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Research Article

Protective Effects of Acetone Extract of *Portulaca oleracea* L. on Liver Histopathology in Hypoxia-Induced Sprague-Dawley Rats

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ABSTRACT

Hypoxia, a condition characterized by insufficient oxygen supply, can cause tissue damage in vital organs, including the liver. The liver is particularly vulnerable to hypoxic injury, which may result in impaired function and hepatocyte death. Purslane (Portulaca oleracea L.) is known for its antioxidant properties, largely attributed to its flavonoid content, which can inhibit the production of reactive oxygen species (ROS). This experimental study aimed to evaluate the effects of acetone extracts of purslane herb on hepatic histopathological changes in hypoxia-induced rats. Five experimental groups were established, each consisting of six rats (n = 6): normal control (N), hypoxia (H), hypoxia treated with dexamethasone (H+DEXA), hypoxia treated with purslane extract at 150 mg/kg body weight (HP1), and hypoxia treated with purslane extract at 300 mg/kg body weight (HP2). Purslane extract was prepared using the acetone maceration method. Hypoxia was induced by exposing rats to a gas mixture of 10% O_2 and 90% N_2 for 10 consecutive days. Liver tissues were processed using paraffin embedding and stained with hematoxylin–eosin for histopathological evaluation, focusing on hepatocyte necrosis, inflammatory cell infiltration, and hemorrhage. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test. The results showed that the 300 mg/kg dose of purslane extract significantly reduced hepatocyte necrosis (35.33 ± 2.52 cells), inflammatory cell infiltration area (2488.51 ± 112.82 µm²), and hemorrhage area (1031.10 ± 17.38 µm²) compared with the hypoxia group ($p < 0.05$). In conclusion, acetone extracts of purslane demonstrated significant hepatoprotective effects in hypoxia-induced rats, suggesting their potential as a natural therapeutic agent for the management of hypoxia-related liver injury.

Keywords: *Acetone extract of purslane; Hemorrhage; Hypoxia; Inflammatory Cell Infiltration; Necrosis.*

Introduction

Hypoxia is a pathological condition characterized by an insufficient supply of oxygen to tissues, resulting in cellular injury, particularly in metabolically active organs such as the brain, kidneys, and liver [1]. The liver, which plays a central role in metabolic homeostasis, is especially susceptible to hypoxic damage; inadequate oxygen availability can disrupt mitochondrial function and induce hepatocyte death [2]. Hypoxic exposure further exacerbates oxidative stress by promoting the formation of unstable free radicals and increasing the production of reactive oxygen species (ROS) [3]. Endogenous antioxidants, such as catalase, mitigate oxidative damage by converting hydrogen peroxide into oxygen and water, thereby limiting cellular injury [4], [5]. However, under sustained hypoxic conditions, these protective mechanisms may become overwhelmed.

Experimental studies in rats have demonstrated that exposure to normobaric hypoxia (10% O_2) for several days can induce hepatocyte necrosis, sinusoidal dilation, and increased levels of inflammatory mediators, reflecting substantial hepatic stress. Antioxidant-based interventions, including quercetin and curcumin, have been reported to ameliorate these effects by reducing oxidative burden and preserving hepatic tissue architecture. These findings underscore the therapeutic potential of natural antioxidants in mitigating hypoxia-induced liver injury [6–11].

Methyl rosmarinate has demonstrated protective effects against hypoxia-induced necrosis through its phenolic antioxidant activity [12]. Similarly, extracts of Acalypha indica and Centella asiatica, which are rich in flavonoids, have been shown to suppress hepatic free radical formation following hypoxic exposure [2]. Despite this growing body of evidence, the exploration of other antioxidant-rich plant sources remains limited.

Purslane (*Portulaca oleracea* L.) contains abundant phenolic compounds, flavonoids (including kaempferol, apigenin, and quercetin), and alkaloids such as adenosine and oleracone, all of which are recognized for their strong antioxidant properties [13], [14]. Despite these attributes, purslane is still widely regarded as a low-value weed, and its potential role in preventing hypoxia-related liver injury remains insufficiently explored. Moreover, most existing studies have employed ethanol or aqueous extracts, whereas data on acetone extracts which may yield distinct antioxidant profiles due to their ability to solubilize more lipophilic compounds are scarce. To date, no study has examined the hepatoprotective effects of acetone extracts of *P. oleracea* in models of hypoxia-induced liver injury.

Therefore, the present study aimed to evaluate the efficacy of acetone extracts of purslane herb on hepatic histopathological alterations in hypoxia-induced Sprague–Dawley rats.

Materials and Methods

Ethical Approval

This study involved experimental procedures using *Portulaca oleracea* L. extracts and laboratory animals. The preparation of purslane extracts (roots, stems, and leaves) was conducted over a two-month period in the Biology Research Laboratory, Ahmad Dahlan University, Yogyakarta, Indonesia. Plant identification was verified at the same institution, and a voucher specimen was deposited under voucher number 182/Lab.Bio/B/V/2021. All animal experimental procedures were reviewed and approved by the Research Ethics Committee of Ahmad Dahlan University (Ethical Clearance No. 012106030 [KEP]) and were carried out in accordance with institutional regulations and internationally accepted guidelines for the care and use of laboratory animals.

Materials

The instruments used in this study included laboratory glassware (Pyrex), a 3 mL gavage needle, a micropipette (Socorex), a complete surgical instrument set (Socorex), a hypoxia chamber, oxygen and hydrogen gas cylinders with regulators, a rotary evaporator, a microtome, and a light microscope. The materials utilized comprised male albino rats (*Rattus norvegicus*) of the Sprague–Dawley strain, aged 8–12 weeks and weighing 150–200 g; purslane (*Portulaca oleracea*); dexamethasone; 0.5% sodium carboxymethyl cellulose (CMC-Na); Whatman No. 1 filter paper; acetone; 70% ethanol; paraformaldehyde; 10% buffered neutral formalin (BNF); picric acid; citrate buffer; xylene; Entellan; phosphate-buffered saline (PBS); hematoxylin–eosin stain; Sirius Red; graded ethanol solutions (96%, 90%, 80%, 70%, 60%, 50%, 40%, and 30%); hydrochloric acid; 0.9% NaCl solution; diethyl ether; and calcium carbonate.

Methods

Preparation of Purslane (Portulaca oleracea) Acetone Extract

The roots, stems, and leaves of purslane (*Portulaca oleracea* L.) were thoroughly washed and air-dried at room temperature for 14 days, after which they were ground into a fine powder. Acetone extraction was performed by macerating the powdered plant material in acetone at a ratio of 1:10 (w/v) for five days. The resulting extract was filtered through Whatman No. 1 filter paper, and the remaining plant residue was subjected to two additional maceration cycles under the same conditions. The combined filtrates were then concentrated under reduced pressure using a rotary evaporator.

Dosage Calculation of Test Preparations

Groups of seven rats, each weighing approximately 170 g, were treated once daily with the designated test preparations. The experimental groups received purslane extract at doses of 150 mg/kg body weight (BW) or 300 mg/kg BW, or dexamethasone as a reference drug. All treatments were prepared in 0.5% sodium carboxymethyl cellulose (CMC-Na), which served as the vehicle.

Animal Preparation

Experimental animals were acclimatized for seven days in cages maintained under controlled environmental conditions, including a room temperature of 24–28°C, relative humidity of 60–75%, and a 12 h light/dark cycle [15]. Standard laboratory feed and water were provided *ad libitum*. Throughout the study, only healthy rats exhibiting clean white fur, normal behavior, clear eyes, and no visible physical abnormalities were included.

Experimental Design

This study employed a completely randomized design (CRD) in an experimental animal model. A total of 28 male albino rats were used and randomly allocated into experimental groups, as shown in Table 1.

Tabel 1. Experimental Animal Groups

No	Treatments	Description
1	N	Normal control
2	H	Hypoxia without drug or extract treatment
3	H + DEXA	Hypoxia with dexamethasone treatment
4	HP1	Hypoxia with purslane (*Portulaca oleracea*) extract, 150 mg/kg BW
5	HP2	Hypoxia with purslane (*Portulaca oleracea*) extract, 300 mg/kg BW

Hypoxia Induction in Experimental Animals

Oxygen concentration inside the hypoxia chamber was continuously monitored using a calibrated digital oxygen

sensor to ensure that the 10% O_2 / 90% N_2 gas composition remained stable throughout the exposure period. The sensor was calibrated before each experimental session, and oxygen levels were verified at multiple time points during the daily 6 h hypoxia induction. The hypoxia induction protocol was adapted from previously published normobaric hypoxia models.

Administration of Purslane (Portulaca oleracea) Extract and Dexamethasone

Following 6 hours of daily hypoxia induction, animals received their respective treatments. Purslane acetone extract was administered orally at doses of 150 mg/kg body weight (BW) and 300 mg/kg BW, while dexamethasone served as the reference drug. All treatments were prepared by serial dilution in 0.5% sodium carboxymethyl cellulose (CMC-Na) and administered once daily for 10 consecutive days using a 3 mL gavage needle.

Preparation of Liver Tissue Specimens

Liver histological specimens were prepared using the paraffin embedding method. Following euthanasia, the liver was excised, rinsed with 0.9% NaCl solution, and fixed in 10% buffered neutral formalin (BNF). Histopathological sections were then prepared and stained with hematoxylin–eosin (H&E) for microscopic examination [16].

***Histological Examination of Liver Tissue* L**

Liver histological sections were examined under a light microscope at 400× magnification (40× objective and 10× eyepiece) in five randomly selected fields of view per slide. The evaluated parameters included normal hepatocytes, necrosis, hemorrhage, and inflammatory cell infiltration. Quantitative analysis of necrotic areas, inflammatory infiltration, and hemorrhage was performed using ImageJ software (NIH, USA). Damaged areas and cell counts were measured in each field of view, and all analyses were independently verified by two blinded observers to ensure consistency and minimize observer bias [16].

Data Analysis

Data were analyzed using the Shapiro–Wilk test and Levene's test to assess normality and homogeneity of variance, respectively. Parametric data are presented as mean ± standard deviation (SD) and were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range post hoc test. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software.

Results and Discussion

Effectiveness of Acetone Extract of Portulaca oleracea on Hepatocyte Necrosis in Rat Liver

Histopathological examination of liver tissue from male Sprague–Dawley rats (Figure 1) revealed that the normal control (N) group exhibited only mild hepatocyte necrosis (Figure 1A), which may reflect normal physiological turnover. In contrast, the hypoxia (H) group showed severe necrotic damage, characterized by nuclear fragmentation and cytoplasmic degeneration, likely resulting from oxidative stress induced by 10 days of hypoxic exposure (Figure 1B). A marked reduction in necrotic lesions was observed in the H+DEXA, HP1, and HP2 treatment groups, with the HP2 group demonstrating the greatest histological improvement (Figure 1C–E).

Necrosis, defined as the irreversible death of hepatocytes, is characterized by nuclear fragmentation and cytoplasmic degeneration and is primarily induced by hypoxic conditions [17–20].

Quantitative analysis of hepatocyte necrosis (Table 2) showed that the normal control (N) group (23.33 ± 3.51) exhibited significantly lower necrosis compared to the hypoxia (H) group (75.33 ± 3.21) ($p \leq 0.05$). In contrast, the H+DEXA (56.33 ± 2.51), HP1 (46.00 ± 2.00), and HP2 (35.33 ± 2.51) treatment groups demonstrated a

significant reduction in necrosis relative to the H group ($p \leq 0.05$). These findings indicate that *Portulaca oleracea* extract effectively reduces hypoxia-induced hepatocyte necrosis, with the HP2 group showing the greatest protective effect.

The liver plays a central role in lipid metabolism, and prolonged exposure to toxic stimuli can disrupt metabolic homeostasis, leading to histopathological damage such as hepatocyte necrosis. The present study demonstrates that acetone extracts of purslane can attenuate hypoxia-induced necrotic damage, likely through the antioxidant activity of its bioactive constituents, particularly flavonoids. Consistent with these findings, previous studies have reported that flavonoids derived from *Swertia chirata* reduce hepatocyte necrosis under hypoxic conditions [21]. Purslane contains both flavonoids and eicosapentaenoic acid (EPA), an omega-3 fatty acid. Flavonoids function as potent antioxidants and can enhance the synthesis and activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), which plays a crucial role in neutralizing free radicals and minimizing cellular damage [22]. Flavonoids also possess hepatoprotective activity, as their polyphenolic structures enable effective scavenging of free radicals and prevention of oxidative injury. These mechanisms help maintain hepatocyte membrane integrity and reduce the risk of liver damage under hypoxic conditions [23]. In addition, flavonoids may interact with immune cells to modulate intracellular signaling pathways that promote tissue repair and recovery [24]. These improvements suggest that the extract may help stabilize mitochondrial function under hypoxic stress, thereby limiting irreversible cellular damage. The observed reduction in necrosis may reflect decreased accumulation of reactive oxygen species (ROS) and improved preservation of hepatocyte viability.

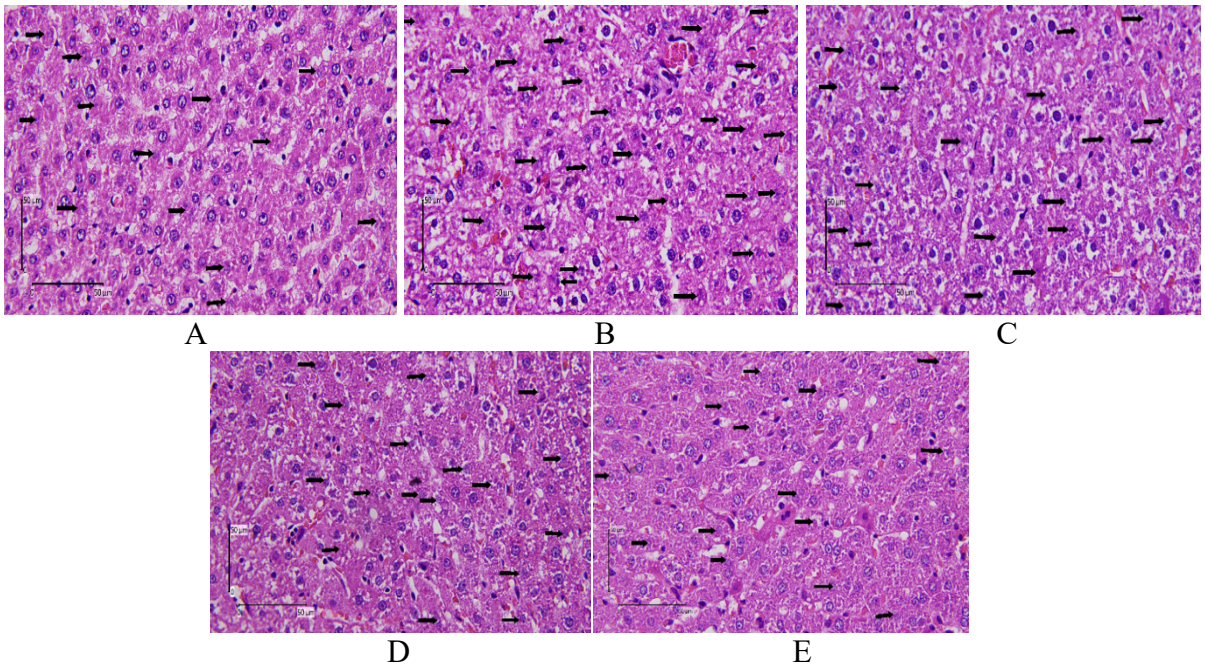

Figure 1. Histopathology of hepatocytes exhibiting necrosis (black arrows) in the liver tissue of male Sprague-Dawley rats (*Rattus norvegicus*). (A) Normal (N); (B) Hypoxia without drug or extract treatment (H); (C) Hypoxia with dexamethasone treatment (H+DEXA); (D) Hypoxia + *Portulaca* extract 150 mg/kg BW (HP1); (E) Hypoxia + *Portulaca* extract 300 mg/kg BW (HP2). Stained with Hematoxylin-Eosin (H&E), 400x magnification (40x objective, 10x eyepiece). Scale bar: 50 µm.

Effectiveness of Acetone Extract of Portulaca oleracea on the Area of Inflammatory Cell Infiltration

Inflammatory cell infiltration represents an early cellular response to harmful stimuli, such as free radicals, and can disrupt normal metabolic processes [25]. These cells contribute to the neutralization and containment of tissue damage while simultaneously promoting tissue repair and regeneration [26]. Figure 2 illustrates inflammatory cell infiltration in liver tissue, characterized by the presence of purple-stained inflammatory cells (indicated by black arrows). Hepatic inflammation is marked by the infiltration of phagocytic cells, including lymphocytes and polymorphonuclear leukocytes, which often originate in the pericentral region around the central vein [25]. Prolonged exposure to toxic stimuli can lead to the extension of inflammatory cell infiltration from the portal triad toward the central vein [27].

Table 2. Quantitative Analysis of Hepatocyte Necrosis in Rat Liver

Group	Mean of Hepatocyte Necrosis (cells) ± SD
N	23.33 ± 3.51^{a}
H	75.33 ± 3.21^{e}
H+DEXA	56.33 ± 2.51^{d}
HP1	46.00 ± 2.00^{c}
HP2	35.33 ± 2.51^{b}

Description: *Different superscript letters (a–e) within the same column indicate statistically significant differences by Duncan's post hoc test ($P \leq 0.05$).*

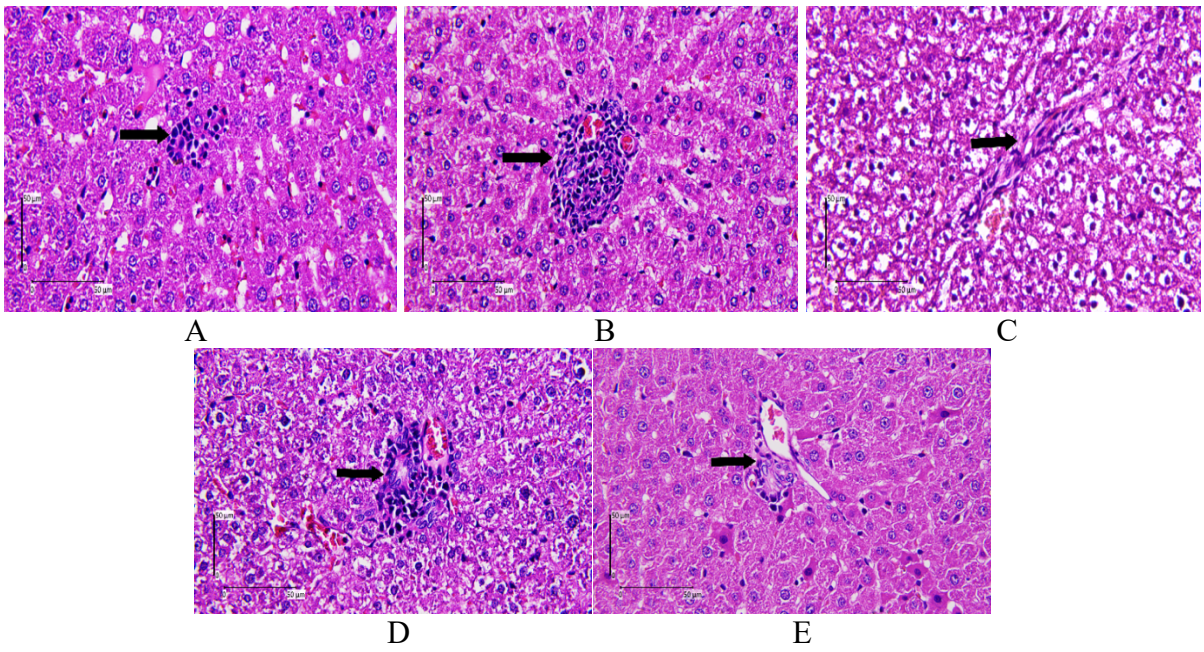

Figure 2. Histopathology of liver tissue showing inflammatory cell infiltration (black arrows) in male Sprague-Dawley rats (*Rattus norvegicus*). **(**A) Normal (N); (B) Hypoxia without drug or extract treatment (H); (C) Hypoxia with dexamethasone treatment (H+DEXA); (D) Hypoxia + *Portulaca* extract 150 mg/kg BW (HP1); (E) Hypoxia + *Portulaca* extract 300 mg/kg BW (HP2). Stained with Hematoxylin-Eosin (H&E), 400x magnification (40x objective, 10x eyepiece). Scale bar: 50 µm.

As shown in Figure 2, the normal control (N) group exhibited the smallest area of inflammatory cell infiltration, whereas the hypoxia (H) group showed the largest. In contrast, the H+DEXA, HP1, and HP2 treatment groups demonstrated markedly reduced areas of inflammatory

infiltration, indicating improved hepatic tissue condition following treatment.

To assess inflammatory cell infiltration, the infiltration area in liver tissue was quantitatively analyzed (Table 3). The normal control (N) group exhibited the smallest infiltration area (2029.02 ± 34.65 µm²), whereas the hypoxia (H) group showed the largest (4658.97 ± 15.06 µm²). The treatment groups (H+DEXA, HP1, and HP2) demonstrated significant reductions in inflammatory infiltration compared with the H group ($p \leq 0.05$). Among these, the HP2 group showed the most pronounced decrease in infiltration area (2488.51 ± 112.82 µm²), indicating substantial histopathological improvement ($p \leq 0.05$). These findings suggest that *Portulaca oleracea* extract effectively mitigates hypoxia-induced inflammation in hepatic tissue.

Table 3. Measurement of Inflammatory Cell Infiltration Area (µm²) in Liver Tissue

Groups	Mean of Inflammatory Cell Infiltration Area (µm²) ± SD
N	2029.02 ± 34.653[a]
H	4658.97 ± 15.062[d]
H+DEXA	3415.99 ± 127.472[c]
HP1	3377.21 ± 84.276[c]
HP2	2488.51 ± 112.817[b]

Description: *Different superscript letters (a–d) within the same column indicate statistically significant differences by Duncan's post hoc test ($P \leq 0.05$).*

Increased inflammatory cell infiltration reflects a hypoxia-induced response mediated by elevated reactive oxygen species (ROS) production and lipid peroxidation, which activate Kupffer cells, macrophages, and monocytes. This activation stimulates the release of proinflammatory cytokines, including interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-α (TNF-α), primarily through the nuclear factor-κB (NF-κB) signaling pathway. The resulting cytokine cascade promotes immune cell recruitment and initiates tissue repair processes [28]. Purslane extract alleviates liver inflammation through its secondary metabolites namely flavonoids, saponins, and alkaloids which exert both anti-inflammatory and antioxidant effects. Flavonoids act as free radical scavengers, inhibit key inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase, reduce arachidonic acid release, and limit immune cell proliferation [29–31]. Saponins reduce vascular permeability and inflammatory exudate formation, whereas alkaloids suppress tumor necrosis factor-α (TNF-α) production by inhibiting activation of the nuclear factor-κB (NF-κB) signaling pathway [32–35]. Collectively, these bioactive compounds mitigate inflammation and promote liver recovery under hypoxic conditions. The observed reduction in inflammatory cell infiltration indicates attenuation of cytokine-driven responses, suggesting improved cellular homeostasis during hypoxic stress. This pattern supports the hypothesis that the extract modulates early inflammatory signaling pathways within hepatic tissue.

Effect of Acetone Extract of Portulaca oleracea on Hemorrhage Area

Hemorrhage refers to the escape of blood from ruptured sinusoidal vessels, resulting in the extravasation of red blood cells into the surrounding hepatic tissue [36]. Microscopically, hemorrhage is characterized by the presence of small hemorrhagic foci (petechiae) or larger areas of bleeding (ecchymoses) [37]. As shown in Figure 3, the normal control (N) group exhibited the smallest area of hemorrhage, whereas the hypoxia (H) group showed the largest. In contrast, the H+DEXA, HP1, and HP2 treatment groups demonstrated reduced hemorrhagic areas, indicating attenuation of vascular damage following treatment. Hemorrhage is commonly caused by sinusoidal congestion, which can lead to vascular rupture and the leakage of red blood cells into the surrounding hepatic

tissue. In addition, chronically elevated intravascular pressure may further contribute to the development of hemorrhagic lesions [19]. To evaluate hemorrhagic damage, the hemorrhagic area in liver tissue was quantitatively measured (Table 4). After 10 days of treatment, the hypoxia (H) group exhibited a significantly larger hemorrhage area (3635.75 ± 136.33 µm²) compared with all other groups ($p \leq 0.05$). In contrast, the H+DEXA, HP1, and HP2 treatment groups showed significant reductions in hemorrhagic area ($p \leq 0.05$), with the HP2 group demonstrating the smallest hemorrhage area (1031.10 ± 17.38 µm²).

Minor hemorrhagic changes observed in the normal control group may be attributable to external factors such as subclinical pathogen exposure, basal immune responses, or mild environmental stressors [38]. The significant reduction in hemorrhagic area observed in the HP2 group (300 mg/kg body weight purslane extract) indicates effective protection against hypoxia-induced vascular damage.

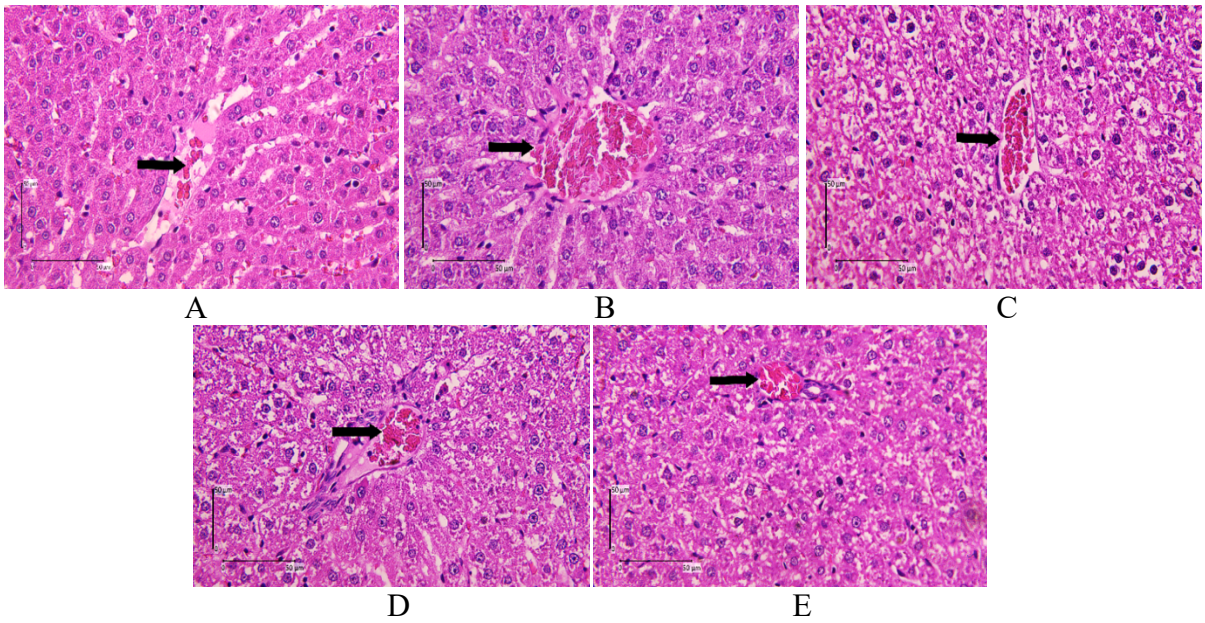

Figure 3. Histopathology of liver tissue showing hemorraghe (black arrows) in male Sprague-Dawley rats (*Rattus norvegicus*). (A) Normal (N); (B) Hypoxia without drug or extract treatment (H); (C) Hypoxia with dexamethasone treatment (H+DEXA); (D) Hypoxia + Portulaca extract 150 mg/kg BW (HP1); (E) Hypoxia + Portulaca extract 300 mg/kg BW (HP2). Stained with Hematoxylin-Eosin (H&E), 400x magnification (40x objective, 10x eyepiece). Scale bar: 50 µm.

Table 4. Measurement of Hemorrhage Area (µm²) in Liver Tissue

Groups	Mean of Hemorrhage Area (µm²) ± SD
N	851.33 ± 10.079[a]
H	3635.75 ± 136.331[d]
H+DEXA	1732.33 ± 70.428[c]
HP1	1668.01 ± 68.646[c]
HP2	1031.10 ± 17.382[b]

Description: *Different superscript letters (a–d) within the same column indicate statistically significant differences by Duncan's post hoc test ($P \leq 0.05$).*

Hemorrhage occurs when severe vascular congestion or inflammation causes blood vessel rupture, permitting the extravasation of red blood cells into the surrounding tissue [39]. This process can be exacerbated by inflammation, which increases endothelial permeability and intravascular pressure, thereby promoting blood extravasation into tissues [40]. The improvement observed following purslane extract administration is likely attributable to its secondary metabolites, particularly flavonoids and tannins. Flavonoids may

help prevent bleeding by promoting vasoconstriction and supporting hemostatic mechanisms, while tannins contribute to rapid blood clot formation and stabilization of damaged vessels [41], [42]. These compounds also exhibit anti-inflammatory and antioxidant properties by reducing proinflammatory cytokine production and oxidative stress, while enhancing the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). In addition, flavonoids may further protect hepatic tissue by inhibiting apoptosis and lipid peroxidation [43]. The observed reduction in hemorrhagic area suggests enhanced stability of sinusoidal vessels under hypoxic conditions. This effect may reflect improved endothelial integrity, thereby reducing susceptibility to vascular rupture.

Conclusion

This study demonstrates that administration of acetone extract of *Portulaca oleracea* at a dose of 300 mg/kg body weight significantly reduced liver necrosis, inflammation, and hemorrhage in hypoxia-induced rats, indicating strong hepatoprotective potential. However, the absence of biochemical markers and molecular pathway analyses limits definitive mechanistic interpretation of these effects. Future studies should incorporate assessments of oxidative stress biomarkers and relevant signaling pathways to further elucidate the underlying mechanisms and to validate the therapeutic potential of *P. oleracea*.

Conflict of interest

The authors declare that no conflict of interest in this study.

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