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Aqueous Portulaca oleracea Leaf Extract Attenuates Methotrexate-Induced Hepatic Glycogen Depletion and Structural Injury in Rats

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Abstract

Methotrexate (MTX), an antifolate drug, is associated with hepatotoxicity. The present study was undertaken to evaluate the hepatoprotective effects of aqueous leaf extract of Portulaca oleracea against MTX-induced biochemical, metabolic and histopathological alterations in rats. Thirty-two male Wistar rats were randomly divided into four groups (n=8): Control, PO400, MTX and MTX+PO400. Extract (400 mg/kg/day, p.o.) was given for 7 days before and 5 days after single injection of MTX (20 mg/kg, i.p.) on Day 8. Animals were assessed on Day 13. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined. Liver injury was assessed using hematoxylin–eosin staining, and glycogen-associated staining was quantified using periodic acid–Schiff (PAS) staining with diastase controls. MTX induced a loss of body weight (−5.6 ± 2.7%) and increased ALT and AST activities to 116.5 ± 15.2 and 137.6 ± 16.3 U/L, respectively. The values were reduced to 69.4 ± 10.7 and 87.9 ± 11.8 U/L for PO400 with reductions of 40.4% and 36.1%, respectively. MTX reduced total PAS-positive area from 20.9 ± 2.1% to 9.2 ± 1.9%. PO400 increased total PAS-positive area to 15.8 ± 2.3%. Similarly, glycogen-associated PAS area was increased from 6.4 ± 1.8% after MTX to 12.8 ± 2.2% after PO400 treatment. Histological examination revealed that PO400 reduced the hepatocellular degeneration, nuclear pyknosis, sinusoidal dilatation, inflammatory infiltration and vascular congestion. These findings point to the therapeutic potential of aqueous *P. oleracea* leaf extract in ameliorating MTX-induced hepatic injury and glycogen depletion, but the underlying molecular mechanisms remain to be elucidated.

Keyword : *Portulaca oleracea; methotrexate; hepatotoxicity; hepatic glycogen; periodic acid–Schiff.*

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INTRODUCTION

Methotrexate (MTX) is a folate antagonist widely employed in oncology and in the treatment of chronic immune-mediated diseases. Despite its established therapeutic value, hepatic toxicity remains clinically and experimentally relevant. MTX-derived intracellular polyglutamates can interfere with hepatic metabolic processes and have been associated with oxidative stress, inflammatory signaling, steatosis, fibrosis, mitochondrial dysfunction, and apoptosis [1].

A single intraperitoneal MTX dose of 20 mg/kg is widely used to reproduce acute hepatic injury in rodents. This model has produced hepatocyte degeneration, hydropic and vacuolar alterations, sinusoidal dilatation, inflammatory-cell infiltration, nuclear pyknosis, vascular abnormalities, elevations in circulating aminotransferases, and reduced hepatic glycogen deposition [2], [3]. More recent studies using comparable experimental exposure have reproduced oxidative, inflammatory, apoptotic, and histopathological abnormalities and demonstrated that these responses can be pharmacologically modified [4]-[8].

The pathological response is not limited to membrane injury as assessed by aminotransferase leakage. MTX can interfere with mitochondrial function and cause cytochrome-c release from hepatocytes [9] suggesting a broader impairment of cellular bioenergetics and survival. Such metabolic disruption is

particularly relevant to hepatic glycogen since the liver is a major glycogen reservoir and central to the maintenance of systemic glucose homeostasis [10].

The periodic acid-Schiff reaction is a well-established technique to visualize carbohydrate-rich material in hepatocytes. Diastase digestion of companion sections helps in interpretation, by eliminating glycogen and leaving diastase resistant PAS-reactive material. PAS staining is not specific for glycogen per se [11]. Reliable demonstration of hepatic glycogen is dependent on standardized fixation and staining conditions, as highlighted by recent experimental studies [12]. Combining total PAS morphometry with PAS-diastase controls therefore offers a stronger histochemical assessment than qualitative PAS staining alone.

Portulaca oleracea L. (purslane; Portulacaceae) is an edible succulent plant with widespread geographical distribution and a long history of nutritional and medicinal use. Contemporary phytochemical reviews describe flavonoids, alkaloids, organic acids, terpenoids, sterols, polysaccharides, fatty acids, vitamins, and other biologically active constituents [13]–[15]. Experimental evidence indicates antioxidant, anti-inflammatory, immunomodulatory, metabolic, and tissue-protective activities, although the chemical composition and biological potency vary substantially with plant material and extraction procedure [13]–[16].

A growing experimental literature also supports hepatic effects of *P. oleracea*. An aqueous leaf preparation administered at 400 mg/kg improved biochemical and histopathological disturbances in aluminium-exposed male Wistar rats [17]. Other preparations have attenuated carbon tetrachloride-induced hepatic injury [18], cholestatic liver injury [19], and acute alcohol-induced hepatic damage [20]. Aqueous *P. oleracea* has also demonstrated hepatoprotective activity in combination with lycopene [21], while additional extract studies have reported reductions in biochemical evidence of chemically induced hepatic injury [22]. The present study was undertaken to evaluate the hepatoprotective effects of aqueous leaf extract of *Portulaca oleracea* against MTX-induced biochemical, metabolic and histopathological alterations in rats.

MATERIALS AND METHODS

Plant Material and Identification

The fresh leaves of Portulaca oleracea L. were collected from Tikrit area, Salah ad-Din Governorate, Iraq in March 2026. The plant material was identified by an expert plant taxonomist at the Department of Biology, College of Education, Tikrit University.
The leaves were thoroughly washed with distilled water, dried at room temperature in the absence of direct sunlight and finely powdered using a mechanical grinder. The powdered material was stored in airtight containers until extraction.

Preparation of Aqueous *Portulaca oleracea* Leaf Extract

Fresh leaves of Portulaca oleracea L. were collected on June 20, 2026, in Iraq / Nineveh. The plant was identified by the Department of Biology, College of Education, Tikrit University. The roots were washed with distilled water, dried in the shade at room temperature, and then powdered.

Extraction in aqueous medium was adapted from Derouiche et al. [17]. Dried powdered leaves (5 g) were added to 50 mL of distilled water and held at 50 °C for 2 h. The mixture was allowed to stand at room temperature for 24 h and filtered on filter paper.

The filtrate was evaporated using a rotary vacuum evaporator. The extract was freshly re-dissolved in distilled water for each dose and kept in a glass container in a refrigerator when not in use. The oral dose was 400 mg/kg body weight/day. This dose was extrapolated from previous studies with the use of 400 mg/kg leaf aqueous extract of P. oleracea in male Wistar rats [17]. The crude extract was obtained and calculated as a yield (%) from the original plant. The extraction yield was 40%.

Experimental Animals

Rats were maintained throughout the experimental period on controlled 12 h light (07:00 h):12 h darkness with free access to commercial standard laboratory rodent feed and water. All experimental groups were maintained under identical nutritional and environmental conditions. Livers were sampled on day 13 in the morning (09:00-11:00) to account for circadian changes in hepatic glycogen metabolism. Animals were not fasted before tissue collection to prevent confounding hepatic glycogen substrate stores with fasting. Terminal sampling times were staggered among treatments to prevent systematic differences in time of collection. Liver samples were harvested and treated identically across groups to minimize variability for later histology and PAS staining.

The animal experiment was reported according to the ARRIVE 2.0 recommendations [23]. All procedures were carried out in accordance with the relevant national and institutional guidelines for the care and use of laboratory animals (**Ref: UM. VET. 2026.168 Date: 15/4/2026).**

Randomization, Anesthesia, and Euthanasia

Following a week of acclimatization, 32 adult male Wistar rats were allocated to four treatment groups (n = 8 per group) via randomly generated group assignment (Microsoft Excel) using a randomization sequence on a computer. Each animal was assigned a unique identification number, and random numbers were generated to assign groups with equal group sizes. The experimental unit was the individual animal.

At experiment termination (day 13), animals were anesthetized by inhalation of isoflurane in oxygen with a calibrated vaporizer. Anesthesia was performed with 4-5% isoflurane, maintained at titrated doses of ~1-2% isoflurane depending on degrees of anesthesia induced. Depth of anesthesia was monitored by loss of the pedal withdrawal reflex and respiration. Terminal blood samples were collected by cardiac puncture when a deep surgical anesthetic plane had been achieved. Exsanguination and bilateral thoracotomy of the animals while still deep under anesthesia were then also performed. The absence of spontaneous respiration, heart beat, and response to any reflex movement was ascertained before collection of the tissues. Liver tissues were quickly removed for biochemical, histochemical, and histological studies.

Experimental Design

Following acclimatization, rats were randomly allocated into four groups of eight animals each.

Table 1. Experimental groups and treatment schedule

Group	Treatment
Control	Distilled water orally once daily and physiological saline i.p. on Day 8
PO400	Aqueous *P. oleracea* leaf extract, 400 mg/kg/day orally, and saline i.p. on Day 8
MTX	Distilled water orally and a single 20 mg/kg MTX dose i.p. on Day 8
MTX + PO400	*P. oleracea* extract, 400 mg/kg/day orally, and a single 20 mg/kg MTX dose i.p. on Day 8

Abbreviations: i.p., intraperitoneal; MTX, methotrexate; PO400, aqueous *Portulaca oleracea* leaf extract administered at 400 mg/kg/day.

A combined preventive and early post-exposure treatment protocol was used. Extract or vehicle was administered on Days 1–7. MTX or physiological saline was administered on Day 8, and oral extract/vehicle administration continued through Day 12. Blood and hepatic tissue were collected on Day 13.

The MTX dose was selected from established hepatotoxicity models using a single 20 mg/kg intraperitoneal exposure [2], [3].

Hence, the present study evaluated the overall hepatoprotective effect of single pre- and early postexposure of aqueous P. oleracea leaf extract in MTX-induced liver injury. The oral dose of 400 mg/kg/day was selected on the basis of past experimental evidence for the beneficial biochemical and

histopathological effects of aqueous P. oleracea leaf extract in rats. The pretreatment period of 7 days (Days 1–7) was chosen to provide sufficient exposure to the extract before MTX treatment, but while still experiencing it in conjunction with it during the first window of postexposure (Days 8–12), which could indicate any possible interactions occurring as injury sets in and develops in the liver. Study termination (Day 13) allowed assessment of biochemical and histopathological outcomes after single i.p. administration of MTX (20 mg/kg) on Day 8. This design was to know the overall protective impact of continuous dosing of extract during MTX exposure rather than differentiating preventive and therapeutic effects.

Body-Weight Assessment and Clinical Observation

Body weight was recorded before initiation of treatment, immediately before MTX exposure, and before euthanasia. Extract dosing was adjusted according to individual body weight.

Animals were observed daily for general activity, grooming, posture, apparent food and water consumption, and visible signs of toxicity or distress.

Methotrexate Administration

Methotrexate was administered as a single intraperitoneal dose of 20 mg/kg body weight on Day 8.

Blood and Liver Collection

Rats were euthanized on Day 13 (~5 days post-MTX administration) with the institutionally approved anesthesia/euthanasia procedure. Blood was collected into plain tubes and allowed to clot before serum was separated by centrifugation. Serum was frozen until biochemical analysis performed. The liver was excised immediately, rinsed carefully with cold 0.9% NaCl, blotted dry, and processed for histological and histochemical examination.

Measurement of Serum ALT and AST

Serum activities of ALT and AST were determined by the use of commercially available diagnostic reagents according to the instructions of the manufacturers. The results were expressed as units per liter (U/L).

Processing of Tissues

Hepatic specimens were fixed in 10% neutral buffered formalin, dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin. Rotary microtome was used to section paraffin blocks at 5 µm. Consecutive sections were stained with H&E to assess general hepatic morphology and PAS to visualize carbohydrate-rich hepatocellular material. The glycogen-associated fraction of PAS reactivity was differentiated by a complementary procedure, diastase digestion of PAS [11], [12].

Histopathological Assessment

To evaluate methotrexate-induced structural changes, hematoxylin and eosin-stained liver sections (5 µm) were examined by light microscopy (×400). To systematize and standardize the analysis, two (50 µm apart) noncontiguous sections and five microscopic fields per section (10 microscopic fields per specimen) were evaluated. Systematic random sampling was used to select regions representative of periportal and centrilobular hepatic zones and to avoid folds, processing artifacts, and regions of poor preservation.

Histological changes were evaluated as hepatocyte degeneration, nuclear pyknosis, sinusoidal diameter, mononuclear inflammatory cell infiltration, and vascular congestion/hemorrhage. Each parameter was graded on a 4-point semiquantitative scale (0, absent; 1, mild; 2, moderate; 3, severe).

Hepatocyte degeneration (vs. cytoplasmic vacuolation) was graded mild, moderate, or severe according to the proportion of hepatocytes exhibiting cellular swelling, hydropic changes, and/or cytoplasmic vacuolation ≤25%, 26–50%, and >50%, respectively. For nuclear pyknosis, the grade, based on the

proportion of hepatocytes exhibiting condensed hyperchromatic nuclei, was scored. Sinusoidal dilatation was graded as mild if focal, limited, and/or mild, moderate if multifocal and obvious, and severe if diffuse, with obvious sinusoidal dilatation and architectural distortion. Mononuclear inflammatory-cell infiltrates were graded as mild when focal inflammatory cells or small focal clusters were present, moderate when well-defined inflammatory aggregates were present, and severe when dense, diffuse, or confluent inflammatory infiltrates were present. Vascular congestion/hemorrhage was graded as mild (focal vascular congestion), moderate (multifocal vascular congestion and focal hemorrhage), or severe (mark vascular congestions or hemorrhagic changes).

PAS Histochemistry and Quantitative Glycogen Assessment

Periodic Acid-Schiff-Diastase Histochemistry of Glycogen in Liver A series of 5-µm sections on paraffin-embedded liver of each experimental subject was cut. One section was stained with PAS, and the serial section was incubated with 1% aqueous α-amylase (diastase) (pH 5.3; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China; cat. no. G1284) at room temperature (20–25 °C) for 30 min before the same PAS staining procedure. The undigested sections served as digestion controls, and a section from a liver rich in glycogen served as a control for enzymatic activity.

Micrographs were obtained at ×400 magnification on a light microscope equipped with a digital imaging system under the same lighting and exposure conditions. Quantitative image analysis was performed with the Color Deconvolution plugin in Fiji/ImageJ2 (National Institutes of Health, Bethesda, MD, USA). Hepatic tissue (representative areas of periportal and centrilobular hepatic tissue) was probed in a systematic random sampling method from 5 non-overlapping microscopic fields per specimen. Fields only included analyzable hepatic parenchyma and omitted large vascular lumina, major portal tracts, tissue folds, sectioning artifacts, and areas that were poorly stained.

H-PAS stain-separation isolated the intense magenta PAS reaction product from hematoxylin counterstaining. The single PAS channel was then converted to 8-bit gray-scale images, and calibration images were selected to set an initial threshold, calculated by the automatic threshold method of Otsu. Based on visual verification with original photomicrographs, the cut-off was established and applied to all images for the avoidance of inter observer-dependent bias. The PAS-positive percentage was calculated as the number of positively segmented pixels over the number of total parenchyma pixels that could be detected as positive for the region of interest (%).

Glycogen-associated staining was determined by comparison to serial diastase-digested sections. Loss or reduction of PAS positivity upon enzymatic digestion was interpreted as glycogen-associated staining, while persistent staining was interpreted as a diastase-resistant PAS-positive substance. Diastase-sensitive fraction was expressed as the percentage difference between PAS-positive areas of untreated and digested sections of structurally identical areas of liver parenchyma.

$$\text{Glycogen-associated PAS area (\%)} = \text{total PAS-positive area (\%)} - \text{PAS-D residual area (\%)}$$

STATISTICAL ANALYSIS

Statistical analyzes were conducted using IBM SPSS Statistics Software (version 32; IBM Corp., Armonk, NY, USA). Based on the result type, data from the 4 experimental groups (n = 8) were analyzed. Shapiro–Wilk test and Q–Q plot analysis was used to check the normality of continuous data, while homogeneity of variance was checked using Levene's test. When the assumptions of normality and homogeneity of variances were met, one-way ANOVA with Tukey's post hoc test was applied to compare serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). When variances were not equal, Welch's ANOVA followed by Games-Howell post hoc analyzes was used. If the results did not meet the assumptions of parametric analysis, Kruskal–Wallis test with an appropriate Dunn's multiple-comparison test was applied. Normally distributed continuous data were presented as mean ± SD, and nonparametric data as median and IQR. All tests were considered statistically significant at the 0.05 probability level.

RESULTS AND DISCUSSION

Body weights were similar among groups at baseline. The overall ANOVA showed no significant baseline group difference (P = 0.993). By the end of the experiment, the Control and PO400 groups had gained 6.5 ± 2.2% and 6.6 ± 2.0%, respectively, of their initial body weight. Conversely, MTX-treated animals exhibited a net decrease of 5.6 ± 2.7 %. Treatment of MTX-exposed rats with PO400 shifted body-weight change toward recovery (mean change in body weight, 1.9 ± 2.5%). Final body weight of Control group was 241.6 ± 13.1 g, of PO400 rats was 243.2 ± 12.8 g, of MTX was 214.7 ± 14.6 g and of MTX + PO400 animals was 230.8 ± 13.5 g.
The hepatic enzyme response was more marked. The ALT increased from 42.3 ± 5.6 U/L in controls to 116.5 ± 15.2 U/L after MTX exposure, approximately 175% increase relative to Control. ALT was reduced to 69.4 ± 10.7 U/L with co-treatment of PO400, about 40.4% below the mean value in the MTX group.

The same pattern was followed by AST. MTX raised AST from 65.7 ± 7.8 U/L to 137.6 ± 16.3 U/L. In MTX + PO400 rats the AST was decreased to 87.9 ± 11.8 U/L, which is about 36.1% lower than after MTX alone.

one-way ANOVA revealed significant overall treatment effects on final body weight, body-weight change, ALT, and AST. Tukey comparisons revealed that ALT and AST were still elevated in MTX + PO400 animals compared with untreated controls, indicating substantial but incomplete biochemical recovery.

Table 2. Body-weight and serum aminotransferase findings

Parameter	Control (n=8)	PO400 (n=8)	MTX (n=8)	MTX + PO400 (n=8)	Overall ANOVA
Initial body weight, g	226.8 ± 12.4^{a}	228.1 ± 11.7^{a}	227.5 ± 13.1^{a}	226.4 ± 12.0^{a}	$P = 0.993$
Final body weight, g	241.6 ± 13.1^{a}	243.2 ± 12.8^{a}	214.7 ± 14.6^{b}	230.8 ± 13.5^{ab}	$P < 0.001$
Body-weight change, %	+6.5 ± 2.2^{a}	+6.6 ± 2.0^{a}	−5.6 ± 2.7^{c}	+1.9 ± 2.5^{b}	$P < 0.001$
ALT, U/L	42.3 ± 5.6^{a}	40.8 ± 5.1^{a}	116.5 ± 15.2^{c}	69.4 ± 10.7^{b}	$P < 0.001$
AST, U/L	65.7 ± 7.8^{a}	64.1 ± 7.4^{a}	137.6 ± 16.3^{c}	87.9 ± 11.8^{b}	$P < 0.001$

ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; MTX, methotrexate; PO400, aqueous Portulaca oleracea leaf extract 400 mg/kg/day; SD, standard deviation. Values are expressed as mean ± SD. Overall ANOVA and Tukey comparisons were based on means, SDs, and equal group sizes reported. Values in the same row with different superscript letters are different (P < 0.05) as determined by Tukey.

Biochemical, metabolic and histological outcomes were consistent within the experimental pattern. Acute MTX exposure was associated with loss of body mass, marked elevations of aminotransferases, extensive depletion of hepatic PAS-detectable glycogen, and marked structural injury of the liver. Oral P. oleracea extract modulated these responses toward the control condition. However, recovery was incomplete, especially for glycogen associated PAS area and several structural lesions, supporting the attenuation rather than full reversal of MTX toxicity.

The MTX regimen generated a phenotype consistent with previous experimental work. Hepatocyte degeneration, vacuolation, pyknosis, sinusoidal dilatation and decreased glycogen accumulation was observed by Akman et al. after a dose of MTX 20 mg/kg [2]. Similarly, Akbulut et al. proved the existence of inflammatory infiltration, hydropic degeneration, vascular abnormalities, oxidative injury and impaired hepatic glycogen deposition [3]. Galangin, empagliflozin, nobiletin, crocin and Andrographis paniculata have subsequently been tested in MTX hepatotoxicity models and have recapitulated the characteristic combination of aminotransferase elevation, oxidative disturbance, inflammation, apoptosis and histopathological injury [4]–[8]. Hence, the present results are consistent with the known biological response to acute systemic MTX exposure.

The response of the aminotransferase was considerable. ALT increased by about 175% relative to Control, while AST increased by about 109%. These changes were associated with obvious morphological hepatocellular damage rather than isolated biochemical abnormalities. PO400 reduced

MTX-induced elevations of ALT and AST by approximately 40.4% and 36.1%, respectively. The extract-alone group was still quite similar to Control, providing no evidence for appreciable hepatocellular injury from the selected dose in either of these two biochemical markers.

Direct comparison should be made with caution since the composition of the extracts and the models are different, but previous work offers a plausible precedent for hepatic protection by P. oleracea. Derouiche et al. [17] used the same dose (400 mg/kg) of aqueous-leaf extract and showed improvement of biochemical and histopathological abnormalities in aluminium-exposed rats. Eidi et al. showed protection against carbon tetrachloride-associated hepatic injury [18]. Moslemi et al. reported lower aminotransferases and inflammatory injury after treatment with P. oleracea in experimental cholestasis [19]. Likewise, Qiao et al. reported improved aminotransferases, antioxidant status, inflammatory markers and liver structure in alcohol-exposed rats [20]. Aqueous preparations in combination with lycopene [21] and other purslane extract fractions have also been reported to exhibit hepatoprotective activity in carbon tetrachloride models [22].

MTX produced pronounced loss of hepatic PAS-positive material. Total PAS-positive area decreased from 20.9 ± 2.1% in the Control group and 21.4 ± 2.0% in PO400 rats to 9.2 ± 1.9% following MTX, representing a reduction of approximately 56.0% relative to Control. Administration of PO400 to MTX-treated animals increased total PAS-positive area to 15.8 ± 2.3%, approximately 71.7% higher than in the MTX group. The value nevertheless remained significantly below that of the untreated groups in the comparison. PAS-D residual staining showed little variation among groups, with mean values between 2.8% and 3.1%, and no significant overall difference. The stability of this diastase-resistant component indicates that the major treatment-related alteration occurred predominantly in diastase-sensitive PAS material. Glycogen-associated PAS area was 17.8 ± 2.0% in Control rats and 18.4 ± 1.9% in PO400 animals. MTX reduced this value to 6.4 ± 1.8%, an approximately 64.0% decline relative to Control. In MTX + PO400 rats, glycogen-associated PAS area increased to 12.8 ± 2.2%, exactly twice the mean recorded after MTX alone. Despite this substantial improvement, glycogen-associated staining remained below control levels.

Table 3. Quantitative hepatic PAS and PAS-diastase morphometry

Variable	Control (n=8)	PO400 (n=8)	MTX (n=8)	MTX + PO400 (n=8)	Overall ANOVA
PAS-positive area, %	20.9 ± 2.1[a]	21.4 ± 2.0[a]	9.2 ± 1.9[c]	15.8 ± 2.3[b]	$P < 0.001$
PAS-D residual area, %	3.1 ± 0.8[a]	3.0 ± 0.7[a]	2.8 ± 0.7[a]	3.0 ± 0.8[a]	$P = 0.879$
Glycogen-associated PAS area, %	17.8 ± 2.0[a]	18.4 ± 1.9[a]	6.4 ± 1.8[c]	12.8 ± 2.2[b]	$P < 0.001$

ANOVA, analysis of variance; MTX, methotrexate; PAS, periodic acid–Schiff; PAS-D, periodic acid–Schiff staining after diastase digestion; PO400, aqueous *Portulaca oleracea* leaf extract at 400 mg/kg/day; SD, standard deviation. Values are mean ± SD. Glycogen-associated PAS area was calculated as total PAS-positive area minus PAS-D residual area. Values within a row with different superscript letters differed at $P < 0.05$ in the Tukey analysis.

The hepatic lobular architecture was preserved in the Control group. Hepatocyte plates were regularly arranged around central veins and hepatic sinusoids were rather narrow and evenly distributed. There was no significant hepatocellular degeneration, inflammatory-cell infiltration, vascular congestion, or hemorrhage. Morphology of liver sections from the PO400-alone group was similar to the Control group. The hepatocytes were seen in their regular arrangement. No consistent evidence of treatment-related structural liver injury was noted.

MTX administration led to evident liver damage. The principal lesions were degeneration of hepatocytes with cytoplasmic vacuolar or hydropic change, pyknotic nuclei, sinusoidal dilatation, infiltration of mononuclear inflammatory cells and vascular congestion/hemorrhage. The median scores for hepatocyte degeneration and inflammatory infiltration were both 3 (IQR, 2 to 3), and hemorrhage/congestion was 3 (3 to 3). Liver injury was less in the MTX + PO400 group. Degeneration and vacuolation of hepatocytes were decreased, sinusoidal dilatation was less prominent, accumulation

of inflammatory cells was decreased and congestion/hemorrhage was much less severe. Residual abnormality was observed, indicating partial rather than complete structural restoration.

Table 4. Semiquantitative hepatic histopathological scores

Histopathological feature	Control	PO400	MTX	MTX + PO400
Hepatocyte degeneration	0 (0–0) [a]	0 (0–0) [a]	3 (2–3) [c]	1.5 (1–2) [b]
Pyknotic nuclei	0 (0–0) [a]	0 (0–0) [a]	1 (1–1) [b]	0.5 (0–1) [b]
Sinusoidal dilatation	0 (0–0) [a]	0 (0–1) [b]	2 (1–3) [c]	1 (0–1) [b]
Mononuclear-cell infiltration	0 (0–0) [a]	0 (0–0) [a]	3 (2–3) [c]	1.5 (1–2) [b]
Hemorrhage/congestion	0 (0–0) [a]	0 (0–0) [a]	3 (3–3) [c]	0.5 (0–1) [b]

Values are expressed as median (interquartile range), with eight animals per group. Histopathological alterations were scored from 0 to 3, corresponding to absent, mild, moderate, and severe changes, respectively. Overall group differences were assessed using the Kruskal–Wallis H test, followed by Dunn's post-hoc multiple-comparison test with Holm adjustment where appropriate. Different superscript letters within the same row indicate statistically significant pairwise differences (adjusted $p < 0.05$). Groups sharing a common letter do not differ significantly.

Quantitative morphometry was strongly supported by PAS staining results. Hepatocytes of the Control group showed intense and relatively homogenous magenta cytoplasmic staining corresponding to the abundant hepatic glycogen. A similar distribution and intensity of PAS-positive material was seen in PO400-alone animals.

Following MTX administration, hepatocellular PAS reactivity became markedly weaker, patchier, and more heterogeneous. In contrast, the MTX + PO400 group showed more extensive and more intense PAS positivity than the MTX group, with a more homogeneous distribution of PAS-reactive material. The staining remained below that observed in Control and PO400 animals, consistent with incomplete restoration of hepatic glycogen.

The hepatic glycogen response contains additional information over and above the conventional serum enzymes. The liver is a major site for glycogen synthesis and storage, and hepatocellular glycogen is regulated dynamically by substrate availability, hormonal cues, cellular energy status, glycogenesis and glycogenolysis [10]. MTX reduced the total PAS-positive area by about 56% and the estimated glycogen-associated component by about 64%. This finding is very similar to the decreased hepatic glycogen described histochemically in earlier experiments with MTX [2], [3].

The addition of PAS-diastase greatly strengthens this interpretation. PAS detects several classes of carbohydrate-containing substances and therefore cannot be considered exclusively glycogen specific on its own. Diastase digestion is routinely used to selectively remove glycogen and differentiate it from the other PAS-positive constituents [11].

From the present measurements a direct mechanism for this effect cannot be established. Mitochondrial dysfunction and cytochrome-c release in hepatocytes have been associated with MTX [9]. Lipid peroxidation, antioxidant depletion, inflammatory signaling and apoptotic activation are typical features of experimental MTX hepatotoxicity [1], [4]–[8].

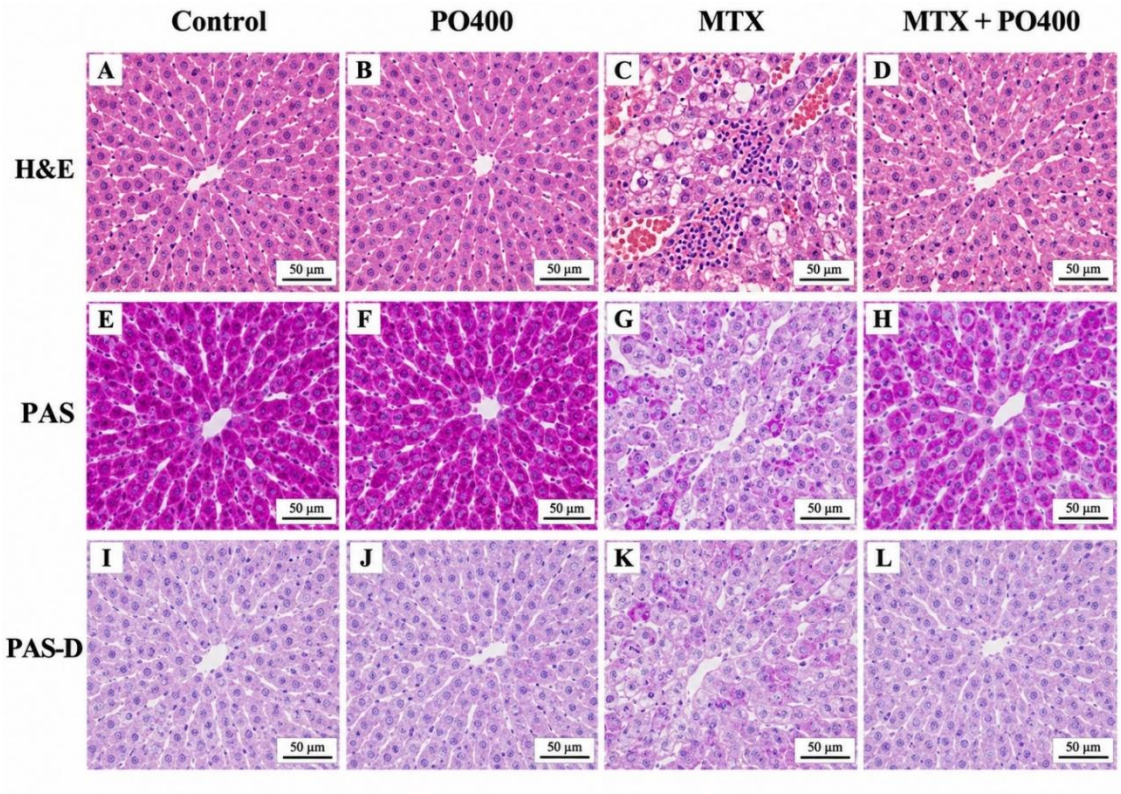


Figure 1. Histopathological and histochemical appearance of hepatic tissue in the experimental groups, Panels (A-D) H & E, (E-H) PAS, and (I-L) PAS-D photomicrographs of liver sections of Control, PO400, MTX, and MTX + PO400 rats. (A) Control liver showing an intact hepatic architecture with organized hepatocyte cords and normal sinusoids. (B) PO400 group showing normal liver architecture with no derangements. (C) Hepatocellular degeneration comprising cytoplasmic vacuolation and nuclear pyknosis (arrows), sinusoid dilation and inflammatory cell infiltration, and vascular congestion in MTX group. (D) MTX + PO400 group showing restoration of the hepatic architecture with only a scant appearance of meager hepatocellular change. (E and F) PAS-positive hepatocytes in control and PO400 groups. (G) MTX group with markedly reduced and variable PAS reactivity. (H) MTX + PO40 group demonstrates almost restored PAS positive staining of hepatocytes. (I-L) Diastase-digested sections showing loss of glycogen-dependent PAS-reactivity, but diastase-resistant staining elements. Images representative of study animals (n = 8 per group) selected on the basis of standard histologic and staining criteria. Original magnification, ×400; bars, 50 µm.

CONCLUSION

The Portulaca oleracea leaf aqueous extract (400 mg/kg/day) significantly protected from methotrexate-induced hepatotoxicity as indicated by decreased serum ALT and AST levels, better hepatic histological architectural features, and partial reactivity of hepatocytes to PAS. These findings suggest the potential hepatoprotective activity of the extract in the pre- and post-exposure treatment protocol studied. However, these results do not speak to the dose response mechanism, optimal duration of dosing, or duration of liver protection, because we studied only 1 dose and 1 time point. Additional dose-ranging, longitudinal, and mechanistic studies will be necessary to determine the magnitude, time course, and biological basis of such effects.

Several limitations of the current study need to be addressed. First, biochemical assessment of liver injury was limited to serum ALT and AST without inclusion of alkaline phosphatase, bilirubin, and hepatic synthetic function. Secondly, the oxidative stress biomarkers and intrinsic antioxidant defense mechanisms were not evaluated directly, hindering conclusive determinations about the role of oxidative processes in the hepatoprotective properties of Portulaca oleracea. Third, PAS staining for glycogen was not accompanied by biochemical measurement of hepatic glycogen content. Thus, such findings should be viewed as 'Cautionary' evidence of hepatoprotection or increased carbohydrate-related staining rather than specificity at the molecular or biochemical level for these mechanisms.

However, other questions remain for further biochemical demonstration, glycogen quantification, and mechanistic exploration.

The other limitation is the pre- and post-exposure dosing schedule. The contribution of each could not be distinguished, because the study did not have separate groups for prophylactic-only and therapeutic-only treatments. Thus, these results reflect the effects of Portulaca oleracea use after MTX induction assuming but not after liver damage.

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المستخلص المائي لأوراق نبات الرجلة (Portulaca oleracea) يخفف من استنزاف الجليكوجين الكبدي والتلف البنيوي الناجم عن عقار الميثوتريكسيت في الجرذان

ملخص

يرتبط عقار الميثوتريكسيت- (MTX) وهو أحد مضادات حمض الفوليك- بحدوث سمية كبدية. هدفت هذه الدراسة إلى تقييم التأثيرات الوقائية للمستخلص المائي لأوراق نبات الرجلة (Portulaca oleracea) ضد التغيرات الكيميائية الحيوية والأيضية والنسيجية المرضية التي يسببها الميثوتريكسيت في الجرذان. قُسّمت 32 جرذًا ذكراً من سلالة "ويستار (Wistar) "عشوائياً إلى أربع مجموعات (n=8): مجموعة ضابطة، ومجموعة عولجت بالرجلة(PO400) ، ومجموعة عولجت بالميثوتريكسيت(MTX) ، ومجموعة عولجت بالميثوتريكسيت مع الرجلة .(MTX+PO400) أُعطي المستخلص (بجرعة 400 ملجم/كجم/يوم، عن طريق الفم) لمدة 7 أيام قبل، و5 أيام بعد، حقنة واحدة من الميثوتريكسيت (بجرعة 20 ملجم/كجم، عن طريق الحقن داخل الصفاق) في اليوم الثامن. خضعت الحيوانات للتقييم في اليوم الثالث عشر. تم قياس مستويات إنزيمي "ألانين أمينو ترانسفيراز (ALT) "و"أسبارتات أمينو ترانسفيراز (AST) " في المصل. كما تم تقييم التلف الكبدي باستخدام صبغة "الهيماتوكسيلين-إيوسين"، بينما قيست كمية الجليكوجين باستخدام صبغة "حمض البيريوديك-شيف (PAS) "مع إجراء اختبارات ضابطة باستخدام إنزيم "الدياستاز". تسبب الميثوتريكسيت في فقدان وزن الجسم (-5.6 ± 2.7%)، ورفع نشاط إنزيمي ALT و ASTإلى 116.5 ± 15.2 و137.6 ± 16.3 وحدة/لتر على التوالي. ومع استخدام مستخلص الرجلة(PO400) ، انخفضت هذه القيم إلى 69.4 ± 10.7 و87.9 ± 11.8 وحدة/لتر، بنسب انخفاض بلغت 40.4% و36.1% على التوالي. كما أدى الميثوتريكسيت إلى تقليل المساحة الكلية الموجبة لصبغة PAS من 20.9 ± 2.1% إلى 9.2 ± 1.9%، بينما ساهم مستخلص الرجلة (PO400) في زيادة هذه المساحة إلى 15.8 ± 2.3%. وبالمثل، ارتفعت المساحة المرتبطة بالجليكوجين (الموجبة لصبغة PAS من 6.4 ± 1.8% بعد التعرض للميثوتريكسيت إلى 12.8 ± 2.2% بعد المعالجة بمستخلص الرجلة .(PO400) أظهر الفحص النسيجي أن مستخلص (PO400) *P. oleracea* قلل من تنكس الخلايا الكبدية، وتكثف النوى (pyknosis)، وتوسع الجيوب الكبدية، والارتشاح الالتهابي، والاحتقان الوعائي. تشير هذه النتائج إلى الإمكانات العلاجية للمستخلص المائي لأوراق نبات *P. oleracea* في تخفيف إصابة الكبد ونضوب الجليكوجين الناجمين عن عقار الميثوتريكسيت(MTX) ، إلا أن الآليات الجزيئية الكامنة وراء ذلك لا تزال بحاجة إلى توضيح.

الكلمات المفتاحية: Portulaca oleracea ؛ ميثوتريكسيت؛ السمية الكبدية؛ جليكوجين الكبد؛ صبغة حمض البيريوديك-شيف (PAS).