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ARTICLE

# Protective effects of black shallot extract against acetaminophen-induced nephrotoxicity in mice

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**ABSTRACT** Acetaminophen (APAP), widely recognized for its analgesic and antipyretic properties, poses a risk of renal toxicity in cases of overdose. Traditional herbal remedies are frequently employed to counteract drug-induced renal damage. This study focuses on black shallot, an innovative food product derived from *Allium ascalonicum*, a plant highly valued in traditional Vietnamese medicine. The aim is to explore the protective effects of black shallot ethanol extract (BSEE) against APAP-induced nephrotoxicity in mice. BSEE was orally administered at various doses (200, 250, and 300 mg/kg) in combination with APAP (3 g/kg). N-acetylcysteine (NAC) (50 mg/kg) served as the reference drug, while saline (10 mL/kg) functioned as the negative control. Evaluations encompassed renal histology, serum and urine renal function tests, antioxidant enzyme concentrations (SOD, CAT, and GPx), lipid peroxidation, and anti-inflammatory cytokines (TNF-$\alpha$, IL-1$\beta$, and IL-6). The findings indicated significant alterations in total protein, albumin, BUN, and serum/urine CRE concentrations ($P < 0.05$), coupled with a decrease in malondialdehyde (MDA) levels ($P < 0.05$) and inflammatory cytokines TNF-$\alpha$, IL-1$\beta$, and IL-6 ($P < 0.05$). Remarkable elevations were observed in antioxidant enzyme concentrations, including renal catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) ($P < 0.05$). The macroscopic and microscopic structures of the kidneys exhibited significant improvement. Consequently, BSEE effectively safeguards against APAP-induced renal damage, as evidenced by enhanced renal structure, reduced inflammation, and biochemical modulation, highlighting its potential therapeutic application in preventing APAP-induced nephrotoxicity.

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## Introduction

Renal toxicity ensues when the detoxification and specific excretory functions of the kidneys operate below optimal levels due to injury or diminished renal function caused by exogenous or endogenous toxic agents. Nephrotoxicity induced by medications remains a noteworthy concern as the administration of drugs leading to renal impairment is an inevitable aspect of clinical practice (Chinnappan et al. 2019). Acetaminophen (APAP) is a widely utilized medication known for its analgesic and antipyretic properties; nevertheless, an overdose can result in hepatic and renal impairment. One of its toxic components lies in its metabolic byproduct, N-acetyl-p-benzoquinone imine (NAPQI), which undergoes a chemical reaction with the sulfhydryl treatment of glutathione (GSH). Elevated APAP doses decrease the concentration of glutathione, causing NAPQI to react with cellular proteins in the liver and kidneys, leading to cellular destruction. Consequently, an increased level of NAPQI may serve as an intermediary for oxidative damage, intensifying hepatocellular and nephrocyte necrosis (Ahmad and Zeb 2020). Despite renal toxicity being less prevalent than hepatic toxicity in cases of APAP overdose, damage to renal tubules and acute renal failure can occur, potentially leading to fatality in both humans and animals (Chinnappan et al. 2019).

Renal protective agents possess the capability to reduce the detrimental effects of toxic substances on the kidneys (Chinnappan et al. 2019). Until now, the utilization of conventional therapeutic drugs to treat or prevent renal toxicity caused by APAP remains limited and is associated with various side effects. As a result, profound scientific efforts are currently confronted with challenges in the exploration of alternative therapeutic agents or supplementary agents for treatment or prevention (Ahmad and Zeb 2020). Herbal medicine's healing properties stem from its intricate chemical composition (Gaikwad et al. 2012). The global adoption of herbal remedies is deeply rooted, driven mainly by the presence of flavonoid and phenolic

compounds in these plants, along with their biological attributes, antioxidant capacities, and ability to neutralize free radicals. As per the World Health Organization, 80% of the global population turns to herbal medicine as a predominant healthcare approach (Rahimi et al. 2022).

Referred to as a bulbous plant, shallot (*Allium ascalonicum* L.) imparts unique culinary characteristics owing to its distinctive aroma, which results from the liberation of organic sulfur compounds during the breakdown of precursor flavor molecules [S-alk(en)yl cysteine sulfoxide compounds]. This process is facilitated by enzymes (allinase and lachrymatory factor synthase) present in shallot cells. The liberation of sulfur compounds during their degradation, along with other bioactive compounds (e.g., flavonols and phenols), demonstrates both preventive and therapeutic characteristics (Moldovan et al. 2022). The pharmacological properties of *A. ascalonicum* operate through redox mechanisms, involving antioxidant, anti-inflammatory, antibacterial, anticancer, antihyperglycemic, and hepatoprotective effects, impacting the liver and kidneys. These features may arise from the presence of diverse phytochemicals and a variety of plant nutrients, such as quercetin and allicin (Ounjaijean and Somsak 2020). Black shallot, a recently introduced food product, is derived from shallot (*A. ascalonicum*), a highly esteemed spice and medicinal herb in Vietnamese culinary practices and traditional medicine. It undergoes a controlled aging process, regulated by temperature and humidity (Tran et al. 2020). Black shallot, distinguished by its deep brown hue, sweet flavor, and gelatinous consistency (Tran and Ngo 2023), has demonstrated heightened anti-cancer and anti-inflammatory properties compared to its fresh shallot (Tran et al. 2020). Despite its recognized pharmacological benefits, there remains a paucity of scientific validation regarding its efficacy in treating nephrotoxicity. Hence, the ongoing research aims to investigate the protective effects of black shallot ethanol extract against acetaminophen-induced renal toxicity.

## Materials and Methods

### *Collection plant material and preparation of* the extract

Shallots (*Allium ascalonicum* L.) were procured from Vĩnh Châu town, Sóc Trăng province, Vietnam, in July 2023. The shallot's meticulous harvesting, thorough washing with clean water, and selective sorting are based on criteria such as freedom from decay, absence of pest damage, and uniform diameter across the bulbs. Following this, they were stored in moisture-resistant bags at 4 °C for use in subsequent experiments.

The entire batch of shallots underwent fermentation in an aging chamber (Shellab, USA) following the protocol outlined by Tran et al. (2020). The black shallots, characterized by their distinct dark brown, sweet-flavored attributes, were sliced and soaked in a 98% ethanol solution at a 5:1 volume ratio for one week. This procedure was repeated twice to maximize the yield. The resulting extracts were combined and filtered using the Whatman No. 4 filter paper. The final filtrate was then concentrated under reduced pressure (130 mbar) at 60 °C, resulting in the black shallot extract (designated as BSEE). Subsequently, the BSEE was stored in moisture-resistant bags at 4 °C, shielded from light, until utilized in subsequent experiments.

### *Evaluation of phytochemical composition of the extract*

The analysis of the black shallot extract aimed to determine the existence of alkaloids, saponins, tannins, flavonoids, steroids, glycosidic compounds, phenolic compounds, and terpenoids. Initial plant chemical profiling followed established procedures outlined by Ekwueme et al. (2015). Qualitative outcomes were denoted as either (+) when the phytochemical was present or (-) when it was absent.

The total phenolic content (TPC) and total flavonoid content (TFC) in BSEE were determined using the Folin–Ciocalteu colorimetric method and the aluminum chloride colorimetric method, respectively. The procedures were conducted following the protocols described by Musdalipah et al. (2021).

### *Experimental animals*

The Swiss albino mice, weighing 30 ± 2 grams, were procured from the Pasteur Institute in Ho Chi Minh City, Vietnam. The animals were accommodated in the animal facility of the Eastern Agriculture and Food Company, Ho Chi Minh City, where the ambient temperature was maintained at 22 ± 2 °C, with a relative humidity of 60 ± 5%, and a 12-hour light-dark cycle. Pelleted rodent feed provided by the Pasteur Institute and filtered reverse osmosis (RO) water ensured their nutritional needs. A seven-day acclimatization period in the facility preceded the commencement of the experiment. Throughout the animal experiments, we strictly adhered to ethical principles governing animal welfare, by the Basel Declaration on Animal Research (Alison 2010) and abided by the regulations stipulated in Livestock Law (2018) (No. 32/2018/QH14) in Vietnam. Our experimental protocols were aligned with the guidelines for clinical and pre-clinical trials in traditional and herbal medicine (Decision 141/QD-K2DT), promulgated in Vietnam in 2015. The use of laboratory animals adhered to the national ethical principles in medical research established by the Ministry of Health of Vietnam (Luyen and Quang 2013). Procedures

for treating the animals followed the guidelines of the World Health Organization (WHO 2000). All preventive measures and safety protocols were implemented following the trial procedures of the Faculty of Biotechnology, Ho Chi Minh City University of Industry. Trained personnel were responsible for the care and treatment of experimental animals, maintaining strict adherence to ethical principles related to animal research and following the guidelines of the Animal Research Ethics Committee at Ho Chi Minh City University of Industry, Vietnam.

### *Experimental design*

Thirty healthy mice were randomly divided into 6 treatments (n = 5; each treatment) and housed in corresponding cages, classified as follows: 1/ Control treatment: mice were orally administered saline (10 mL/kg) daily for 7 consecutive days; 2/ Negative control treatment (APAP treatment): mice received saline (10 mL/kg) for 7 consecutive days, followed by a single dose of APAP (3 g/kg); 3/ Positive control treatment (APAP+NAC treatment): mice were given N-acetylcysteine (NAC) at a dose of 50 mg/kg daily for 7 days, followed by a dose of APAP (3 g/kg); 4/ BSEE treatments ($BSEE_{200}$, $BSEE_{250}$, and $BSEE_{300}$ treatments): mice were pretreated with BSEE (200, 250, and 300 mg/kg, respectively) for 7 consecutive days, followed by a dose of APAP (3 g/kg) via oral administration. APAP was given to the selected treatments one hour following the final treatment dose on the $7^{th}$ day, and euthanasia of all animals occurred on the $8^{th}$ day of the experiment.

### *Analysis of hematology and biochemical parameters in serum and urine*

After a week of experimentation, blood samples were obtained from all experimental treatments using the retro-orbital method. Red blood cell (RBC) and white blood cell (WBC) counts were analyzed using an automated hematological analyzer (Drew D3, USA). Following centrifugation at 3000 rpm at 30 °C for 15 min, the resulting serum was employed for biochemical assessments. Total protein (TP), albumin (AB), blood urea nitrogen (BUN), and serum creatinine (CRE) levels were quantified using an automated biochemical analyzer (COBAS C111-Roche, USA). Additionally, urine collected over 24 h from mice treated with APAP was utilized to determine creatinine excretion levels using the 9180 Electrolyte Analyze (Roche Diagnostics, USA).

### *Determination of antioxidant enzymes activity*

The homogenized kidney tissue from treated mice was analyzed for the activity of the enzymes Superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx) using methods described by Marklund and Marklund (1974), Sinha (1972), and Rotruck et al. (1973), respectively.

### *Lipid peroxidation*

The malondialdehyde (MDA) level serves as an indicative measure of lipid peroxidation. MDA undergoes a reaction with thiobarbituric acid (TBA), forming the thiobarbituric acid reactive substances (TBARS) complex, resulting in a red coloration. Subsequently, a 1 mL homogenized sample was introduced into a complex solution containing TBA/trichloroacetic acid (TCA)/hydrochloric acid (HCL) (2 mL) and subjected to boiling in a water bath for 40 min. Following cooling to room temperature, the solution underwent centrifugation at 1000 g for 10 min, and the absorbance was measured at a wavelength of 535 nm (Mohebbati et al. 2016).

### *Measure levels of inflammatory cytokines*

TNF-$\alpha$, IL-1$\beta$, and IL-6 were measured using ELISA kits following the method described by Tran et al. (2023). TNF-$\alpha$, IL-1$\beta$, and IL-6 antibodies were added to individual wells of a 96-well plate and incubated overnight. The next day, another set of biotin-labeled antibodies was incubated with samples or standard antigens on the plate before adding streptavidin-HRP. TNF-$\alpha$, IL-1$\beta$, and IL-6 levels were determined at a wavelength of 450 nm to quantify their amounts. The levels of TNF-$\alpha$, IL-1$\beta$, and IL-6 were expressed as protein pg/mg.

### *Histopathology*

For histopathological evaluation, kidney specimens from all mice underwent immediate rinsing with phosphate-buffered saline (pH 7.4) to eliminate any residual blood and blood clots. Subsequently, the specimens were placed in a 10% formalin solution for 24 h for fixation. After fixation, the specimens were processed, sectioned into 4-5 μm slices, and stained with hematoxylin and eosin (H&E). Finally, the tissues were subjected to microscopic examination and characterization using an Olympus CX2 light microscope.

### *Statistical analysis*

The data are expressed as mean ± SD. One-way analysis of variance (ANOVA) was applied to assess significant differences among treatments for each parameter. Post hoc comparisons were conducted using Dunnett's test in the presence of a significant ANOVA result. A $P$-value of $< 0.05$ was deemed statistically significant. The statistical analysis was executed using Centurion XIX software (Statgraphics Technologies, USA).

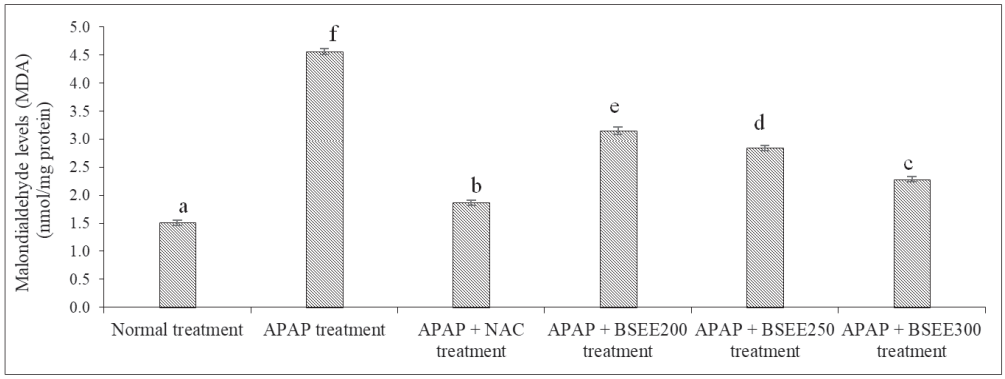


**Figure 1.** Effect of black shallot extract on malondialdehyde (MDA) levels of acetaminophen-administered mice. Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatments ($P < 0.05$).

## Results

### *Phytochemical analysis and quantification of TPC and TFC in the extract*

Conducting phytochemical screening of the black shallot extract yielded positive results, as evidenced by notable color changes. In the current study, we identified the presence of tannins, flavonoids, steroids, alkaloids, saponins, terpenoids, and phenolics in the extract, while cardiac glycosides were not detected (Table 1). Furthermore, the extract exhibited a rich content of bioactive compounds such as polyphenols and flavonoids, measuring 59.42 ± 2.05 mg GAE/g DW and 38.72 ± 1.79 mg QE/g DW, respectively (Table 2). These findings align with prior research by Tran et al. (2020) and Son et al. (2022), where the authors reported the presence of bioactive compounds including flavonoids (quercetin 3,4'-diglucoside, isorhamnetin 3,4'-diglucoside, quercetin 3-glucoside, quercetin 4'-glucoside, isorhamnetin 4'-glucoside, quercetin aglycone, isorhamnetin), phenols (methyl (2-hydroxyphenyl) acetate, trans-p-hydroxycoumaric acid, umbelliferone, and 8-hydroxy-3,4-dimethylisocoumarin).

### *The effect of black shallot extract on hematology and serum/urine biochemical parameters*

Mice in the APAP treatment exhibited significantly decreased levels of RBC, TP, and AB compared to the control treatment. Conversely, treatment with BSEE prevented the reduction in these parameters, with BSEE 300 mg/kg bringing these values close to normal levels (Table 3). Table 3 indicates a significant increase in serum urea and creatinine in the PCM-only treatment compared to the control treatment. Notably, mice treated with BSEE 300 mg/kg showed a significant elevation in WBC, BUN, and CRE concentrations compared to the treatment receiving only APAP. Similar results were observed for the level of CRE excretion. There was a significant decrease in CRE excretion in the treatment using only APAP compared to the control treatment (Table 3). These changes were reversed in the treatments treated with BSEE, with a significant decrease in serum WBC, BUN, and CRE, and a significant increase in CRE excretion in the BSEE treatments compared to the APAP-only treatment (Table 3). These results suggest that BSEE treatment may protect renal tissue, reducing damage caused by the effects of APAP. Data from the BUN/CRE ratio also show no significant changes in all examined treatments after treatment (Table 3).

### *Effect of black shallot extract on the activity of antioxidant enzymes and lipid peroxidation of the kidney*

The findings presented in Table 4 demonstrate that the

**Table 1.** Chemical composition of black shallot extract.

| Phytochemicals | BSEE | Phytochemicals | BSEE |
|---|---|---|---|
| Tannins | + | Alkaloids | + |
| Flavonoids | + | Saponins | + |
| Steroids | + | Phenolics | + |
| Terpenoids | + | Cardiac glycosides | - |

Notes: "+" denotes "present" and "–" denotes "absent"

**Table 2.** Quantification of total phenolic and flavonoid content in black shallot extract.

| Sample | Total phenolic content (mg GAE/g DW) | Total flavonoid content (mg QE/g DW) |
|---|---|---|
| BSEE | 59.42 ± 2.05 | 38.72 ± 1.79 |

Note: GAE - Gallic acid equivalent, QE - quercetin equivalents, DW - dry weight.

administration of APAP led to a reduction in GPx activity and an increase in CAT activity compared to the control treatment ($P < 0.05$). Furthermore, a marked increase in SOD activity was observed in the APAP+BSEE$_{(200\text{-}300)}$ and APAP+NAC treatments compared to the APAP treatment ($P < 0.05$). Notably, the utilization of BSE (200, 250, and 300 mg/kg) also resulted in a substantial improvement in the activities of GPx and CAT enzymes ($P < 0.05$).

Figure 1 illustrates the impact of BSEE and NAC on the lipid peroxidation process in the kidneys of mice with acetaminophen-induced injury. The MDA level in the APAP treatment (4.56 ± 0.05 nmol/mg protein) was significantly higher than in the normal treatment (1.51 ± 0.04 nmol/mg protein). Simultaneous administration of BSEE extract (200, 250, and 300 mg/kg) normalized the kidney MDA values (3.15 ± 0.07, 2.84 ± 0.04, and 2.28 ± 0.05 nmol/mg protein, respectively).

### *Influence of black shallot extract on inflammatory cytokines*

The levels and expression of inflammatory cytokines in the serum, such as TNF-$\alpha$, IL-1$\beta$, and IL-6, were measured. As shown in Table 5, the levels of inflammatory cytokines in mice treated with APAP were significantly higher than those in the control group. Conversely, treatment with black shallot extract (BSEE) reduced the levels of these cytokines compared to the APAP group. These results suggest that BSEE may have an anti-inflammatory effect, reducing the expression of inflammatory cytokines in the serum of mice induced by APAP.

### *Effects of black shallot extract on renal morphology and histology in mice with APAP-induced nephrotoxicity*

Assessment of mouse kidney morphology revealed significant changes (Fig. 2A). In the case of APAP-induced injury, the kidney exhibited alterations in color, a rough surface, shrinkage, and hardening (Fig. 2Ab). Conversely, the normal macroscopic structure of the kidney is healthy, with a smooth surface and a soft texture (Fig. 2Aa). Following treatment with BSEE (Fig. 2Ac) and NAC (Fig. 2Ad), the kidney was protected from APAP-induced damage, displaying a structure and color like the control treatment.

Histological examination of mouse kidney sections in the APAP group (Fig. 2Bb) revealed a compromised renal tissue morphology, with severe tubular degeneration, dilation of tubules, renal corpuscle damage, peritubular

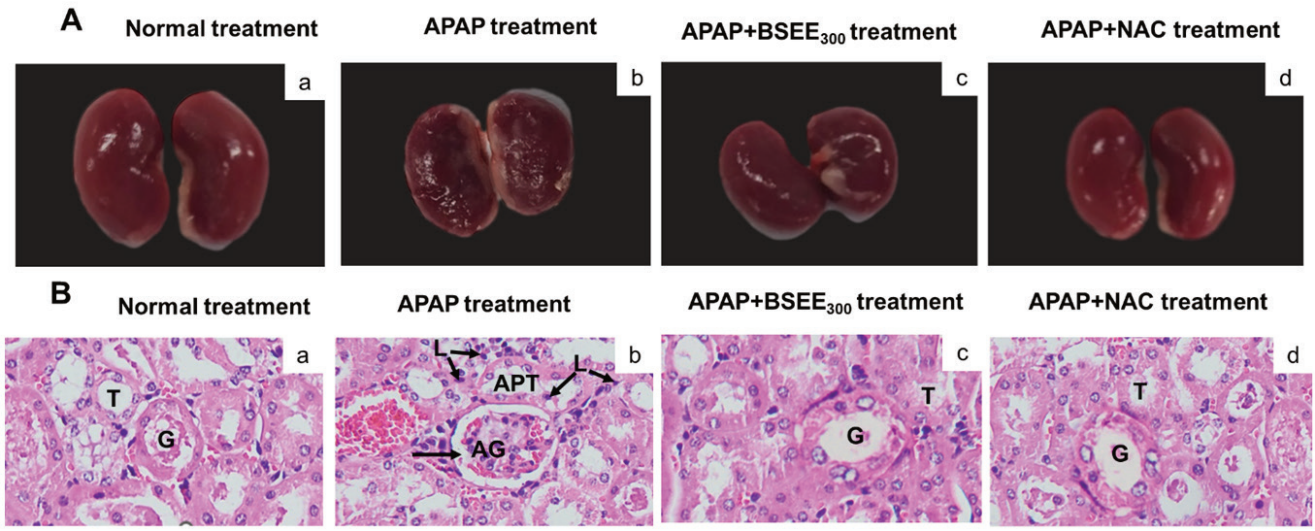


**Figure 2.** Displaying renal macroscopic (A) and microscopic (B) structures (200× magnification, H&E staining) from the control treatment and treatments treated with BSEE and NAC. (G) Normal renal glomeruli, (T) Normal renal tubules, (APT) alteration of proximal tubules and dilatation of urinary space, (AG) atrophy of the glomerulus, (L) Lymphocytes.

**Table 3.** Influence of black shallot extract on hematology and serum/urine biochemistry in mice with acetaminophen-induced nephrotoxicity.

| Parameters | Normal treatment | APAP treatment | APAP + NAC treatment | APAP + $BSEE_{200}$ treatment | APAP + $BSEE_{250}$ treatment | APAP + $BSEE_{300}$ treatment |
|---|---|---|---|---|---|---|
| RBC ($\times 10^6$cells/$mm^3$) | 8.02 ± 0.07$^f$ | 5.49 ± 0.04$^a$ | 7.74 ± 0.03e | 6.52 ± 0.06$^b$ | 6.98 ± 0.05$^c$ | 7.25 ± 0.04$^d$ |
| WBC ($\times 10^3$cells/$mm^3$) | 4.24 ± 0.03$^a$ | 10.75 ± 0.04$^f$ | 4.81 ± 0.04$^b$ | 6.82 ± 0.05e | 6.14 ± 0.04$^d$ | 5.35 ± 0.04$^c$ |
| Serum TP (g/dL) | 5.04 ± 0.12$^f$ | 2.72 ± 0.08$^a$ | 4.51 ± 0.11e | 3.68 ± 0.11$^b$ | 4.03 ± 0.09$^c$ | 4.31 ± 0.11$^d$ |
| Serum AB (g/dL) | 1.66 ± 0.04e | 0.89 ± 0.05$^a$ | 1.49 ± 0.05$^d$ | 1.21 ± 0.06$^b$ | 1.25 ± 0.04$^b$ | 1.42 ± 0.06$^c$ |
| Serum BUN (mg/dL) | 8.18 ± 0.06$^a$ | 16.98 ± 0.06$^f$ | 9.09 ± 0.05$^b$ | 11.37 ± 0.02e | 10.43 ± 0.05$^d$ | 9.62 ± 0.05$^c$ |
| Serum CRE (mg/dL) | 0.36 ± 0.04$^a$ | 0.65 ± 0.04$^d$ | 0.41 ± 0.07$^{ab}$ | 0.51 ± 0.07$^c$ | 0.46 ± 0.04$^{bc}$ | 0.43 ± 0.06$^b$ |
| CRE clearance (mL/min) | 0.28 ± 0.01$^a$ | 0.19 ± 0.02$^d$ | 0.25 ± 0.01$^c$ | 0.21 ± 0.02$^b$ | 0.220 ± 0.02$^b$ | 0.24 ± 0.01$^c$ |
| BUN/CRE ratio | 10.36 ± 1.05$^a$ | 18.75 ± 2.13$^d$ | 11.51 ± 1.19$^a$ | 14.59 ± 1.81$^c$ | 13.81 ± 1.75$^{bc}$ | 12.19 ± 1.04$^{ab}$ |

Values are expressed as mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatments (P < 0.05). Red blood cells (RBC), White blood cells (WBC), Total protein (TP), Albumin (AB), Blood urea nitrogen (BUN), Creatinine (CRE).

capillary congestion, and degeneration of parenchyma, accompanied by a substantial infiltration of inflammatory cells into the renal tissue. Conversely, the kidneys of animals treated simultaneously with BSEE 300 mg/kg (Fig. 2Bc) and NAC (Fig. 2Bd) exhibited noticeable improvements in renal corpuscles and tubules, with no presence of inflammatory cells in renal tissue. These results were comparable to the renal tissue structure of the control group (Fig. 2Ba), displaying nearly normal appearances of renal corpuscles, interstitium, and tubules within the kidney. This indicates the significant renal protective potential of BSEE (Fig. 2).

## Discussion

The renal damage resulting from the adverse effects of frequently prescribed medications is strongly linked to the occurrence of both acute and chronic kidney failure. Among these medications, acetaminophen (APAP) stands out as a prominent inducer of nephrotoxicity. APAP undergoes primary metabolism, forming sulfate and glucuronide conjugates; however, a minor fraction transforms N-acetyl-p-benzoquinone imine (NAPQI) through the action of cytochrome P450 enzymes. Typically, NAPQI is counteracted by GSH. In cases of excessive APAP absorption, cellular GSH reserves are depleted, leading to the generation of nephrotoxic compounds (Ansari et al. 2020). Numerous traditional herbal remedies have been employed to treat drug-induced kidney damage. This study marks the first investigation into the influence of black shallot extract on APAP-induced nephrotoxicity in mice. Our results highlight the positive impact of plant compounds present in black shallot extract (BSEE) on renal health. Flavonoids have shown the capacity to prevent or mitigate kidney damage by reducing blood pressure and exerting a direct influence on renal tissue. They interfere with various signaling pathways that contribute to renal injury (Vargas et al. 2018). Additionally, polyphenols play a role in protecting against renal damage by engaging in oxidative phosphorylation (OXPHOS), regulating renal mitochondria function, overseeing adenosine triphosphate (ATP) production, neutralizing free radicals, mitigating programmed cell death, and reducing inflammation (Ashkar et al. 2022).

Exposure to a toxic dose of APAP results in a range of metabolic disruptions, causing changes in serum components like urea, creatinine, albumin, and total protein. The considerable reduction in total protein and albumin levels observed in the serum of the APAP-only group (Table 3) is likely attributable to the arylating impact of NAPQI on proteins (Chinnappan et al. 2019). Elevated serum

**Table 4.** Effect of extract black shallot activity of antioxidant enzymes in the kidney of mice with APAP-induced nephrotoxicity.

| Parameters | Normal treatment | APAP treatment | APAP + NAC treatment | APAP + $BSEE_{200}$ treatment | APAP + $BSEE_{250}$ treatment | APAP + $BSEE_{300}$ treatment |
|---|---|---|---|---|---|---|
| SOD (U/mg protein) | 2.08 ± 0.04$^e$ | 1.05 ± 0.04$^a$ | 1.72 ± 0.05$^d$ | 1.48 ± 0.05$^b$ | 1.57 ± 0.04$^c$ | 1.62 ± 0.05$^c$ |
| CAT (mM/min/g tissue) | 63.41 ± 7.91$^a$ | 32.03 ± 4.94$^d$ | 59.43 ± 6.22$^b$ | 48.29 ± 4.52$^{cd}$ | 52.67 ± 5.86$^{cd}$ | 56.78 ± 5.53$^{bc}$ |
| GPx (nM/mg tissue) | 6.54 ± 0.03$^a$ | 3.16 ± 0.04$^f$ | 5.87 ± 0.04$^e$ | 4.22 ± 0.05$^b$ | 4.99 ± 0.05$^c$ | 5.39 ± 0.05$^d$ |

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatments ($P$ < 0.05).

creatinine and BUN levels, as noted in the APAP group, have also been reported in investigations of renal toxicity in animal studies (Ahmad and Zeb 2020). Creatinine originates from the metabolism of muscle proteins, with most of it being filtered by the kidneys and excreted in urine. The Glomerular Filtration Rate (GFR) serves as a well-established metric for assessing the kidneys' excretory functionality (Chinnappan et al. 2019). A sudden decline in urinary creatinine is indicative of renal impairment, especially when induced by APAP toxicity. The findings from the present study indicate that a 3 g/kg dose of APAP induces renal toxicity, leading to notable alterations ($P < 0.05$) in RBC, WBC, PT, AB, BUN, serum CRE, and a decrease in urinary CRE compared to the NC group. The protective efficacy of BSEE against APAP-induced toxicity in mice was evaluated in comparison with NAC. Utilizing three treatment doses (200, 250, and 300 mg/kg) of BSEE ensured varying therapeutic potentials, and it exhibited the capacity to modulate hematological and serum/urine biochemical parameters in mice throughout the experimental duration. With its flavonoid content and inherent antioxidant and anti-inflammatory properties, BSEE demonstrated protective effects, suggesting its potential as an optimal alternative for preventing APAP-induced toxicity (Rahimi et al. 2022). The biochemical findings were further validated through histological examination of the kidneys, illustrating the preservation of glomerular, tubular, and vascular structures (Fig. 2). Most drugs induce renal damage primarily affecting proximal tubules, glomeruli, or various segments of the nephron (Chinnappan et al. 2019). The administration of APAP to the APAP-treated mice led to significant renal damage, characterized by tubular degeneration, expanded spaces, glomerular injury, constricted interlobular arteries, and parenchymal degeneration. Pre-treatment with BSEE demonstrated a substantial and dose-dependent protective effect against APAP-induced nephrotoxicity.

The body's defense system against free radicals faces disruption from toxins, free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS), leading to oxidative stress. The kidneys, highly exposed to drugs and toxins, are particularly vulnerable to drug-induced ROS. Some studies propose oxidative stress, specifically ROS production, as a potential factor in APAP-induced organ toxicity. Primary defenses against ROS involve antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). The decline in antioxidant levels partly indicates the mechanism behind APAP-induced nephrotoxicity. Among various antioxidants, CAT and SOD play pivotal roles in eliminating ROS. The drop in SOD levels in the APAP group may be linked to increased superoxide anion levels. SOD mitigates free radical harm by converting superoxide anions into hydrogen peroxide. Thiol groups act as sensitive markers of oxidative stress in proteins and GSH. APAP-induced renal damage stems from NAPQI reacting with sulfhydryl groups and protein thiols, depleting the GSH reserve and subsequently neutralizing cellular molecules following APAP overdose (Ansari et al. 2020). In the current study, the administration of APAP at a dose of 3 g/kg significantly reduced the concentrations of the enzymes SOD, CAT, and GPx. Conversely, treatment with BSEE significantly increased the concentrations of SOD, CAT, and GPx, respectively.

The administration of APAP can result in significant peroxidation of lipid membranes and depletion of antioxidant substances in renal tissue. Commonly used drugs like acetaminophen and gentamicin can cause severe renal damage by triggering the production of highly reactive free radicals. The impaired antioxidant status in renal tissue partially elucidates the mechanism of APAP-induced nephrotoxicity through the generation of free radicals (Ahmad et al. 2012). Plants rich in flavonoids, steroids, and alkaloids demonstrate diuretic and substantial renal protective effects. Additionally, plant extracts containing ancaloit, alongside the antioxidant properties of alkaloids, may contribute to shielding the kidneys from APAP-induced toxicity (Chinnappan et al. 2019). These constituents have been identified in BSEE.

TNF-$\alpha$, IL-1$\beta$, and IL-6 are pivotal pro-inflammatory cytokines that trigger apoptosis through receptor binding on cell surfaces. These cytokines are elevated in various diseases where apoptosis is implicated, such as renal injury, acute, and chronic kidney failure. Oxidative stress, resulting from APAP overdose, significantly contributes to the upregulation of pro-inflammatory cytokines, leading to increased inflammation and necrosis rates in the kidneys (Rahimi et al. 2022). The findings of the current study reveal that APAP raises the concentrations of TNF-$\alpha$, IL-1$\beta$, and IL-6 compared to the control group. Treatment with black shallot extract has effectively lowered the levels of TNF-$\alpha$, IL-1$\beta$, and IL-6, thereby alleviating APAP-induced renal damage.

In summary, these findings indicate that black shallot extract has the potential to safeguard the kidneys from APAP-induced harm and could emerge as a promising therapeutic option for addressing PCM-induced nephrotoxicity.

## Conclusions

Based on the study findings, black shallot extract (BSEE), rich in alkaloids, flavonoids, steroids, and phenolics, shows significant potential in preventing acetaminophen (APAP)-induced kidney damage. BSEE efficiently reduced

biomarker levels in serum and urine while concurrently increasing the concentrations of antioxidant enzymes (CAT, SOD, GPx) in renal tissue and decreasing malondialdehyde (MDA), a key indicator of oxidative stress. These results underscore BSEE's capacity to alleviate oxidative stress and enhance the body's antioxidant defenses. Moreover, BSEE markedly decreased pro-inflammatory cytokine concentrations (TNF-$\alpha$, IL-1$\beta$, IL-6), highlighting its anti-inflammatory properties. Observable improvements in both macroscopic and microscopic kidney structures were noted. The absence of inflammatory cells in renal tissue indicates BSEE's effective protection against APAP-induced damage.

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