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Original Article

Sitagliptin protects against bile duct ligation-induced cholemic nephropathy in male rats through modulation of inflammation, sestrin2, and the Nrf2/SOD pathway

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Abstract

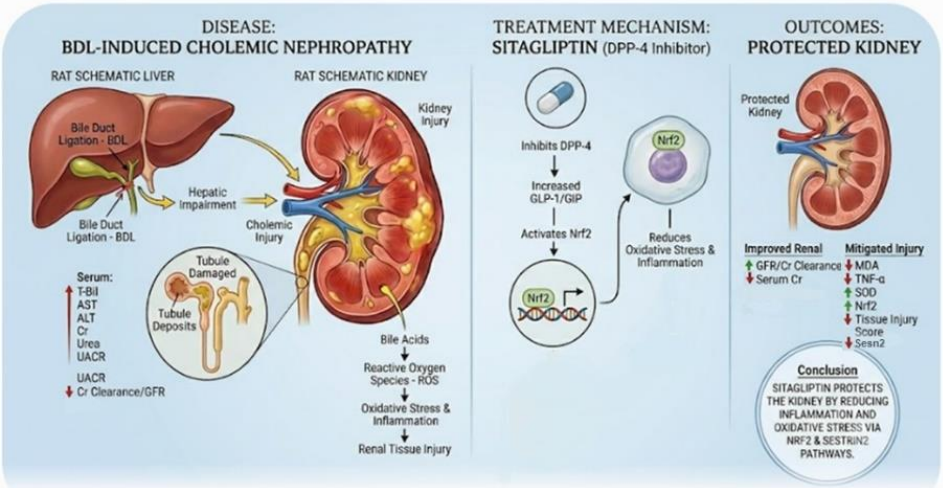
Background and purpose: Cholestasis or bile duct ligation (BDL) could develop cirrhosis and may lead to other organ dysfunction, including the kidneys, which is called cholemic nephropathy (CN). Hyperbilirubinemia, bile acid accumulation, renal hypoperfusion, oxidative stress, and inflammation are implicated in the pathogenesis of CN. Sitagliptin is an oral antihyperglycemic drug with anti-inflammatory and anti-oxidative effects. Its effects on CN were unknown.

Experimental approach: The BDL-induced CN model was performed in 35 male Wistar rats (200-250 g), which were divided into five groups (n = 7) as follows: sham group receiving distilled water as vehicle (sham + Veh), BDL group receiving vehicle (BDL + Veh), and BDL groups receiving sitagliptin at the doses of 10, 50, or 100 mg/kg/day by gavage for 14 days named BDL + Sit 10, BDL + Sit 50, and BDL + Sit 100, respectively. Aspartate transferase, alkaline phosphatase, total bilirubin (T-Bil), renal function biomarkers, creatinine (Cr) clearance, redox system status, TNF-α, and renal histopathology were evaluated.

Findings/Results: BDL increased serum liver enzymes, T-Bil, Cr, urea, urine Cr, albumin to Cr ratio, and decreased Cr clearance. Also, BDL elevated renal sestrin2, malondialdehyde, TNF-α, renal index, and kidney tissue injury score, while reducing superoxide dismutase (SOD) activity, total antioxidant capacity, and nuclear factor erythroid 2-related factor 2 (Nrf2). Sitagliptin, especially at the low dose, reversed these effects.

Conclusion and implications: Sitagliptin improved renal injury and function *via* ameliorating inflammation and oxidative stress by activating the Nrf2/SOD pathway in BDL rats. Sitagliptin might help treat renal complications in cirrhosis and liver diseases.

Keywords: Cholemic nephropathy; Inflammation; Nrf2; Oxidative stress; Sestrin2; Sitagliptin.



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INTRODUCTION

Severe hepatic disorders due to alcoholic or non-alcoholic liver disease may lead to other organ dysfunction, including the kidneys, among vital organs, which is known as hepatorenal syndrome (HRS) (1). Renal dysfunction is a common manifestation of advanced cirrhosis that is associated with significant mortality and morbidity (2). The exact incidence and prevalence of HRS in cirrhosis are currently unknown (3). HRS incidence might be 18% and 39% at 1-year and 5-year periods, respectively, for patients with liver cirrhosis and ascites (4).

Cholestasis or bile duct ligation (BDL) caused liver impairment is a clinical complication that could develop cirrhosis, fibrosis, and liver failure (5,6). These advanced stages of liver damage usually become apparent 2-4 weeks after BDL (7). Renal damage caused by the accumulation of substances in bile, including bile acids, can be observed in the nephron collecting ducts of BDL model mice after three days (8). Mechanistically, renal injury as a consequence of cirrhosis is due to circulatory and hemodynamic alterations. The changes, such as splanchnic arterial vasodilation, reduced total peripheral resistance, blood volume depletion, and hypotension, activate several mechanisms to maintain blood pressure, including the sympathetic nervous system, the renin-angiotensin system, and hormone secretion (1). The accumulation of bile acids and bilirubin in cholestasis could play a cytotoxic role for renal cells (9). The main mechanisms of bilirubin toxicity can be caused by mitochondrial membrane dysfunction, oxidative stress, apoptosis, necrosis, and endoplasmic reticulum stress in organs (10). Literature review supported the influences of oxidative stress and inflammation on hepatic and renal damage in cholestasis (11). Cholemic nephropathy (CN) causes a decrease in mitochondrial dehydrogenase activity, mitochondrial depolarization, as well as a decrease in ATP stores, lipid peroxidation, and mitochondrial permeability (12). An increase in the production of reactive oxygen species (ROS), lipid peroxidation, and protein carbonylation, as well as the elevation of pro-inflammatory cytokines in liver and renal tissues of cholestatic rats, has been reported (13). Acute kidney injury occurs in 20-50% of cirrhotic hospitalized patients (14). Accompanied by the progression of liver disease, renal vasoconstriction has been reported, which could damage renal function in late stages, reduce glomerular filtration rate (GFR), and increase serum creatinine (Cr) (15). Cholestasis increases HRS susceptibility through the elevation of portal vein pressure and cirrhosis (16), so it is an acceptable experimental model to assess the pathophysiology of cirrhosis and HRS.

Sitagliptin is a dipeptidyl peptidase-4 (DPP4) enzyme inhibitor, which is prescribed to treat type 2 diabetes mellitus (T2DM) (17). Sitagliptin prolongs the action of incretin hormones, including glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide, by inhibiting their breakdown (18). Also, the administration of sitagliptin reduces the serum levels of inflammatory cytokines in patients with T2DM (19). By preventing the inactivation of incretin hormones, sitagliptin improves glycemic control in patients with type 2 diabetes, primarily by suppressing glucagon levels and increasing endogenous insulin secretion (18). In a kidney injury model, gentamicin initiated apoptotic pathways and disrupted normal mitochondrial membrane function (20), whereas sitagliptin could prevent renal tubular apoptosis by reducing apoptotic mediators (21). In the literature, several documents have reported the nephroprotective effects of DPP4 inhibitors like sitagliptin in diabetic animals and other renal injuries through the elimination of oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress (21,22). Hence, this study was designed to determine whether sitagliptin could ameliorate CN in experimental cholestatic rats, and also to assess the antioxidant and anti-inflammatory action of sitagliptin as possible mechanisms.

MATERIALS AND METHODS

Animals

To evaluate the possible protective effects of sitagliptin on cholestasis-induced renal injury, 35 male Wistar rats (200-250 g) were purchased from the animal house of Kerman University of

Medical Sciences (Kerman, Iran). The animals were housed in cages with free access to food and water under 24 ± 2 °C temperature and a 12-h light/dark cycle. The Ethics Committee of Kerman University of Medical Sciences approved the experimental protocol (Ethical No. IR.KMU.AH.REC.1401.264).

Study design

Experimental groups and surgical procedures

The animals were divided into five groups (n = 7) as follows: sham + Veh, BDL + Veh, BDL + Sit 10, BDL+ Sit 50, and BDL+ Sit 100 groups (Fig. 1A). To perform the BDL, rats were anesthetized with the injection of a ketamine (60 mg/kg) and xylazine (2 mg/kg) cocktail intraperitoneally (IP) (23). The abdomen was opened with a midline incision (approximately 2 cm). After observation of the bile duct, it was ligated with two surgical knots with surgical silk 5-0 at the proximal and distal ends. After BDL, 1 mL of 0.9% sodium chloride solution was injected into the peritoneal cavity to maintain the physiological condition. Then, the two layers of the abdomen (muscle and skin) were sutured separately with 4-0 silk thread (24). In the sham group, the laparotomy was performed without the BDL. Sitagliptin (99% purity, Jalinous Pharmaceutical Co., Iran) was given at the doses of 10, 50, or 100 mg/kg/day for two weeks to the treatment groups through gavage with special needles (25). The sham + Veh and BDL + Veh groups received distilled water in equal volume *via* gavage during the experimental period. On day 14, the animals were placed in metabolic cages to collect the 24-h urine samples. On day 15, after measuring the body weight (BW) with a digital scale, the animals were anesthetized by the injection of ketamine/xylazine (100/10 mg/kg, IP), followed by collecting blood samples from the heart (26). After centrifuging, the serum samples were separated and frozen at -80 °C for assessment. Finally, the animals were sacrificed. Then, the kidney tissues were removed and weighed immediately. The right kidneys were placed in 10% buffered formalin (pH 7.4) for pathological examination. Also, the left kidneys were stored at -80 °C to examine tissue parameters (Fig. 1B). The percentage change of BW and kidney weight (KW) index were calculated using equations **(1)** and **(2)**, respectively:

$$BW\ change\ (\%) = \frac{BW2\ (g)\ -\ BW1\ (g)}{BW1\ (g)} \times 100 \quad (\mathbf{1})$$

where BW_1 and BW_2 express BW on days 1 and 15 of the experiment period, respectively.

$$KW\ index = \frac{KW\ (mg)}{BW\ (g)} \quad (\mathbf{2})$$

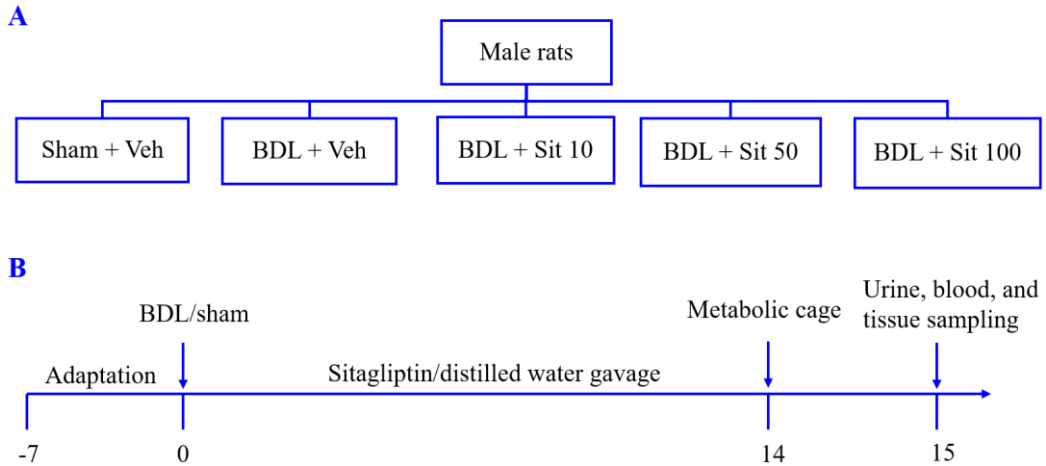


Fig. 1. Schematic representation of (A) the experimental groups and (B) timeline of the study. In the sham group, the laparotomy was performed without the BDL. Sitagliptin was given at the doses of 10, 50, or 100 mg/kg/day for two weeks through gavage. The sham + Veh and BDL + Veh groups were given distilled water in equal volume through gavage during the experimental period. Sit, Sitagliptin; BDL, bile duct ligation; Veh, vehicle.

Histopathological examination

The left kidney tissues were fixed with 4% buffered paraformaldehyde and embedded in paraffin. After preparing 2-µm-thick sections, hematoxylin and eosin (H&E) staining was used to evaluate tissue damage and inflammation. The sections were examined with an Olympus microscope (CX41, Tokyo, Japan) by an expert pathologist who was blind to the groups. For quantitative damage scoring, six fields in each sample were selected and scored from 0-5 (magnification ×100 or ×400). Histopathological changes in the kidney tissues were graded according to the predominance of tubulointerstitial changes, which include degeneration of tubular epithelial cells and loss of brush border, thickening of the basement membrane, dilation of tubules and the presence of protein or cellular casts, and tubular atrophy, along with edema and inflammation of the interstitial space as follow: 0, no change; 1, 10%; 2, 10-25%; 3, 25-50%; 4, 50-75%; 5, 75-100% of tubulointerstitial injury (27).

Assessment of serum and urine urea, Cr, and albumin

The urine and serum levels of urea, Cr, and albumin were measured by Commercial kits (Pars Azmoon, Tehran, Iran) using an autoanalyzer (Selectra-XL, Vital Science, Netherlands). The urine albumin to Cr ratio (UACR) was calculated using the equation **(3)**:

$$\text{UACR} = \frac{\text{Urine albumin (mg}/dL)}{\text{Urine Cr (mg}/dL)} \quad (3)$$

To estimate GFR, the renal clearance of Cr was determined. Accordingly, urinary and serum Cr concentrations and urine flow were used, and GFR was calculated using equation **(4)**, then reported as µL/min/mg KW(28):

$$GFR\ (mL/min) = \frac{Ucr\ (mg/dL) \times UF(mL/min)}{Scr\ (mg/dL)} \quad (4)$$

where UCr and SCr express urinary and serum Cr concentrations, respectively. Also, UF expresses urine flow.

Assay of liver enzymes

The activity of liver enzymes, such as aspartate transferase (ALT) and alkaline transferase (AST) and total bilirubin (T-Bil) in serum was measured using the standard kit (Pars Azmoon, Tehran, Iran) by the enzymatic method according to the manufacturer's instructions.

Determination of renal oxidant/antioxidant activity

To measure the tissue levels of malondialdehyde (MDA), superoxide dismutase (SOD), and total antioxidant capacity (TAC), the kidney tissues were homogenized in 1.5% potassium chloride (KCl) solution and then centrifuged for 10 min at 1000 rpm. Finally, the supernatants obtained were used to determine renal oxidant/antioxidant activities.

The thiobarbituric acid (TBA) method was used for MDA assessment. Briefly, the supernatant was mixed with trichloroacetic acid and TBA, then placed in a boiling water bath for 45 min. After cooling and adding n-butanol, the absorbance was measured at 534 nm (29).

The TAC was measured by the ferric reducing/antioxidant power (FRAP) method. Briefly, 5 µL of the supernatant and 70 µL of FRAP reagent were mixed. Distilled water was used as a blank; the mixture was incubated at 37 °C for 5 min, then the absorbance was read at 593 nm (30).

The SOD activity was determined according to the Randox kit instructions (Cat. No. RS504, Randox Labs, UK). The absorbance of SOD was measured at 570 nm.

All absorbances of MDA, SOD, and TAC were measured with an Epoch™ spectrophotometer (BioTek® Instrument, Inc, Winooski, VT 05404-0998, USA).

ELISA assay

Renal levels of nuclear factor erythroid 2-related factor 2 (Nrf2), sestrin2 (Sesn2), and tumor necrosis factor alpha (TNF-α) were measured using commercial ELISA kits (R&D, USA) according to the manufacturer's instructions. The NFE2L2 ELISA kit was used to quantitatively determine the concentration of Nrf2 in renal tissue. The measurement of TNF-α and Sesn2 was performed by DuoSet ELISA and Sesn2 polyclonal antibody (Cat. No. E-AB-64956), respectively.

Statistical analysis

Data were expressed as mean ± SEM and analyzed using GraphPad Prism version 8. The data normality was checked with the Shapiro-Wilk test. One-way ANOVA followed by Tukey post-hoc test was used for normally distributed data. Kruskal-Wallis and Mann-Whitney U tests were used for non-normally distributed data. $P < 0.05$ was considered statistically significant.

RESULTS

The effects of sitagliptin on BW and the KW index

Two-week induction of BDL in the animals decreased BW, which was significant in the BDL + Veh group compared to the sham + Veh group (Fig. 2A). The administration of sitagliptin at the dose of 10 mg/kg insignificantly decreased the BW loss, but the other doses of sitagliptin did not show any effects on BW loss compared to the BDL + Veh group (Fig. 2A).

KW index significantly increased in the BDL + Veh group in comparison to the sham + Veh group. However, none of the sitagliptin doses was effective in preventing the increment (Fig. 2B).

The effects of sitagliptin on serum biomarkers

Serum liver enzymes

The serum AST level was significantly increased in the BDL + Veh group compared to the sham + Veh group. Treatment with sitagliptin at the dose of 10 mg/kg lowered the serum level of AST compared to the BDL + Veh group, reducing it to nearly the levels observed in the sham + Veh group. However, sitagliptin at the doses of 50 and 100 mg/kg did not result in a significant reduction of the serum AST level (Fig. 2C).

The serum level of ALT was increased in the BDL + Veh group compared to the sham + Veh group, significantly. Like AST, the only low dose of sitagliptin significantly reduced ALT serum level, while sitagliptin at the medium and high doses caused a significant increase in ALT serum level in comparison to the BDL + Veh group (Fig. 2D).

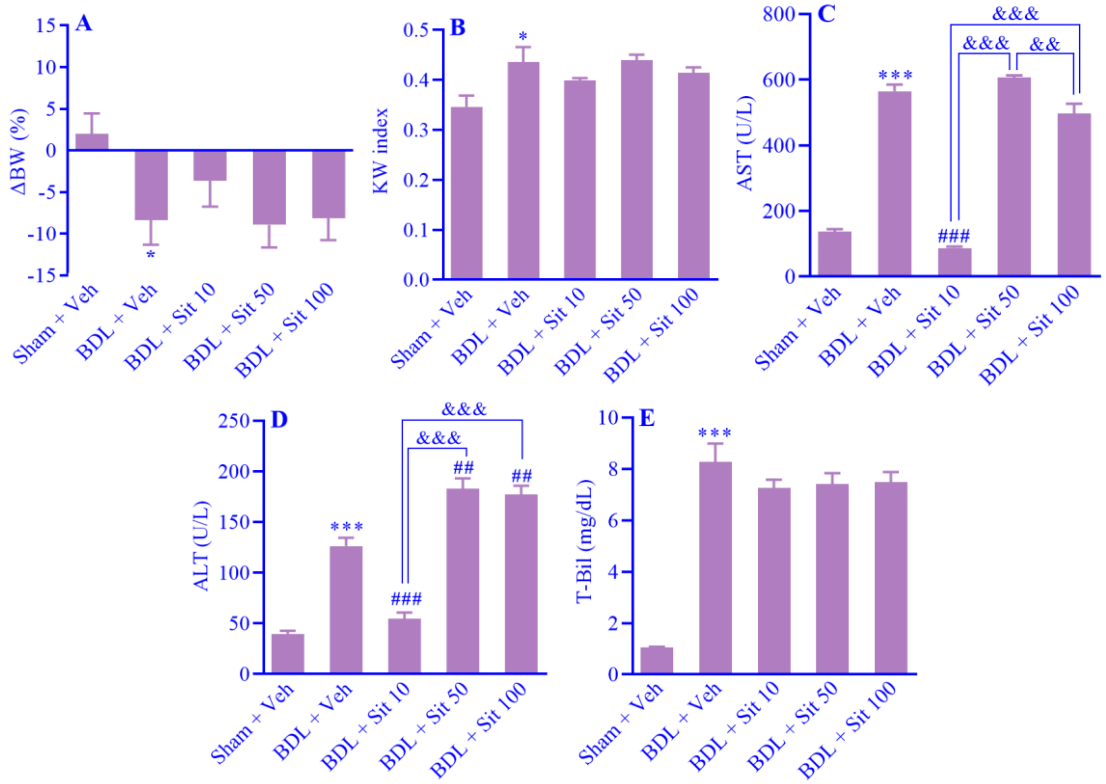


Fig. 2. Effect of sitagliptin on (A) the percentage of BW change, (B) the KW index, the serum levels of (C) AST, (D) ALT, and (E) T-Bil. Sitagliptin was given at the doses of 10, 50, or 100 mg/kg/day for two weeks through gavage. The sham + Veh and BDL + Veh groups were given distilled water in equal volume through gavage during the experimental period. Data were expressed as mean ± SEM, n = 7. $^{*}P < 0.05$ and $^{***}P < 0.0001$ demonstrate significant differences compared to the sham + Veh group; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ *versus* the BDL + Veh group; $^{\&\&}P < 0.01$ and $^{\&\&\&}P < 0.001$ represent the significant differences between the Sit-treated groups. BW, Body weight; KW, kidney weight; AST, aspartate aminotransferase; ALT, alkaline transferase; T-Bil, total bilirubin; Sit, sitagliptin; BDL, bile duct ligation; Veh, vehicle.

Serum T-Bil levels

In consequence of two-week BDL, the serum T-Bil levels were significantly increased in the BDL + Veh group in comparison to the sham + Veh group. None of the sitagliptin doses could reduce the serum level of the marker compared with the BDL + Veh group (Fig. 2E).

Serum levels of Cr, urea, and albumin

The serum levels of Cr and urea were increased in the BDL + Veh group compared to the sham + Veh group, significantly (Fig. 3A and B). Although the serum level of urea was significantly reduced by all doses of sitagliptin (Fig. 3B), the reduction of the serum Cr level was seen only in the BDL + Sit 10 group in comparison to the BDL + Veh group (Fig. 3A). Neither induction of BDL nor sitagliptin treatment affected the serum albumin levels (Fig. 3C).

The effects of sitagliptin on urine biomarkers

Urine Cr and UACR

Urine Cr level was significantly reduced in the BDL + Veh group compared to the sham + Veh group. Sitagliptin treatment at the dose of 100 mg/kg significantly increased the parameter compared to the BDL + Veh group (Fig. 3D). UACR was significantly increased in the BDL + Veh group compared to the sham + Veh group. On the other hand, sitagliptin at the doses of 50 and 100 mg/kg significantly reversed the ratio compared to the BDL + Veh group (Fig. 3E).

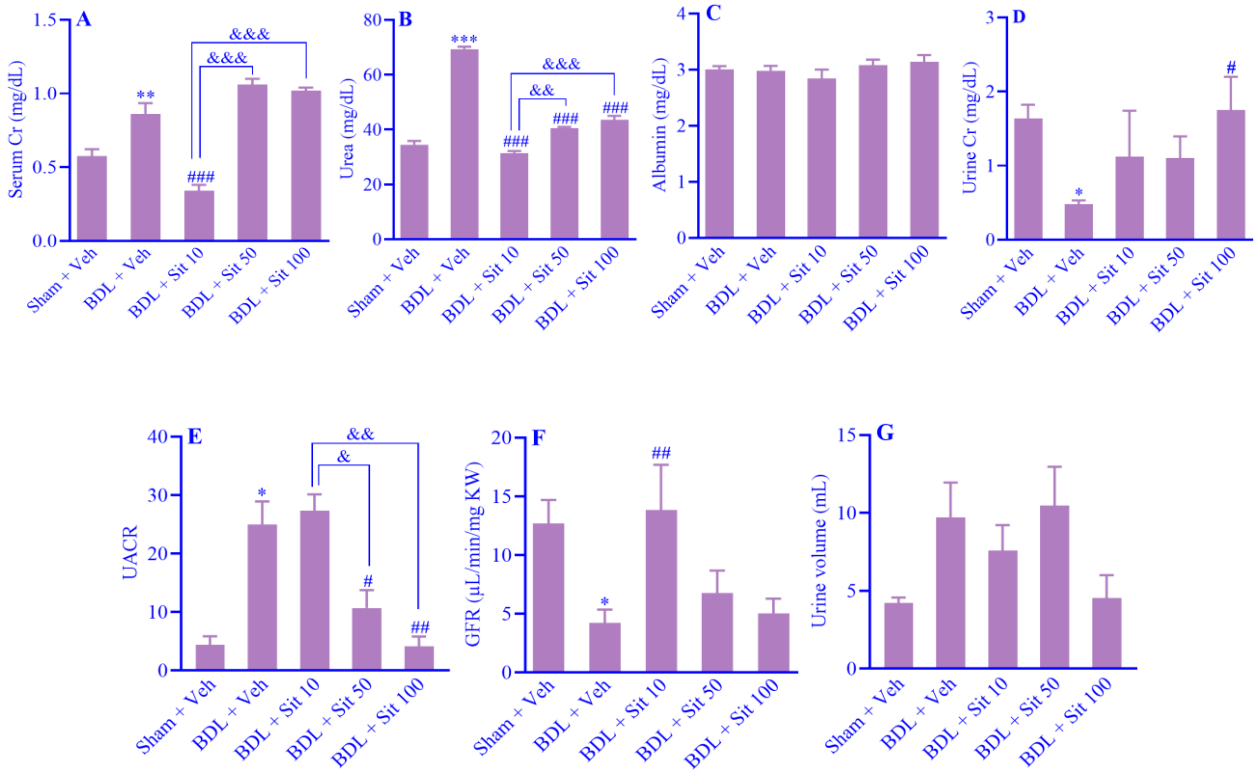


Fig. 3. Effect of sitagliptin on renal function in BDL-induced cholemic nephropathy in the experimental groups. Serum levels of (A) Cr, (B) urea, and (C) albumin; (D) urine level of Cr; (E) UACR; (F) GFR; and (G) 24-h urine volume. Sitagliptin was given at the doses of 10, 50, or 100 mg/kg/day for two weeks through gavage. The sham + Veh and BDL + Veh groups were given distilled water in equal volume through gavage during the experimental period. Data were expressed as mean ± SEM, n = 7. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ demonstrate significant differences compared to the sham + Veh group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus the BDL + Veh group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, and $^{\&\&\&}P < 0.001$ represent the significant differences between the Sit-treated groups. Sit, Sitagliptin; BDL, bile duct ligation; Veh, vehicle; Cr, creatinine; UACR, urine albumin to Cr ratio; GFR, glomerular filtration rate.

GFR and urine volume

Induction of BDL significantly reduced GFR in the BDL + Veh group compared to the sham + Veh group. The administration of sitagliptin at the dose of 10 mg/kg significantly improved GFR compared to the BDL + Veh group. The other doses of sitagliptin had no significant effects on GFR (Fig. 3F). Moreover, there were no significant differences in urine volume among the experimental groups over 24 h (Fig. 3G).

The effects of sitagliptin on the renal activity of the redox system, Nrf2, and Sesn2 levels

The alleviation of TAC, TAC/MDA ratio, SOD, and Nrf2 in the kidney was significantly seen in the BDL + Veh group compared to the sham + Veh group (Fig. 4A-D). Also, the renal levels of MDA and Sesn2 were significantly increased in the BDL + Veh group compared to the sham + Veh group (Fig. 4E and F). On the other hand, sitagliptin in all doses significantly improved the parameters, except Nrf2 and MDA (Fig. 4A-F). The renal level of Nrf2 was significantly increased by sitagliptin at the doses of 50 and 100 mg/kg in comparison to the BDL + Veh group (Fig. 4D). In addition, the renal MDA level was significantly affected by sitagliptin administration only at the dose of 10 mg/kg in comparison to the BDL + Veh group (Fig. 4E).

The effect of sitagliptin on renal tissue TNF-α level

The renal TNF-α level was significantly elevated in the BDL + Veh group compared to the sham + Veh group. sitagliptin at all doses significantly reduced the inflammatory cytokine compared to the BDL + Veh group (Fig. 4G).

The effect of sitagliptin on renal histopathology

The histopathologic alterations, including tubular cell swelling, brush border loss, protein cast, deposition of bile pigments, interstitial inflammation, vascular congestion, and edema, were observed in H&E-staining assessment of kidney tissue in the cholestatic rats (Fig. 5A$_{1\text{-}6}$). BDL significantly enhanced the kidney tubular injury score (KTIS) in the BDL + Veh group in comparison to the sham + Veh group. KTIS was significantly reduced in the BDL + Sit10 group compared to the BDL + Veh group (Fig. 5B).

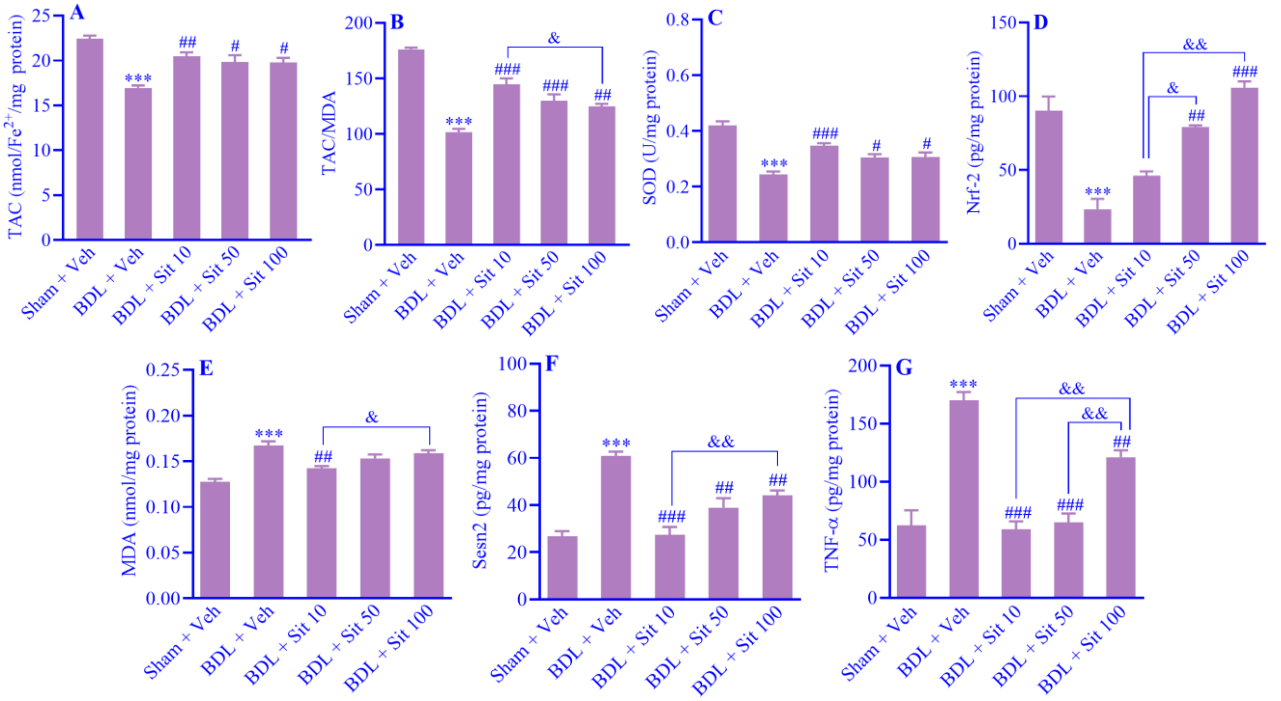


Fig. 4. Effect of sitagliptin on kidney level of (A) TAC, (B) TAC/MDA, (C) SOD activity, (D) Nrf2, (E) MDA, (F) Sesn2, and (G) TNF-α. Sitagliptin was given at the doses of 10, 50, or 100 mg/kg/day for two weeks through gavage. The sham + Veh and BDL + Veh groups were given distilled water in equal volume through gavage during the experimental period. Data were expressed as mean ± SEM, n = 7. $^{***}P < 0.001$ demonstrates significant difference compared to the sham + Veh group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus the BDL + Veh group; $^{\&}P < 0.05$ and $^{\&\&}P < 0.01$ represent the significant differences between the Sit-treated groups. Sit, Sitagliptin; BDL, bile duct ligation; Veh, vehicle; TAC, total anti-oxidant capacity; MDA, malondialdehyde; SOD, superoxide dismutase; Nrf2, nuclear factor erythroid 2-related factor 2; Sesn2, sestrin2; TNF-α, tumor necrosis factor alpha.

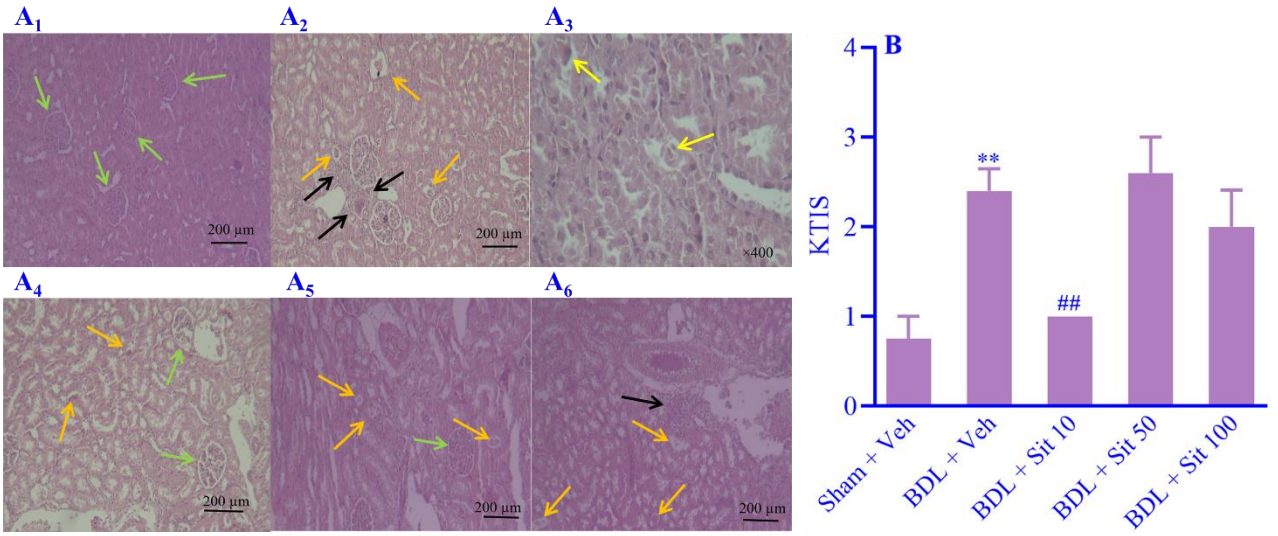


Fig. 5. The effect of sitagliptin on kidney damage in BDL-induced cholemic nephropathy in the experimental groups. (A_1) Sham + Veh, (A_2) BDL + Veh, (A_3) BDL + Veh (×400 magnification), (A_4) BDL + Sit 10, (A_5) BDL + Sit 50, (A_6) BDL + Sit 100 groups. The kidney sections were stained with hematoxylin-eosin (×100 magnification except A3). Green arrows, Glomeruli; black arrows, inflammatory cells; orange arrows, protein casts inside the tubules; yellow arrows, deposition of pigments in the tubule. (B) Quantitative analysis of KTIS. Sitagliptin was given at the doses of 10, 50, or 100 mg/kg/day for two weeks through gavage. The sham + Veh and BDL + Veh groups were given distilled water in equal volume through gavage during the experimental period. Data were expressed as mean ± SEM, n = 5. $^{**}P < 0.01$ demonstrates significant difference compared to the sham + Veh group; $^{\#\#}P < 0.01$ *versus* the BDL + Veh group. Sit, Sitagliptin; BDL, bile duct ligation; Veh, vehicle; KTIS, kidney tubular injury score.

DISCUSSION

Cholestasis-induced nephropathy, also called CN, is a prevalent disorder in cholestasis patients. The primary role of oxidative stress in the pathogenesis of CN has been documented in the literature review (31). Thus, the present study used BDL-induced nephropathic rats to investigate the potential mechanism of sitagliptin on renal injury and oxidative stress induced by CN. This study highlighted several important results as follows: (1) BDL induction increased the serum levels of AST, ALT, and T-Bil, as well as BW loss and the KW index; (2) BDL increased the serum levels of Cr and urea, as well as UACR, while decreasing the urine Cr levels and Cr clearance; (3) BDL-induced injury decreased renal TAC, SOD, TAC/MDA, and Nrf2 levels, while increasing MDA, Sesn2, and inflammatory cytokine TNF-α in renal tissue; (4) histopathological changes, such as epithelial cell swallowing, brush border loss, cast formation, tubular necrosis, and inflammation, resulted in an overall increase of KTIS following 14-day BDL; (5) treatment with sitagliptin, especially at the low dose reversed the alterations.

Hyperbilirubinemia in cholestasis develops renal injury and structural disturbances. Bile acids inhibit electrolyte transporters in the renal tubules in a way that increases tubular acidity, which favors cast formation and leads to tubular obstruction (32). Another mechanism of CN is due to renal hemodynamic alteration. High serum bilirubin exerts a negative effect on cardiac contractility and rhythmicity, which contributes to poor renal perfusion (33). In addition, bile acid accumulation is cytotoxic, impairs mitochondrial function, and elevates ROS formation (34). Oxidative stress and inflammation play an important role in the development of renal injury (35,36). Direct nephrotoxicity of bilirubin and bile salts has been proven. The presence of 66% acute kidney injury was reported in renal biopsy of bile cast-positive patients who suffered from severe liver disease (5).

In the current study, the changes, such as the deposition of bile pigments, tubular injury, and interstitial inflammation, were seen in the kidney tissues. Also, the alterations, including the enhancement of oxidative stress biomarkers, lipid peroxidation, and inflammatory cytokine TNF-α, were observed in the kidneys of cholestatic groups. The observations were in line with

findings that mentioned the involvement of oxidative stress in the pathogenesis of CN (4).

It has been reported that DPP-4 inhibitors like sitagliptin, an oral anti-hyperglycemic drug, have antioxidant, anti-inflammatory, and nephroprotective effects. The protective effect of sitagliptin has been revealed as an antioxidant agent in diabetic and non-diabetic conditions in the kidney tissue (22). In an experimental model of xenobiotic-induced nephrotoxicity, sitagliptin ameliorated renal injury by augmentation of antioxidant defense and Nrf2/HO-1 pathway up-regulation (37). The Kelch-like ECH-associated protein 1 (Keap1)-Nrf2-antioxidant response element system is the main pathway activated in oxidative stress states. The ablation or disrupted activation of Nrf2 accelerates oxidative stress conditions and disrupts normal cellular redox balance, leading to cell dysfunction (38). In severe acute pancreatitis-related acute lung injury, sitagliptin could mitigate oxidative stress by activating the Keap1-Nrf2 signaling pathway (39). Nrf2 is a transcription factor that up-regulates downstream antioxidant enzymes, SOD and catalase, in stress states (40). Nrf2-deficiency in mice aggravates oxidative stress, inflammation, and renal injury in diabetic models (41). Moreover, increased Nrf2 activity preserves mitochondrial function and morphology (42). The latter observations supported renal protection mediated by Nrf2 through antioxidant and anti-inflammatory functions (43). In agreement with other research, the present data revealed an increment in Nrf2 level, SOD activity, TAC, and TAC/MDA ratio, accompanied by a reduction of TNF-α and histopathologic alterations in cholestatic rats receiving sitagliptin. In addition, the beneficial effect of sitagliptin administration on cardiac performance and histology has been reported in a 5/6 nephrectomized model of chronic kidney disease (44). The cardio-protective action of sitagliptin could help restore renal circulation and improve renal function and histology in CN.

In the present study, sitagliptin at three doses showed antioxidant and anti-inflammatory effects in terms of improving antioxidant factors (TAC, SOD, and Nrf2) and reducing inflammatory factor (TNF-α), although some markers were not significant. The results revealed a dose-dependent and mechanistically distinct efficacy pattern of sitagliptin in CN. The low dose (10 mg/kg) revealed nephroprotection by a significant reduction of serum Cr and improving GFR to near the sham + Veh group levels, accompanied by hepatoprotection (ALT and AST), while the high doses (50 and 100 mg/kg) failed to improve serum Cr and GFR (versus BDL + Veh group) and paradoxically elevated liver enzymes above levels in both the sham + Veh and BDL + Veh groups. Although Nrf2-SOD activation was preserved at all doses, indicating the presence of an antioxidant effect, this was not enough for hepatotoxicity prevention at high doses. This loss of hemodynamic and hepatic benefit at high doses might be due to glucagon-like peptide-1 (GLP-1) receptor desensitization. As indicated in the literature, long-term treatment of β-pancreatic cell lines with GLP-1 (same as using sitagliptin), desensitized the GLP-1 receptors (45). In line with the dose-dependent results, Chuo *et al*. suggested a J-shaped and dose-dependent effect of DPP4 inhibitors in colorectal cancer risk (46). Consistent with the present findings, previous studies have reported the protective effects of 10 mg/kg sitagliptin against diabetic nephropathy (47) and doxorubicin-induced nephropathy (48). Additionally, sitagliptin has shown protective roles at lower doses (5 and 10 mg/kg) in 5-fluorouracil-induced nephropathy (49), as well as at the doses of 2.5 and 10 mg/kg in adenine-induced kidney disease models (50). Furthermore, the nephroprotective effects of sitagliptin have been noted at higher doses (100 mg/kg for diabetic nephropathy and 300-600 mg/kg for ischemia-reperfusion injury) (51,52), confirming the efficacy of sitagliptin through its antioxidant and anti-inflammatory properties across various types of renal injury, independent of its hypoglycemic effects. Aligning with the current results, Asakawa *et al*. reported that sitagliptin at a dose of 50 mg failed to reduce ALT levels (53). A review study also stated that the ineffectiveness of sitagliptin on ALT and AST was not unexpected (54).

DPP4 inhibitors, such as sitagliptin, increase the circulating level of GLP-1 by inhibiting its breakdown, thereby improving glucose metabolism in diabetic mellitus (55).

The literature suggested that sitagliptin, through the elevation of GLP-1 and GLP-1 receptor levels, may potentiate anti-inflammatory and anti-oxidant effects, thereby protecting kidney tissue from acute kidney injury (25) and cardiovascular tissue from endothelial dysfunction and atherosclerosis (56).

Sesn2, a member of a family of highly conserved antioxidant proteins, regulates intracellular ROS levels in hypoxia, oxidative stress, and DNA damage (57). Yang *et al*. explained the contribution of renal Sesn2 in the maintenance of redox balance (57). The overexpression of Sesn2 in BDL mouse liver and bile acid-treated HepG2 cells revealed the hepatoprotective effect of Sesn2 against bile acid-induced endoplasmic reticulum stress (58). The results of this study confirmed the involvement of Sesn2 in oxidative stress induced by cholestasis in renal tissue. Sitagliptin might improve renal damage *via* the accretion of Sesn2 levels in BDL rats.

CONCLUSION

Collectively, the findings revealed a dose-dependent nephroprotective effect of sitagliptin in cholestasis, as evidenced by a decrease in serum levels of Cr, urea, and a decrease in urine Cr and UACR, and an increase in GFR in association with a reduction of inflammation, oxidative stress, and Sesn2 through the Nrf2/SOD pathway. As sitagliptin is an FDA-approved drug and used to treat diabetic patients, we suggested considering it to alleviate renal injury and dysfunction in cirrhosis and severe liver disease, provided that the therapeutic dose is carefully optimized to achieve maximal protection without loss of efficacy or safety concern.

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Authors' contributions

M. Akhbari participated in the experimental procedures, statistical analysis, and drafting; S. Saberi participated in the design and coordination of the study, drafting of the manuscript, statistical analysis, interpreting the findings, and final revision of the manuscript; M. Khaksari participated in the design and coordination of the study; M. Askaripour participated in drafting; E. Jafari participated in pathology scoring.

All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

Conflict of interest statement

All authors declared no conflict of interest in this study.

AI declaration

During the preparation of this work, the authors used ChatGPT AI to improve readability and language. After using this tool, the authors reviewed and edited the content and take full responsibility for the published content. Artificial intelligence was used at the end of the work and solely for paraphrasing, and then it was re-reviewed by the authors. Moreover, AI-assisted design tools were employed to prepare the graphical abstract, followed by manual refinement by the authors.

Data availability

The data supporting the findings of this study are documented and available from the corresponding author upon reasonable request. Researchers interested in accessing the data should contact the corresponding author to discuss the terms and conditions of access.

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