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Purslane Seed Extract Mitigates Thioacetamide-induced Acute Kidney Injury in Rats by Suppressing Renal Necroptosis and Inflammation

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ABSTRACT

Background: Acute kidney injury (AKI), a global public health challenge with limited treatment options, involves necroptosis and inflammation as key pathological mechanisms. Purslane seed extract (PSE), known for its antioxidant and anti-inflammatory properties, may offer therapeutic potential, but its mechanisms in AKI remain unexplored. **Methods:** A rat model of thioacetamide (TAA)-induced AKI was used to evaluate PSE's renoprotective effects. Rats were pretreated with PSE (400 mg/kg) for 15 days before TAA single dose administration (300 mg/kg). Serum biomarkers (creatinine, BUN, KIM1, NGAL, TNF-α) and renal tissue markers (RIPK1, RIPK3, MLKL, ZBP1, HMGB1) were analyzed. Histopathological examination of kidney tissues was also performed. **Results:** PSE pretreatment restored renal function by significantly reducing serum creatinine and BUN. PSE attenuated tubular injury, lowering KIM1 and NGAL levels and inhibited necroptosis, via suppressing renal levels of RIPK1, RIPK3, and MLKL. It also reduced renal ZBP1 concentration, indicating blockade of non-canonical necroptosis and diminished inflammation through decreasing TNF-α and HMGB1 levels. PSE pretreatment also managed to alleviate the histopathological changes caused by AKI. **Conclusion:** PSE protects against TAA-induced AKI by dual inhibition of RIPK1-dependent and ZBP1-dependent necroptosis pathways, coupled with anti-inflammatory effects. These findings highlight PSE as a promising multi-targeted natural therapy for AKI, warranting further clinical exploration.

Keywords: *Acute kidney injury; Purslane seed extract; Necroptosis; ZBP1; RIPK1/MLKL; Inflammation.*

INTRODUCTION

Acute kidney injury (AKI), which is associated with significant morbidity and death, is defined by a sharp reduction in and/or loss of renal function. AKI's annual incidence has been rising [1]. Over 50% of patients in the intensive care unit and approximately 10% to 15% of hospitalized patients develop AKI [2].

Thioacetamide (TAA) is a well-known nephrotoxicant[3]. TAA-induced changes in trace elements and structural kidney damage resulted in collagen deposition in the renal medulla and fibrin in the renal tubules. TAA also accelerates cell death in the proximal renal tubules' terminal portion. TAA administration impairs renal function, resulting in severe tubular epithelial cell death and glomerular congestion. TAA also demonstrated severe renal tissue infiltration of inflammatory cells, degeneration, sclerosis, and necrosis of the glomeruli, interstitial fibrosis, dilated tubules with necrotic tubular cells, and epithelial shedding [4].

The main focus of AKI management is the supportive care, such as modifying volume hemodynamic status and avoiding nephrotoxins. Other than renal replacement therapy, there are currently no medical or surgical options available to prevent or treat AKI. Since AKI is a worldwide public health issue, it is imperative to find new therapeutic targets and medications to prevent and treat it [2].

Although the treatment of kidney diseases has been advanced significantly, there are still few effective medications available. The search for novel natural medicines, especially those made from plants, has been prompted by the high expense of pharmaceuticals and their severe side effects. Because of their anti-inflammatory and antioxidant qualities, which can reduce toxin-induced nephrotoxicity and prevent renal tissue damage, research on natural treatments for the prevention and treatment of renal diseases has grown in popularity [5].

Portulaca oleracea, also known as purslane, is a nutrient-dense vegetable that has been used since the pharaohs' time according to the Egyptian texts [6]. Its traditional use in Chinese medicine to promote health is thought to be due to its effective active ingredients. Omega-3 fatty acids and antioxidants are abundant in *Portulaca oleracea* [7]. Purslane is rich in bioactive compounds, such as phenolic acids, flavonoids, alkaloids, and vitamins. It has renoprotective effects against oxidative stress, inflammation, infection, drugs, and toxins [8].

In recent decades, a number of regulated cell death (RCD) mechanisms have been identified, including apoptosis, ferroptosis, pyroptosis and necroptosis [9]. Necroptosis is a lytic form of RCD that is rely on receptor-interacting protein kinase 1 and 3 (RIPK1/RIPK3) and mixed lineage kinase domain like pseudokinase (MLKL) nevertheless independent of caspases [10,11]. Numerous investigations have implemented tumor necrosis factor-α (TNF-α) to trigger necroptosis in order to recognize the signaling pathway that underlies this type of RCD [12]. Additionally, Z-DNA binding protein 1 (ZBP1), a sensor for endogenous Z-nucleic acids, stimulates necroptosis by activating RIPK3 [13]. Several inflammatory disorders including AKI has been linked to necroptosis, as a lytic form of cell death that releases various inflammatory cellular components known as damage-associated molecular patterns (DAMPs) for example, the high-mobility group box 1 (HMGB1) [14,15]. Necroptosis is therefore a key therapeutic target for a variety of inflammatory illnesses.

Although some researches have examined purslane's chemical composition and potential health benefits, its impact on kidney function remains poorly understood. This study aimed to investigate how purslane seed extract (PSE) may protect against TAA-induced AKI, with a focus on underlying mechanisms and therapeutic potential.

MATERIAL AND METHODS

Materials

Thioacetamide (TAA) was obtained from Sigma–Aldrich Chemicals Co. (St. Louis, MO, USA). As a common vegetable, purslane was purchased from Al-Naqiti Herbs Company, Mansoura, Egypt. Each assay included a description of the kit/reagent supplier. All further chemicals were of high analytical quality.

Methods

Plant material and extract preparation

The extract was prepared at NAWAH Scientific Labs, Al-Mokattam, Cairo, Egypt. The plant stalks, stones, and seeds were cleaned manually and dried. The plant seeds were subsequently subjected to direct sunlight. Then, the purslane seeds were oven dried for 12 h at 50°C, and the powder was made with a grinder. The prepared powder with a particle size of 0.5–1 mm was kept in a vacuum dryer until use. Ground samples of 3125 g were mixed with 6 L of methanol and then homogenized for 15 min via a T 50 digital Ultra-Turrax® (IKA Labortechnik, Germany). The above procedure was repeated a second time. Later, the extract was filtered and collected and then dried under vacuum at 40°C, producing a brown residue weighing 138.125 g which is then stored at -20 °C for further use. The extraction process was performed according to [16], with some modifications.

Animals

Adult male Wistar albino rats, weighing between 130 g and 170 g, were obtained from the animal house of the National Research Center, Cairo, Egypt. Rats were maintained in an environmentally controlled room with a relative humidity of 35 to 75%, a 12-hour light/dark cycle, and unrestricted access to food and water. Prior to the experiment, rats were given a week to get used to the above conditions. The Principles of Laboratory Animal Care (NIH Publication No. 86-23) were followed when handling the animals.

Ethical statement

The Research Ethics Committee for Animal Experimentation of Cairo University's Faculty of Pharmacy (approval no. BC3926) authorized all animal usage procedures. Every attempt was made to reduce animal discomfort and suffering.

Acute oral toxicity study

The acute toxicity of the PSE was assessed using Lorke method [17] to establish the dose regimen for the study. Together with a control group, four groups of four rats each, received oral gavages of the extract once daily at doses of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, and 2000 mg/kg body weight (bw). The groups were observed for 72 h. Weight change, grooming, hyperactivity, sedation, respiratory arrest, convulsions, increased and decreased motor activity, and mortality, if any, were among the observations that were noted.

Experimental design

The rats were randomly separated into 4 groups (n=6/group): a normal control (NC) group received oral normal saline (1 ml/100 g); an AKI (TAA) group injected intraperitoneally with a single dose of TAA (300 mg/kg) [18] dissolved in normal saline to establish the AKI animal model; and two groups (PSE and PSE+TAA) orally pretreated with PSE (400 mg/kg) [19,20] for 15 consecutive days with rats in PSE+TAA group received a single intraperitoneal injection of TAA (300 mg/kg) on day 15. One day after 24 h of TAA injection, measurements of body weights were achieved and blood samples were obtained from the retro-orbital plexus under mild diethyl ether anesthesia (1.9% (0.08 ml/L of container volume)) through inhalation. The rats were subsequently euthanized via spinal dislocation while they were in a fasting state, and their kidneys were collected. Afterwards, kidneys were weighted and allocated randomly for estimation of biochemical parameters or histopathological examination.

Assessment of relative kidney weight

Kidneys were quickly dissected, rinsed with ice-cold saline, dried between filter papers, and weighed separately. The relative kidney weight was calculated as total kidney weight (g)/animal body weight (g)×100 [21].

Blood biochemical assays

The blood samples were incubated at room temperature for one hour. Next to centrifugation (4000 rpm, 15 min at room temperature) via a Hettich centrifuge (Germany), the sera were isolated and then stored at -80°C for subsequent analysis. Quantitative colorimetric determinations of serum blood urea nitrogen (BUN) and creatinine were carried out using BUN Colorimetric Assay Kit (Catalog No. E-BC-K183-M, Elabscience, Wuhan, China) and QuantiChrom™ Creatinine Assay Kit (Catalog No. DICT-500, BioAssay Systems, USA). Serum kidney injury molecule 1 (KIM1), neutrophil gelatinase associated lipocalin (NGAL) and TNF-α levels were determined using ELISA kits, following the supplier's protocols (Catalog No. MBS355395, MyBioSource, USA, Catalog No. E-UNEL-R0040, Catalog No. E-EL-R2856, Elabscience, Wuhan, China, respectively).

Preparation of kidney homogenate

Rats from various groups had their kidney tissue removed, cleaned with ice-cold normal saline, and homogenized using a homogenizer (Tissue Lyser II, Qiagen, Netherlands) in an ice-cold buffer (0.25 M sucrose, 1 m M EDTA, and 1 mM Tris-HCl, pH 7.4). At 4 °C, this homogenate was centrifuged for 30 min at 1000 rpm. After that, the supernatant was utilized for additional biochemical analyses. Renal RIPK1, RIPK3 and MLKL contents were evaluated using ELISA kits supplied from LifeSpan BioSciences (USA) (Catalog No. LS-F9518, Catalog No. LS-F35895 and Catalog No. LS-F55370 , respectively), in accordance with manufacturer's instructions. ZBP1 and HMGB-1 concentrations in kidney homogenate were also quantified using ELISA kits purchased from MyBioSource, (USA) (Catalog No. MBS725570) and Elabscience, Wuhan, China (Catalog No. E-EL-R0505), respectively, according to manufacturer's guidelines.

Histopathology

The kidney was promptly immersed in 10% neutral-buffered formalin for 24 h, subsequently dried by a graded ethanol series, and prepared for paraffin embedding for light microscopic analysis. Serial 5-µm thick sections were stained with haematoxylin and eosin (H&E). Sections were inspected and photographed using an Olympus BX40 light microscope (Olympus, Hamburg, Germany) coupled to a Canon Powershot A640 digital camera (Canon Inc., Tokyo, Japan).

Statistics

All the results are expressed as the mean ± standard error of mean (SEM). Using the Shapiro–Wilk test, we determined whether the results were normally distributed. Multiple comparisons between groups were achieved via one-way ANOVA followed by post hoc analysis (Tukey). The significance level was set at $p < 0.05$. Statistical analyses were made via Graph Pad Prism software version 8.4.2 (San Diego, CA, USA).

RESULTS

Extract yield percent

The extraction of *Portulaca oleracea* (purslane) seeds using methanol yielded 4.5% (v/w) (**Table 1**).

Table 1. Percent yield of purslane seed extract

Solvent type	Weight of sample used (g)	Extract yield (%)
Methanol	3125	4.5

Acute toxicity study

The control rats exhibited normal behavior. PSE given at four dosage levels (500, 1000, 1500, and 2000 mg/kg bw) shown that, after 72 h, no grooming behavior was seen at any of the dosage levels. The administered dose levels did not influence the activity of the rats, which remained normal. However, at doses of 1000, 1500, and 2000 mg/kg, all rats displayed drowsiness, respiratory arrest, convulsions, decreased motor activity, and mortality. At a dose of 500 mg/kg bw, 50% of the rats died, but at doses of 1000, 1500, and 2000 mg/kg bw, 100% of the rats died, showing that a dose of less than 500 mg/kg bw is safe for further study.

Effect of PSE pretreatment on body weight and relative kidney weight in TAA-induced AKI in rats

The rat body weight reduced significantly in the TAA group compared to the NC group ($p = 0.0072$). Whereas the PSE+TAA group showed no significant increase in animal body weight compared to the TAA group ($p = 0.4555$), as described in Table 2. On the other side, TAA treatment resulted in significantly higher relative kidney weight (kidney weight/animal weight) compared to the NC ($p = 0.0001$) and PSE control groups ($p < 0.0001$). However, the PSE+TAA group had a significantly lower relative kidney weight than the TAA group ($p = 0.0384$) (**Table 2**).

Table 2. Effect of PSE pretreatment on body weight and relative kidney weight in TAA-induced AKI in rats

	Parameters		
Groups	IBW (gm)	FBW (gm)	RKW (%)
NC	164.3± 8.09	185.7± 6.17	0.35±0.01
TAA	155± 2.88	145.7± 2.33[a]	0.55±0.009[a]
PSE	140± 3.61	182.3± 5.69[b]	0.33±0.012[b]
PSE+TAA	157.7± 5.54	159± 8.51	0.47±0.03[abc]

Data are presented as the mean ± SEM, and a significant difference was considered at $p < 0.05$. The letters (a, b, and c) represent significant differences from the normal control (NC) group, thioacetamide (TAA) group, and purslane seed extract (PSE) control group, respectively. FBW, final body weight; IBW, initial body weight; RKW, relative kidney weight.

Effect of PSE pretreatment on kidney function in TAA-induced AKI rats

Figure 1 showed that, in TAA group, serum BUN and creatinine levels were significantly elevated compared to NC group ($P<0.0001$). Whereas, PSE pretreatment abrogated the TAA-induced AKI while significantly decreasing the serum BUN level ($P<0.0001$) as compared with the untreated rats and normalizing serum creatinine level ($P=0.4801$).

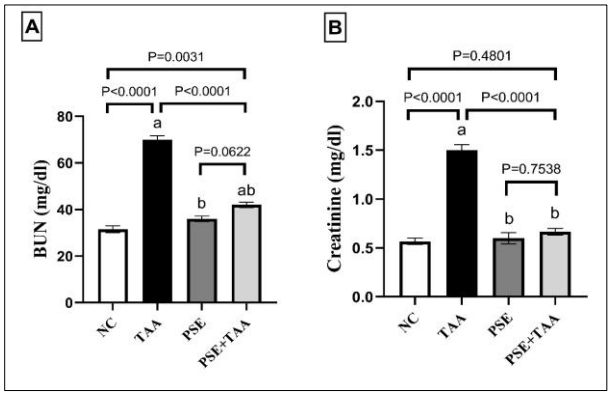

Figure 1. Effect of PSE pretreatment on kidney function in TAA-induced AKI rats: A) BUN and B) creatinine. Data are presented as the mean ± SEM, and a significant difference was considered at $p < 0.05$. The letters (a, b, and c) represent significant differences from the normal control (NC) group, thioacetamide (TAA) group, and purslane seed extract (PSE) control group, respectively. *AKI, acute kidney injury; BUN, blood urea nitrogen*

Effect of PSE pretreatment on kidney damage markers in TAA-induced AKI rats

Further analysis exhibited that induction of AKI caused significant increase in kidney damage markers, KIM1 and NGAL ($P<0.0001$) when compared to NC group. On the other side, PSE pretreatment significantly reduced serum KIM1 and NGAL levels in TAA+PSE rats ($P<0.0001$) compared to TAA group as shown in **Figure 2**.

Effect of PSE pretreatment on necroptosis markers in TAA-induced AKI rats

TAA administration to rats showed significant higher renal content of RIPK1, RIPK3, MLKL and ZBP1 ($P<0.0001$) than in NC animals, indicating necroptosis activation. PSE pretreatment managed to significantly declined the renal contents of RIPK1/RIPK3 and MLKL ($P<0.0001$) during TAA-induced AKI of PSE-treated rats against untreated group. Interestingly, PSE pretreatment was able to normalize RIPK1 renal levels ($P=0.0523$). Furthermore, PSE pretreatment significantly suppressed the TAA-induced elevation in ZBP1 ($P<0.0001$) as compared with that in the kidneys of rats from the untreated TAA-induced AKI group (**Figure 3**).

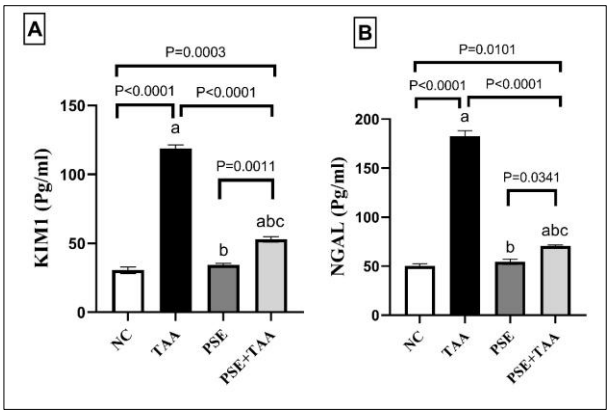

Figure 2. Effect of PSE pretreatment on kidney damage markers in TAA-induced AKI rats: A) KIM1 and B) NGAL. Data are presented as the mean ± SEM, and a significant difference was considered at $p < 0.05$. The letters (a, b, and c) represent significant differences from the normal control (NC) group, thioacetamide (TAA) group, and purslane seed extract (PSE) control group, respectively. AKI, acute kidney injury; KIM1, kidney injury molecule 1; NGAL, neutrophil gelatinase associated lipocalin

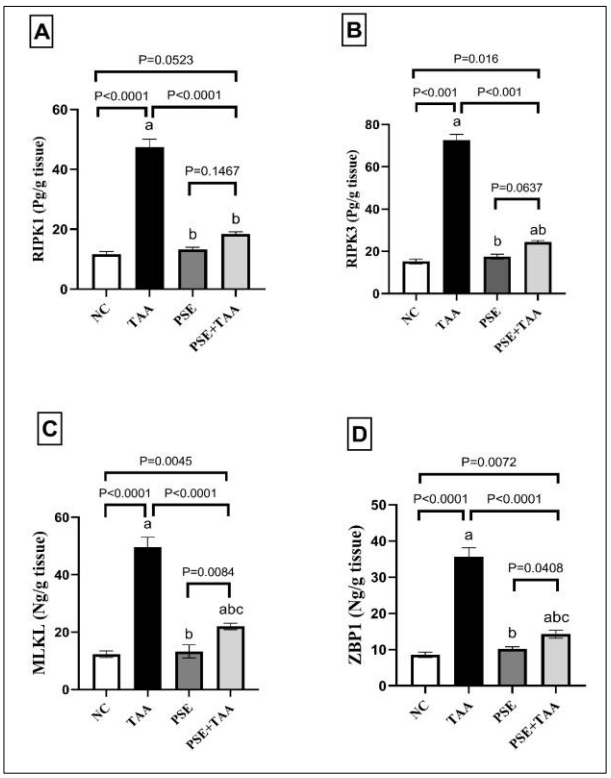

Figure 3. Effect of PSE pretreatment on necroptosis markers in TAA-induced AKI rats: A) RIPK1, B) RIPK3, C) MLKL and D) ZBP1. Data are presented as the mean ± SEM, and a significant difference was considered at $p < 0.05$. The letters (a, b, and c) represent significant differences from the normal control (NC) group, thioacetamide (TAA) group, and purslane seed extract (PSE) control group, respectively. *AKI, acute kidney injury; MLKL, mixed lineage kinase domain like pseudokinase; RIPK1, receptor-interacting protein kinase 1; RIPK3, receptor-interacting protein kinase 3; ZBP1, Z-DNA-binding protein 1.*

Effect of PSE pretreatment on inflammation in TAA-induced AKI rats

TAA-induced AKI rats showed significant increase in renal HMGB1 content and serum TNF-α level ($P<0.0001$) compared to NC group. In contrast, the renal HMGB1 and the pro-inflammatory cytokine, TNF-α levels were found to be significantly diminished ($P<0.0001$) following PSE pretreatment in TAA-induced AKI rats (**Figure 4**).

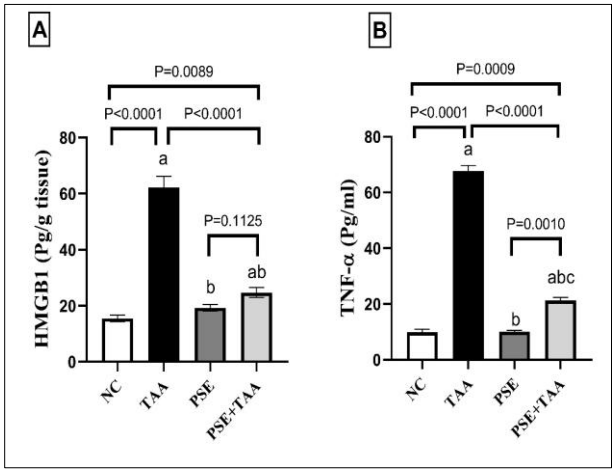

Figure 4. Effect of PSE pretreatment on inflammation in TAA-induced AKI rats: A) HMGB1 and B) TNF-α. Data are presented as the mean ± SEM, and a significant difference was considered at $p < 0.05$. The letters (a, b, and c) represent significant differences from the normal control (NC) group, thioacetamide (TAA) group, and purslane seed extract (PSE) control group, respectively. *AKI, acute kidney injury; HMGB1, High Mobility Group Protein B1; TNF-α, tumor necrosis factor-α.*

Effect of PSE pretreatment on histopathological changes in TAA-induced AKI rats

As indicated in **Figure 5**, no histopathological alterations were found in the NC group. On the contrary, the TAA group exhibited a variety of changes in both the renal cortex and the medulla. Renal tubular degeneration and necrosis were commonly detected. In addition to mononuclear inflammatory cells infiltrations were seen in the renal cortex and medulla. The PSE group had apparently normal renal components. The PSE+TAA group showed significant improvement; practically all evaluated individuals had apparently normal kidney sections, with the exception of a few sections that indicated mild degeneration and inflammatory cells infiltration in renal tubules.

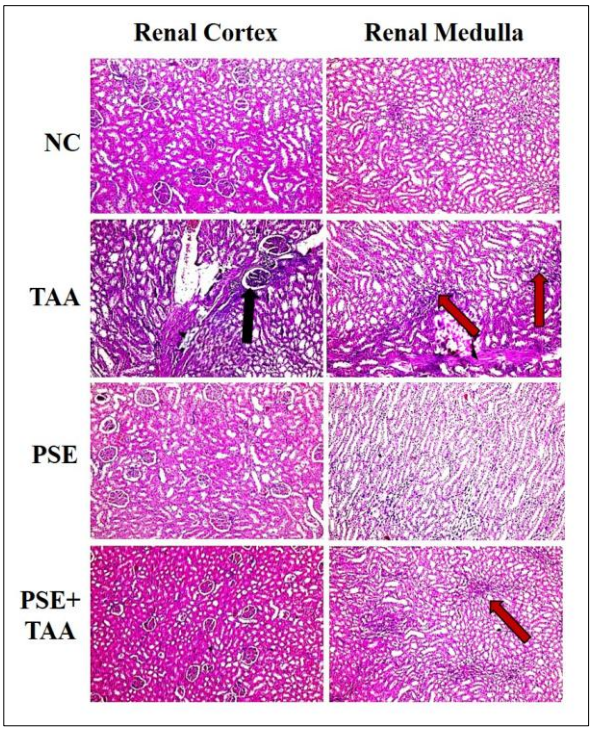

Figure 5. Effect of PSE pretreatment on histopathological changes in TAA-induced AKI rats. Photomicrograph of H&E stained-kidney tissues (100X), displaying normal renal cortex and medulla in the NC group, tubular degeneration (black arrow) and inflammatory cells infiltration (red arrows) in the TAA group, almost normal kidney sections in the PSE group besides mild tubular degeneration and renal inflammation (red arrow) in the PSE+TAA group.

DISCUSSION

Our study elucidates that PSE confers protection against TAA-induced AKI by concurrently targeting canonical necroptosis mediators (RIPK1/RIPK3/MLKL) and the nucleic acid sensor ZBP1, a key alternative activator of programmed necrosis. This dual inhibition disrupts a self-amplifying cycle of tubular cell death and inflammation, offering a novel mechanistic basis for PSE's renoprotective effects.

Consistent with our hypothesis, PSE pretreatment significantly reduced serum BUN and creatinine levels, indicating improved renal filtration capacity. This aligns with previous studies reporting the nephroprotective properties of *Portulaca oleracea* extracts in kidney injury [8,22]. This functional improvement was further supported by the downregulation of KIM1 and NGAL, which are highly sensitive biomarkers of tubular damage [23]. The strong correlation between reduced KIM1/NGAL and improved renal function [14] suggests that PSE preserves tubular epithelial integrity, thereby preventing the leakage of waste products into circulation. This aligns with previous studies showing that KIM1 and NGAL are not only diagnostic markers but also actively participate in tubular injury and repair processes [24,25].

Moreover, TAA-induced histopathological alterations as evidenced by the tubular degeneration, necrosis and inflammatory cells infiltration, confirming establishment of the kidney injury. These findings are in line with prior studies demonstrating the toxic effect of TAA on kidney tissues [26–28]. PSE pre-administration effectively countered these changes with almost normal kidney tissues rending PSE an effective nephroprotective therapy.

A key novel finding of this study is the inhibition of the necroptotic pathway, RIPK1/RIPK3/MLKL, by PSE. TAA-induction of AKI triggers activation of necroptosis [29,30]. Necroptosis, a regulated form of inflammatory cell death, contributes to AKI pathogenesis [31] by releasing DAMPs such as HMGB1, which further amplify renal inflammation and injury, and its suppression has been proposed as a therapeutic strategy [32,33]. The observed decrease in RIPK1, RIPK3, and MLKL in PSE-pretreated rats suggests that PSE disrupts necroptotic signaling, thereby limiting renal cell death and thereby reducing tubular epithelial loss. This mechanism has not been previously reported for PSE, highlighting its potential as a multi-targeted agent in AKI. This is particularly significant because necroptosis has been linked to KIM1 upregulation in AKI models [34,35]. Thus, PSE-mediated inhibition of necroptosis may explain the concurrent reduction in KIM1 levels, highlighting a direct mechanistic link between cell death pathways and tubular injury markers.

The reduction in TNF-α and HMGB1 levels following PSE pretreatment underscores PSE's anti-inflammatory role [36,37] in modulating inflammation-driven kidney injury. TAA causes inflammatory cell infiltration, and cell death [38,39], proved herein by the significant increase in TNF-α and in the prototypic DAMPs protein, HMGB1, which is released from dead, or damaged cells [40]. Furthermore, TNF-α is a key cytokine that promotes necroptosis via RIPK1 activation [41,42], while HMGB1, a DAMP released during necroptosis, fuels a self-continuing cycle of inflammation and cell death [43]. HMGB1, also exacerbates AKI by promoting inflammation and fibrosis [44–46]. Its downregulation, along with TNF-α, suggests that PSE modulates both early and late inflammatory mediators. These effects may synergize with necroptosis inhibition to confer comprehensive renal protection.

While our data confirm PSE-mediated suppression of the canonical RIPK1/RIPK3/MLKL pathway, the elevated renal ZBP1 in TAA-AKI and its suppression by PSE reveal an unexplored mechanism in toxin-induced AKI. Emerging evidences suggest ZBP1-dependent necroptosis may contribute to renal tubular injury [47,48]. ZBP1 senses endogenous nucleic acids as

mitochondrial DNA released, herein during TAA-induced oxidative stress, potentially triggering RIPK3/MLKL activation independently of RIPK1 [13]. This aligns with the observed elevation of HMGB1 (as HMGB1 promoting ZBP1 activation [49]) in untreated TAA-AKI rats and findings linking ZBP1 to sepsis-induced AKI [50]. PSE's downregulation of ZBP1 may occur via mitochondrial stabilization [51] and reduction of mitochondrial DNA release or through direct modulation of ZBP1-nucleic acid sensing. This proposal is clinically significant, as ZBP1-driven necroptosis may be resistant to RIPK1 inhibitors [13], even though it require further investigation. Moreover, TNF-α promotes RIPK1 activation [52], while HMGB1 enhances ZBP1 oligomerization [53]. PSE breaks this cycle by concurrently suppressing upstream triggers (TNF-α) and downstream effectors (HMGB1/ZBP1).

CONCLUSION

PSE protects against TAA-induced AKI by improving renal function, blocking necroptosis via RIPK1-dependent and ZBP1-dependent pathways, and reducing inflammation. These findings highlight PSE's potential as a novel, natural inhibitor of both canonical and alternative necroptosis in AKI. Future studies should validate these effects in clinical-relevant settings. Further studies should also explore PSE molecular targets and synergistic potential with existing AKI therapies.

Abbreviations

AKI, acute kidney injury; BW, body weight; BUN, blood urea nitrogen; DAMPs, damage- associated molecular patterns; HMGB1, High Mobility Group Protein B1; KIM1, kidney injury molecule 1; MLKL, mixed lineage kinase domain like pseudokinase; NC, normal control; NGAL, neutrophil gelatinase associated lipocalin; PSE, purslane seed extract; RIPK1, receptor-interacting protein kinase 1; RIPK3, receptor-interacting protein kinase 3; RCD, regulated cell death; SEM, standard error of mean; TAA, thioacetamide; TNF-a, tumor necrosis factor-a; ZBP1, Z-DNA- binding protein 1

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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