity>98%, Sigma-Aldrich Co., St Louis, MO, USA) for 28 days.

Trigonelline (50 mg/kg)-treated group: Rats were subjected to the surgical procedure for CBD ligation. They were then treated

with trigonelline (50 mg/kg, p.o., purity>98%, Sigma-Aldrich Co., St Louis, MO, USA) for 28 days.

Trigonelline (100 mg/kg)-treated group: Rats were subjected to the surgical procedure for CBD ligation. They were then treated with trigonelline (100 mg/kg, p.o.) for 28 days.

Trigonelline (200 mg/kg) treated group: Rats were subjected to the surgical procedure for CBD ligation. They were then treated with trigonelline (200 mg/kg, p.o.) for 28 days.

Previous reports were used to determine the treatment doses of trigonelline (50, 100, 200 mg/kg).[16,20]

Serum biochemistry

On day 28, at the end of the reperfusion period (24 hr), blood was withdrawn by the retro-orbital plexus and serum was obtained by centrifugation at 8350 g for 10 min at 4°C. Serum albumin, Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Aminotransferase (ALT), Gamma-Glutamyl Transferase (GGT), total bilirubin and direct bilirubin levels were measured using reagent assay kits (Accurex Biomedical Pvt. Ltd., Mumbai, India) and an ultraviolet-visible spectrophotometer (JASCO-V-530, JASCO Corp., Tokyo, Japan).

Biochemical estimation

Tissue homogenate preparation and estimation of oxidative stress

All animals were sacrificed under ethereal anesthesia at the end of the study, that is, on the 29th day, the liver was immediately isolated. One portion of the liver was used to prepare homogenate using 0.1 M Tris-HCl buffer (pH 7.4) and supernatant of homogenates was employed to estimate Superoxide Dismutase (SOD), reduced Glutathione (GSH), lipid peroxidation (MDA, i.e., Malondialdehyde) and Nitric Oxide (NO) content as described previously.[21]

Reverse Transcriptase PCR

Another portion of liver was used to estimate mRNA expressions of α-SMA (Forward: 5′-GTTTGACTTCAATGTCCC-3′, Reverse: 5′-CGATCTCACGCTCAGCAGTGA-3′, base pair (bp): 334), Collagen-1 (Forward: 5′-GAGCGGAGAGTACTGGATCG-3′, Reverse: 5′-GG TTCGGGCTGATGTACCAG-3′, bp: 218), TGF-β (Forward: 5′-GTTCTTCAATACGTCAGACATTCG-3′, Reverse: 5′ CATTATCTTTGCTGTCA CAAGAGC-3′, bp: 309), Smad-3 (Forward: 5′-AGCACACAATAACTTGGACC-3′, Reverse: 5′-TAAGA CACACTGGAACAGCGGATG-3′, base pair (bp): 368), β-actin (Forward: 5′-GCCATGTACGTAGCCA TC-3′, Reverse: 5′-GAACCGCTCATTGCCGAT-3′, bp: 375) using quantitative reverse transcription-polymerase chain reaction (qRT–PCR) according to a method described elsewhere.[22] PCR was performed using 1 X forward and reverse primers and 2.5 U Taq polymerase (MP Biomedicals India Pvt Ltd., India). β-actin served as a control for sample loading and integrity.

Histological analysis

Histopathological analysis of the liver tissue was carried out using Hematoxylin and Eosin (H&E) staining under a light microscope (Nikon E200, Japan) and was graded as grade 0 (not present), grade 1 (slight/minimal), grade 2 (mild), grade 3 (moderate), or grade 4 (severe). Cirrhosis Ishak scoring was performed using a previously described method.[19]

Statistical analysis

GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA) was used for data analysis. Data are expressed as mean±Standard Error Mean (SEM) and were analyzed using One-Way ANOVA followed by Tukey's multiple range post hoc analysis (for parametric tests) and the Kruskal-Wallis test for post hoc analysis (non-parametric tests). Correlation analysis was performed using a two-sided Fisher's exact test. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of trigonelline on body weight, liver index in rats

Compared to the sham group, the induction of obstructive jaundice did not cause any significant change in body weight in the vehicle control group. Treatment with curcumin and trigonelline (50, 100 and 200 mg/kg) also resulted in no significant changes in body weight (Table 1). However, compared to the sham group, the induction of obstructive jaundice caused a significant increase ($p<0.001$) in liver weight (absolute liver weight) and liver weight to body weight ratio (relative liver weight) in the vehicle control group. In contrast, treatment with curcumin resulted in a significant attenuation ($p<0.001$) in absolute and relative liver weights compared to the vehicle control group. When compared with vehicle control rats, trigonelline (200 mg/kg)-treated rats also showed a significant decrease ($p<0.001$) in absolute and relative liver weights. Administration of trigonelline (100 and 200 mg/kg) did not show any significant protection against increased hepatic weights (Table 1).

Effect of trigonelline on hepatic function tests in rats

On the 28th day, serum ALP, AST, ALT, GGT, total bilirubin and direct bilirubin levels in the vehicle control group showed a significant ($p<0.001$) increase, whereas serum albumin levels showed a significant ($p<0.001$) decrease when compared to the sham group. On the other hand, treatment with curcumin showed a significant ($p<0.001$) decrease in serum ALP, AST, ALT, GGT, total bilirubin and direct bilirubin, as well as a significant ($p<0.001$) increase in serum albumin, compared to the vehicle control group. Treatment with trigonelline (200 mg/

kg) significantly attenuated obstruction-induced alterations in serum albumin, ALP, AST, ALT, GGT, total bilirubin and direct bilirubin levels compared with the vehicle control group. Trigonelline (50 and 100 mg/kg) failed to significantly inhibit obstruction-induced alterations in serum albumin, ALP, AST, ALT, GGT, total bilirubin and direct bilirubin levels compared with the vehicle control group (Table 1).

Effect of trigonelline on hepatic protein and oxido-nitrosative stress in rats

The hepatic SOD and GSH levels in the vehicle control rats were significantly lower ($p<0.001$) than those in the sham rats. SOD and GSH levels in the hepatic tissue of curcumin-treated rats were significantly higher ($p<0.001$) than those in the vehicle control rats. Treatment with trigonelline for 28 days (200 mg/kg) significantly attenuated ($p<0.001$) this obstruction-induced decrease in the levels of SOD and GSH in hepatic tissue compared to vehicle control rats (Table 2).

In contrast, there was a significant increase in hepatic total protein, MDA and NO levels in vehicle control rats compared to sham rats. Compared with vehicle control rats, total protein, MDA and NO levels in the hepatic tissue of curcumin rats were significantly decreased ($p<0.001$). Administration of trigonelline (200 mg/kg) significantly ($p<0.001$) reduced the levels of total protein, MDA and NO in hepatic tissue compared to vehicle control rats. However, trigonelline (50 and 100 mg/kg) treatment failed to produce any significant changes in obstruction-induced alterations in total protein, SOD, GSH, MDA and NO levels in hepatic tissue compared with vehicle control rats (Table 2).

Effect of trigonelline on hepatic α-SMA, Collagen-1, TGF-β and Smad-3 mRNA expressions in rats

CBD obstruction caused significant upregulation ($p<0.001$) in hepatic α-SMA, Collagen-1, TGF-β and Smad-3 mRNA expression in the vehicle control group when compared to the sham group on day 28. Curcumin treatment significantly ($p<0.001$) downregulated hepatic α-SMA, Collagen-1, TGF-β and Smad-3 mRNA expression levels compared to those in the vehicle control group. Treatment with trigonelline (200 mg/kg) also significantly ($p<0.001$) downregulated hepatic α-SMA, Collagen-1, TGF-β and Smad-3 mRNA expression compared to that in the vehicle control group on day 28. Trigonelline (50 and 100 mg/kg) showed a non-significant downregulation in hepatic α-SMA, Collagen-1, TGF-β and Smad-3 mRNA expression compared to the vehicle control group on day 28 (Figure 1).

Serum total bilirubin levels linearly correlated with hepatic α-SMA ($R^2=0.8334$, $p<0.001$), Collagen-1 ($R^2=0.8689$, $p<0.001$), TGF-β ($R^2=0.864$, $p<0.001$) and Smad-3 ($R^2=0.8697$, $p<0.001$) mRNA expression (Figure 2).

Table 1: Effect of trigonelline treatment on body weight, liver index and serum biochemistry in rats.

Parameters	Treatment					
	Sham	Vehicle control	C (20)	T (50)	T (100)	T (200)
Body weight (g)	236.80±1.30	234.00±1.16	232.70±1.61	234.80±1.56	236.50±0.92	235.03±1.76
Liver Weight (g)	5.44±0.24	8.85±0.17$^{\#\#\#}$	6.15±0.14***	8.39±0.18	7.81±0.25	7.12±0.19***
Liver Index ($x10^{-3}$)	22.94±0.95	37.81±0.74$^{\#\#\#}$	26.43±0.65***	35.71±0.61	33.03±1.02	30.22±0.61***
AST (IU/L)	92.49±2.42	301.80±2.50$^{\#\#\#}$	108.80±2.34***	300.30±3.01	277.30±3.07	159.80±2.40***
ALT (IU/L)	31.58±1.71	141.90±2.51$^{\#\#\#}$	48.99±3.06***	129.50±2.67	119.30±1.86	93.69±1.27***
ALP (IU/L)	60.33±2.08	199.30±4.55$^{\#\#\#}$	99.46±1.53***	181.90±3.36	183.60±3.99	140.00±3.97***
Albumin (g/dL)	6.03±0.17	2.47±0.14$^{\#\#\#}$	5.07±0.22***	2.00±0.13	2.67±0.20	4.41±0.26***
Total bilirubin (mg %)	0.13±0.01	0.42±0.01$^{\#\#\#}$	0.17±0.01***	0.38±0.01	0.35±0.01	0.22±0.01***
Direct bilirubin (mg %)	0.20±0.01	0.71±0.01$^{\#\#\#}$	0.21±0.01***	0.71±0.01	0.67±0.01	0.40±0.01***
GGT (IU/L)	14.48±1.12	70.65±0.74$^{\#\#\#}$	29.81±0.65***	66.74±0.85	64.25±0.54	41.36±0.81***

Data are presented as the mean±SEM ($n=6$). Data were analyzed using one-way ANOVA variance followed by Tukey's multiple range test. $^{\#\#\#}p<0.05$, compared to the sham group and ***$p<0.05$, compared to the vehicle control group. The figures in parentheses indicate the oral doses in mg/kg. VC, vehicle control; C (20), curcumin (20 mg/kg) treated; T (50), trigonelline (50 mg/kg) treated; T (100), trigonelline (100 mg/kg) treated; T (200), trigonelline (200 mg/kg)-treated rats; AST: Aspartate transaminase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase.

Table 2: Effect of trigonelline treatment on total hepatic protein and oxidative stress levels in rats.

Parameters	Treatment					
	Sham	Vehicle control	C (20)	T (50)	T (100)	T (200)
Total protein (mg/mL)	38.38±3.08	75.38±1.44###	50.62±2.03***	72.91±2.94	71.69±2.30	55.11±2.01***
SOD (U/mg of protein)	6.52±0.09	2.54±0.12###	5.36±0.11***	2.61±0.17	3.21±0.12	4.78±0.14***
GSH (µg/mg of protein)	0.17±0.01	0.05±0.01###	0.15±0.01***	0.07±0.02	0.07±0.01	0.14±0.01***
MDA (nM/mg of protein)	0.25±0.02	0.95±0.01###	0.35±0.02***	0.73±0.03	0.67±0.02	0.49±0.02***
NO (µg/mL)	106.50±2.58	252.40±2.04###	134.50±2.88***	248.80±2.40	238.70±3.88	179.90±3.36***

Data are represented as mean±SEM (n=6) and were analyzed by one-way ANOVA followed by Tukey's multiple range test. ###p<0.05, compared to the sham group and ***p<0.05, compared to the vehicle control group. Figures in parentheses indicate oral dose in mg/kg. VC: Vehicle control; C (20): Curcumin (20 mg/kg) treated; T (50): trigonelline (50 mg/kg) treated; T (100): trigonelline (100 mg/kg) treated; T (200): trigonelline (200 mg/kg) treated rats; SOD: Superoxide dismutase; GSH: Glutathione; MDA: Malondialdehyde; NO: Nitric oxide.

Effect of trigonelline on hepatic histopathology of rats

The normal central vein with portal triads, without any evidence of necrosis or edema (grade 0), reflected the normal architecture of the liver tissue from sham rats (Figure 3A). However, a mild inflammatory infiltration was observed. Histopathological studies of the liver tissue from vehicle control rats showed significant (p<0.001) inflammatory infiltration around the centrilobular veins, edema and a higher cirrhosis Ishak score (Figure 3B). Liver tissue from curcumin-treated rats showed effective (p<0.001) amelioration of obstruction-induced congestion, edema, inflammatory infiltration and cirrhosis Ishak score (Figure 3C). Furthermore, trigonelline (200 mg/kg)-treated rats also showed moderate damage to the liver architecture, as revealed by a significant (p<0.001) reduction in necrosis, congestion, cirrhosis Ishak score and inflammatory cell infiltration (Figure 3D).

DISCUSSION

Obstructive jaundice results from blockage of bile ducts and thus prevents bile flow into the intestines.[10] The resultant accumulated bile in the liver caused increased bilirubin in serum. Chronic obstructive jaundice results in nutritional deficiency, acute renal failure, infectious complications and impaired cardiovascular function.[1,3] Thus, timely diagnosis and treatment are critical for managing obstructive jaundice and preventing complications. While current treatment options for obstructive jaundice can be effective, especially in benign cases such as gallstones, significant challenges remain, particularly in malignant or chronic conditions.[5] The risks as invasive procedures, recurrent obstruction, complications from surgery and liver dysfunction, which require careful management.

The current study evaluated the protective efficacy of trigonelline against obstructive jaundice by assessing serum albumin, AST, ALT, ALP, GGT, total bilirubin and direct bilirubin levels, providing insights into the systemic effects of the constricted common bile duct and the potential mitigating role of trigonelline. To unravel the molecular mechanisms, this study delved into the levels of SOD, GSH, MDA and NO in hepatic tissue homogenates, followed by quantification of hepatic fibrotic markers such as α-SMA, Collagen-1, TGF-β and Smad-3 mRNA expression to shed light on the specific influence of trigonelline on molecular pathways implicated in obstruction-induced jaundice. Finally, histopathological evaluation of hepatic tissues elucidates the morphological changes induced by obstruction and the efficacy conferred by trigonelline.

During obstructive jaundice, elevated levels of toxic bile acids, along with impaired bile flow, result in oxidative stress, a major contributor to liver damage, apoptosis, inflammation and fibrosis.[5] Thus, oxidative stress plays a major role in the pathogenesis of liver injury during obstructive jaundice and contributes to the progression of liver dysfunction, inflammation and cellular damage.[6] The impaired liver function reduces the ability of the liver to produce and regenerate key antioxidant enzymes (such as SOD and catalase).[22,23] Glutathione, one of the most important antioxidants in the liver, is depleted in obstructive jaundice and impairs the liver's capacity to neutralize ROS. Furthermore, excessive ROS generated during obstructive jaundice leads to lipid peroxidation, which damages cellular membranes, mitochondrial DNA, proteins and other cellular components, leading to apoptosis and necrosis of hepatocytes.[19,24] This imbalance between ROS production and the ability of the liver to detoxify harmful molecules results in oxidative damage. Obstructive jaundice is also associated with vascular dysfunction in the liver and other organs, partly due to oxidative stress.[19] Nitric oxide, a key regulator of vascular tone, can react with ROS to form peroxynitrite, a highly reactive and damaging molecule that contributes to endothelial cell injury.[25-27] This further impairs blood flow to the liver, worsening the overall hepatic dysfunction. Thus, managing oxidative stress through antioxidant therapy and

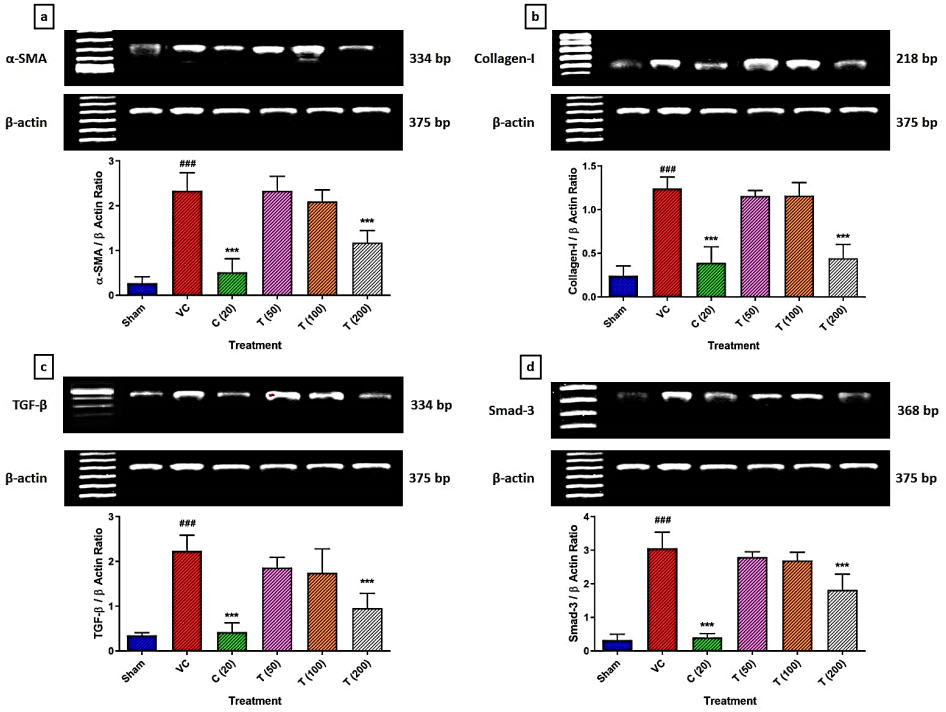

Figure 1: Effect of trigonelline treatment on hepatic α-SMA (a), Collagen-1 (b), TGF-β (c) and Smad-3 (d) mRNA expressions in rats.

Data were represented as Mean±SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple range test. ###p<0.05 as compared with sham group and ***p<0.05 as compared with Vehicle control group. Figures in parentheses indicate oral dose in mg/kg. VC: Vehicle control; C (20): Curcumin (20 mg/kg) treated; T (50): trigonelline (50 mg/kg) treated; T (100): trigonelline (100 mg/kg) treated; T (200): trigonelline (200 mg/kg) treated rats; α-SMA: alpha-smooth muscle actin; TGF-β: Transforming growth factor beta; Smad-3: Mothers against decapentaplegic homolog 3.

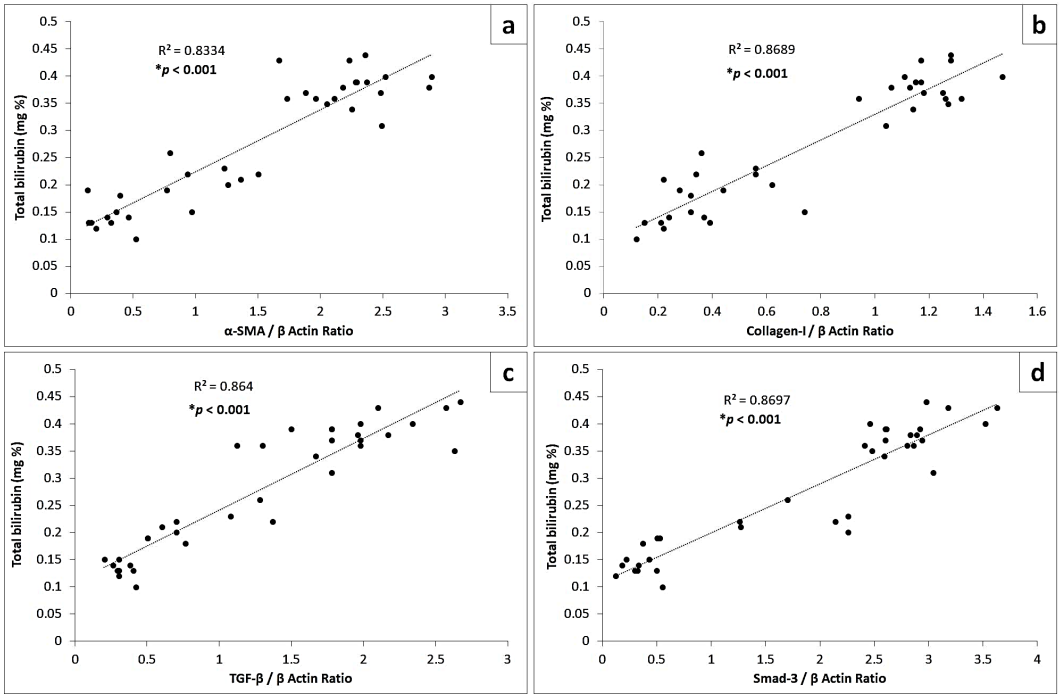

Figure 2: Simple regression of hepatic α-SMA (a), Collagen-1 (b), TGF-β (c) and Smad-3 (d) mRNA expression levels of total bilirubin in rats.

Correlation coefficients were determined using a two-sided Fisher's test.

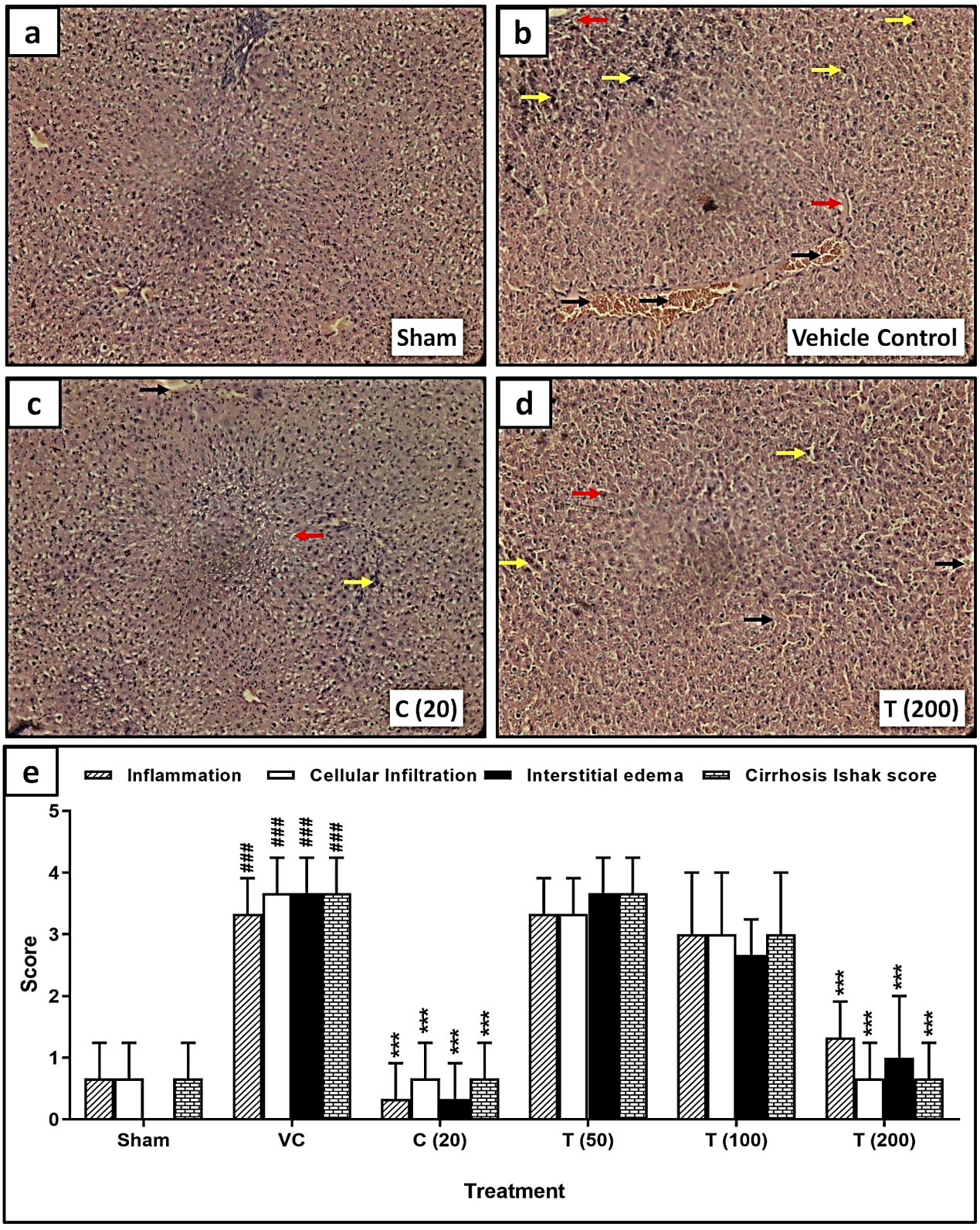

Figure 3: Effect of trigonelline treatment on hepatic histopathology in rats.

Photomicrograph of hepatic tissue sections from sham (a), vehicle control (b), curcumin (20 mg/kg)-treated (c) and trigonelline (200 mg/kg)-treated (d) rats (H&E stain). Quantitative representation of the histological score (e). Data are expressed as mean±SEM (n=3) and one-way ANOVA followed by the Kruskal-Wallis test was applied for *post hoc* analysis. ###p<0.05, compared to the sham group and ***p<0.05, compared to the vehicle control group. VC: Vehicle control; C (20): curcumin (20 mg/kg) treated; T (50): trigonelline (50 mg/kg) treated; T (100): trigonelline (100 mg/kg) treated; T (200): trigonelline (200 mg/kg) treated rats. Congestion (red arrow), edema (yellow arrow), inflammatory infiltration (black arrow).

improving bile flow is the primary focus of researchers as they aim to mitigate liver injury and improve patient outcomes. In the present study, chronic obstruction of the common bile duct resulted in elevated oxido-nitrosative stress, which was alleviated by trigonelline treatment, as reflected by diminished MDA and nitric oxide levels along with elevated SOD and GSH levels. A previous study also reported the hepatoprotective efficacy of trigonelline via inhibition of elevated oxidative stress,[16,17,28] and the results of the present study are in accordance with the findings of these investigators.

Obstructive jaundice can trigger a cascade of molecular events leading to liver injury and fibrosis and one of the key players in this process is α-SMA.[29] In the context of liver damage caused by obstructive jaundice, α-SMA is primarily associated with activating Hepatic Stellate Cells (HSCs), which play a central role in liver fibrosis.[30] Upon activation, HSCs differentiate into myofibroblast-like cells, characterized by the expression of α-SMA, a marker of myofibroblast activity.[31] These activated HSCs are critical in ECM production, primarily secreting collagen and other fibrotic components.[32,33] This leads to liver fibrosis. Oxidative stress plays a major role in promoting fibrosis during obstructive jaundice.[29] Accumulated hepatic ROS drive HSCs activation and upregulation of α-SMA expression. Additionally, TGF-β is a key cytokine involved in the activation of HSCs and induction of α-SMA expression.[34,35] In obstructive jaundice, liver injury up-regulates TGF-β, promoting the differentiation of HSCs into myofibroblasts. TGF-β stimulates the production of ECM proteins such as Collagen-1, enhancing the fibrotic response.[36] Furthermore, α-SMA-positive myofibroblasts are the primary drivers of fibrosis. These cells deposit type I collagen and other ECM proteins, resulting in the stiffening of the liver tissue. In the present study, α-SMA, a critical marker of hepatic fibrosis, was reflected by its elevated expression in the obstruction-induced vehicle control rats. In contrast, trigonelline treatment downregulated this expression, suggesting its antifibrotic potential. Subsequently, trigonelline ameliorated TGF-β and collagen-I expression in the hepatic tissue. Recently, trigonelline treatment inhibited renal fibrosis via amelioration of elevated α-SMA and collagen expressions in diabetic kidneys.[31] The findings of the current investigation corroborate with the results of a previous researcher.[31]

One of the key molecular pathways implicated in hepatic fibrosis in obstructive jaundice is the TGF-β/Smad signaling pathway, particularly involving Smad-3.[37] TGF-β signaling is primarily mediated through the Smad family of proteins, particularly Smad-2 and Smad-3.[38,39] Upon activation by TGF-β, these proteins translocate to the nucleus and regulate the expression of genes involved in ECM production and fibrosis.[40] Smad-3 plays a crucial role in the activation of HSCs via binding of TGF-β to its receptor that further triggers the phosphorylation of Smad-3, which then forms a complex with Smad-4 and translocates to the nucleus to induce the expression of fibrogenic genes such as collagen-I and fibronectin.[41] Thus, Smad-3-mediated transcription promotes the production of ECM proteins, contributing to the development of liver fibrosis during obstructive jaundice.[37] Previously, several studies have shown that bile duct obstruction exhibits upregulation of TGF-β and Smad-3, which is associated with the progression of liver fibrosis,[37,42] and in the present investigation also, vehicle control rats exhibited up-regulated TGF-β and Smad-3 mRNA expressions. Gong *et al.* (2023) and Zhang *et al.*, (2024) showed that trigonelline attenuated renal and pulmonary fibrosis via inhibition of TGF-β/Smad signaling activation,[31,43] and the results of the present study are in line with these findings, where trigonelline inhibited liver fibrosis during obstructive jaundice via inhibition of the TGF-β/Smad signaling pathway.

Herbal medicine has been explored as a complementary or alternative treatment option for managing liver disorders, including obstructive jaundice.[2,11] Several herbs have been traditionally used for their hepatoprotective, anti-inflammatory and antioxidant properties, which may help alleviate the symptoms of obstructive jaundice and support liver function.[6,10,11] A case study demonstrated the efficacy of Ayurvedic preparation containing *Picrorhiza kurroa* against obstructive jaundice.[11] In another study, patients with malignant obstructive jaundice (n=52) received Inchinkoto, a traditional Japanese medicine and showed improved bile flow.[44] Trigonelline, a plant alkaloid widely present in fenugreek, showed its therapeutic benefits in various clinical studies, including type 2 diabetes,[45-48] lipidemia[49] and metabolic syndrome.[50] Thus, trigonelline can be considered for further evaluation for obstructive jaundice treatment in clinical settings.

CONCLUSION

The findings of the present study suggest that trigonelline ameliorates obstructive jaundice via elevated oxido-nitrosative stress and up-regulated mRNA expression of α-SMA, Collagen-1, TGF-β and Smad-3 in hepatic tissue. Thus, trigonelline can be considered a valuable therapeutic moiety for the management of obstructive jaundice.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ABBREVIATIONS

α-SMA: Alpha-smooth muscle actin; **ALT:** Alanine aminotransferase; **ALP:** Alkaline phosphatase; **AST:** Aspartate transaminase; **GGT:** Gamma-glutamyl transferase; **GSH:** Glutathione; **MDA:** Malondialdehyde; **NO:** Nitric oxide; **ROS:** Reactive Oxygen Species; **RT-PCR:** Reverse transcription-polymerase chain reaction; **Smad-3:** Mothers against decapentaplegic homolog 3; **SOD:** Superoxide dismutase; **TGF-β:** Transforming growth factor beta.

AUTHOR CONTRIBUTION

The authors declare that all data was generated in-house and no paper mill was used.

Xiaoning Fu: Concepts, Design, Manuscript preparation, Manuscript editing, Manuscript review.

Ying Liu: Experimental studies, Data acquisition, Manuscript editing, Manuscript review.

ETHICAL STATEMENTS

The experimental protocol was approved (approval no. 478261SY) by the Institutional Animal Ethics Committee of Kangping Experimental Animal Research Institute, China and performed in accordance with the guidelines of the National Institute of Health Guide for Care and Use of Laboratory Animals.

SUMMARY

Trigonelline, a plant alkaloid from fenugreek seeds, has been extensively studied for its hepatoprotective potential. Obstructive jaundice is characterized by excessive accumulation of bilirubin due to blockage of the bile ducts. Chronic obstruction may result in various complications. Bearing this evidence in mind, this study aimed to investigate the putative mechanism of action of trigonelline against an experimental model of obstructive jaundice. These findings emphasize that trigonelline ameliorated obstructive jaundice via elevated oxido-nitrosative stress and up-regulated the mRNA expression of α-SMA, Collagen-1, TGF-β and Smad-3 in hepatic tissue.

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