

Sitagliptin ameliorates liver fibrosis through reducing inflammation and sestrin2 through Nrf2/SOD in cholestasis rats

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Article

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Abstract

Cholestasis, caused by impaired bile flow, can progress to severe liver injury and fibrosis. Oxidative stress and inflammation are major drivers of this process. Sitagliptin, a dipeptidyl peptidase-4 (DPP4) inhibitor used for hyperglycemia, has also been reported to show antioxidant and anti-inflammatory properties. This study evaluated whether sitagliptin can attenuate bile duct ligation (BDL)-induced liver fibrosis in rats and examined related mechanisms. 35 male Wistar rats were randomized into five groups: sham, BDL, and BDL treated with sitagliptin at 10, 50, or 100 mg/kg. After BDL surgery under anesthesia, rats received saline or sitagliptin by oral gavage for 14 days. Serum and liver tissues were collected for biochemical and molecular assessment. Liver injury were evaluated using H&E and Masson's trichrome staining. BDL increased serum alkaline phosphatase, lactate dehydrogenase, and total bilirubin, and elevated hepatic oxidative and inflammatory markers, including MDA, TNF- α , TGF- β , and Sestrin2, along with higher hydroxyproline content and fibrosis scores. Antioxidant defenses (SOD activity, total antioxidant capacity, and Nrf2) were decreased. Sitagliptin reversed these changes, reducing oxidative stress and inflammation and improving antioxidant responses, with some effects more evident at 10 mg/kg. In conclusion, sitagliptin mitigated BDL-induced fibrosis by suppressing oxidative stress and inflammatory signaling.

1. Introduction

Cholestasis is defined as a disorder of bile formation or bile flow, which is clinically known as fatigue, itching and clearly jaundice [1]. The prevalence of cholestasis varies around the world, Latin America is among the first countries in terms of prevalence. Factors such as genetic disorders or viral infections, drug poisoning, bile duct tumor, cyst formation or injuries caused by surgery can cause cholestasis[2]. Biliary obstruction causes acute liver cell damage and leads to progressive fibrogenesis [3]. Although the mechanism of this damage is not well defined, oxidative stress and releasing of inflammatory cytokines has been suggested as a process involved in the occurrence of liver damage [3]. Also, in the cholestasis model due to bile duct obstruction, it has been suggested that bile acids (BAs) act as inflammatory agents and may activate signaling pathways in hepatocytes that regulate the production of pro-inflammatory cytokines and chemokines [3]. There are many experimental and clinical data that show the important role of reactive oxygen species in the pathogenesis of liver damage and cholestasis caused by biliary obstruction [4]. Therefore, anything that can increase the antioxidant capacity will naturally cause more protection of the liver tissue against oxidants [5]. In cholestasis, inflammation is characterized by the increase of pro-inflammatory factors, interleukins and TNF- α , which if not reduced or stopped, cause irreparable damage to the tissue and advance it to the stage of liver cancer [6].

Sitagliptin is a drug that inhibits the Dipeptidyl peptidase-4 (DPP4) enzyme and is used in the treatment of type II diabetic patients [7]. DPP4 inhibitors have a protective role against oxidative stress and protect against mitochondrial membrane depolarization in diabetic patients and diabetic rats [8]. On the other hand, it is believed that DPP-4 plays a role in regulating cytokines and signaling functions so that the protective effects of sitagliptin on the liver have been proven in the experimental fatty liver model [9].

Based on the antioxidant and anti-inflammatory properties of sitagliptin, this study aims to investigate the effects of sitagliptin in the biliary obstruction model that induces external cholestasis.

2. Results

2 – 1. The effects of sitagliptin on liver weight

Induction of two weeks BDL in animals, increased liver weight ($P < 0.001$). The groups were treated by sitagliptin showed a slight insignificant reduction in tissues weight (Fig. 2A).

2–2. The effects of sitagliptin on serum biomarkers

2-2-1. Serum glucose and total bilirubin levels

In considering the usage of sitagliptin as an anti-hyperglycemic agent, blood glucose was assessed. Serum glucose level was failed in animals of BDL groups compare to the sham group significantly ($P < 0.001$). Administration of sitagliptin in all three doses prevents this fail (Fig. 2B).

In consequence of two weeks BDL, serum T-bil level were increased ($P < 0.001$). None of sitagliptin doses could reduce this marker (Fig. 2C).

2-2-2. Serum liver enzymes

Administration of 10 mg/kg sitagliptin reduced elevated serum ALP level, although doses of 50 and 100mg/kg were not effective (Fig. 2D).

BDL induction increased serum level of LDH ($P < 0.001$), treatment of animals with sitagliptin improved this factor dose-dependently and dose of 100mg/kg has the best effect (Fig. 2E).

2–4. The effects of sitagliptin on liver activity of redox system, Nrf2 and sestrin2 levels

As figure presented, BDL causes liver TAC and SOD activity reduction (Fig. 23A,B). Sitagliptin treatment restored these antioxidant parameters. It was found that the most effective dose was 10mg/kg.

Sitagliptin treatment increased the Nrf2 level reduction was caused by cholestasis (Fig. 3C).

Liver lipid peroxidation elevated after 2 weeks BDL. Although sitagliptin at all doses succeeded to reduce MDA level, this factor was reduced more in the BDL+Sit10 group (Fig. 3D).

Sestrin2 level as a stress-induced protein was increased by induction of cholestasis model. Sitagliptin in all three doses could reduce this factor (Fig. 3E).

2–5. The effects of sitagliptin on inflammation and fibrosis biomarkers in liver

The figure is reflected, TNF- α , TGF- β , and hydroxyprolin levels were reduced by cholestasis, whilst sitagliptin treatment reduced the profibrotic cytokines and collagen deposition (Fig. 4A,B,C).

2–6. The effect of sitagliptin on liver histopathology

Necroinflammatory in the injury group showed an increase compared to the sham group, but significance was only observed in the 50 and 100 groups compared to the sham group. (Fig. 5A). In examining the degree of liver tissue fibrosis, the obstruction group showed a significant increase compared to the control group ($P < 0.01$), while fibrosis in the BDL + Sit 10 group was significantly reduced compared to the injury group ($P < 0.01$) (Fig. 5B).

3. Discussion

Cholestasis is a clinical disorder that affects liver function. Severe or long-term cholestasis may lead to liver fibrosis, cirrhosis or liver failure [10]. Cholestasis and the accumulation of bile compounds that are potentially cytotoxic, such as hydrophobic bile acids and bilirubin, can severely damage liver cells [11]. Also, in the cholestasis model due to bile duct obstruction, it has been suggested that BAs act as inflammatory agents and may activate signaling pathways in hepatocytes that regulate the production of pro-inflammatory cytokines and chemokines that induce neutrophil recruitment since liver cells are the first target of bile acids, it is obvious that liver damage will follow [3]. Although the mechanism of this injury pattern is not well defined but several studies have shown that oxidative stress plays an important role in cholestasis-related disorders [12]. Today we know that DPP4 inhibitors improve oxidative stress and protect against mitochondrial membrane depolarization in diabetic patients[8] because of this sitagliptin has been shown to protects the liver in experimental model-induced steatohepatitis or fatty liver [9] and methotrexate-induced hepatotoxicity by inhibiting inflammation and apoptosis [13]. Recently, the anti-inflammatory potential of sitagliptin has received attention [14]. The results of our study showed that two weeks of BDL blockage caused damage to liver cells, this is confirmed by the increase in the level of liver enzymes ALP and LDH, which are known as indicators of hepatocyte damage. These findings agree with the results of other studies [12]. The increase in liver weight index and its histopathological changes in the BDL group are other signs of liver inflammation, which have also been mentioned by other researchers [15]. In the damage group, glucose should decrease, while sitagliptin causes a significant increase in glucose, and this shows the moderating role of this drug, not just lowering blood sugar. In the present study, the increase of serum bilirubin increased in BDL groups and this result is very close to mind because the injury model is a physically applied disorder [16], but

sitagliptin prevented its strong increase. Sitagliptin increases the TAC through the increase antioxidant enzymes like SOD that were decreased in liver damage groups, and reduces MDA that this feature of the drug is one of its most effective effects.

The protective effect of sitagliptin against oxidative and nitrosative stress caused by ischemia-reperfusion (IR) of liver tissue has been reported due to its antioxidant mechanism [17]. Sesn2 protein, as explained, its expression increases against stress conditions, since sitagliptin has anti-stress properties, so it is natural to reduce stress conditions, which we see with the decrease of Sesn2 protein, similar to this finding Ha Yang et al also reported the involvement of renal Sesn2 in maintaining the balance of the redox system[18]. But if we want to examine the upstream of these events, we must mention the protective role of sitagliptin by increasing the activity of Nrf2, which causes its antioxidant and anti-inflammatory properties. Nrf2 is a nuclear redox translation factor that activates antioxidant factors and cellular protective genes in response to ROS production and oxidative stress [19]. The transfer of Nrf2 from the cytosol to the nucleus increases the expression of antioxidant and anti-inflammatory factors, which in turn strengthens the cell defense against stress and inflammation. It is claimed that the anti-inflammatory effect of gliptins is even stronger than their antioxidant effect [20]. In this study, the reduction of the inflammatory cytokine TNF- α , which was increased by BDL, was observed in the liver tissue of rats treated with a low dose of sitagliptin. In agreement with our results, the role of TNF- α in liver damage caused by cholestasis [15] and the protective effect of sitagliptin in liver damage caused by acetaminophen, methotrexate and reperfusion ischemia have been reported in previous studies [17]. In addition, sitagliptin inhibited the increase of TGF- β factors, which is a key cytokine in liver fibrogenesis [21], in line with that, hydroxyproline and collagen deposition also decreased, which is an indicator of fibrosis score in the histopathology of the BDL group.. All three doses of sitagliptin reduced TGF- β and hydroxyproline content in liver tissue in cholestasis group, while only administration of 10 mg/Kg dose of sitagliptin reduced the fibrosis score in histological examination. In line with our results, other researchers have also reported increased expression of TGF- β and increased percentage of collagen deposition following cholestasis [15]. Overall, sitagliptin through its anti-inflammatory and antioxidant effects it reduced liver fibrosis in BDL model rats.

Conclusion: Cholestasis causes liver tissue damage by activating oxidation and causing inflammation. Administering sitagliptin, especially low dose, strengthens the antioxidant system, reduces inflammation and, as a result, improves liver function.

4. Material and methods

In the present study, 35 Wistar rats were purchased from the animal farm of Kerman University of Medical Sciences and kept in the animal house of Kerman Medical School for one week. During this one week, the animals had free access to food and water and were subjected to standard conditions (temperature 25°C and light cycle 12 hours). All procedures were performed in accordance with relevant guidelines and regulation and this project was approved by the Ethics Committee in Kerman Medical School with ethics code IR.KMU.AH.REC.1401.264.

4 – 1. Experimental groups and surgical procedures

The animal divided into 6 groups (n = 7): Sham, bile duct ligation (BDL), BDL+ sitagliptin 10 mg/kg, BDL+ sitagliptin 50 mg/kg, BDL+ sitagliptin 100 mg/kg. To perform the bile duct ligation (BDL), Animals were anesthetized by intraperitoneal (IP) injection of ketamine (60 mg/kg) and xylazine (2 mg/kg)[22].

The abdominal surface of the rat is opened with an incision in the midline (approximately 2 cm), and after viewing the bile duct, the beginning and end of this duct are closed with two knots with 5 – 0 silk surgical thread. After BDL, 1 ml of 0.9% sodium chloride solution was injected into the peritoneal cavity to maintain physiological conditions, and then the two abdominal layers (muscle and skin) were sutured separately with 0–4 surgical thread[23]. In the Sham groups, the laparotomy was performed without ligation of the bile duct. In animals receiving sitagliptin, doses of 10, 50 and 100 mg/kg of sitagliptin were given orally (PO) for two weeks. The animals of sham groups physiological serum was given to in the same period and volume. After the end of two weeks on the 14th day. On day 15, after measuring body weight with a digital scale, animals were anesthetized with higher doses of ketamine/xylazine (10.100 mg/kg, IP), and blood samples were collected from the heart, centrifuged, and the serum was frozen at – 80°C for the assessment of serum parameters. Finally, the liver tissue of the animals was removed from the body and after weighing, a part of the liver was fixed in 10% buffered formalin (pH 7.4) for pathological examination, and remaining parts were stored at -80°C for examining tissue parameters. Liver weight index was calculated with the following formula:

$$\text{liver weight index} = \frac{\text{liver weight (mg)}}{\text{body weight (gr)}}$$

The Fig. 1 shows a schematic representation of the experimental groups and timeline of the study (Fig. 1A, B).

4 – 2. Histological examinations

The liver tissues were fixed with 10% paraformaldehyde buffered in paraffin, and 2 µm thick sections were prepared. Hematoxylin and eosin (H&E) staining was used to evaluate tissue damage and inflammation. Sections were examined with an Olympus microscope (CX41, Tokyo, Japan) by an expert pathologist blinded to the groups.

In the pathological changes of the liver tissue, the parameters of necrosis and lobular inflammation, the presence of necrosis around the portal spaces and portal inflammation were investigated and scored, for inflammation of the portal spaces if less than one third of the portal spaces are involved, mild (score 1) and If the inflammation is one half of the port spaces, it is moderate (score 2) and if it is more than two thirds, it is severe (score 3). Regarding lobular inflammation, if less than 10% of the liver parenchyma is involved: score 1, between 10 and 50%: score 2, and if it is more than 50% of the liver parenchyma, score 3 was given. Also, for the scoring of liver parenchyma necrosis, if less than 10% of liver

parenchyma score is 1, between 10 and 25% of liver parenchyma, score of 2 and in case of necrosis of more than 25% of liver parenchyma, score of 3 is given. A necroinflammatory score was given for liver tissue[24]. Also, other histopathological changes in liver failure, including the loss of the normal structure of liver tissue, steatosis (fat storage in hepatocytes), liver cell damage as hydropic degeneration, cholestasis, central and portal vein congestion, sinusoidal congestion and dilatation, increased of binucleated hepatocytes, interface hepatitis, proliferation of Kupffer cells, proliferation of bile ducts in the port space were investigated[25].

4 – 3. Masson trichrome staining to investigate liver fibrosis

After preparing 5-micron sections of liver tissue, Masson's trichrome staining was done to evaluate fibrosis. The stained slides were examined by a pathologist who did not know how to group the study, using a light microscope (Olympus, CX33, Tokyo, Japan) with a magnification of 100 and the amount of fibrosis based on collagen deposition, which is seen in blue color graded. For the quantitative grading of liver fibrosis damage, ten fields were observed in each slide and numbered according to Knodell et al.'s article in the following order: zero = no fibrosis, one = fibrosis limited to the port space, two = extensive fibrosis in the port space and foci of the bridging, three = bridging fibrosis (between two port spaces or the port space with the central vein) widely, and four = cirrhotic view[26].

4–4. Liver enzymes assay

The activity of liver enzymes (ALP, LDH) in serum with T-BIL was measured using the standard kit (Pars Azmoon, Tehran, Iran) by enzymatic method.

4–5. Investigating the amount of oxidant/antioxidant activity in the liver

First, the liver tissue was homogenized in 1.5% KCL solution and then centrifuged at 1000 rpm for 10 minutes. The thiobarbituric acid method was used to evaluate malondialdehyde (MDA). Homogenous liver tissues were mixed with trichloroacetic acid and thiobarbituric acid (TBA) and then placed in a boiling water bath for 45 minutes, and after cooling and adding n-butanol, absorbance at 534 nm wavelength was measured [27]. Superoxide dismutase (SOD) and total antioxidant capacity (TAC) activities were determined according to the instructions of the Randox kit (UK; Cat NO.RS504; and ferric reducing/antioxidant power (FRAP), in liver tissue homogenates, respectively. SOD absorbance at 570 nm was measured.

4–6. ELISA assay

Hepatic levels of sestrin2, Nrf2 and TNF- α were measured using commercial ELISA kits (R&D, USA).

4–7. Statistical Analysis

In this study, SPSS software version 26 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. Data normality was checked by Shapiro-Wilk test. Also, one-way ANOVA with Tukey's post hoc test was used for normal distribution data. Kruskal-Wallis test followed by Mann-Whitney U test was used for non-normally distributed data. Data expression was reported as mean $\pm$ SEM. $P < 0.05$ was considered statistically significant.

4–8. 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.

Declarations

Declaration of Interest Statement

The authors didn't have any declaration of interest.

Author Contribution

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; 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.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. 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.

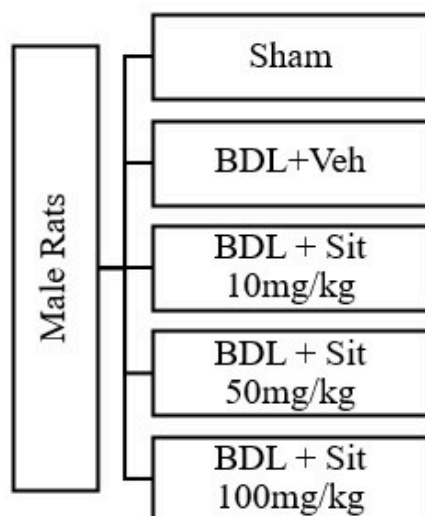
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Figures

A



B

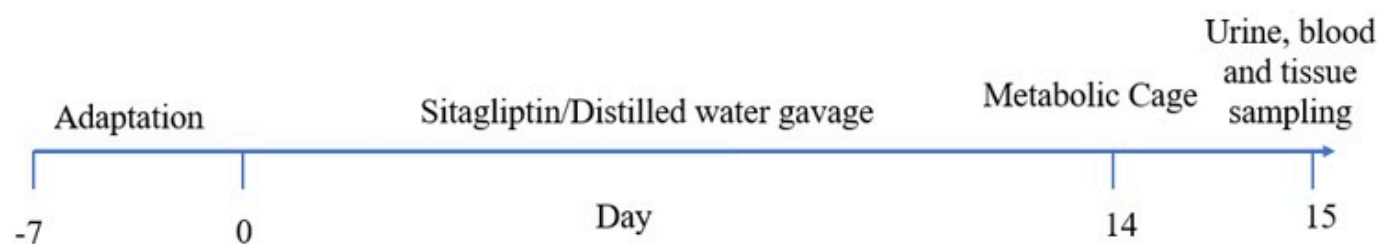


Figure 1

Schematic representation of the experimental groups (A), and timeline of the study (B). Sit: sitagliptin, BDL: bile duct ligation, Veh: vehicle.

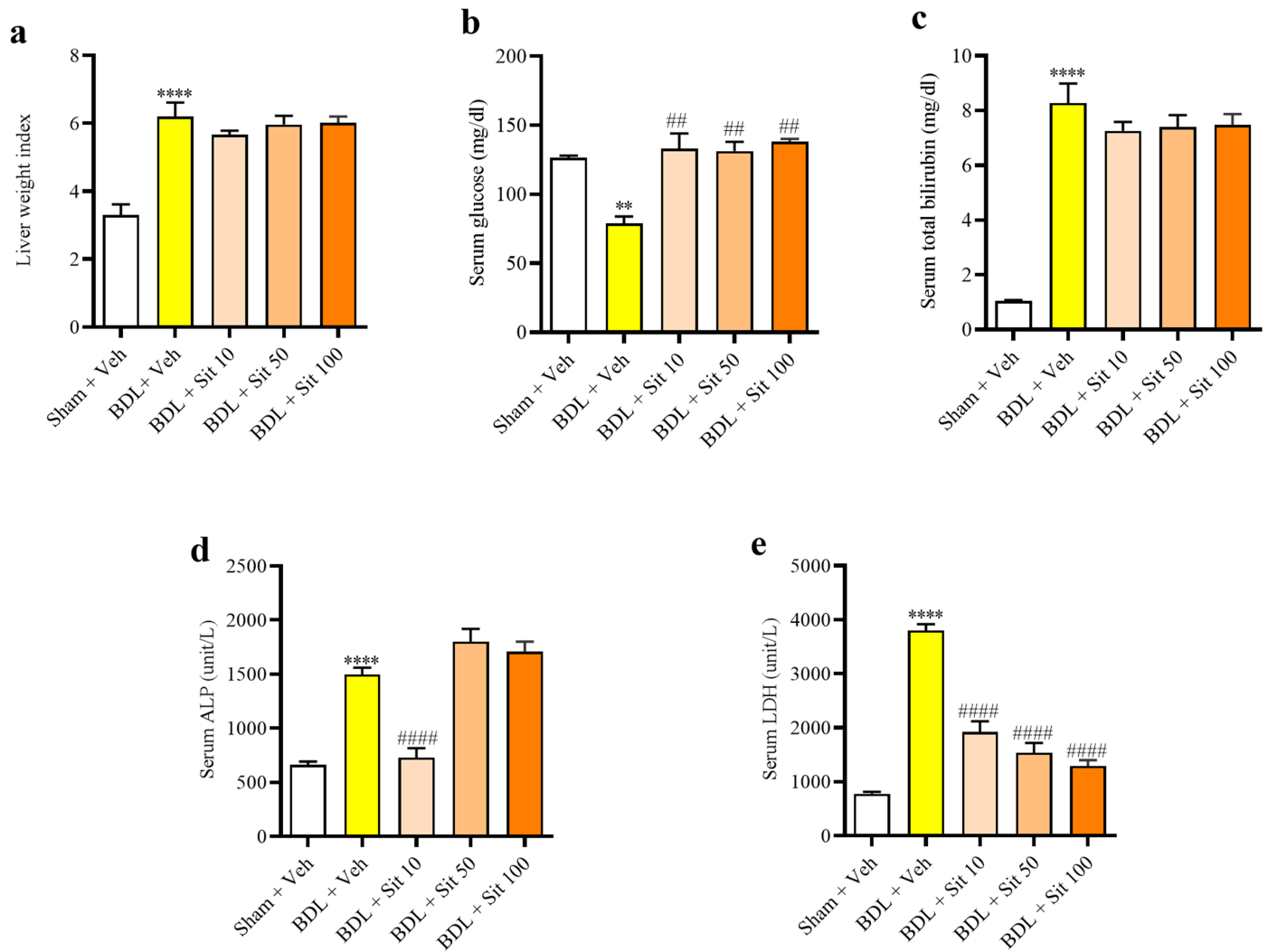


Figure 2

Effect of sitagliptin on the liver weight index, serum levels of glucose, total bilirubin (T-bil), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). Statistical significance is indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ VS Sham + veh, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$ VS BDL+veh; (mean $\pm$ SEM, $n = 7$). Sham+vehivle (Sham+Veh), Bile duct ligation + vehicle (BDL+Veh), BDL+sitagliptin 10, 50, or 100 mg/kg (BDL+Sit 10, 50, 100).

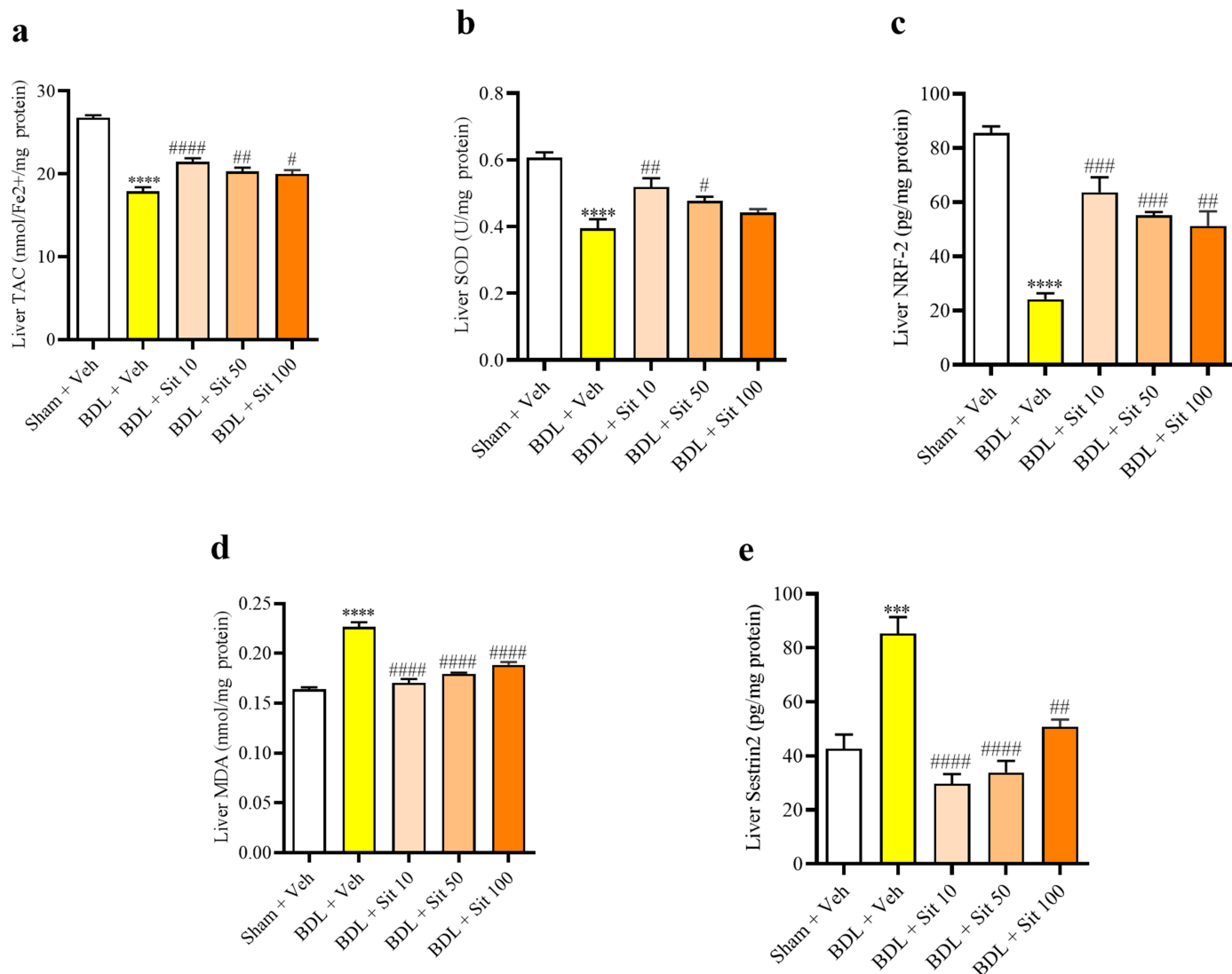


Figure 3

Effect of sitagliptin on liver level of total anti-oxidant capacity (TAC), superoxide dismutase (SOD) activity, Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2), malondialdehyde ratio (MDA), and sestrin2 (sesn2). Statistical significance is indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ VS Sham + veh, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$ VS BDL+veh; (mean $\pm$ SEM, $n = 7$). Sham+vehivle (Sham+Veh), Bile duct ligation + vehicle (BDL+Veh), BDL+sitagliptin 10, 50, or 100 mg/kg (BDL+Sit 10, 50, 100).

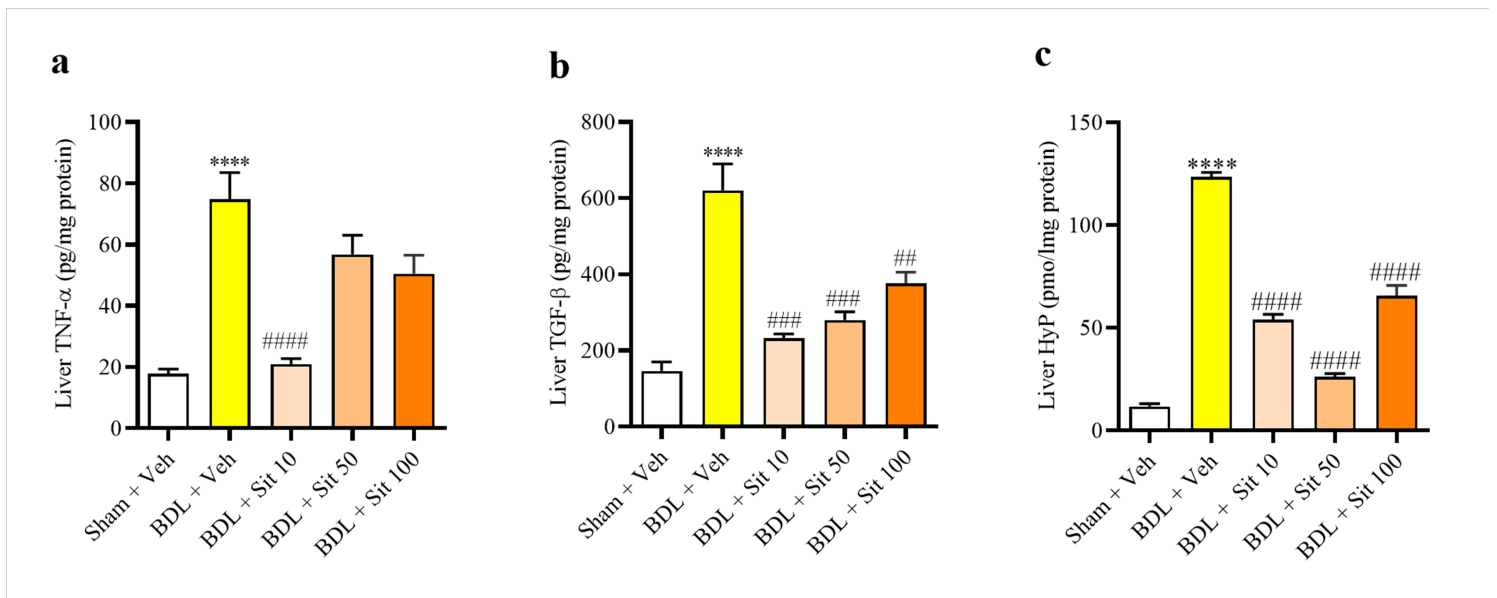


Figure 4

Effect of sitagliptin on liver level of tumor necrosis factor alpha (TNF-α) levels, transforming growth factor (TGF)-β, and hydroxyproline. Statistical significance is indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ VS Sham + veh, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$ VS BDL+veh; (mean $\pm$ SEM, $n = 7$). Sham+vehivle (Sham+Veh), Bile duct ligation + vehicle (BDL+Veh), BDL+sitagliptin 10, 50, or 100 mg/kg (BDL+Sit 10, 50, 100) and Sham+ sitagliptin 50 mg/kg (Sham+Sit 50).

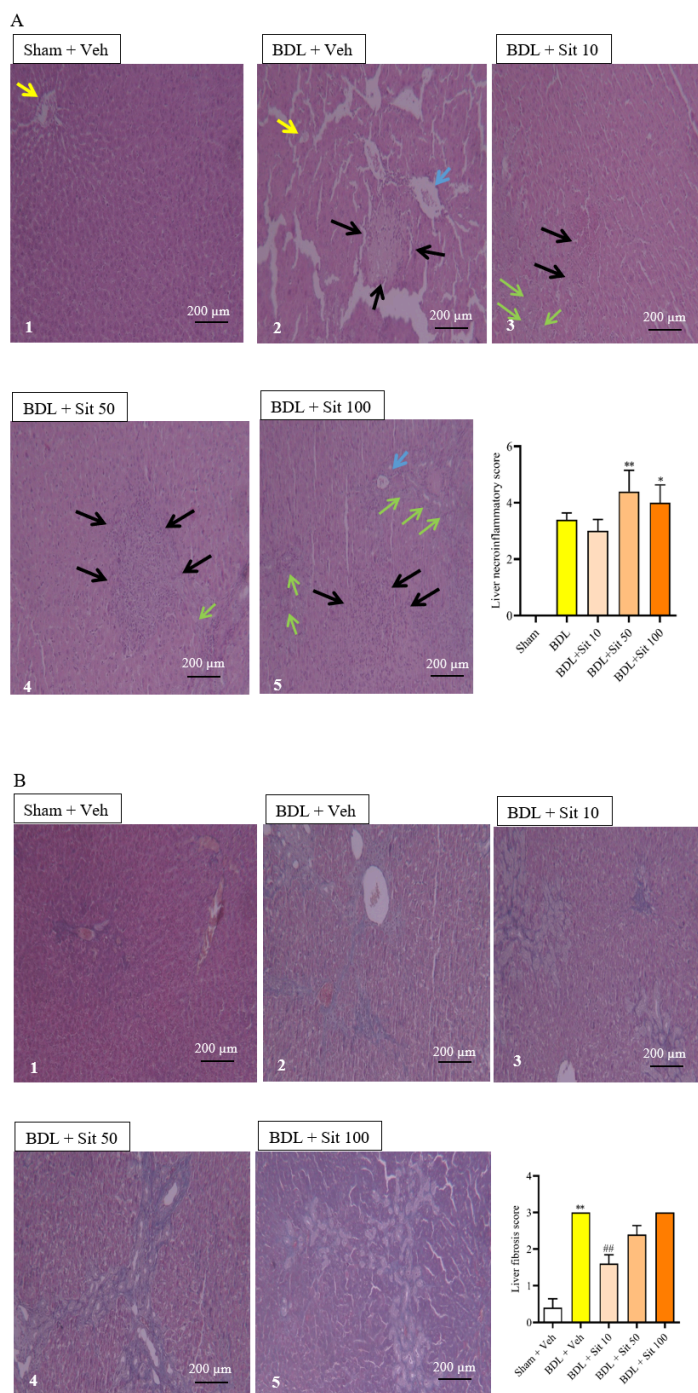


Figure 5

Representative necrosis and inflammation by H&E staining (A) and fibrosis by trichrome staining (B) of liver sections from studied groups. Yellowish arrow shows central vein, lobular necrosis and inflammation show with black arrows, bluish arrow represents portal tract with portal vein congestion and bile duct proliferation show with green arrows. (all images are at $\times 100$ magnification). Quantitative analysis of the liver necrosis score. Statistical significance is indicated as follows: $**P < 0.01$ VS Sham +

veh, ## $P < 0.01$ VS BDL + veh; (mean $\pm$ SEM, n=5). Sham+vehivle (Sham+Veh), Bile duct ligation + vehicle (BDL+Veh), BDL+sitagliptin 10, 50, or 100 mg/kg (BDL+Sit 10, 50, 100).