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Therapeutic effect of purslane (*Portulaca oleracea*) shoot powder in treating liver fibrosis through the regulation of inflammation and oxidative stress

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Abstract: **Background**: Liver fibrosis causes increased mortality rate combined with substantial disabilities throughout the population. The bioactive moieties in purslane promote liver health by shielding hepatic cells while aiding cellular regeneration, by improving overall hepatic functions. **Objectives**: The present study examined the nutritional characterization of purslane shoot powder and evaluated its therapeutic effect through bio- evaluation trial. **Methods**: 60 male Wistar rats were randomly divided into six groups, namely normal control (NC), bile duct ligation (BDL) induced liver fibrosis groups i.e., (PC, STD, P1, P2 and P3). The STD (Standard Drug Group) received silymarin at 50 mg/kg bw, while other groups received different doses of purslane shoot powder P_3 (1200 mg/kg), P_2 (800 mg/kg) and P_1 (400mg/kg) respectively for 04 weeks. **Results**: Purslane shoots exhibited a considerable antioxidant potential in addition to important minerals and fatty acid content. Current intervention reduces serum cholesterol with dose P_3 (1200 mg/kg), followed by P_2 (800 mg/kg) and P_1 (400mg/kg), resulting in 101.0±4.75 mg/dL, 109.9±4.35 mg/dL and 110.1±4.22 mg/dL, respectively. While increase in HDL was also observed as P_1 (12.26±0.35), P_2 (13.21±0.39) and P_3 (15.09±0.56) mg/dL, respectively. A remarkable reduction in LFT was observed in experimental group with maximum reduction in P_2 as 135.7±2.38 IU/L for ALP, 41.9±2.09 IU/L for ALT and 93.4±4.67 IU/L for AST, respectively. The results showed a constant reduction in malondialdehyde (MDA) and nitric oxide (NO) levels. Administration of purslane shoot powder significantly showed a marked inclination towards the normalization of all measured biochemical parameters in BDL rats. **Conclusion**: Thus, the current study supports that purslane shoot had a curative effect in inhibiting oxidative stress and reducing inflammation in managing liver fibrosis and its associated complications by the activation of hepatic stellate cells.

Keywords: Cellular regeneration; Liver fibrosis; Malondialdehyde; Nitric oxide

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INTRODUCTION

Purslane (*Portulaca oleracea*) has been traditionally used in various cultures worldwide, with its increasing consumption as a dietary staple. The succulent plant contains bioactive compounds like flavonoids, alkaloids and omega-3 fatty acids along with terpenoids, β-carotene, amino acids, α-tocopherols, ascorbic acid, glutathione (GSH) that provide various health advantages (Kadkhoda *et al*., 2022). Researchers recommend purslane as a medical intervention to improve liver fibrosis due to its immense amount of bioactive compounds which further aid in scavenging reactive oxygen species, reducing inflammation in the body, possessing anti-fibrotic properties and, preventing tissue scarring (Ali *et al*., 2016; Samarghandian *et al*., 2017; Gu *et al*., 2022;Zhang *et al*., 2024). The inhibition of hepatic stellate cell activation through anti-fibrotic action becomes the key factor contributing to reducing excessive collagen progression throughout liver fibrosis treatment. Purslane controls the activity of cytokines as well as growth factors and matrix metalloproteinases (MMPs) since all these substances participate in fibrosis development and anti-fibrotic capacity is owing to its potent antioxidant potential (Rauf *et al*., 2022). Through its ability to counteract reactive oxygen species (ROS) in the body, purslane protects liver cells and prevents both their destruction and tissue scarring (Bhargava *et al*., 2021).

Purslane supports liver health by exerting hepatoprotective effects through antioxidant and anti-inflammatory mechanisms, safeguarding hepatocytes from oxidative stress–induced injury, promoting cellular regeneration and thereby enhancing overall hepatic function (Singh *et al*., 2020). The plant's anti-inflammatory mechanisms help decrease chronic liver inflammation since this condition drives fibrosis development. The inflammatory response mechanisms of purslane help to decrease liver destruction while inhibiting the advancement of fibrosis. A sustained inflammatory state together with relentless liver cell damage leads to progressive liver fibrosis, which develops into excessive scarring tissues in the liver. Many different hepatic conditions lead to liver fibrosis, such as viral hepatitis, alcoholic disease, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis and additional hepatic disorders (Din *et al*., 2021).Liver fibrosis becomes a serious medical condition and leads to cirrhosis if left

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untreated, substantially increasing the incidence of chronic hepatic failure. Several essential points establish the justification for studying purslane as a treatment for liver fibrosis. The current therapeutic options have shown weak results, yet new alternative treatment solutions prove necessary for this condition. People have traditionally used the popular medicinal plant Purslane (*Portulaca oleracea*) to receive multiple health advantages (Jalali & Rahbardar, 2022). Furthermore, the potential benefits of purslane in liver fibrosis are significant. If proven effective, purslane could offer a natural, affordable and accessible alternative or complementary treatment for liver fibrosis patients, especially for those who may not tolerate or respond well to conventional therapies. It may also have the potential to reduce the progression of fibrosis, prevent cirrhosis and improve liver function, thus improving the quality of life and prognosis for patients with liver fibrosis. Additional research is needed to determine the extended safety dynamics of purslane use while reviewing its substance responses to pharmacological agents and possible health risks primarily affecting expectant mothers and breastfeeding individuals, along with people having existing medical conditions (Abdulhussein & Mutlag, 2022).

Several studies have highlighted the need for cautious use of herbal remedies like purslane due to limited toxicological data and undefined dosing guidelines (Rahimi *et al*., 2019). Moreover, while some traditional medicine practices endorse its use during pregnancy, scientific evidence on its safety in gestational and lactation stages remains scarce and certain extracts have shown uterine stimulant effects in animal models (Kakouri *et al*., 2021). Furthermore, its high oxalate content and potential to interfere with drug metabolism necessitate careful evaluation in patients on medication regimens or with kidney disorders (Uddin *et al*., 2014).

In conclusion, the research on the therapeutic potential of purslane in liver fibrosis is based on a compelling rationale. Purslane's availability, safety profile, anti-inflammatory, antioxidant and anti-fibrotic properties, as well as its potential benefits in liver fibrosis management, form a promising therapy for further investigation. Understanding its mechanisms of action and evaluating its safety and efficacy could contribute to our understanding of liver fibrosis pathophysiology, promote the use of natural products in liver disease management and potentially offer a valuable alternative or complementary treatment option for patients with liver fibrosis (Oguntibeju & Okaiyeto, 2021).

The present research is planned with the intent to investigate the therapeutic potential of purslane shoots in managing liver fibrosis. The relationship between the antioxidant activity present in purslane shoots and its curing effect as hepatoprotective effect.

MATERIALS AND METHODS

Procurement of raw material

A botanist from the Botany Department of Minhaj University Lahore verified the species and taxonomy of Purslane, acquired from agricultural fields near Lahore.

Proximate analysis

AACC Method No. 44-01 was used to determine moisture content (AACC, 2000). To determine protein content Kjeldahl apparatus was used by AACC Method No. 46-10 (AACC, 2000). Crude fat used by AACC Method No. 30-10 through an HT2 1045 Extraction unit manufactured by Hoganas Sweden. The fiber analysis system produced by Labconco Corporation, based in Kansas, USA, performed the AACC Method No. 32-10 (AACC, 2000). To measure ash content muffle furnace (Model: MF-1/02, PCSIR, Pakistan) per AACC Method No. 08-01 procedures (AACC, 2000). The nitrogen-free extract (NFE) measurement process required the following calculation:
NFE % = 100 - (% moisture+% crude fiber + % crude protein + % crude fat + % ash)

Minerals and fatty acid content

The AOAC documented procedures were used for mineral analysis. Sodium (Na) and potassium (K) measurement required a Flame Photometer-410 manufactured by Sherwood Scientific Ltd., based in Cambridge (AOAC, 2006). The Atomic Absorption Spectrophotometry machine model AA240 from Australia measured mineral concentrations of Mg, Mn, Ca and Fe. Gas chromatography was used to analyze purslane shoot fatty acids after applying a modified Bligh and Dyer method (Bligh & Dyer, 1959).

Determination of total phenolic and flavonoid content

The Folin-Ciocalteu method (Singleton *et al*., 1999) was used for measuring total phenolic content (TPC) while the TFC analysis followed the protocol of (Ordonez *et al*., 2006. The determination of free radical scavenging activity through DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Muller *et al*., 2011). The extracts' ferric reducing power was evaluated at 700 nm wavelength, while increased absorbance signified enhanced reducing power (Yuan *et al*., 2003). The researchers conducted the ABTS assay, measuring 734 nm absorbance through a Spectrophotometer (CECIL CE7200) (Bohm *et al*., 2002).

Experimental trial and induction of liver fibrosis

A bio-evaluation trial was planned to explicate the effect of purslane formulations on hepatic fibrosis. From the National Health Institute, Islamabad, 60 healthy male Wistar rats weighing 200-215 grams were purchased and housed in the Animal Room of the College of Pharmacy, Government College University, Faisalabad. The rats were being fed a basic diet (10% corn oil, 10% proteins, 66% corn starch, 3% minerals, 10% cellulose in addition to 1% vitamins combination). The rats were divided into six

groups including, NC (Normal Control having basic diet), PC (Positive Control having basic diet), STD (Standard Drug Control basic diet + silymarin 50mg/kg b.w), P_1 (Basic diet + Purslane shoot powder 400 mg), P_2 (Basic diet + Purslane shoot powder 800 mg) and P_3 (Basic Diet + Purslane shoot powder 1200 mg) having 10 rats in each group (Charan & Kantharia, 2013) to detect moderate treatment effects while controlling variability. They were subjected to bile-duct ligation (BDL)-induced liver fibrosis (Tag *et al*., 2015) and the effect was measured at 04 weeks. The investigation was conducted under the same environmental conditions, which comprised a 12-hour light-dark cycle, a temperature set at 23±2°C and a relative humidity of 55±5%. During the research, food and water consumption as well as changes in body weight were tracked. The rats in each group were decapitated at the end of the experiment and samples of blood were collected in vials coated with EDTA. Additionally, the serum sample was isolated after the vials containing blood were centrifuged for 6 minutes at 4000 revolutions per minute using a centrifuge machine (Centrifugal Machine, China).

Serum lipid profile

At the specified times, the serum lipid profile was completed, paying particular attention to triglycerides, cholesterol, as well as LDL. The cholesterol CHOD-PAP technique (kim *et al*., 2011) was employed for the calculation of cholesterol content in all of the obtained samples of the sera. Consequently, to evaluate the high-density lipoproteins the Cholesterol Precipitant Method was used (Alshatwi *et al*., 2010).

Kidney functioning tests

To assess renal functionality, the collected sera were tested to determine the concentration of urea using the GLDH technique and for creatinine using the Jaffe process (Jacobs *et al*., 1996; Thomas, 1998).

Hepatic efficiency tests

ALT, AST and ALP were measured to determine the liver's functionality (Basuny *et al*., 2009). Dinitrophenylhydrazine (DNPH) was utilized to evaluate the alkaline phosphatase and alanine transferase concentrations. Sigma Kits 58-50 and 59-50 were used in order. Alkaline Phosphatase-DGKC was applied to analyze the ALP concentrations.

Determination of oxidative stress biomarkers

Malondialdehyde (MDA) was measured according to the protocol followed by (Ohkawed *et al*., 1979). Glutathione (GSH) was determined according to protocols (Beutler *et al*., 1963). Nitric oxide (NO) was measured according to the method (Montgomery & Dymock, 1961).

TNF- α

Tumor necrosis factor α (TNF-α) was measured using polymerase chain reaction following the manufacturer's instructions (Aerts & Vandekerckhove, 2002).

Statistical analysis

The information was collected applying a completely randomized design (CRD) and then statistical evaluation was completed applying the Prism pad 9.2.0 software. Subsequently, post hoc significant Duncan multiple range tests were applied through the use of Costat 2.0, especially when groups show significant differences from each other, with the level of significance $p \leq 0.05$.

RESULTS

Proximate analysis of purslane shoot powder

The examined purslane shoot powder showed an immense amount of all the essential nutrients, as shown in table 1.

Antioxidant assay

The mean values concerning total phenolic content (TPC) of solvent extracts depict that the maximum TPC and TFC were observed in aqueous extract (Table 2), measuring (40±3.23 mgGAE/100g**)** and (12±1.02 mgGAE/100g), respectively. While, IC50 value of DPPH was 51.9 and 46.3 in methanol and aqueous extract, respectively. The extracts were further analyzed for FRAP and ABTS and it was shown that maximum values were observed in aqueous in comparison with the methanol extract.

Minerals and fatty acid contents

The mineral content of dried shoot powder (Table 3) includes potassium, sodium, magnesium, manganese, calcium and iron as 4082.13, 652.13, 796.5, 3.90, 1148.21 and 149.81 mg/kg, respectively (Table 3). The fatty acid profile of purslane shoot powder showed different percentages of fatty acids, including Oleic acid, Linoleic acid, Linolenic acid, Stearic acid and Palmitic acid 7.65, 29.87, 10.57, 3.16, 14.80%, respectively.

Body and liver weight

In each of the trials, body mass changed considerably with meals and investigation durations, according to data analysis (mean square). Means values showed that final body mass 222±9.9, 232±10.2 and 244±11.2 g/rat were significantly different from initial body mass 211±9.2, 212±8.3 and 214±9.2 g/rat, respectively, for treatments, P1, P2 and P3 (Table 4). The initial body weight for the positive control group was 210±9.3 g at the end of the experiment, the final body weight for the positive control group was 214±9.1, with significant increase in weight.

Lipid profile

Results showed that (Fig. 1) throughout 04 weeks intervention period, purslane shoot powder was more effective in lowering total cholesterol levels with different doses, when compared to the positive control group. LDL values in the positive control group (PC) increased, while P1, P2 and P3 groups showed a decrease in LDL values, respectively. At the completion of the experiment, the average HDL levels remarkably improved in all treatment groups. While the PC group has a low HDL level with a 4.31±0.2 mg/dL value. Triglyceride average values

indicated potential modifications in intervention-based groups with readings of 85.05±4.2, 86.70±4.3 and 84.70±3.9 mg/dL for P1, P2 and P3, respectively. The results of the purslane shoot on the parameters of lipid profile were found to be positive, which could be attributed to the bioactive components of the plant present in the shoot. These antioxidants are essential for reducing inflammation in the body by improving lipid metabolism.

Kidney and liver functioning test

Significant outcomes related to therapies and investigation duration were checked (Table 5) for ALP as being 136.5±5.9, 137.3±6.1, 135.7±IU/L for treatment groups P1, P2, P3, respectively. The STD group also showed decreased levels of ALP 146.9±6.5 IU/L, compared with PC group. As a result of the therapies, substantial changes were seen in ALT. The ALT suggested scores ranged 39.1±1.9, 41.5±1.8, 41.9±1.7 IU/L for treatment groups P1, P2, P3 respectively. Reduced ALT readings were recorded in the standard drug group, 37.7±1.8 IU/L, administering 50mg/kg drug for 04 weeks, which can significantly lessen the degree of hepatic fibrosis ($P<0.05$). The concentration of AST in current research was recorded as 161.8±7.0 IU/L (PC) to 90.1±4.2, 91.8±4.1, 93.4±4.38 IU/L with the ingestion of diets P1, P2 and P3, respectively. The bio-evaluation trial revealed significant variations in creatinine levels, these disparities were caused by the medications and interval periods. The average value for creatinine clearance in the positive control was experimentally 0.97±0.03 mg/dL, although rats given purslane showed a slight reduction in total creatinine levels P1, P2 and P3, as 0.96±0.04, 0.94±0.04 and 0.94±0.03 mg/dL, respectively. Mean squares for blood urea explained that substantial variations were influenced by interventions, time periods and their interactions. At the conclusion of the investigation, values for urea in P_1, P2 and P_3 groups were 29.8±1.2, 30.2±1.4 and 29.0±1.2 mg/dL, respectively. As compared to the normal positive control group, 33.4±1.2 mg/dl, the urea level decreased to 29.0±1.3 mg/dl.

Oxidative stress parameters

After the completion of 04 weeks treatment period purslane shoot powder showed a significant reduction in overall levels of oxidative stress (Fig 2). Malondialdehyde (MDA) levels were significantly lower in purslane shoot-treated groups, but were found to be increased as compared to the NC groups. The current finding showed a great potential of purslane shoot powder to attenuate oxidative stress by improving the antioxidant defense mechanism. Glutathione content for the PC group was lower than that of P1 (22.8±0.4mg/g) and P_2 (23.0±0.5mg/g) groups. Likewise, in P3 group, the glutathione value (25.0±0.3mg/g) was increased. Nitric oxide level in the positive control group is elevated (102.4±1.72nmol/L), as compared to the other intervention groups P1 (54.2±1.42nmol/L), P2 (57.0±1.07nmol/L) and P3 (57.3±1.1nmol/L), respectively.

TNF-α

TNF-α content for positive control (83.1±3.8) was higher than that of P_1 (51.8±1.9), P_2 (56.1±2.1) and P_3 (57.7±2.3) groups. The TNF-α values (Fig. 3) in the drug treatment group were also reduced to (56.2±2.7) as compared to the positive control group.

DISCUSSION

In Chinese traditional medicine, *Portulaca oleracea* (PO), often called the "vegetable for long life", has gained attention for its health benefits. In this study, the shoots of PO were found to contain valuable nutrients such as minerals, fatty acids and antioxidants. Earlier research has also shown that this plant is a good source of vitamins, omega-3 fatty acids and many natural compounds like alkaloids, terpenoids, flavonoids and organic acids (Nemzer *et al*., 2020). These natural substances help protect body cells by stopping harmful oxidation processes, which in turn prevent fat damage and protect vital molecules. In animal experiments, liver fibrosis is often produced by a method called bile duct ligation (BDL), which causes chemical and functional changes in the liver similar to chronic liver disease. In this study, we tested the protective effect of *Portulaca oleracea* (PO) shoot powder on liver damage caused by bile duct ligation (BDL) in rats. Liver fibrosis is determined by the overproduction of extracellular matrix (ECM) protein, especially collagen due to the conditions of chronic liver diseases. Progressing further, at its advanced stages, fibrosis may develop into liver failure, portal hypertension and cirrhosis which may require liver transplant (Bataller & Brenner, 2005; Friedman, 2008). Distortion of hepatic architecture due to deposits of ECM proteins formed in the diseased liver leads to the formation of fibrous lesions. The combination of the scarring and formation of regenerative nodules of hepatocytes is the hallmark of cirrhosis (Kisseleva *et al*., 2021). Liver fibrosis was traditionally viewed as a passive process with an irreversible outcome caused by the hepatic parenchyma collapse and the replacement of this tissue with collagen-containing one (El-Sayed *et al*., 2019). Free radicals are atoms or groups of atoms with one or more unpaired electrons, thus being unstable and very reactive. In biological systems, they are typically divided into two categories: reactive oxygen species (ROS), whose formation is based on oxygen and reactive nitrogen species (RNS) based on nitrogen. Any compromise of this balance towards an increase of ROS/RNS is called oxidative stress (Muriel, 2009). Reactive oxygen species (ROS) play roles in the normal physiology, including cellular maturation, maintenance of signaling pathways and host defense against invading pathogens. In bile duct obstruction (BDL), fibrosis advances as a result of liver cell death, proliferation of bile duct epithelial cells and activation of hepatic stellate cells (HSCs). ROS can induce the activation of hepatic stellate cells (HSCs), leading to the deposition of extracellular matrix (ECM) proteins, fibrosis, cirrhosis and hepatocellular carcinoma (HCC).

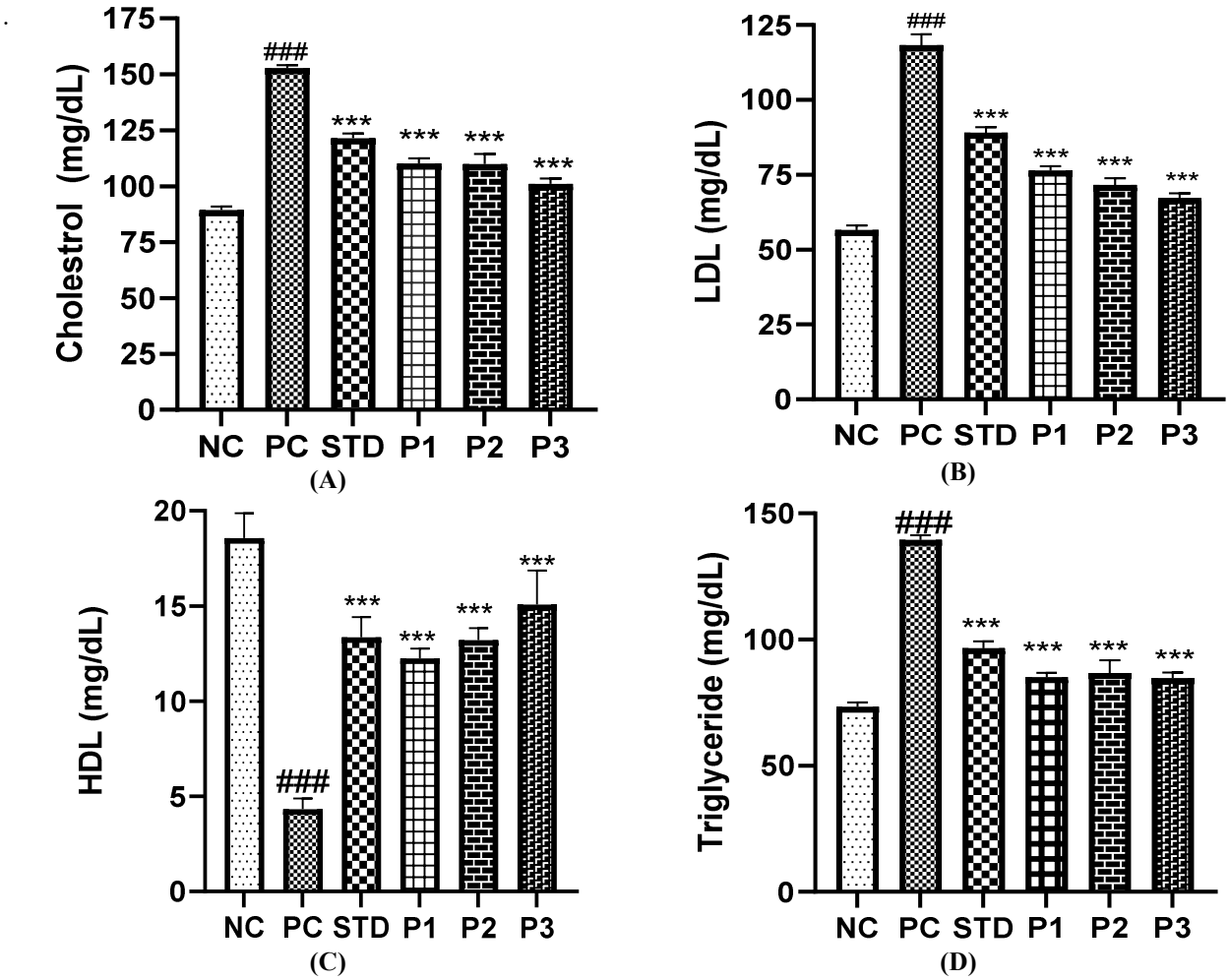

Fig. 1: Lipid Profile (a) Serum Cholesterol (b) High Density Lipoprotein HDL mg/dl (c) Low Density Lipoprotein (d) Triglycerides (TG) levels in the NC (normal control), PC (positive control), STD group (50mg/kg) and different purslane shoot powder based treatment groups: P1(400mg/kg body weight), P2 (800mg/kg body weight), P3(1200 mg/kg body weight) for 04 week period in bile duct ligation induced rats.

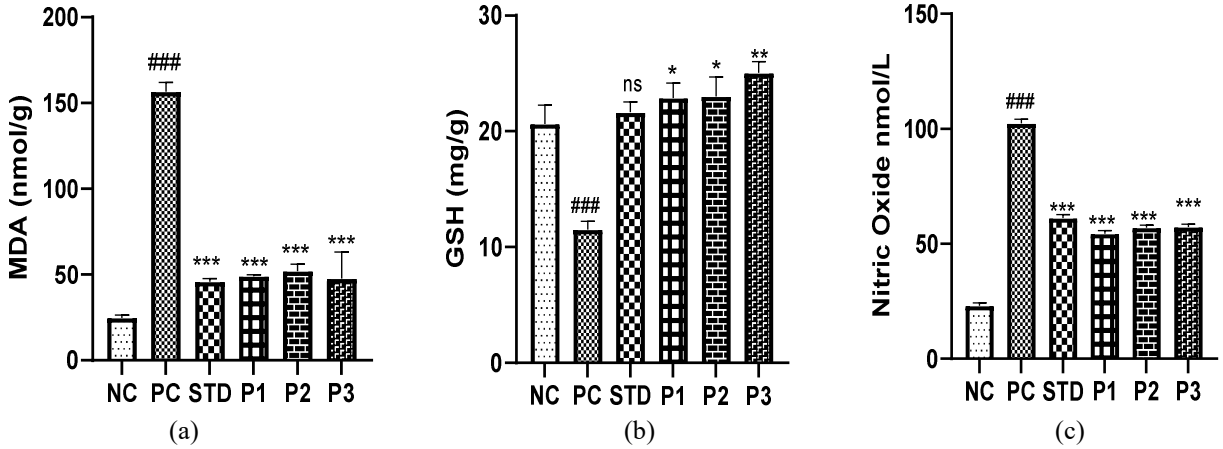

Fig 2: Effect of purslane shoot powder in comparison with silymarin on the Oxidative stress parameters, values are (mean± SE).*significantly different from normal control group.**significantly different from positive control group at p <0.05.(a) Malondialdehyde (nmol/g), (b) Reduced glutathione (mg/g),(c) Nitric oxide nmol/L levels in NC (normal control), PC (Positive Control), STD (standard drug 50mg/kg), P1(400mg/kg body weight),P2 (800mg/kg body weight weight), P3 (1200mg/kg body weight) for 04 week period in bile duct ligation induced rats.

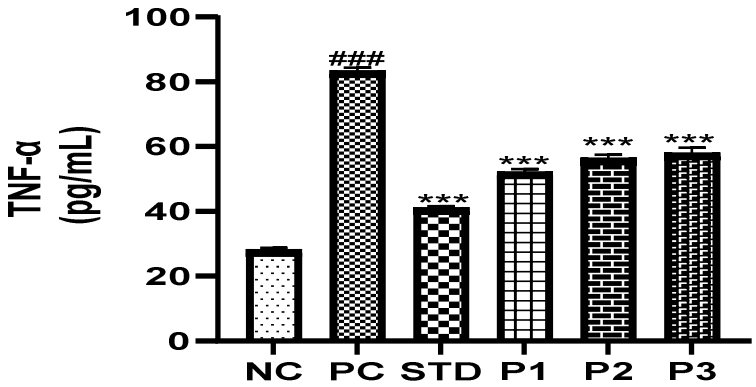

Fig. 3: Effect of Purslane P1 (400mg/kg 4 weeks), P2 (800mg/kg4 weeks), P3 (1200 mg/kg 4 weeks) in comparison with silymarin (50 mg/kg) on the TNF- α serum level.

Table 1: Percentages of proximate components found in purslane shoot powder.

Parameters	Results (%)
Moisture	10.20±0.4
Protein	17.13±0.7
Fat	3.42±0.1
Fiber	4.03±0.2
Ash	16.46±0.7
Carbohydrate	36.86±1.9
NFE	8.26±0.3

The table shows the proximate analysis of purslane shoot powder *i.e,* moisture, protein, fat, fiber, Carbohydrate, ash, NFE% the values are expressed as mean ± standard deviation.

Table 2: Phytochemical analysis of purslane shoot extract

Phytochemical analysis	Solvents		*P*-value
	Methanol	Aqueous	
TPC mgGAE/100g	26±1.23	40±3.23	0.01
TFC mgGAE/100g	6.5±0.56	12±1.02	0.02
DPPH IC50	51.9±0.57	46.3±0.32	0.05
FRAP mgTE/g	3.28±0.09	5.53±0.08	0.02
ABTS mgTE/g	53.64±0.56	67.18±0.82	0.01

Values are expressed as mean ± standard deviation (SD). The p-values ($P<0.05$) indicate the statistical significance of differences between the two samples. Total Phenolic Content: TPC, Total Flavonoid Content: TFC, 2,2-diphenly-1-picrylhydrazyl: DPPH, Fluorescence Recovery after Photo bleaching: FRAP, 2,2'-azino-bis(3-ethylbenzothianzoline-6-sulfonic acid): ABTS.

Table 3: Minerals and fatty acid composition of purslane shoot powder

Nutrient	Value
Potassium (K) (mg/kg)	4082.13
Sodium (Na) (mg/kg)	652.13
Magnesium (Mg) (mg/kg)	796.51
Manganese (Mn) (mg/kg)	3.90
Calcium (Ca) (mg/kg)	1148.21
Iron (Fe) (mg/kg)	149.81
Oleic acid (C18:1) (%)	7.65%
Linoleic acid (C18:2) (%)	29.87%
Linolenic acid (C18:3) (%)	10.57%
Stearic acid (C18:0) (%)	3.16%
Palmitic acid (C16:0) (%)	14.80%

The mineral (mg/kg) and fatty acid (%) composition were determined using Gas Chromatography (GC).

Table 4: Effect of purslane powder on body weight, liver weight and liver index in normal and BDL-induced rats.

Groups	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Liver weight (g)	Liver weight / body weight × 100	P-value
NC	211 ± 9.8	246 ± 11.8	35 ± 1.7	4.0 ± 0.1	1.62 ± 0.07	0.007
PC	210 ± 9.3	214 ± 9.1	4 ± 0.2	5.3 ± 0.2	2.47 ± 0.10	0.001
STD	209 ± 8.9	225 ± 10.9	16 ± 0.8	4.5 ± 0.2	2.00 ± 0.10	0.010
P1	211 ± 9.2	222 ± 9.9	11 ± 0.5	4.3 ± 0.1	1.93 ± 0.07	0.035
P2	212 ± 8.3	232 ± 10.2	20 ± 1.0	4.2 ± 0.2	1.81 ± 0.08	0.008
P3	214 ± 9.2	244 ± 11.2	30 ± 1.5	4.1 ± 0.2	1.68 ± 0.08	0.005

This table shows the effects of different treatments on body weight and liver parameters. NC is the normal control, while PC is the disease-induced group without treatment. STD received a standard drug, and P1, P2, P3 were given different treatment doses. Values are Mean ± SD, with P-values comparing each group to NC using ANOVA and post hoc analysis.

Table 5: Effect of purslane shoot powder on kidney and liver functioning test

Parameters	NC	PC	STD	P1	P2	P3
ALP IU/L	123.8±4.3^{c}	167.4±3.88^{a}	146.9±1.94^{b}	136.5±2.54^{b}	137.3±1.80^{b}	135.7±2.38^{b}
ALT IU/L	24.1±1.20^{c}	69.2±3.46^{a}	37.7±1.88^{b}	39.1±1.95^{b}	41.5±2.0^{b}	41.9±2.09^{b}
AST IU/L	62.4±3.12^{c}	161.8±8.09^{a}	86.1±4.3^{b}	90.1±4.50^{b}	91.8±4.59^{b}	93.4±4.67^{b}
Creatinine mg/dL	0.81±0.04^{c}	0.97±0.04^{a}	0.89±0.04^{b}	0.96±0.04^{b}	0.94±0.04^{b}	0.94±0.04^{b}

This table shows the effects of different treatments on biochemical parameters i.e. ALP (Alkaline Phosphatase), ALT (Alanine Tranaminase) AST (Aspartate Aminotransferase) IU/L and Creatinine mg/dLn experimental groups. Values are presented as mean ± standard deviation. Different superscript letters (a, b, c) within a row indicate significant differences ($p < 0.05$).

ROS promotes the activation of HSCs from a quiescent state, which is the ECM producing phenotype; in turn, ECM deposition leads to fibrosis, cirrhosis and eventually HCC. Antioxidants, nuclear factor-E2-related factor-2 (NRF2) and nitric oxide (NO) seem to play antifibrotic roles by inhibiting ROS-induced HSC activation. Inducible nitric oxide synthase (iNOS) can synthesize large amounts of NO in the liver, utilizing L-arginine as a substrate. Nitric oxide (NO) is mainly produced by a hepatocyte-inducible NO synthase (iNOS) as a result of enhanced inflow of endotoxins to the liver and also by accumulation of bile salts in hepatocytes and subsequent hepatocellular injury (Lopez-Sanchez & Laura 2010; Ramos-Tovar & Muriel 2020).

Oxidative stress is one of the key features of liver injury and occurs when there is an imbalance between oxidants and the antioxidant defense system. Disruption of this balance plays an important role in BDL-induced liver damage (Hamza, 2010). In the current study, rats with BDL that received purslane supplements showed a clear reduction in oxidative stress markers compared with untreated BDL rats, which agrees with earlier reports. The therapeutic effect of purslane supplementation was more noticeable than its preventive effect, as it resulted in a marked reduction of liver enzyme levels (El-Swefy & Hassanen, 2009). Purslane is rich in natural antioxidants such as flavonoids, saponins, tannins, minerals, melatonin and vitamin C (Erkan, 2012; Rahimi *et al*., 2019; Derouiche *et al*., 2017). These compounds can block lipid peroxidation, neutralize free radicals and bind metal ions, thereby reducing oxidative damage. Malondialdehyde (MDA), a toxic product of lipid peroxidation, is often used as a marker of oxidative damage (Qiao *et al*., 2019). MDA can also bind with biomolecules and disrupt cell functions. Studies have demonstrated that purslane reduces MDA levels while increasing glutathione (GSH), an important antioxidant, in diabetic rat models (Samarghandian *et al*., 2017). In agreement, our study showed that purslane shoot powder significantly reduced MDA levels in BDL rats (Zhou *et al*., 2015).

Another useful marker of fibrosis progression is hydroxyproline, which reflects collagen production in liver tissue. Bile duct ligation causes bile acid reflux into the liver, damaging hepatocytes and triggering inflammation through activation of Kupffer cells and infiltration of leukocytes. These immune cells release ROS, which promote collagen deposition (Sadeghi *et al*., 2019). Current findings were further supported by earlier research showing that free radicals play a major role in liver injury by attacking unsaturated fatty acids in cell membranes, which initiates lipid peroxidation and eventually damages proteins and DNA (Milkarizi *et al*., 2024). In different parts of the Purslane plant, the flavonoid content varies, with the highest levels present in the root, followed by the stem and the leaf. Purslane plant contains seven different flavonoids, including kaempferol, myricetin, luteolin, apigenin, quercetin, genistein and genistin (Zhu *et al*., 2010). In *portulaca oleracea* plant, the presence of quercetin shows positive effects against liver damage, having anticancer, antioxidant and antiviral features (Hashemzaei *et al*., 2016). (Nakhaee *et al*., 2021) findings showed improvement of oxidant/antioxidant agents (MDA, SOD and NOx) in various tissues, including the heart, liver, adrenal and kidney in a rat model of tramadol intoxication,

which received 100mg/kg of quercetin for 14 days. Purslane shows strong antioxidant activity in vitamin A-deficient rats by lowering oxidative markers (MDA, NO, lipid peroxidation) in liver, heart, kidney and testis. It boosts key antioxidant enzymes (GPx, GR, GST, SOD, CAT) and improves glutathione balance. These effects reduce liver enzyme leakage (ALT, AST, γ-GT, ALP), indicating protection against liver injury. Overall, purslane helps maintain antioxidant defenses and protects tissues from oxidative stress. Furthermore, catechins were found to inhibit the production of TNF-α and nitric oxide by lipopolysaccharide-activated macrophages (Arruda *et al*., 2004).

The Present Study found that purslane improved the blood fat profile in the rats. This positive effect may be due to its high amount of omega-3 fatty acids, more than most other leafy vegetables, and its flavonoid compounds (Nemzer *et al*., 2021). Flavonoids can help lower triglycerides (TG) by increasing a molecule called cAMP, which activates enzymes that break down TG, reducing their levels in blood and liver. Flavonoids also help the body use LDL receptors better, which improves cholesterol balance (Deng *et al*., 2021). The Present study also showed that silymarin has clear effects on total cholesterol TC, HDL and LDL in BDL rats. Other research agrees with this, showing that silymarin can lower TC and increase HDL, likely by reducing how much cholesterol is absorbed (Marková *et al*., 2021). Another study also found that silymarin lowers total lipids, TG, TC and lipoproteins (Morsy *et al*., 2021). Likewise, many studies support our results, showing that purslane can reduce TC and TG. This may be because it slows down the enzymes that make cholesterol or reduces fat breakdown in the body (Jung *et al*., 2021).

Biochemical markers of liver fibrosis include raised levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST), which reflect altered bile acid metabolism in the liver (Samarghandian *et al*., 2017). In agreement with this, BDL in our study significantly elevated the plasma levels of these enzymes. According to (Pishva *et al*., 2018), increased ALT, AST and ALP levels are linked with structural damage of the liver, as these enzymes leak into the bloodstream following cellular injury. In general, elevated AST indicates hepatocellular damage, while ALT is more closely associated with liver necrosis (Arya *et al*., 2021). Likewise, BDL is well known to increase ALP activity, which is a hallmark of cholestasis. Since ALP is a membrane-bound enzyme, this increase is thought to be due to bile salt accumulation that disrupts the cell membrane, causing cell breakdown and release of ALP into circulation (Sadeghi *et al*., 2019). It was shown that oral administration of the ethanolic extract of purslane in vivo rodent models lowered serum enzymatic activities of AST, ALT and increased serum albumin and total protein levels (Alazabi & Abdelhameed, 2025). These effects may be due to the antioxidant properties of purslane that give protection against free radicals and damage to the liver.

The beneficial role of purslane has also been confirmed in human studies. In one trial, (Milkarizi *et al*., 2024) showed the positive effect of consuming a 700 mg *portulaca oleracea* supplement for 08 weeks to improve the condition of hepatic steatosis and fibrosis in patients with NAFLD. No serious side effects were reported, suggesting that purslane is safe for up to 8–12 weeks of use (Saraf *et al*., 2007). In addition, purslane also showed anti-inflammatory effects by lowering TNF-α levels in BDL rats. These findings are supported by (Lee *et al*. 2008), who reported that the omega-3 fatty acids in purslane possess anti-inflammatory activity. Altogether, these results highlight that purslane has protective properties that may help slow or prevent liver complications caused by cholestatic disorders and could potentially reduce the risk of progression to fibrosis and cirrhosis.

CONCLUSION

The research establishes that purslane shoots are an antioxidant-rich plant that exhibits strong hepatoprotective properties through their enhancing liver function and their capacity to reduce oxidative stress markers, along with their ability to regulate inflammatory responses. The tested benefits revealed stronger impacts during preventive use compared to therapeutic usage, which demonstrates its ability to prevent liver fibrosis.

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Authors' contribution
Sana Azher: writing-original draft. Conceptualization, data collection, statistical analysis,
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The authors have no conflict of interest.

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