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Carvedilol versus Urolithin A as a hepatoprotective agent against Thioacetamide induced liver fibrosis in adult male albino rats (Histological and Immunohistochemical study)

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Background
The prevalence of liver fibrosis has increased. It is defined as the reaction to the chronic injury of the liver by which the hepatic parenchymal cell is distorted and replaced by extracellular matrix.

Objective
To investigate the probable therapeutic properties of Carvedilol and Urolithin A on liver fibrosis evedinced by thioacetamide (TAA) in rats.

Materials and methods
The study was conducted on 36 mature male albino rats which was distributed in four groups (nine rats each): The control group, (TAA) group, which received 200 mg/kg of (TAA) by (Ip) injection three times a week, Urolithin A group, received both (TAA) and urolithin A solution (50 mg/kg) by (Ip) injection every day, and Carvedilol group, given (TAA) and carvedilol (10 mg/kg) orally. All treatments were given for eight weeks. Serum concentrations of (AST), (ALT), and (ALP) were determined and the histopathological and immunohistochemical staining were used to evaluate the hepatic tissues.

Results and conclusion
TAA-treated rats showed pathological hepatic damage and substantial elevations in serum liver enzymes and hepatic malondialdehyde (MDA) concentration. Additionally, it reduced the activity of superoxide dismutase (SOD) and catalase (CAT) in the liver tissue. When urolithin A or carvedilol were given in combination with (TAA), they markedly diminished the (MDA) concentration in the liver tissues and the serum liver enzymes while augmenting the activity of the liver tissue's (SOD) and (CAT). The histopathological and biochemical alterations prompted by (TAA) administration were ameliorated under the influence of Urolithin A and Carvedilol. Carvedilol exhibited a significantly superior hepatoprotective and anti-fibrotic effect compared to Urolithin A.

Keywords: Thioacetamide, Urolithin A, carvedilol, (PCNA), (α- SMA), hepatic fibrosis.

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Introduction

The prevalence of liver fibrosis has increased worldwide According to the most recent estimates released in 2020, there are about 1.5 billion cases of liver fibrosis worldwide, representing a 13% increase from 2000 [1].

Hepatic fibrosis results from a chronic inflammatory and tissue repair process, by which hepatic parenchymal cells are distorted and replaced by extracellular matrix or scar [2]. A well-known mechanism in the pathophysiology of liver fibrosis is the stimulation of the hepatic stellate cell (HSC), and its transformation into a myofibroblast which generates alpha smooth muscle actin (α-SMA) [3].

Thioacetamide (TAA) is utilized in different industry sectors, including the leather processing, paper and textile, rubber, and pesticide industries. It is also used as a motor fuel stabilizer [4]. Additionally, it is shown to cause liver fibrosis in experimental animals; a centrilobular hepatocyte necrosis can be brought on by a single dosage of (TAA) [5-6].

Carvedilol is a nonselective β-blocker having anti-fibrotic properties in addition to its antihypertensive

effects [7]. However, the exact mechanisms of its antifibrotic role and its effectiveness in a separate experimental model remain unclear [8].

In the liver studies, researchers have revealed that carvedilol anti-fibrotic effect is owed to the antioxidant and anti-inflammatory properties [9]**.** Furthermore, by preventing (HSCs) activation and proliferation as well as by lowering the (TGFβ1/SMAD) pathway, carvedilol can reduce collagen synthesis in (HSCs) and subsequently improve liver cirrhosis [10].

Urolithins are a kind of polyphenol that are natural metabolites formed by the microbiota of the gut through enzymatic hydrolysis of ellagic acid and ellagitannins [11-12].

Urolithin A exhibits potent antioxidant properties, making it a potential treatment for pathological conditions induced by oxidative stress [13].

This investigation aims to analyze the (TAA) effect on the liver of rats and the possible protective benefits of urolithin A and Carvedilol through biochemical, histological, and immunohistochemical techniques.

Materials and methods

Drugs and chemicals

Thioacetamide (TAA) was obtained from Sigma Aldrich (St. Louis, MO, USA) in the form of a brownish powder of bad offensive odor. It dissolved in 10% Tween 20 then, mixed for total dissolution.

Urolithin

(Urolithin A in the form of powder with Cat. No. SML1791) and dimethyl sulfoxide (DMSO) with Cat. No. (D2650) were also obtained from Sigma Aldrich (St. Louis, MO, USA). To make a solution, it was liquefied in PBS which contains 5% DMSO by a concentration of 20 mg/mL and kept at 4 °C in darkness. Just before intraperitoneal injection, an appropriate amount from the solution was taken, then diluted in PBS to the needed concentration [14].

Carvedilol was provided from Multiapex Pharma Sidi Gaber, Alexandria Governorate, Egypt with the commercial name (CARVID) 12.5 mg tablets. The tablets were crushed and dissolved in distilled water forming a suspension.

Experimental animals

36 mature male albino rats 2 months old, weighting 180–210 g, were utilized in this research. The rats were provided by the experimental animal farm in Helwan, Cairo, Egypt. These animals were caged in separate plastic cages and received a standard diet and tap water. The lab conditions were ambient, at a temperature of 20 ± 2°C , moisture of approximately 55–65 %, and exposed to a 12 h light/dark rotation.

Experimental design

After one week of adaptation, the rats were divided into four groups, consisting of nine rats each.

Group I (control group): Subdivided into three equal subgroups:

• **Group Ia**: A regular diet was given to three animals.

• **Group Ib**: Three animals were injected (Ip) with 10% Tween 20 (5 mL/k.g) three times a week for eight weeks.

• **Group Ic:** The last three animals were injected (Ip.) with 5% DMSO/PBS solution (the same amount and duration as urolithin treated group).

Group II (TAA group): Rats injected (Ip) with 200 mg/kg body weight (b.w.) TAA, after dissolved in 10% Tween 20, three times a week for 8 weeks [15].

Group III (urolithin A group): Rats received both (TAA)(200 mg/kg) as in group II, and urolithin A solution at a dosage of 50 mg/kg through (Ip) injection every day for 8 weeks [16].

Group IV (carvedilol group): Rats were given (TAA) (200 mg/kg) as in group II, with concurrent management of carvedilol (10 mg/kg b.w.) through oral gavage daily for 8 weeks [17].

With the end of the experiment time (8 weeks), animals were fasted for 24 h, then they were sacrificed using an overdose of ketamine and xylazine anesthesia. Intracardiac puncture was used to obtain blood samples, which were placed in clot-activated tubes for analysis of the biochemical parameters [18].

Biochemical parameters (liver function test)

Centrifugation was used to separate the blood in clot-activated tubes for 15 minutes at 2500 rpm. By the spectrophotometry method, a spectrophotometer is used to detect the liver enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) [19].

Gross appearance of the liver

Evaluation of liver tissue was performed by opening through the abdominal and the thoracic cavities. The liver tissues were separated, washed using cold phosphate-buffered saline (PBS), dried using filter sheets, and examined for obvious pathological variations. [20].

Liver oxidative stress indicators

Before being homogenized, one gram of each liver was submerged in 10 mL of 10% PBS at pH 7.2. The liver tissue homogenates were made using a Teflon homogenizer (on ice), and any cell debris was excluded by centrifugation of the mixture for 15 minutes at 4 ◦C at 4500 rpm. The collected supernatant was used to detect the antioxidant activity using catalase (CAT), superoxide dismutase (SOD), and Malondialdehyde (MDA) analysis kits (Cayman Chemical Company, USA) [21].

Histological examination

The collected livers were cut into 2-centimeter cubes, preserved in 10% phosphate-buffered formalin, and then embedded in paraffin. 5 µm thickness slices were regularly stained by hematoxylin and Eosin (H&E) to analyze the overall histological structure and Masson trichrome staining was utilized to highlight the collagen fibers [22].

Immunohistochemistry

Glass slides containing Poly-L-lysine were used for (PCNA and α-SMA) staining processes. Briefly, hydrogen peroxide, sodium azide 0.03%, was used for chunk endogenous peroxidase for five min; then, slices were washed away. After that, slices that had been cleaned were incubated for 15 minutes with biotinylated primary antibody against PCNA (1:200, ab18197,abcam), as well as rabbit anti-rat α-smooth muscle actin antibody (1:100, ab5694,abcam) for 30 min. After three minutes of gentle rinsing in distilled water, tissue slices were set aside in the moist compartment within a buffer bath. Streptavidin (HRP) treated slices were incubated for 15 min, then washed in twice distilled water for three min. Biopsy slices were incubated with diaminobenzidine (DAB) substrate Chromogen for five min, then washed and counterstained with hematoxylin for 5 sec. [9].

Morphometric study

The Leica Qwin 500 image analysis computer system was utilized to morphometrically analyze serial sections stained with α-smooth muscle actin and PCNA, revealing the percentage of brown color in each section. Additionally, Masson's trichrome-stained sections were evaluated to determine the percentage of collagen fibers at a magnification of (x 200). Five non-overlapping fields were used for these procedures, and each group of rats had five different sections from five different rats.

Statistical analysis

The whole data were accessible as mean ± standard deviation. The Statistical Package for Social Science application (SPSS program; version 20.0 for windows, SPSS Inc., Chicago,IL,USA) was used to evaluate the data obtained from the biochemical records and the image analyzer. Using one-way ANOVA for statistical analysis.When the (P) value ≤ 0.05, the results were considered significant.

Results

Control groups

Group Ia, Control group Ib and Control group Ic, showed a non-significant difference ($p > 0.05$). Therfore, the mean of all control groups was chosen as a representative group for the three control groups to be compared with the results of the test groups.

Biochemical results

When compared to the control rats, the biochemical analysis of the liver enzymes showed a substantial rise ($P \leq 0.05$) in the serum level of AST, ALT, and ALP in the animals exposed to TAA. Treatment with either urolithin A or carvedilol considerably decreased ($P \leq 0.05$) these enzymes' levels in comparison to the TAA group. Furthermore, liver enzyme concentration in the carvedilol group did not vary significantly from the control group (Table 1).

Hepatic tissue homogenate analysis showed a noticeable increase in the level of MDA ($P \leq 0.05$) in the TAA exposed rats relative to the control group. While the MDA level was significantly reduced ($P \leq 0.05$) in the urolithin A group or the carvedilol group in relation to the TAA group. Conversely, the levels of (SOD) and (CAT) in the hepatic tissue were considerably decreased ($P \leq 0.05$) in the TAA group comparable to the control rats. When urolithin A or carvedilol were given in combination with (TAA), these levels were reversed (Table 2).

Table 1 The effect of urolithin A or carvedilol on the serum liver enzymes.

	Group I **(Control group)**	Group II **(TAA group)**	Group III **(Urolithin A group)**	Group IV **(Carvedilol group)**
AST	75± 1.1	135.5 ± 1.9 [a,c & d]	90. 6 ± 1.9 [a& b]	79.5± 1.7 [b]
ALT	43.6 ± 1.5	96.6± 1.4 [a,c & d]	53.9 ± 1.2 [a& b]	48.5 ± 1.1 [b]
ALP	68.6 ± 1.8	153.5 ± 2.6 [a,c & d]	88.2 ± 1.7 [a& b]	78 ± 2 [b]

The statistics available as mean±SD, and analysed by using one -way ANOVA.

*significance ≤ 0.05

a: Significance vs Control,

b: Significance vs (TAA) group, c: Significance vs Urolithin A group, d: Significance vs Carvedilol group

Table 2 The influence of urolithin A or carvedilol on the oxidative/antioxidative parameters in liver tissue.

	Group I (Control group)	**Group II (TAA group)**	**Group III (Urolithin A group)**	**Group IV (Carvedilol group)**
Malondialdehyde (MDA)(nmol/mg protein)	7.1± 0.3	22.2±0.9 [a,c&d]	9.2±0.6 [b]	7.9±0.4 [b]
Catalase (CAT) (U/mg protein)	17.3 ± 0.6	7.6 ±0.6 [a,c&d]	13.1±0.8 [a & b]	15±0.4 [b]
Superoxide dismutase (SOD) (U/mg protein)	38.3 ± 0.6	8.3±0.6 [a,c&d]	26.4±1 [a & b]	32.4±1.5 [b]

The statistics are available as mean±SD, and analysed by using one -way ANOVA.
*significance ≤ 0.05
a: Significance vs Control, b: Significance vs (TAA) group,
c: Significance vs Urolithin A group, d:Significance vs Carvedilol group.

Gross liver appearance

Gross appearance of the liver from the control rats was a uniform liver appearance with smooth surface and regular lobes (Figure 1A). While the macroscopic appearance of the liver exposed to TAA displayed an irregular outer surface with several coarse macro nodules (Figure 1B). Treatment with urolithin A improved the liver construction, but a few occasional nodules were still observed on the surface (Figure 1C). The Carvedilol group showed a clear morphological improvement in the liver with a nearly regular surface which was comparable to the control liver (Figure 1D).

Histological findings

Hematoxylin and eosin stain

Liver sections from group I revealed normal hepatic lobule histological architecture. Hepatocytes were organized into plates that radiated from the central vein, and the portal triad was clearly seen, containing a branch from the portal vein, a branch from the hepatic artery, in addition to the bile duct (Figure 2A). On the other hand, group II displayed disorganized hepatic cords with marked vacuolar degenerative changes. Periportal fibrosis, thick fibrous stands with inflammatory cell infiltration, congested dilated portal vein, thicker hepatic artery wall, and bile duct hyperplasia were observed in the portal area (Figures 2B & 2C). Group III displayed enhancement of the liver tissue's histological structure, but few pyknotic nuclei, scanty cytoplasmic vacuolations. Portal region showed decreased periportal fibrosis and inflammatory cellular infiltration, however congested portal vein was detected (Figure 2D). Group IV showed reconstruction of the liver architecture with regular arrangement of hepatic cords around the central veins. Portal area appeared closely similar to the control liver sections with normal portal vein, hepatic artery and very few inflammatory cellular infiltrations (Figure 2E).

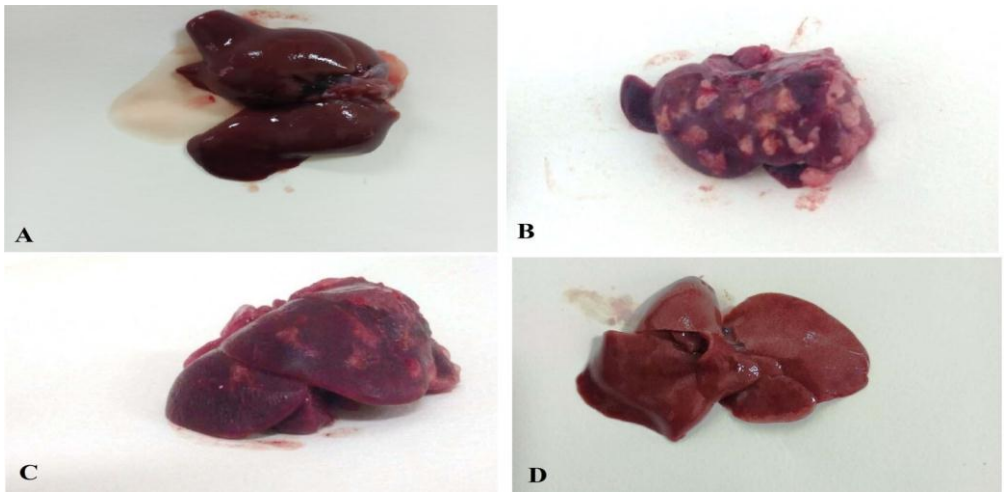

Fig. 1 Photographs of macroscopic appearance of the liver from different groups; (A) control rat liver with uniform appearance, regular lobes and smooth surface. (B) (TAA) Exposed liver displayed irregular outer surface with evenly distributed coarse external nodules. (C) Urolithin A group demonstrated improved liver construction with few occasional nodules. (D) Carvedilol group showed a clear morphological improvement in the liver with nearly regular surface that was similar to normal.

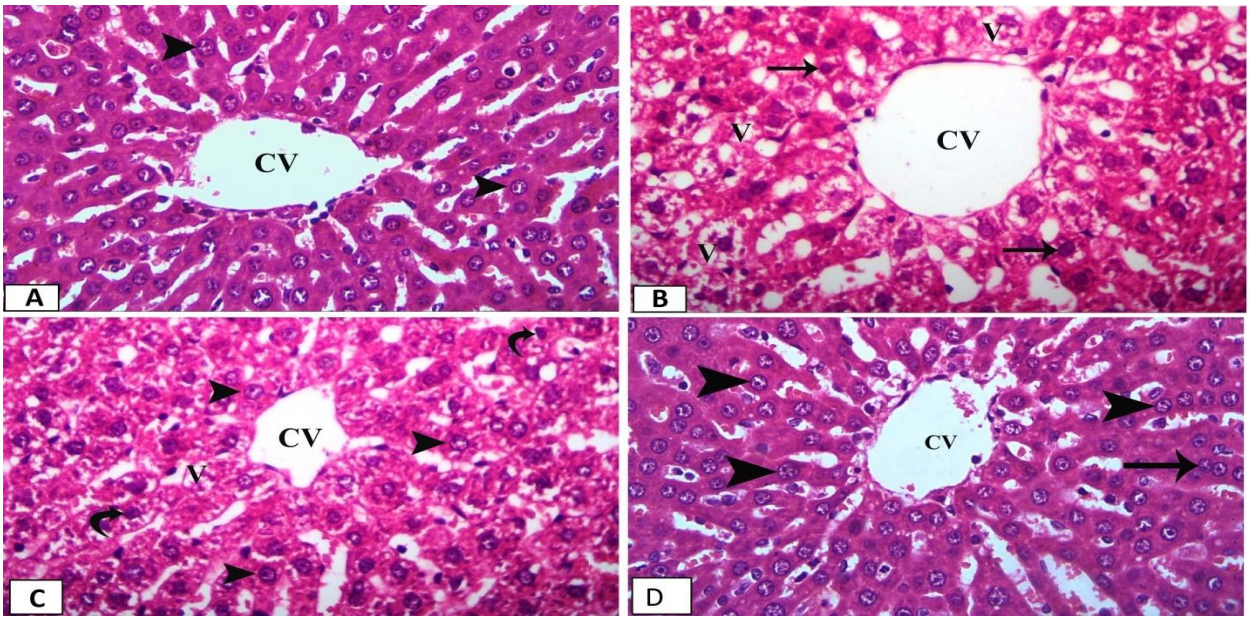

Fig. 2a Hematoxylin and Eosin -stained liver slices photomicrographs; (A): control group showing normal liver architecture with normal central vein (CV), hepatocytes arranged into plates with vesicular nuclei and acidophilic cytoplasm (arrow head). (B):TAA group presenting disorganization of hepatocyte cords , most hepatocytes with vacuolated cytoplasm (V) and deep pyknotic nuclei (arrow), dilated central vein (CV). (C):Urolithin A group showing most of hepatocytes have vesicular nuclei and acidophilic cytoplasm (arrow head), few hepatocytes appeared with deeply stained nuclei (curved arrow),scanty cytoplasmic vacuolations (V). (D): Carvedilol group displaying restoration of the hepatic architecture with arrangement of hepatic cords (arrow head) around the central vein (CV), some hepatocytes are binucleated (arrow) (H&E X 400)

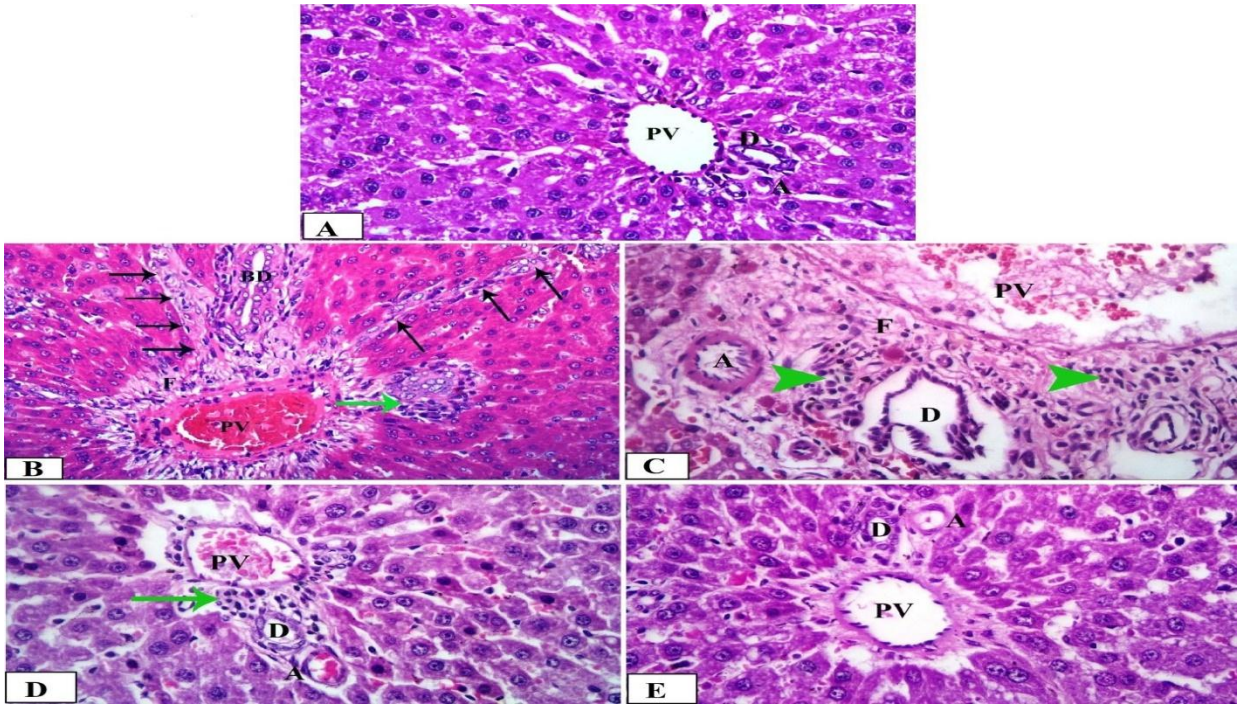

Fig. 2b Hematoxylin & Eosin -stained liver slices photomicrographs; (A): control group presenting normal hepatic architecture with portal triad comprising portal vein branch (PV), hepatic artery branch (A), in addition to bile duct (D). (B): TAA group showing congested dilated portal vein (PV) with periportal fibrosis (F), bile duct hyperplasia (BD), inflammatory cellular infiltration (green arrow), and fibrous strands (black arrows) stretched between the portal area and the central vein . (C): TAA group showing portal region with congested expanded portal vein (PV) , periportal fibrosis (F), hepatic artery has thick wall (A) , bile duct (D) hyperplasia , and inflammatory cellular infiltration (arrow head). (D): Urolithin A group displaying portal region with congested portal vein (PV), branch of hepatic artery (A) in addition to the bile duct (D), and reduced inflammatory cellular infiltration (green arrow). (E): Carvedilol group showing portal region closely similar to control group, containing branch from the portal vein (PV), hepatic artery (A), and the bile duct (D) (A, C, D & E X 400; B X 200).

Masson trichome stain

Liver sections from the control group showed normal distribution of a little amount of collagen fibers surrounding the central veins and in the sinusoidal spaces between hepatocyte plates (Figure 3A). However, TAA treated group presented extensive deposition of collagen fibers in the portal region surrounding the portal vein (Figure 3B). The Urolithin A-treated group displayed mild periportal distribution of collagen fibers (Figure 3C). While a minimal amount of collagen fibers was noticed in Carvedilol- treated group (Figure 3D).

Immunohistochemical results

(α- SMA) immunostained hepatic sections from the control rats revealed very minimal expression of (α-SMA) (Figure 4A). On the other hand, The TAA treated group presented diffuse intense expression of (α- SMA) in the liver sinusoids wall and among the hepatocytes indicating extreme stimulation of hepatic stellate cells (Figure 4B). Treatment with urolithin A showed moderate reduction in α- SMA immune expression (Figure 4C). While treatment with carvedilol showed minor α- SMA immune reaction in between the hepatocytes (Figure 4D).

PCNA immunostained hepatic sections from the control rats displayed a negative immune reaction for PCNA proving no cell regeneration occurring (Figure 5A). In contrast, The TAA group displayed a vastly positive immuno expression for PCNA in the nuclei of hepatocytes indicating their proliferation (Figure 5B). Urolithin A showed mild downregulation in PCNA expression (Figure 5C). While The Carvedilol group displayed a weak immune reaction for PCNA in few hepatocyte nuclei (Figure 5D) vastly.

Morphometric results

The average value of the area percentage of collagen dispersion was considerably higher ($P \leq 0.05$) in the TAA-exposed animals contrary to the control rats. While The Urolithin A group or The Carvedilol group displayed a substantial reduction in the collagen area percentage ($P \leq 0.05$) relative to the TAA group (Figure 6).

Figure 7 exhibited that the average area percentage of αSMA and PCNA immune-reactivity in the TAA exposed group was significantly raised ($P \leq 0.05$) comparable to the control animals. Co-administration of either urolithin A or carvedilol with TAA produced a substantial ($P \leq 0.05$) reversal of the increased parameters. Moreover, there was a significant difference ($P \leq 0.05$) between the Carvedilol group and Urolithin A group with regard to the area percentage of αSMA and PCNA immunoexpression.

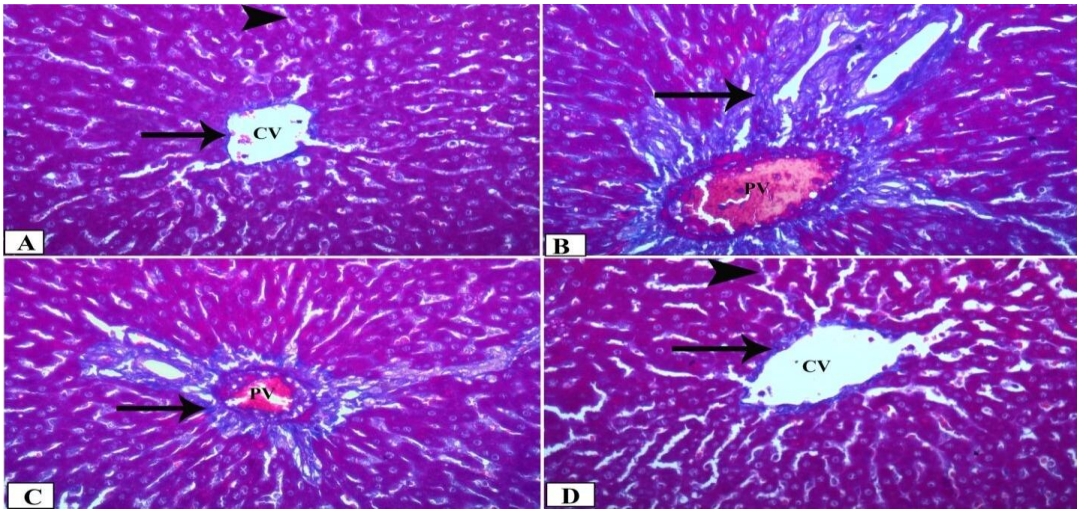

Fig. 3 Photomicrographs of Masson's trichome stained hepatic sections from all groups ; (A):Control group displaying typical distribution of little collagen fibers that surround the central vein (CV) (arrow) and in the sinusoidal spaces between hepatocyte plates (arrow head).(B): TAA group presenting coarse collagen fibers deposition in the portal region (arrow) surrounding the portal vein (PV) .(C): Urolithin A group displaying mild periportal deposition of collagen fibers (arrow) .(D): Carvedilol group viewing minimal collagen fiber quantity around the central vein (CV) (arrow) and among the hepatocyte plates (arrow head) (Masson's trichome staining X 200).

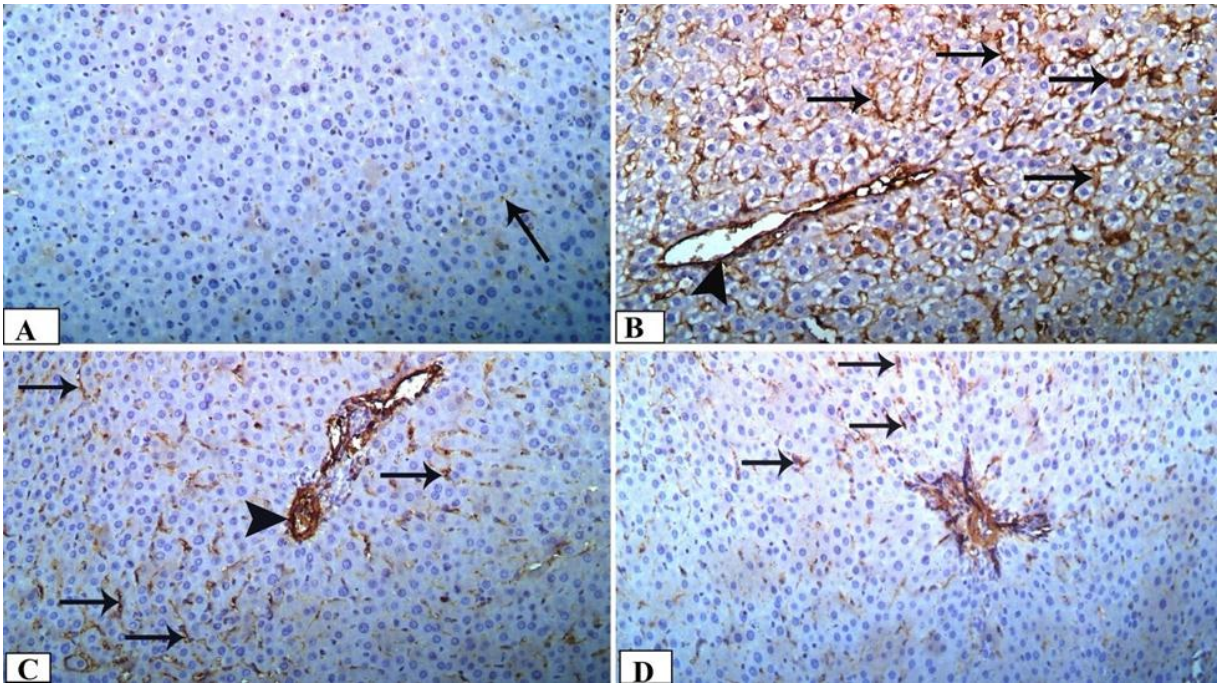

Fig. 4 Photomicrographs of (α-SMA) immunostained liver sections; (A): Control group presenting very minimal (α-SMA) immune expression (B): (TAA) treated group presenting diffuse intense expression of (α-SMA) immune staining in between the hepatocytes (arrows) and in the wall of liver sinusoids (arrow head). (C): Urolithin A treated group viewing moderate (α-SMA) immune expression among the hepatocytes (arrows) and in the portal vein wall (arrow head). D: Carvedilol treated group showing minor (α-SMA) immune reaction (arrows) in between the hepatocytes (α-SMA immunostaining X 200).

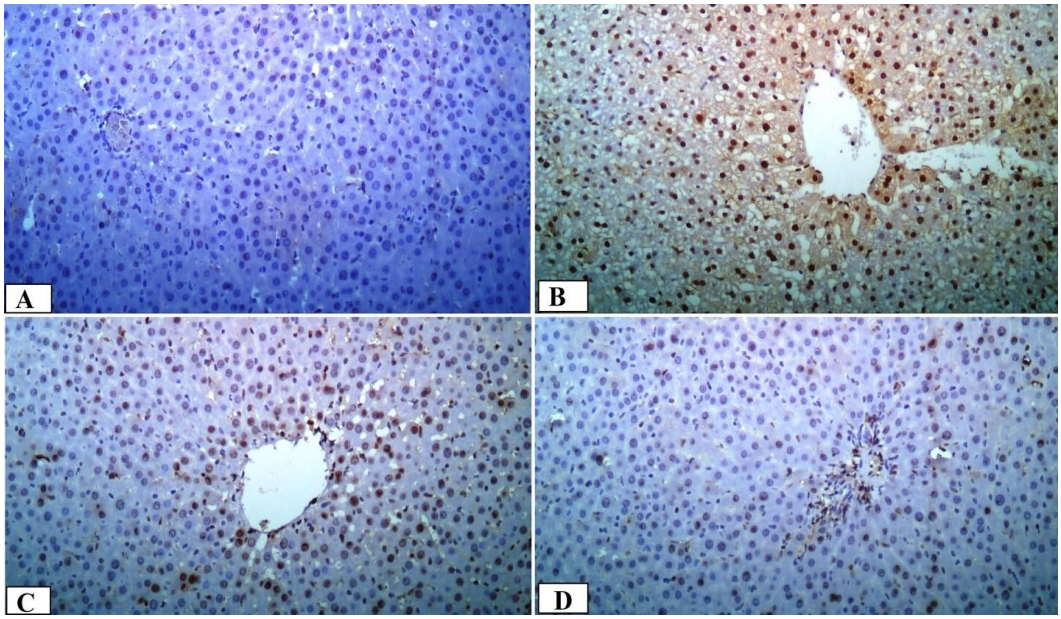

Fig. 5 photomicrographs of (PCNA) immune stained liver sections from different groups; A: showing negative immune reaction for (PCNA). B: TAA group presenting extensive positive immune reactivity for (PCNA) in the nuclei of hepatocytes. C: Urolithin A viewing mild down regulation of (PCNA) expression in some hepatocyte nuclei. D: Carvedilol group displaying weak immune reaction for (PCNA) in few hepatocyte nuclei (PCNA immunostaining X 200).

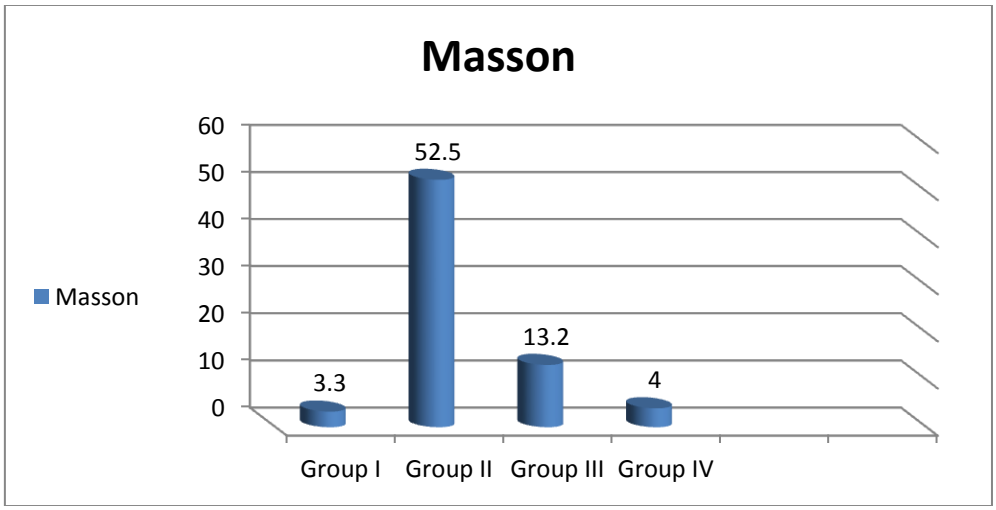

Fig. 6 The area percentage of collagen fiber distribution in all groups.

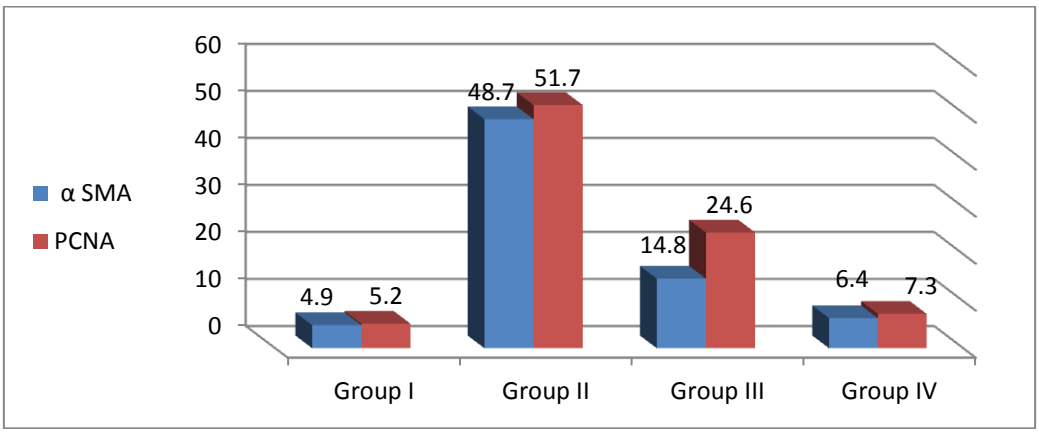

Fig. 7 The area percentage of α SMA and PCNA immune reactivity in all groups.

Discussion

Liver fibrosis is the body's reaction to prolonged liver injury and may progress organ failure [8].

Numerous medications have been developed to treat liver fibrosis; however, their efficacy is questionable. Therefore, there is a pressing need for novel therapeutics to inhibit liver fibrosis.

The purpose of the present investigation was to evaluate the effect of Carvedilol and Urolithin A in eliminating thioacetamide induced hepatic fibrosis.

The biochemical analysis of the liver enzymes in our investigations showed that contrary to the control group, rats exposed to TAA had considerably greater serum level of AST, ALT, and ALP ($P \leq 0.05$). These outcomes are consistent with the findings in [23]. which reported that due to TAA's oxidative stress capability, the liver enzymes present in the cytoplasm escape to the blood stream in quantities correlated with the severity of liver impairment, and this surpasses the body's defense and antioxidant systems [24]. In our study, we demonstrated that Urolithin A treatment protects against liver injury caused by TAA, as indicated by an important ($P \leq 0.05$) reduction of AST, ALT, and ALP serum concentrations. These outcomes agree with those of Shahid et al. [25], who discovered that supplementing with Urolithin A at two distinct dosages (2.5 and 5 mg/kg) had a considerable protective influence against Doxorubicin hepatotoxicity. Urolithin A also has a crucial function for controlling insulin resistance, obesity, and metabolism which may enhance liver hemostasis, according to [12]. The results of this experiment documented a marked rise ($P \leq 0.05$) in the MDA level with a simultaneous decline in the antioxidant enzyme activity (CAT and SOD) in the liver tissues of TAA-treated rats in comparison to control rats. This is consistent with Alamri [26] who stated that TAA produced oxidative pressure, as evidenced by the significant drop in the antioxidant blood levels of GST, SOD and CAT, as well as an increase in the oxidative stress indicator (MDA).

On the other hand, co-dministration of Urolithin A or carvedilol to TAA exposed rats noticeably diminished oxidative stress by lowering MDA levels and increasing CAT and SOD activity. Cytoprotective benefits of Urolithin A can be attributed to its capacity to increase cell survival, decrease ROS production, and increase the physiological activity of numerous antioxidant defense systems [14]. Similarly, carvedilol co-treatment significantly mitigated hepatotoxicity triggered by carbon tetrachloride and restored the antioxidant activity of the hepatic tissues, also, the carbazole component in Carvedilol structure is the source of the drug's antioxidant action, which prevents free radical damage [27].

In the current study, (H & E)-stained sections from the TAA group revealed significant histopathological abnormalities, which were demonstrated as irregular liver architecture and noticeable vacuolar degenerative alterations. Also, there were strong fibrous stands, deep pyknotic nuclei, and inflammatory cell infiltration. This is in line with [26] that showed that, TAA-treated mice had septa of dense, fibrous connective tissues dividing the liver to pseudo lobules and there was leucocytic cell infiltration. TAA causes an inflammatory response which sets off a dynamic chain reaction of immunological response linked to the production of copious levels of inflammatory cytokines, including IL-6 and TNF-a [28].

According to our findings, the co-administration of Urolithin A in conjunction with (TAA) resulted in less damage to the hepatic cellular composition, reduced periportal fibrosis and inflammatory cellular infiltration. These findings support the outcomes of [16]**,** who reported that Urolithin A co-administration may be able to lessen the hepatic pathological alterations brought on by repeated D-galactose injections. The documented hepatoprotective benefits may be due to Urolithin A's anti-inflammatory action through decreased production of (TNF- and IL-6), two significant inflammatory cytokines [12]. Additionally, it is well-known that the underlying mechanism of drug induced hepatotoxicity involves the nuclear transcription factor NF-κB. Urolithin A inhibits the production of proinflammatory cytokines and the activation of NF-kB [29]. Our research showed that the Carvedilol group had restored the hepatic architecture, with the hepatic cords arranged regularly around the central vein. These findings are in accord with those of [30].who discovered that co-treating with Carvedilol significantly lessened the architectural damage brought on by carbon tetrachloride and declared that, the hepatic defensive influence of Carvedilol might be accredited to its capacity to reduce the deposition of fibrous tissues, which decreases liver cell destruction.

This study's MassonTrichome stained slices from the TAA-treated rats presented an important collagen fiber deposition surrounding the congested portal vein. The findings of [31].confirm our result. On the contrary, the levels of inflammatory cells and collagen fiber distribution were much lower in the Urolithin A group and the Carvedilol group. These results are reliable with those of [32].who found that carvedilol enhanced liver histology and hindered CCl4-induced collagen deposition.

Liver injury stimulates hepatic stellate cell (HSC), which might be activated to the myofibroblasts that express (α-SMA) via numerous stimuli as inflammatory mediators and cytokines. The activated (HSCs) migrate, proliferate, and produce extracellular matrix in liver injury sites, upregulating the (α-SMA) expression levels. Consequently, it is thought that (α-SMA) is a significant indicator for evaluating (HSCs) proliferation and activity [33]. Histomorphometric analysis of the immunohistochemical stained sections from (TAA) exposed animals exhibited a significant elevation ($P \leq 0.05$) in the area percentage of α-SMA immune-reaction comparable to the control rats. These results match with those of [15]. An additional confirmation of Uorolithin A and Carvedilol's anti-fibrotic efficacy was obtained in our experiment through minimal expression of (α-SMA) immunostaining in the liver sections from Carvedilol or Urolithin A treated animals in relation to TAA intoxicated animals. This was supported by Ying et al. [32] who explained that Carvedilol inhibited HSCs progression and mitochondrial apoptosis via lowering (Bcl-2/Bax) ratio in HSC.

The most effective collaborative way to comprehend how tissues reproduce is to use immunohistochemistry to detect PCNA. In our study, the liver cells in the normal control group did not exhibit (PCNA) discoloration, a sign of no cell renewal. On the other hand, the (TAA) group's hepatic cells exhibited upregulation of (PCNA), suggesting that the significant liver structure injuries had been restored. This is consistent with findings of [33] which showed that hepatocyte proliferation is stimulated in response to TAA induced liver damage as a coping mechanism for hepatic tissue regeneration.

Both the Uorolithin A group and the Carvedilol group exhibited reduced hepatocyte cell renewal in contrast to the TAA group. This was demonstrated by a significant drop ($P \leq 0.05$) in (PCNA) immune expression, which suggests a decreased degree of necrotized hepatocyte propagation. However, Carvedilol was found to considerably increase both the (PCNA) positive nuclei number and the average area percentage of the (PCNA) positive immunological reaction [34] The majority of hepatocytes nuclei of the rats treated with isoprenaline exhibited a negative PCNA reactivity, demonstrating that acute heart failure (AHF) convinced hepatic ischemia and hepatocytes degeneration [34]. When these animals were treated with Carvedilol for two weeks, there was an increased hepatocyte reproduction and regeneration rate causing higher PCNA immune reactivity. Additional studies have demonstrated that Urolithin A has antihepatotoxic effects against CCl4-induced liver damage by upregulating anti-apoptotic proteins and downregulating pro-apoptotic ones [35].

Hence, we can conclude that Carvedilol or Urolithin A might be promising medications to inhibit liver fibrosis triggered by TAA. However, carvedilol displayed a significantly superior hepatoprotective influence which is proven through improved histopathological construction, better biochemical parameters, increased antioxidant activity, inhibiting (HSCs) propagation, down-regulation of α-SMA and PCNA immunostain.

Conclusion

Both Carvedilol and Urolithin A showed hepatoprotective effect after alterations in liver tissue produced by TAA administration. However, Carvedilol treatment showed superior hepatoprotective and anti-fibrotic effect than Urolithin A treatment.

Conflicts of interest

The authors declare no conflicts of interest.

Authors' contributions

All authors contributed to the study conception and design; material preparation, data collection, and analysis. All authors read and approved the final manuscript.

Ethical considerations

Ethics approval and consent to participate: according to the guidelines for animal care and the Ethics Committee of the Faculty of medicine at Benha University , this experiment was conducted in the Anatomy department of Benha University's Faculty of Medicine with (Approval No.: RC 16-11-2023) October 2024.

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