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Hypolipidemic and Collagenolytic Effects of *Portulaca Oleracea* Extract in a Rat Model of Cholestasis and Liver Fibrosis

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Abstract: Hyperlipidemia, a condition normally observed in cholestatic liver disease, is a risk factor for cardiovascular diseases and end-stage liver disease. Using bile duct ligation (BDL) in rats as a model of cholestasis and liver fibrosis, we examined serum lipid profile, the hepatic hydroxyproline (HYP) content, mRNA expression of tissue inhibitor metalloproteinase (TIMP-1), liver index (LI) and histopathological change of the liver in both sham-operated and BDL rats. Significant increases in liver weight were observed in BDL rats. BDL caused an increase in levels of TC, TG, LDL-c, VLDL-c, and atherogenic indices (TC/HDL-c and LDL-c/HDL-c), while the level of HDL-c was decreased. In addition, the hepatic HYP content and mRNA expression of TIMP-1 was increased by 13 and 37 folds in BDL rats. BDL induced several histopathological changes, such as BD proliferation, edema, inflammation, and necrosis. To examine the hypolipidemic and collagenolytic effects of purslane on a rat model of cholestasis and liver fibrosis, it was administered at a dose of 400mg/kg/day, p.o for 4 weeks immediately after surgery. Administration of purslane ameliorated most of the histopathological alterations, improved the lipid profile, and reduced hepatic collagen deposition, which BDL previously induced. In conclusion, BDL leads to an abnormal lipid profile and increased hepatic collagen deposition. These factors showed significant improvement following the administration of purslane extract. Thus, purslane showed hypocholesterolemic and collagenolytic activity. The current preliminary study recommends the use of purslane as at least a safe and effective agent of cholestasis-induced hyperlipidemia and liver fibrosis.

Keywords: purslane; hydroxyproline; tissue inhibitor metalloproteinase; hyperlipidemia; histopathological changes.

1. Introduction

Portulaca oleracea L. (Portulacaceae), commonly known as purslane, is an annual summer plant that is grown as a vegetable in many parts of the world. The World Health Organization lists it as one of the most commonly used medicinal plants and has been given the term "a vegetable for long life" [1]. Purslane is a rich source of omega-3 fatty acids, glutathione, gallotannins, kaempferol, quercetin, apigenin, monoterpenoids, alkaloids, coumarins, and flavonoids [2]. It has been used previously as an antiseptic, antispasmodic, diuretic, antihelmintic, antipyretic, and anti-scorbutic for treating urinary disorders and reducing pain and swelling [3]. Several studies have demonstrated various pharmacological

effects of this plant, including hypoglycemic [4], hypolipidemic [5], antioxidant [6], analgesic and anti-inflammatory [7], neuroprotective [8], and wound healing effects [9]. In addition, purslane is safe for daily use without cytotoxicity or genotoxicity [10].

The liver has a central role in controlling various aspects of lipid metabolism. Primarily, the liver produces bile, constituents of which are required for efficient intestinal fat absorption. Additionally, biliary secretion of cholesterol (as such or in the form of bile salts) and phospholipids from the liver into the intestine is of major importance in body lipid homeostasis. The liver is a major source of plasma lipoproteins and their clearance: it synthesizes apoproteins (i.e., apo A-I, apo B, apo E) that regulate many complex metabolic interconversions between lipoprotein classes, as well as lipoprotein lipid constituents as cholesterol, triacylglycerols, and phospholipids [11]. Also, the liver synthesizes enzymes (e.g., Lecithin-cholesterol acyltransferase, Phospholipid Transfer Protein, and Lipoprotein lipase) involved in lipoprotein metabolism in the plasma compartment. Finally, the liver is the site of active synthesis, metabolism and/or oxidation of various lipid classes, including long-chain polyunsaturated fatty acids [12].

In some cholestatic diseases, hyperlipidemia occurs due to impaired metabolic degradation and excretion of cholesterol [13]. Since cholesterol regulates the function of many proteins, either directly by interacting with them or indirectly by its effects on membrane fluidity, thus increasing membrane cholesterol content and reducing membrane fluidity and function, cholesterol retention can exacerbate cholestasis [14]. As a result of cholestasis, hydrophobic bile salts are retained, damaging hepatocytes and resulting in liver fibrosis. The present study aims to assess the possible protective effects of omega-3 fatty acids and combined antioxidant compounds found in purslane in reducing experimental cholestasis and liver fibrosis.

2. Materials and Methods

2.1.Preparation of purslane hydro-ethanolic extract.

The fresh aerial parts of purslane were collected, washed, dried, and weighed, then refluxed with (70%) ethanol for 4 hrs. The resulting slurry was filtered, and the residue was refluxed with fresh solvent. The filtrate was dried under reduced pressure at 40°C using a rotary evaporator. The concentrated extract was transferred to dishes and dried in an oven at 60°C till constant weight. The yellowish-brown hygroscopic solid purslane extract was then kept in a vacuumed desiccator until use [15].

2.2.Quantitative determination of fatty acid content as fatty acid methyl ester using gas-liquid chromatography.

Standard oil/extract (1 gram) was refluxed with methanol, benzene, and sulphuric acid (200: 100: 10 v/v) for 2 hrs. The filtrate was mixed with distilled hexane and water in a separating funnel. After removing the lower layer, the upper layer was washed with 50ml sodium bicarbonate, shaken, and then the lower layer was removed, too. After washing twice with saturated sodium chloride solution, the upper layer was saved and went through anhydrous sodium sulfate. Gas chromatography was used to analyze the residue obtained from evaporating the extract. Sample (1µL) was injected into the Hps column (12 mm, 0.2 mm, 0.3 µm) with the initial column temperature of 120 °C programmed at 20°C/min and a final temperature of 300°C using a flame ionization detector (275°C) and injector (180°C). The

fatty acid profile of each standard oil and extract was identified by comparing the retention time of their component peaks with those of authentic methyl esters of fatty acids [16].

2.3.Quantitative determination of total phenolic and flavonoid content.

First, to determine the total phenolic content, 1mL of the extract was mixed with 5 ml Folin-Ciocalteu reagent (2N) and 4 mL Na2CO3 (0.7 M). After 2 hrs, the absorbance was spectrophotometrically measured at 765 nm. To determine total flavonoid content, 1 mL of the extract was mixed with 0.15 mL of 5% NaNO2. After 10 minutes, mix with 1 mL 2% AlCl3. The absorbance was spectrophotometrically measured at 415 nm, using quercetin as standard [17].

2.4.Experimental animals.

Adult female albino rats (n=96, 150-200 g) were supplied by NODCAR, Giza, Egypt. They were housed in stainless steel cages at a constant temperature of 24 ± 2˚C with a 12h light-dark cycle. Animals were fed a standard diet and water. All animal experiments were carried out by the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.4.1.Establishment of cholestasis and liver fibrosis.

BDL was carried out according to the previous method [18]. The effect of purslane (400 mg/kg) [19] was tested against a reference agent, silymarin extract (50 mg/kg, daily for 4 weeks immediately after surgery [20], Sigma Chemical Co, St. Louis MO, USA). Rats in the untreated BDL group and the sham group received a vehicle once a day for the same period. A total of 48 rats were divided randomly into equal 4 groups (12 rats each). At the end of the 4th week, animals were sacrificed. Blood was collected, and part of each liver was fixed in 10% buffered formalin, while other hepatic specimens were immediately snapped frozen and stored at -80˚C.

2.4.2.Measurement of lipid profile.

Serum triacylglycerols (TG) [21], total cholesterol (TC) [22], and high-density lipoprotein cholesterol (HDL-C) [23] were evaluated using commercial kits (BIO diagnostic co., Egypt). Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) concentrations were calculated using by means of the Friedewald equation [24], and atherogenic indices (TC/HDL-C and LDL-C/HDL-C) were also calculated.

2.4.3.Measurement of hepatic hydroxyproline (Hyp) content.

Hepatic Hyp content was determined according to a former technique [25]. The total protein is needed for other tissue parameters calculation and was determined by the method described before [26].

2.4.4.Detection of TIMP-1(tissue inhibitor of metalloproteinase-1) gene expression by reverse transcription-polymerase chain reaction (RT-PCR).

Total RNA was isolated from homogenates of whole livers. RNA extraction was performed using the acid guanidinium thiocyanate-phenol-chloroform method. RNA concentrations were determined by measuring the absorbance at 260 nm, and RNA quality was

verified by electrophoresis on an ethidium bromide-stained 2% agarose gel. The total RNA was thermally denatured and reverse transcribed by incubating at 42ºC for 90 min with Moloney murine leukemia virus (MMLV) reverse transcriptase, human placental ribonuclease inhibitor (HPRI), deoxy-nucleotide triphosphate mixture, and random hexamers. In order to terminate the reactions, samples were heated at 95ºC for 10 min and then cooled on ice. The cDNA samples were amplified using Taq DNA polymerase, deoxy-nucleoside 5'-triphosphate, and 1 nM of each primer of the appropriate primer pairs. The steps of the PCR process were as follows; initial denaturation at 97ºC for 5 min, repeated amplification cycles that consisted of denaturation at 96ºC for 1.5 min, annealing for 1.5 min, and extension at 72ºC for 3 min. A final extension step includes heating at 72ºC for 15 min. Total RNA was reversed transcribed in RT buffer, Superscript II RNase H- reverse transcriptase, random primers, oligo dT, and total RNA. Samples were incubated at 20˚C for 10 min followed by 42˚C for 30 min, and reverse transcriptase was inactivated by heating at 99˚C for 5 min and cooling to 5˚C for 5 min [27].

2.4.5. Liver index determination.

To calculate the liver index, the formula described by Yang *et al*. was applied [28].

2.4.6. Histopathological examination.

Liver tissues were fixed in formalin/phosphate buffer (10%), dehydrated in alcohols then embedded in paraffin. Tissue sections were stained with hematoxylin and eosin stain (H&E) [29] and Crossman stain.

2.5. .Statistical analysis.

Results are presented as means ± S.E. Statistical evaluation was done using a one-way analysis of variance (ANOVA) (Senedecor and Cochran, 1981). Values of $P< 0.05$ were considered significant.

3. Results and Discussion

3.1. Fatty acid content.

The fatty acid profile of the extract was identified by comparing the retention time of its component peaks with those of authentic methyl esters of fatty acids, as shown in Table 1.

Table 1. The fatty acid profile of the extract.

Fatty acid	Content %
Palmitic acid, C16 %	17.9%
Stearic acid, C18 %	2.98%
Oleic acid, C18 $_{(1)}$ %	8.69%
Linoleic acid, C18 $_{(2)}$ (ω_6) %	33.7%
Linolenic acid, C18 $_{(3)}$ (ω_3) %	11.49%
Others	25.24%

3.2. Total phenolic and flavonoids content.

The total amount of phenolic content of the hydro-ethanolic extract of purslane was 151.4 mg GAE /g, while the total amount of flavonoid content was 6.25 mg QE/g.

3.3. Lipid profile

The current results showed a marked increase in serum TC, TG, LDL-C, VLDL-C, and atherogenic indices in BDL-rats 2.5, 1.5, 1.9, 2.5, and 10.4 fold, respectively, versus the sham-operated group. BDL significantly decreased levels of HDL-C by 5.4-fold in BDL rats relative to values in the sham-operated group. The present data reported a significant improvement in all lipid parameters and atherogenic indices after purslane treatment (Table 2). The BDL rats treated with purslane showed greater serum levels of HDL-c by 2.9-folds while exhibiting lower levels of serum TC and LDL-c by about 28 and 34%, respectively, versus BDL rats. On the other hand, silymarin failed to significantly affect TC, HDL-c, and LDL-c levels ($P>0.05$) compared with untreated BDL- rats. In contrast, the serum VLDL-c levels were lower in rats receiving either purslane or silymarin (by 1.6 and 1.4-folds, respectively) versus untreated BDL rats. There is an observed decrease in both atherogenic indices in BDL rats treated with either purslane or silymarin by about 4.5-folds and 2.8-folds, respectively, versus untreated BDL rats.

Table 2. Effect of purslane (400 mg/kg, 4wks) in comparison with silymarin (50 mg/kg, 4wks) on lipid profile BDL-rats.

Parameter/Groups	Sham	BDL	BDL+ purslane	BDL+ silymarin
TG (mg/dl)	53.9 ± 3.27	136.9 ± 6.80 [a]	87.8 ± 2.92 [#]	98.7 ± 4.84 [#]
TC (mg/dl)	92.8 ± 5.13	141.1 ± 6.77 [a]	101.7 ± 6.14 [#]	122.9 ± 11.67
HDL-C (mg/dl)	22.9 ± 1.81	4.2 ± 0.27 [a]	12.3 ± 0.89 [#]	10.0 ± 0.86
LDL-C (mg/dl)	59.1 ± 5.76	109.5 ± 7.22 [a]	71.8 ± 6.02 [#]	93.1 ± 8.82
VLDL-C (mg/dl)	10.8 ± 0.65	27.3 ± 1.36 [a]	17.5 ± 0.58 [#]	19.7 ± 0.97 [#]
(TC/HDL-C)	4.1 ± 0.38	33.6 ± 3.17 [a]	8.2 ± 0.53 [#]	12.3 ± 1.22 [#]
(LDL-c/HDL-C)	2.6 ± 0.25	26.1 ± 2.99 [a]	5.8 ± 0.47 [#]	9.3 ± 0.90 [#]

Values are (mean ± SE), a: significantly different from the sham-operated group, and #: significantly different from the BDL group at $p < 0.05$ level.

BDL-rat model in the present study showed a disturbed lipogram pattern represented by a significant ($P<0.05$) increase in serum TC, TG, LDL-C, VLDL-C, and atherogenic indices and decreased level of HDL-C relative to values in the sham group throughout the study periods. A possible explanation by which BDL-induced cholestasis provoked dyslipidemia may be related to; 1. The absence of specific bile components at their sites of action, particularly in the intestine. 2. Disruption of the continuous flux of lipids from the liver into the bile and intestine results in the accumulation of toxic and non-toxic bile components in the body. 3. Characteristic alterations in plasma lipoprotein composition associated with cholestatic liver diseases. This deleterious impact is further emphasized in previous clinical trials illustrating that BDL causes complete blockage of cholesterol excretion and that hyperlipidemia develops in obstructive jaundice [30]. In addition, there is a marked elevation in free cholesterol and phospholipids in obstructive liver disease [31]. Evidence reveals that cholestatic liver disease in humans is associated with lipid abnormalities, including high TC and TG and low HDL-C levels [32].

3.4. Hepatic Hyp content.

The increased level of liver HYP in BDL-rats by more than 14-fold was significantly reduced by treatment with either purslane or silymarin by about 3-fold (Figure 1).

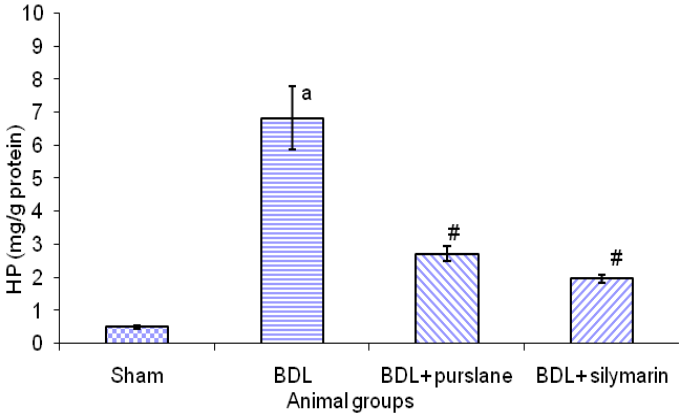

Figure 1. Effect of purslane (400 mg/kg, 4wks) in comparison with silymarin (50 mg/kg, 4wks) on the liver content of HYP in BDL-rats. Values are (mean ± SE), a : significantly different from the sham-operated group and # : significantly different from the BDL group at $p < 0.05$ level.

The current work revealed that both HYP quantification estimated BDL-induced fibrosis in rats as a marker for collagen in addition to Crossman's staining. This study established the increased level of liver HYP in BDL rats that revealed severe liver fibrosis. It is recognized that oxidative events, particularly lipid peroxidation, can play a key role in collagen accumulation regulation. As oxidative stress activates hepatic stellate cells, leading to the initiation of unbalanced synthesis of collagen, proteoglycan, hyaluronate, and cytokines (transforming growth factor-beta and interleukin-1) are involved [33].

3.5. TIMP-1 gene expression.

This study demonstrated a 37-fold increase in mRNA levels for TIMP-1 expression was decreased by 60-80% after treatment with either purslane or silymarin in both rat models (Figures 2 and 3). Enhanced matrix synthesis and diminished breakdown of connective tissue proteins induce extracellular matrix deposition and, consequently hepatic fibrosis [34]. The current study assessed the changes in TIMP-1 expression and activity during the exacerbation of liver fibrosis in BDL rats, consistent with other outcomes which concluded the increase in TIMP-1 mRNA expression in response to BDL [35]. Finally, the present study revealed a significant increase in the liver index in BDL rats due to body weight loss and liver enlargement.

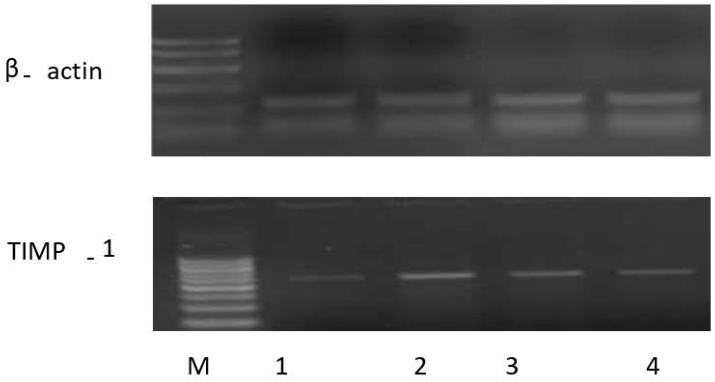

Figure 2. Representative profile of agarose gel electrophoresis showing PCR products of hepatic TIMP-1 gene and control gene (β-actin) in all rats. Lane M: 100 bp DNA ladder, lane 1: PCR products of TIMP-1 in a sham-operated control group, lane 2: TIMP-1 in BDL group, lane 3: TIMP-1 in [BDL+purslane (400 mg/kg, 4wks)] group, lane 4: TIMP-1 in [BDL+silymarin (400 mg/kg, 4wks)] group.

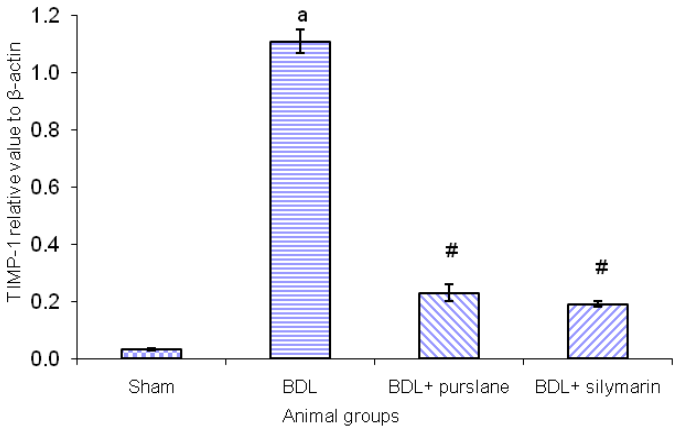

Figure 3. Effect of purslane (400 mg/kg, 4wks) in comparison with silymarin (50 mg/kg, 4wks) on TIMP-1 level in BDL-rats. Values are (mean ± SE) and a : significantly different from the sham-operated group and # : significantly different from the BDL group at $p < 0.05$ level.

3.6. Liver index.

Significant increases in liver weight were observed in BDL rats only, and non-significant results were in purslane-treated groups (Figure 4). On the other hand, the BDL rats supplemented with silymarin exhibited significantly lower ($P<0.05$) LI by about 21% versus untreated BDL- rats.

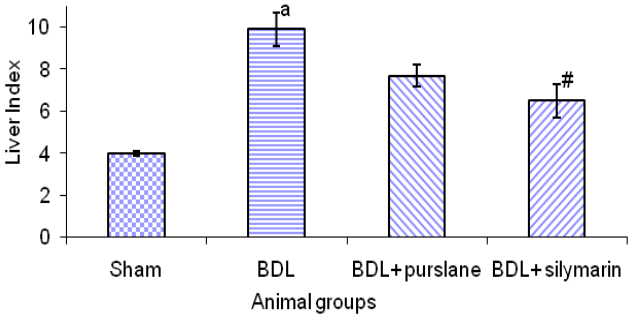

Figure 4. Effect of purslane (400 mg/kg, 4wks) in comparison with silymarin (50 mg/kg, 4wks) on liver index in BDL-rats. Values are (mean ± SE) and a : significantly different from the sham-operated group and # : significantly different from BDL group at $p < 0.05$ level.

3.7. Histopathological results.

After 4 weeks of BDL, jaundice was observed in the visceral and parietal peritoneum of all animals, the livers of animals were markedly enlarged, and the bile ducts above the obstruction point were dilated.

Table 3. The histopathological alterations in liver tissues in the examined groups.

Histopathological appearance Group	Portal inflammation	Bile duct proliferation	Necrosis	Fibrosis	Edema
Sham (-ve) control	-	-	-	-	-
BDL (+ve) control	++++	++++	++++	++++	+++
BDL + purslane	++	++	+++	+	++
BDL + silymarin	++	++	++	+	++

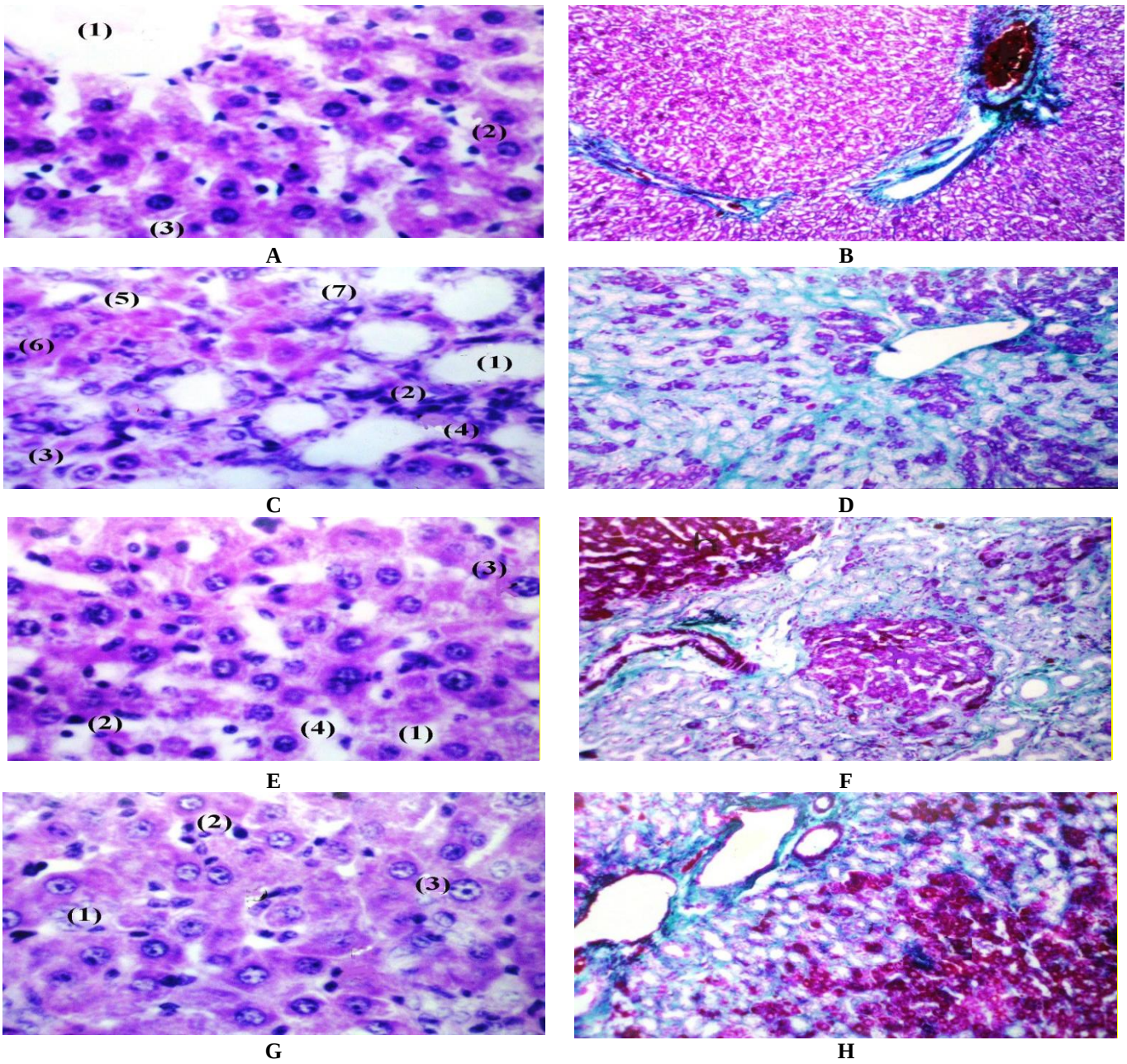

Figure 5. Histological sections of liver tissue stained with H&E or Crossman's stain. (**A**) A histological section in liver tissue of sham-operated negative control rat (H&E *160); (**B**) Histological section in liver tissue of sham-operated negative control rat (Crossman *40); (**C**) Histological section in liver tissue of BDL positive control rat (H&E *160); (**D**) Histological section in liver tissue of BDL positive control rat (Crossman *40); (**E**) Histological section in liver tissue of BDL rat treated with purslane (400 mg/kg, 4wks) (H&E *160); (**F**) Histological section in liver tissue of BDL rat treated with purslane (400 mg/kg, 4wks) (Crossman *40); (**G**) Histological section in liver tissue of BDL rat treated with silymarin (50 mg/kg, 4wks) (H&E *160); (**H**) Histological section in liver tissue of BDL rat treated with silymarin (50 mg/kg, 4wks) (Crossman *40). Values are (mean ± SE) and a: significantly different from the sham-operated group and #: significantly different from BDL group at $p < 0.05$ level. Representative photomicrographs of sham-operated control (A and B) displaying normal hepatic architecture with normal hepatocytes, parenchyma, and sinusoids. BDL rats (C and D) showed severe bile duct proliferation(1), prominent inflammatory cell infiltration(2), karyomegaly(3), multiple vacuoles(4), and severe necrosis(5). BDL rats treated with purslane/silymarin (E-H) showed some bile duct proliferation(1), less inflammatory cells infiltration(2), fewer karyomegaly(3), fewer vacuoles(4), moderate focal necrosis(5) and mild degree of periportal fibrosis**. (A**) A photomicrograph showing the normal histological structure of the central vein(1) and surrounding hepatocytes(2) and sinusoids(3). (**B**) A photomicrograph showing no fibrosis, only very few naturally occurring collagen fibers "blue color". (**C**) A photomicrograph showing severe bile duct proliferation(1), massive inflammatory cellsinfilteration(2), hepatocytes necrosis(3), fibrous tissue proliferation(4), several vacuoles(5), pyknosis(6) and hepatocytes with marginal chromatin(7)**. (D)** A photomicrograph showing

aggregates of hepatic cells "purple color" separated by a wide area of fibrous tissue "blue color" harboring multiple proliferated bile ductules. (**E)** A photomicrograph showing marked reduced bile duct proliferation(1), mild inflammatory cell infiltration (2) karyomegaly(3), and dilated sinusoids(4). (**F)** A photomicrograph showing mild fibrous tissue bands "blue color" between well-formed hepatic lobules and a few bile ductules could be seen. (**G)** A photomicrograph showing very little bile duct proliferation(1), mild inflammatory cell infiltration (2), karyomegaly(3), and well-formed hepatic architecture. (**H)** A photomicrograph showing nearly normal fibrous tissue distribution "blue color" between well-formed hepatic lobules with mild congestion.

Purslane administration drastically modified the collagen fiber deposition profile in BDL rats and was able to ameliorate most of the histopathological alternations (Figure 5). Table 3 displays the histological alterations in liver tissues in the examined groups.

The animal model of common BDL in our study stimulated many histopathological features; marked liver enlargement, extreme bile duct proliferation, severe edema, inflammation, hepatocytes necrosis, and loss of normal architecture due to accumulated collagen fibers deposition as compared to sham control rats. Regarding the Crossman stain, consecutive fibrosis led to the formation of Porto-portal septa, impaired blood supply, and disappearance of fine branches of the biliary tree, in harmony with previous results [36].

A novel approach was addressed in this study, where purslane exerted more beneficial effects regarding BDL-induced hyperlipidemia as compared to untreated BDL rats. Consistently, our data reported marked significant amelioration of lipogram pattern and atherogenic indices after purslane pretreatment of BDL-rats than in curative one. This effect may be displayed due to the highest content of omega-3 fatty acids found in purslane as compared to other leafy vegetables and flavonoid contents [37]. Indeed, the TG-lowering effect of flavonoids seems to be done through the activation of cAMP synthesis; cAMP activates protein kinase, and this enzyme increases TG hydrolysis and hence reduces its levels in the blood and liver. Flavonoids also activate LDL receptors [38]. In the current study, silymarin had no significant effect on TC, HDL-C, and LDL-C levels in BDL rats, but there is conflicting information regarding the hypolipidemic effect of silymarin. It was reported that silymarin caused a significant decrease in serum TC and an increase in HDL-C while the levels of TG and VLDL-C were unaffected. This suggests cholesterol absorption suppression may be a contributing mechanism [39].

Conversely, another experimental study conducted showed that silymarin reduced blood total lipids, TG, TC, and lipoproteins [40]. There is accumulating evidence, in agreement with our data, that serum TC and TG levels decreased significantly after purslane supplementation due to low activity of cholesterol biosynthesis enzymes or low levels of lipolysis [41]. The decreased level of the hepatic HYP after treatment with either purslane or silymarin in BDL-rats throughout the current study is related to a decrease in collagen formation compared to untreated BDL-rats, indicating the improvement of fibrosis extent. This may be attributed to the effect of these natural products on MMP-TIMP balance via direct mechanism or by expression of various cytokines and other mediators [42].

Our investigation demonstrated that purslane was associated with a decrease in expression of TIMP-1 in liver homogenate throughout the study period relative to that in untreated BDL groups, and that is the mechanism by which the observed degradation of the febrile liver matrix is mediated during recovery from liver fibrosis. This effect suggested that such treatment favored collagenolytic activity [43]. These results agree with those declared that silymarin suppresses the expression of profibrogenic procollagen a1(I) and TIMP-1, most likely via downregulation of TGFb1 mRNA in rats with biliary fibrosis [44]. Likewise, another study displayed that the lipid-lowering diet and nutraceuticals reduce the expression and

proteolytic activity of interstitial collagenase (MMP-1), reducing oxidative stress and activating endothelial cells and the thrombotic potential [45]. Finally, a recent study exhibited the role of purslane in decreasing lipid profile due to its contents as sterols and fatty acids [46].

The histopathological assessment in the current experimental study revealed that the purslane and silymarin were able to alleviate the signs of tissue degeneration and attenuated the collagen fiber deposition in BDL rats, as compared to untreated BDL-ones. Moreover, this was accompanied by remarkable improvement of necrosis and portal inflammation, mild bile duct proliferation, limited areas of fatty change and lymphocyte infiltration, and restoration of cells near normal. Flavonoids and omega-3 fatty acids may cause collagen accumulation reduction in harmony with previous significant improvements in diverse biochemical parameters [47].

4. Conclusions

In conclusion, purslane extract showed beneficial hypocholesterolemic and collagenolytic activity in experimental cholestasis and liver fibrosis. However, more experimental and clinical studies are required to assess the active dose of this plant for humans without any side effects.

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Conflicts of Interest

The authors declare no conflict of interest.

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