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Possible Protective Effect of Papaverine on ANIT Induce Cholestasis in Rat [#]

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Abstract

Intrahepatic cholestasis is a clinical syndrome caused either by a defect in synthesis or flow of bile acid. pathophysiology of cholestasis is complicated by many variables, including oxidative stress, inflammatory response, and dysregulation of the bile acid transporter. Rats, mice, and guinea pigs are utilized as experimental animals, and alpha-naphthylisothiocyanate (ANIT) is administered to them to create a model that closely resembles intrahepatic cholestasis in humans. This study examined the protective effects of papaverine, a non-narcotic opium alkaloid derived from papaver somniferum and discovered as a farnesoid X receptor (FXR) agonist, on alpha-naphthylisothiocyanate (ANIT)-induced cholestasis in rats. White albino rats utilized in this study were divided into 3 groups (10 rats per group), Group I (control) or vehicle group rats administered corn oil (1ml/kg) once daily 48 hours before sacrifice. Group II rats orally administered ANIT100mg/kg single dose 48 hours before sacrifice. Group III rats were administered 100mg/kg papaverine orally for 7 consecutive days and on day 5 rats were administered ANIT. The results showed that papaverine treatment decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bile acid, as well as increased antioxidant enzyme and decreased inflammatory mediators. In conclusion, papaverine may have a protective effect to alleviate ANIT-induced cholestasis and may be a therapeutic target to treat cholestasis.

Keywords: Intrahepatic cholestasis, ANIT, papaverine

التأثير الوقائي المحتمل لجرعه منخفضه من بابافرين على الركود الصفراوي المحفزبالفانفثيل ازوثايوسياناييد في الجرذان[#]

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الخلاصة

الركود الصفراوي داخل الكبد هو متلازمة سريرية سببها إما خلل في صناعة أو تدفق حمض الصفراء ، وهناك العديد من الاسباب التي تسبب الركود الصفراوي مثل الالتهاب ، والإجهاد التأكسدي ، وعدم انتظام ناقل حامض الصفراء. إعطاء ANIT لحيوانات التجارب ، مثل الجرذان والفئران وخنازير غينيا ، يستخدم لإنشاء نموذج يحاكي بدقة الركود الصفراوي داخل الكبد . بابافيرين أفيون غير مخدر معزول من papaver somniferum وتم تحديده بانه يرتبط بمستقبلFXR وتهدف هذه الدراسة إلى دراسة تأثيره الوقائي على الركود الصفراوي الناجم عن(alpha-naphthylisothiocyanate (ANIT في الجرذان. تم تقسيم الجرذان المستخدمة في هذه الدراسة إلى ٣ مجموعات (عشرة فئران لكل مجموعة).المجموعة الأولى (مجموعة السيطرة) التي تم إعطاؤها زيت الذرة (١ مل / كجم) مرة واحدة قبل ٤٨ ساعة من التضحية بالمجموعة. اما المجموعة الثانية فتم اعطاء ١٠٠ ملغ/كغم كجرعه واحده عن طريق الفم alpha-naphthylisothiocyanate (ANIT) ٤٨ ساعة قبل التضحية بالمجموعة اما المجموعة الثالثة فتم اعطاء ١٠٠ مجم / كجم من بابافيرين لمدة ٧ أيام متتالية وفي اليوم الخامس تم اعطاء جرعه ANIT١٠٠ملغ/كغم وتم التضحية بالمجموعة بعد ٤٨ ساعه. اظهرت نتائج المجموعة الثالثة ان هناك انخفاضا معنويا في ALT ,AST,ALP, GGT إجمالي البيليروبين ، إجمالي حمض الصفراء وأيضًا ادى الى زيادة إنزيم مضادات الأكسدة GPX وتقليل TNF-α, MDA, و interleukin IL1-β. في الختام ، يمكن توفير تأثير وقائي بواسطة بابافيرين لعلاج الركود الصفراوي المستحث ب ANIT ، وقد أشارت هذه النتائج إلى أنه يمكن اعتبار البابافيرين هدفًا علاجيًا لعلاج الركود الصفراوي.

الكلمات الافتتاحيه:الركود الصفراوي ,ANIT,بابافرين FXR,

Introduction

The largest organ in the human body is the liver, which represents 2% of total body weight; it is crucial for clearing waste and poisons [(1,2)]. Cholestasis can be caused by a problem with bile production or bile secretion and flow [(3)].

In recent years, cholestasis prevalence has increased significantly, raising serious public health concerns. Cholestasis can be classified as intra- intrahepatic or extrahepatic cholestasis and is caused by stones, tumors, parasitic infections, immune-mediated conditions, drugs like steroids, nonsteroidal anti-inflammatory drugs, antibiotics, and anti-diabetic agents [(4)].

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There are currently very few clinical options for cholestasis therapy. The Food and Drug Administration (FDA) has approved two therapeutic medications that can be used to treat cholestatic liver diseases: obeticholic acid (OCA) and ursodeoxycholic acid (UDCA), fifty percent of patients do not respond to UDCA treatment; hence, the clinical outcome is unsatisfactory, OCA also causes side effects, including fatigue, itching, and abdominal pain [(5)]. Thus, it is essential to seek a more effective medication.

Bile acids are crucial regulators of cholesterol and triglyceride homeostasis and are essential for the efficient absorption of lipids and fat-soluble vitamins, and they also aid in digestion [(6)]. Nuclear farnesoid X receptor (FXR) is triggered by bile acid; FXR is essential for preserving the homeostasis of bile acids and is largely expressed in the liver, kidney, and gut; FXR controls target gene transcription by binding to FXR response elements (FXREs) through heterodimerization with the retinoid X receptor (RXRs) [(7)]. FXR regulates bile acid synthesis in the liver via promoting transcription of small heterodimer partner (SHP), which in turn suppresses LRH-1-mediated expression of cholesterol-7alpha-hydroxylase (CYP7A1), the major enzyme of bile acid synthesis, During the reabsorption of bile acids in the ileum, FXR is activated in the intestine, increasing fibroblast growth factor 15/19 (FGF15/19) after entering the portal circulation, stimulates the liver's FGF receptor 4 (FGFR4) to inhibit the production of bile acids, mediating link between the liver and gut[(8)]. Elevated blood levels of gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) are biochemical indicators of cholestasis; these enzymes are found in the hepatocytes' plasma membrane [(9)]. Heme breakdown produces unconjugated bilirubin, which then conjugates by the liver and excretes in bile. Cholestasis is closely correlated to conjugated hyperbilirubinemia, which can occur when the liver loses 50% of its ability to secrete bile [(10)]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are biochemical indicators of hepatocellular liver injury [(11)].

Hepatotoxic substance α-naphthylisothiocyanate (ANIT, C10H7N C S) has mostly been utilized as a tool to research the etiology of chemically induced cholestasis, Cytochrome P450 enzymes break down ANIT and subject it to GSH conjugation [(12)]. The conjugate of ANIT and GSH is carried into the bile, where it quickly dissociates and results in injured bile duct epithelial cell, which lead to Reduced bile flow, bile acid buildup in the liver, and lead to necrosis of the hepatocytes [(12)]. Hence, by giving ANIT to experimental animals like rats, mice, and guinea pigs, it may be possible to create a model that closely resembles human intrahepatic cholestasis and liver injury [(13)]. According to reports, ANIT activates neutrophils, which raises levels of interleukin-6 and tumor necrosis factor-alpha. Lipid peroxidation carried by reactive oxygen species (ROS) has also been linked to the development of the cholestasis that was generated by ANIT; therefore, oxidative stress, dysregulation of bile acid transporters, and inflammation are major factors to the liver damage carried by ANIT [(14)].

Papaver somniferum contains the non-narcotic opium alkaloid papaverine, Papaverine is a smooth muscle relaxant used to treat erectile dysfunction and vasospasm; it works by blocking phosphodiesterase 10A [(15)]. According to reports, papaverine is an FXR agonist [(16)].

This study aims to determine whether papaverine may have protective effects on ANIT-induced cholestasis in rats.

Materials and Methods

Chemicals and reagents

Alpha-naphthylisothiocyanate (ANIT) and papaverine (PPV) obtained from sigma Aldrich St Louis USA, standard assay rat's kits for alkaline phosphatase (ALP), total bilirubin (TBIL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were obtained from linear chemicals S.L.U Spanish and rat gamma-glutamyl transferase (GGT), tumor necrosis factor-alpha (TNF-α), interleukin1β (IL-1β) Elisa kit were purchased from elabscience U.S.A. total bile acid (TBA) colorimetric assay kit also obtained from elabscience U.S.A, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX) Elisa kit were purchased from MyBioSource U.S.A.

Experimental animals

Thirty male albino rats, 4-6 weak old, with an average body weight of 100–150g, were obtained from the College of Pharmacy/university of Baghdad's animal house. Before the treatment, the animals had a two-week acclimatization period. Water and a regular diet were freely available. They were kept in regular conditions of humidity, temperature (25 C), and light/dark cycles. Experimental studies were carried out under the care and standard experimental animal methodology. This study separated the animals into three groups, each including ten rats. Each group received the following: (1) Group I (control), in which rats were orally administered 1ml/kg of corn oil 48 hours before sacrifice.

(2) Group II single dose of ANIT (100mg/kg) [(17)] orally administered to rats 48 hours before sacrifice.

(3) Group III rats were administered 100mg/kg [(18)] papaverine (PPV) orally for 7 consecutive days and at day 5, ANIT 100mg/kg was orally administered to rats 48 hours before sacrifice. At the end of the experiment, the animals in each group were euthanized by diethyl ether and sacrificed.

Biochemical assessment

Blood was collected in the gel tubes and then centrifuged for 20 min at 3600 rounds per minute (rpm). Serum was obtained and stored in Eppendorf tubes at – 20 °C to determine AST, ALT, ALP, GGT, total bilirubin, and total bile acid.

preparation of liver tissue homogenate

The liver was removed from the rat after euthanized by diethyl ether, then rinsed in ice-cold buffer phosphate saline pH 7.4 to remove extra blood, and weighed before homogenization (1g of tissue and 9 ml of PBS). The tissue was then homogenized using a homogenizer. The homogenate was then spun using a cold centrifuge for 20 minutes at 10000 rpm, and the supernatant was used to calculate the amounts of GPX, SOD, MDA, TNF-α, and IL-1β.

Enzyme-Linked Immunosorbent Assay

ELISA kits use to measure TNF-α, IL-1β, GPX, SOD, and MDA. Briefly, the sample, standards, and blank were added to the precoated antibody plate and incubated for 1 h at 37°C then, biotinylated-specific antibody, enzyme conjugate, chromogenic substrate, and stop solutions were added in sequential order. The optical density (OD) at 450 nm was measured by a microplate reader.

Statistical analysis

Using graph prism version 8, data were examined. The mathematical information was presented as mean ± standard error of the mean. It was done using an analysis of variance (ANOVA) and the Tukey multiple comparison test. All of the results given in this study were considered significant if the p-value was less than 0.05 ($p<0.05$).

Results

Effects on alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Rats orally administered ANIT (group II) at a dose of 100mg/kg showed significant elevation ($p<0.05$) in the level of ALT and AST compared to the level in the control (group I) rats, mean ± SEM of ALT and AST were respectively 38.76 ± 1.607 and 38.54 ± 2.288 compare to 19. 01 ± 0.348 and 19. 22 ± 1.036, as shown in Figure 1 (A, B).

Upon papaverine treatment, the plasma level of ALT and AST was a significant decrease compared to the level in group II rats, where mean ± SEM of ALT and AST were respectively 28.03 ± 0.6955 and 27.9 ± 0.5965 compared to 38.76 ± 1.607 and 38.54 ± 2.288 as shown in figure 1(A, B).

Effects on total bilirubin and total bile acid

ANIT administration caused significant elevation ($p<0.05$) in the level of total bile acid and total bilirubin in **group II** compared to the level in control (group I) rats, mean ± SEM of total bile acid and total bilirubin were respectively 171.4 ± 3.002 and 1.762 ± 0.0727 compared to 48.42 ± 1.709 and 0.415 ± 0.02 as shown in figure 1 (C, D).

Rats treated with papaverine cause a significant decrease ($p<0.05$) in total bile acid and total bilirubin compared to the level in group II rats where mean ± SEM of total bile acid and total bilirubin were respectively 102.7 ± 4.947and 0.85 ± 0.0469 compared to 171.4 ± 3.002 and 1.762 ± 0.0727 as shown in table 1 as shown in figure 1 (C, D).

Effects on alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT)

ALP and GGT were significantly increased ($p<0.05$) in ANIT-treated rats compared to the level in the control (group I) rats; the Mean ± SEM of ALP and GGT were respectively 74. 39 ± 3.246 and 165.1 ± 11.48compare to 42. 59 ± 1.394 and 15. 79 ± 3.6, as shown in Figure 1 (E, F).

Papaverine led to a significant decrease ($p<0.05$) in ALP and GGT in group III compared to the level in group II rats where mean ±SEM of ALP and GGT were respectively 55. 52 ± 1.021 and 60. 4 ± 3.557 compared to 74. 39 ± 3.246 and 165. 1 ± 11.48, as shown in Figure 1 (E, F).

Effects on interleukin 1β (IL-1β) and tumor necrosis factor α(TNF-α)

ANIT caused significant elevation ($p<0.05$) in the level of IL-1β and TNF-α of group II compared to the level in control (group I) rats. The mean ± SEM of IL1β and TNF-α were respectively 1161 ± 35. 23 and 386.9 ± 28.12 compared to 374 ± 47. 17 and 164. 9 ± 10.75, as shown in Figure 2 (A, B).

Treatment with papaverine for seven days caused a significant decrease ($p<0.05$) in IL1β and TNFα compared to the level in group II rats where mean ± SEM of IL1β and TNFα were respectively 621. 7 ± 22.37 and 220 ± 17. 2 compare to 1161 ± 35. 23 and 386. 9 ± 28.12, as shown in Figure 2 (A, B).

Effects on malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX)

Group II administered oral ANIT showed significant elevation ($p<0.05$) in the level of MDA compared to the level in control (group I) rats. The mean ± SEM of MDA was 40.92 ± 2.393 compared to 9.564 ± 1.291, as shown in Figure 2 (C). papaverine caused a significant decrease ($p<0.05$) in MDA in group III compared to the level in group II rats where mean ± SEM of MDA were respectively 20.91 ± 1.513 compared to 40.92 ± 2.393, as shown in figure 2 (C).

ANIT group showed a significant decrease ($p<0.05$) in the level of SOD and GPX compared to the level in the control (group I) rats, mean ± SEM of SOD and GPX were respectively 3.4 ± 0.319 and 11.96 ± 1.311 compared to 41.45 ± 2.898 and 51.52 ± 2.21 as shown in figure 2 (D, E).

There was a significant increase ($p<0.05$) in GPX but not SOD in group III rats treated with papaverine compared to the level in group II rats where mean ± SEM of SOD and GPX were respectively 8.937 ± 0.3144 and 18.49 ± 1.676 compared to respectively 3.4 ± 0.319 and 11.96 ± 1.311 as shown in figure 2 (D, E).

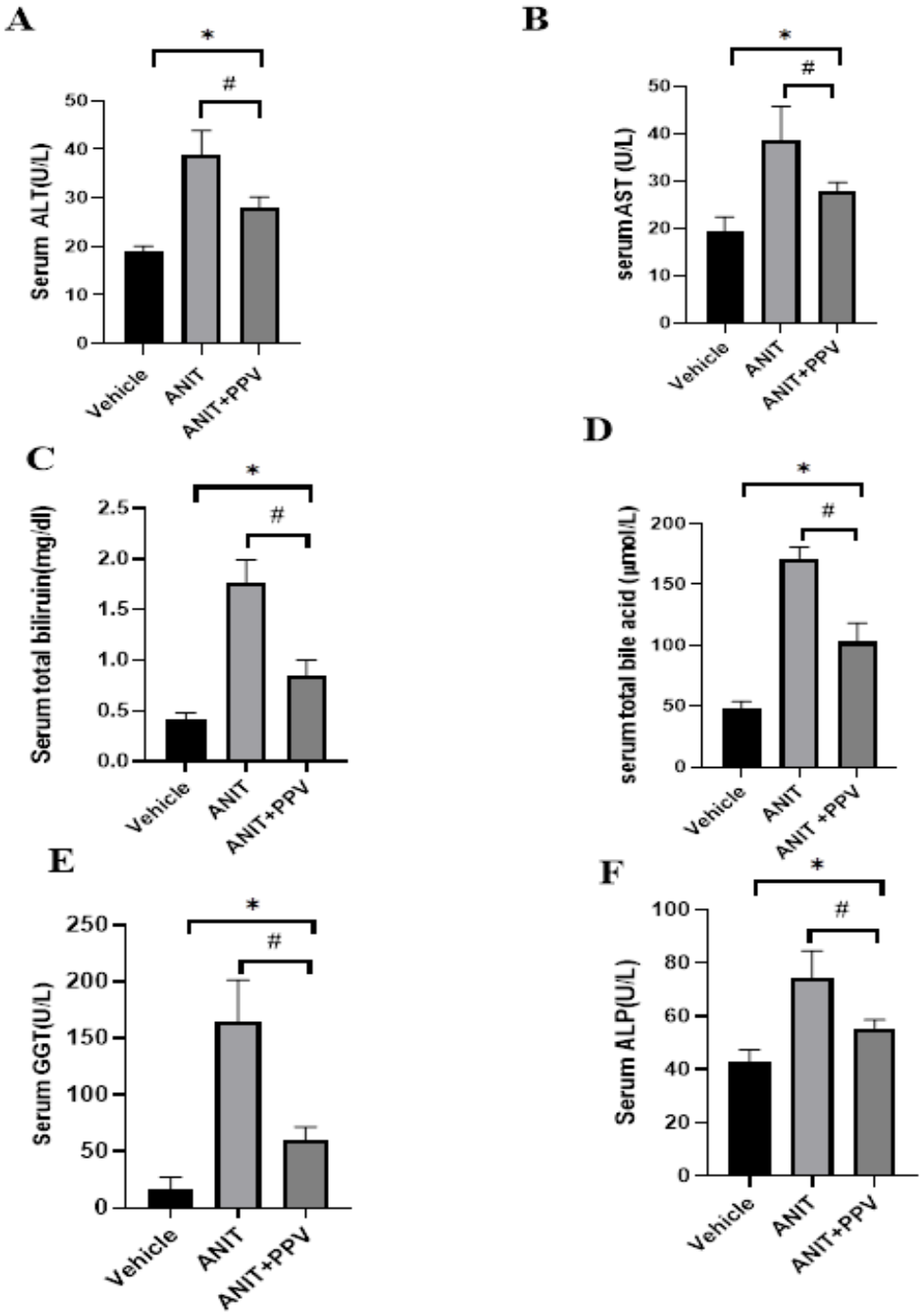

Figure1. Papaverine effect on the biochemical parameter in ANIT-induced cholestasis. Serum levels of (A) ALT, (B) AST, (C) serum total bilirubin, (D) serum total bile acid, (E) GGT, (F)ALP. Data Represented as mean ±S.E., n=10 animals. *P<0.05 vs. control; #P < 0.05 vs. the ANIT-treated rats.

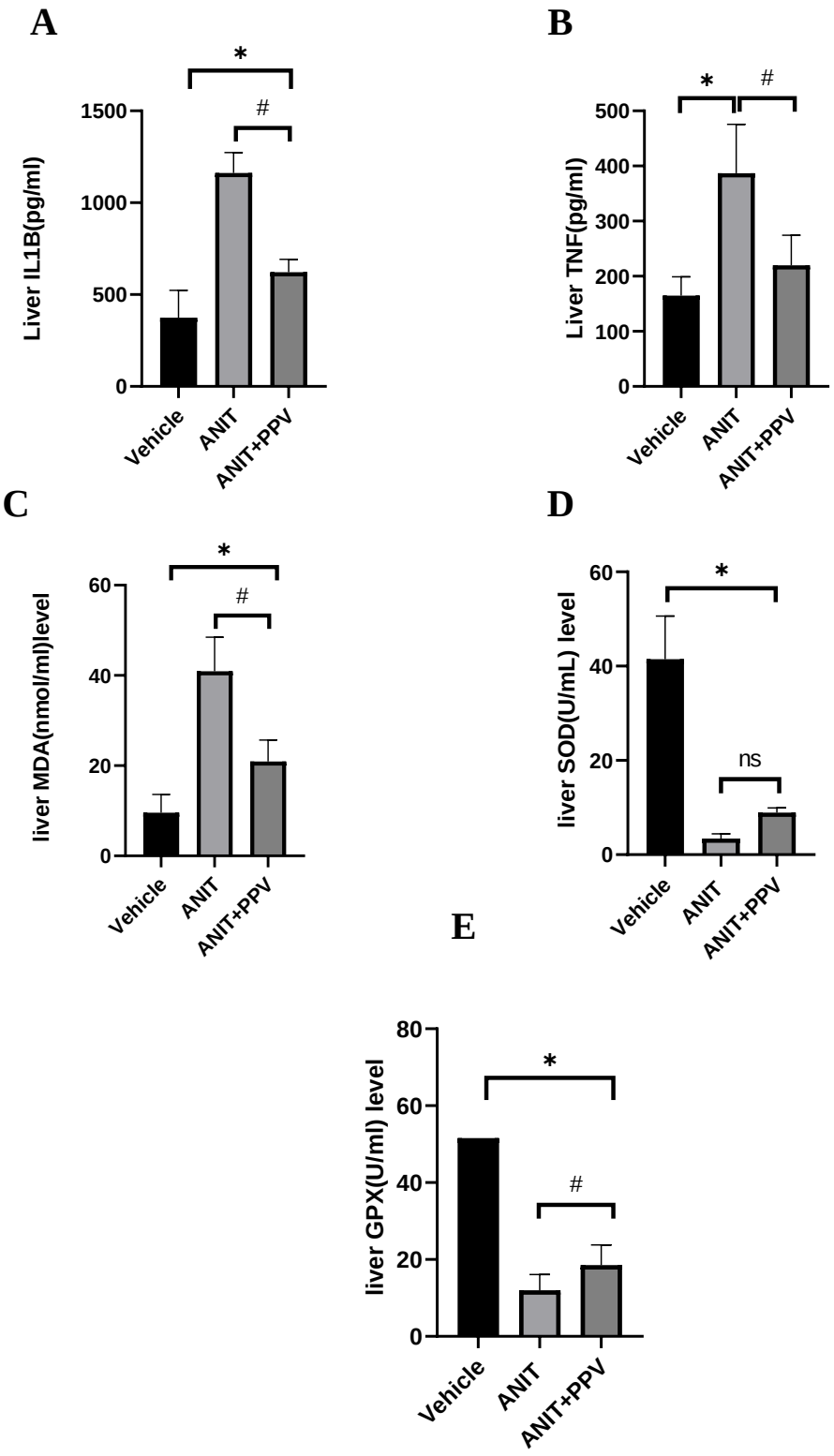

Figure2. Papaverine effect on inflammatory markers and some antioxidant parameters in ANIT-induced cholestasis. Tissue levels of (A) IL-1β, (B) TNF-α, (C) MDA, (D) SOD, (E)GPX Data Represent as mean ±S.E., n=10 animals. *P<0.05 vs. control; #P < 0.05 vs. the ANIT-treated rats.

Histopathologic examination

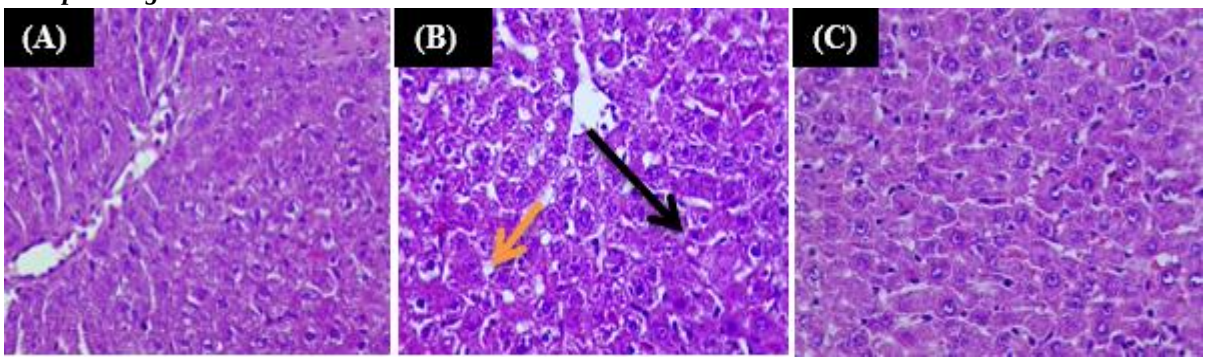

Figure 3. The effect of papaverine on histological changes in the liver tissue of ANIT-induced cholestasis in rats. Image of H & E stained liver sections (20x magnification) at 48 h after ANIT administration was shown. (A) control group: no histological change was observed, (B) ANIT group: revealed sinusoidal congestion was marked by a yellow arrow and dead cells were marked by a black arrow, (C) PPV treated group: mild dilation of sinusoids and depletion of glycoprotein.

Discussion

UDCA and OCA are frequently used in treating cholestasis, but the results are unsatisfactory. Cholestasis is characterized by an intrahepatic accumulation of bile acids either due to a defect in formation or bile flow, which leads to hepatocyte toxicity [19]. AST and ALT, extensively used clinical indicators of liver function and a biochemical diagnostic of hepatocellular damage, are present in the cytoplasm [11,20]. 0ur study shows a significant increase in ALT and AST in group II, inconsistent with previous study [21]. Also, the administration of papaverine caused a significant decrease in ALT and AST, which can attenuate liver damage caused by ANIT.

Elevated blood levels of gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) are biochemical indicators of cholestasis; these enzymes are found in the hepatocytes' plasma membrane. Bile acids act as detergents, releasing enzymes from the plasma membrane of hepatocytes as they build up in the liver; Bile acids also facilitate the production of ALP [22].

In this study, we found rats that were orally administered ANIT exhibited liver injury and cholestasis as an indicator by the serum activity level of GGT and ALP; this agrees with a previous report [23,24], Also, administration of papaverine caused a reduction in ALP and GGT suggesting a protective effect of papaverine.

Elevated serum bilirubin, particularly direct bilirubin, can result from cholestasis due to irregularities in bilirubin synthesis, conjugation, and excretion; hepatocellular injury can increase both direct and indirect bilirubin; however, the rise of direct bilirubin is typically more noticeable than that of indirect bilirubin [25].

In addition to serving as lipid absorption detergents, bile acids also function as signaling molecules that are crucial for maintaining the homeostasis of lipid, glucose, and energy levels [26]. The production and clearance of BAs can be affected in various liver diseases, which may result in changes to the concentration and makeup of BAs in the liver; BA buildup has the potential to cause hepatotoxicity and even liver necrosis [27]. Therefore, BAs have been considered biomarkers of cholestasis in pregnancy [28].

Figure 1 shows a significant increase in total bilirubin and total bile acid in the ANIT group, and this agrees with other studies [29,30], Also, administration of papaverine causes a significant decrease in total bilirubin and total bile acid, suggesting a protective effect of papaverine.

A crucial component of ANIT-induced cholestasis is oxidative damage, which may result from one or more sulfhydryl-reactive intermediates conjugated with glutathione [31, 32]. Reactive oxygen species are produced mostly by mitochondria, especially after cholestasis, when their antioxidant properties are depleted [33]. Reactive oxygen species can damage proteins, DNA, and phospholipids in the mitochondrial membrane, uncoupling oxidative phosphorylation and changing the rate at which ATP is synthesized [34].

The major feature of cholestasis is oxidative stress; 24 hours after bile duct ligation (BDL), laboratory rats' plasma, kidneys, brain, and hearts show an increase in malondialdehyde (MDA); rodents and humans with bile duct abnormalities have elevated levels of oxidative stress, MDA is a biomarker of lipid peroxidation ([35, 36].

The results exhibited a significant increase in MDA in the ANIT group, in agreement with other studies [37,38]. Also, administration of papaverine causes a significant decrease in MDA, suggesting an antioxidant effect of papaverine.

Like other studies, ANIT causes a significant decrease in SOD and GPX [39]. Also, administration of papaverine causes a significant increase in GPX with non-significant changes in

SOD activity, suggesting an antioxidant effect of papaverine and may require higher doses of papaverine to induce SOD level.

The main cells implicated in oxidative hepatic damage are parenchymal hepatic cells. Parenchymal cells have mitochondria, microsomes, and peroxisomes that can generate ROS. The endothelial cells of the bile duct, hepatic stellate cells, and Kupffer cells are all extremely sensitive to chemicals associated with oxidative stress. TNF and other inflammatory cytokines are produced by Kupffer cells in response to oxidative stress, which worsens hepatic inflammation and apoptosis [(40)].
Macrophages secrete proinflammatory cytokine tumor necrosis factor; many disorders have been linked to TNF dysregulation [(41,42)]. Papaverine, a potent vasodilator and opium alkaloid, is believed to have antioxidant properties by inhibiting the mitochondrial glutamate oxidase system, which lowers oxidative stress and lipid peroxidation [(43)].

High-mobility group box 1 (HMGB1) is a nuclear protein required for the regulation of gene transcription; HMGB1 is released from necrotic cells or activated immune cells and extracellular HMGB1 regulates the release of pro-inflammatory cytokines such as interleukin IL-1β, IL-6, and tumor necrosis factor TNF- α by activating the receptor for advanced glycation end products (RAGE) and toll-like receptors (TLRs) [(44)].

Recent studies identified papaverine as a RAGE inhibitor That suppressed the HMGB1-mediated production of pro-inflammatory cytokines (IL-6 and TNF-α) in macrophages [(45)].

This study shows a significant increase in TNF-α and IL1β in the ANIT group, which agrees with other studies [(46,47).] Also, administration of papaverine causes a significant decrease in TNF and IL1β, suggesting an anti-inflammatory effect of papaverine.

Conclusion

According to the findings of this study, it could be concluded that the protective effect of papaverine at a dose of 100mg/kg when administered with 100 mg/kg ANIT can attenuate the ANIT-induced cholestasis in rats.

Funding

None

Ethics Statements

The University of Baghdad/ College of Pharmacy Animal Research Local Ethics Committee accepted this study with protocol #: 2006 at 15/2/2022.

Conflict of Interest

non conflict of interest.

Author Contributions

Conception and Design of the study by Dr. Munaf H. Zalzala. Conducted animal experiment and manuscript writing by Doaa Adnan Atshan.

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