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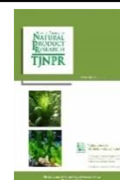

Counteracting Paracetamol-Induced Hepatotoxicity with Black Shallot Extract: An Animal Model Investigation

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ABSTRACT

Black shallot is a novel product of shallots (*Allium ascalonicum* L.), a traditional plant used worldwide for both culinary and folk medicinal purposes through controlled temperature and humidity fermentation. Paracetamol, a commonly used pain reliever and fever reducer, is a leading cause of poisoning in children and can result in liver failure. This study investigates the hepatoprotective effects of black shallot against acute paracetamol-related toxicity. Swiss albino mice were divided into six treatments and administered normal saline (10 mL/kg), N-acetylcysteine (50 mg/kg), and black shallot extract (EAAS) at doses of 200, 300, and 400 mg/kg for seven days, followed by paracetamol (PCM, 3000 mg/kg). Liver toxicity assessments were conducted 48 hours after PCM administration. The total phenolic and flavonoid contents of EAAS were found to be 22.74 mg/100g and 38.3 mg/100g, respectively. EAAS significantly reduced ($p < 0.05$) WBC counts, serum bilirubin levels (0.44 ± 0.02 µmol/L), and liver enzyme activities (AST, ALP, and ALT), as well as MDA levels (1.87 ± 0.07 nmol/mg protein) mediated by PCM. Similarly, EAAS significantly increased ($p < 0.05$) the activities of CAT (13.73 ± 1.07 mM/min/g tissue), GSH (4.01 ± 0.04 nM/mg tissue), and SOD (22.39 ± 4.27 mM/min/mg tissue) in PCM-intoxicated mice. Morphological and histological changes related to PCM-induced liver toxicity were also improved by EAAS. Overall, the oxidative and histological evaluations suggest that black shallot extract may have a preventive effect against acute paracetamol-induced liver damage.

Keywords: *Allium ascalonicum* L., Liver injury, Paracetamol, Hepatotoxicity, Hepatoprotective effects.

Introduction

The liver, the largest and most vital internal organ in the human body, plays a crucial role in regulating essential biochemical and physiological processes.[1] It is involved in various key functions, including metabolism, the synthesis of important immune-related molecules, and the detoxification of harmful substances. As a result, the liver is vulnerable to damage from a wide range of harmful agents such as inflammatory mediators, toxins, drugs (including antibiotics, antipyretics, and analgesics), and other contributing factors, which can have detrimental effects on its structure and function.[2]

Paracetamol (PCM), commonly known as Tylenol® in the United States, is the most widely used over-the-counter pain reliever and fever reducer, particularly in pediatric care. It is easily accessible without a prescription in many countries and is a staple in household medicine cabinets.[3] Paracetamol is generally considered safe when administered according to recommended dosing guidelines (10-15 mg/kg).[4] However, paracetamol overdose is responsible for a significant 44% of self-poisoning cases among adults.[2] Paracetamol-induced hepatotoxicity is a leading cause of acute liver failure in many countries, characterized by severe liver damage, hepatocyte necrosis, and kidney dysfunction.[3]

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Excessive paracetamol consumption leads to the formation of a dangerous compound called "N-acetyl-p-benzoquinone-imine" (NAPQI) through the enzymatic metabolic pathway. NAPQI depletes the liver's limited glutathione reserves, resulting in irreversible binding to liver cells, which can lead to hepatotoxicity and potential liver necrosis.[5]

Public apprehension surrounding paracetamol-induced liver toxicity has spurred extensive research aimed at uncovering its mechanisms. Oxidative stress has been closely linked to paracetamol's toxic effects.[3] N-acetyl cysteine (NAC), a well-known antioxidant, is commonly used to mitigate paracetamol-induced liver toxicity. NAC protects the liver by preventing the depletion of cellular glutathione (GSH), thus inhibiting oxidative reactions within the cells. However, NAC treatment can lead to adverse effects, and its efficacy diminishes if administered more than 8 hours after paracetamol overdose. Therefore, there is a pressing need to investigate alternative or adjunct therapeutic agents, particularly those derived from traditional botanical sources known for their historical contributions to medicine.[2]

Shallot (*Allium ascalonicum* Linn.), a member of the Liliaceae family, has a rich history in traditional cuisine. It offers antibacterial, antifungal, and antioxidant properties, along with the ability to neutralize peroxynitrite.[6] Shallots contain essential proteins, dietary fiber, minerals, and vitamins (A, B, C), as well as phenolic and flavonoid compounds like gallic acid, apigenin, quercetin, and tannic acid, which confer antioxidant benefits for liver and kidney health.[7] Additionally, shallots exhibit anti-cancer properties, inhibiting the growth of cancer cell lines, including HeLa and MCF-7, and promoting apoptosis in cervical cancer cells.[6] Innovative techniques have transformed pungent purple shallots into sweet black shallots rich in bioactive compounds like quercetin and branched-chain amino acids. These components offer antioxidative, anti-inflammatory, anti-cancer, and liver and kidney detoxification benefits.[7] Despite research on black shallot's potential, its effectiveness in addressing paracetamol-induced liver toxicity remains

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unexplored. This study investigates the hepatoprotective properties of black shallot extract in mice with paracetamol-induced liver injury. It aims to validate the traditional use of this remedy for liver disorders and explore new therapeutic avenues as alternatives or complements to NAC in managing paracetamol overdoses.

Materials and Methods

Collection plant material
In June 2023, we acquired shallots (*Allium ascalonicum* L.) from Vĩnh Châu town, Sóc Trăng province, Vietnam. These specimens, designated as AS210623VST, are currently maintained in the Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry, Vietnam. The shallots were meticulously hand-picked, thoroughly cleansed with distilled water, and selectively sorted based on several criteria (absence of rot, pest damage, and even diameter). Subsequently, they were stored in moisture-resistant bags at a temperature of 4°C for future experimental use.

Preparation of the extract
The complete fermentation of black shallots was carried out in a controlled aging chamber (Shellab, USA) at three distinct temperature settings (60, 70, and 80°C), with a constant relative humidity of 70%, spanning 21 days.[7] This process aimed to yield dark brown or black shallot products. Subsequently, the black shallots were sliced and immersed in a 98% ethanol solution in a 5:1 ratio for one week. This ethanol immersion step was repeated twice to maximize the extraction yield. The resultant extracts were pooled and subsequently filtered through the Whatman No. 4 filter paper. The final filtrate was concentrated under reduced pressure (130 mmBar) at 60°C to obtain the concentrated extract, referred to as EAAS. The black shallot extract was stored at 4°C until required for further experimentation.

Phytochemical screening and determination of total phenolic content (TPC) and total flavonoid content (TFC) of extract
Phytochemical screening analysis: The black shallot extract underwent qualitative phytochemical analysis, following established international protocols and methodologies, as previously detailed by Ilukho *et al*.[8] This analysis aimed to identify the presence of various chemical constituents, including alkaloids, flavonoids, tannins, terpenoids, saponins, anthraquinones, cardiac glycosides, steroids, phenolics, and phlobatannins.
The alkaloid examination involved dissolving 5 mL of the extract in 3 mL of acidified ethanol, gently warming, and then filtering. A few drops of Mayer's reagent and 1 mL of Dragendroff's reagent were added to 1 mL of the filtered solution, and turbidity appeared. Flavonoid analysis involved the addition of a few drops of 1% aluminum solution to a 5 mL extract. The appearance of a yellow color signified the presence of flavonoids. Tannin examination involved adding a few drops of 0.1% ferric chloride to the extract solution and observing the development of a brown or blackish-green color, indicating the presence of tannins. Saponin examination involved boiling 2 g of the sample in 20 mL of distilled water, followed by filtration. Ten milliliters of the filtrate were mixed with 5 mL of distilled water, and vigorously shaken to generate stable foam. This foaming portion was then combined with 3 drops of olive oil, and vigorously shaken again, and the subsequent formation of emulsion was observed. The formation of a red precipitate during the boiling of the plant sample's water extract solution with 1% hydrochloric acid is indicative of the presence of phlobatannins. 2 mL of acetic anhydride was added to 0.5 g of the ethanolic extract solution of each sample with 2 M H_2SO_4. A color change from purple to blue or green in some samples indicated the presence of steroids. 5 mL of each extract solution was mixed with 2 mL of chloroform, and concentrated H_2SO_4 (3 mL) was added carefully to form a layer. The formation of a brownish-red color on the surface indicated a positive result for the presence of terpenoids. Cardiac glycoside test: 5 mL of the plant sample was mixed with 2 mL of glacial acetic acid containing a drop of ferric chloride solution. This was then intensified with 1 mL of concentrated sulfuric acid. The brown ring observed at the interface signified the presence of deoxysugar, a characteristic of cardenolides. A potential purple ring might emerge beneath the brown ring, and in the acid layer, the gradual development of a greenish-blue ring could be observed throughout the thin layer. Phenolic testing: Mixing 1 mL of the extract solution with 2 mL of 10% lead acetate solution and observing a brown precipitate indicates a positive reaction. Anthraquinone test: 3 mL of each extract solution was mixed with 3 mL of chloroform, and the chloroform layer was separated. A 5% potassium hydroxide solution was added to this mixture. Samples showing a yellow color with green fluorescence upon treatment with a drop of 6% hydrogen peroxide, and the development of a red color, were regarded as positive for anthrone derivatives.

Plant chemical components quantification: To determine the chemical composition of the black shallot extract, a range of methods and techniques, as outlined by Ilukho *et al.* were utilized.[8] Total phenolic content determination via spectrophotometric method: The sample underwent boiling with 50 mL of ether to extract phenolic components within 15 minutes. Subsequently, 5 mL of the extract was pipetted into a 50 mL volumetric flask, and 10 mL of distilled water was added. The sample underwent a 15-minute boiling process with 50 mL of ether to extract its phenolic components. Next, 5 mL of the extract was transferred to a 50 mL volumetric flask, followed by the addition of 10 mL of distilled water. Subsequently, 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were introduced. The samples were made up to mark and allowed to react for 30 minutes to develop color, with measurements taken at a wavelength of 505 nm. Flavonoid assessment using the Bohm and Kocipai-Abyazan method: 10 mL of the solution was combined with 100 mL of 80% methanol solution at room temperature. The entire solution was then passed through a Whatman No. 1 filter. The filtered solution was subsequently transferred to an evaporation dish and evaporated to a constant weight using a water bath, followed by weighing.

Experimental animals
Healthy Swiss albino mice, weighing between 29 - 32 grams, were obtained from the Pasteur Institute in Ho Chi Minh City, Vietnam. These mice were housed in a breeding facility at the East Agriculture and Food Company, Ho Chi Minh City, where the room temperature was maintained at 25-28°C with a relative humidity ranging from 50% to 60%. A 12-hour light and dark cycle was established. The mice were placed in glass cages measuring 60 x 30 x 30 cm, with each cage accommodating five mice. Stainless steel hanging hooks were installed on the cage walls to provide food and RO-filtered water. Before the experiments, the mice were acclimated to the laboratory environment for 7 days. During the research endeavor, we adhered to ethical principles about animal welfare, as outlined in the Basel Declaration on Animal Research and we also complied with Livestock Law (No. 32/2018/QH14) in Vietnam .[9,10] Our testing protocols adhered to the Clinical and Pre-clinical Testing Guidelines for Traditional and Herbal Medicine (Decision 141/QD-K2ĐT) introduced in Vietnam in 2015.[11] The use of experimental animals adhered to the National Ethical Guidelines in Medical Research established by the Ministry of Health in Vietnam.[12] The animal treatment was conducted under the guidelines provided by the WHO in 2000.[13] All experimental animals were cared for and treated by trained personnel who adhered to ethical principles regarding animal research, following the guidelines of the Ethics Committee for Animal Research at Ho Chi Minh City University of Industry, Vietnam.

Experiment design
The research followed the experimental protocol outlined by Henneh *et al.*[2] Six treatments of experimental mice (n = 6) underwent a 24-hour fasting period before commencing the treatment. Subsequently, the animals received daily treatments for seven days. The normal control treatment and negative control treatment were given normal saline (10 mL/kg), while the positive control treatment received N-acetylcysteine (NAC) at a dose of 50 mg/kg. The treatments $EAAS_{200}$, $EAAS_{300}$, and $EAAS_{400}$ were administered EAAS at doses of 200, 300, and 400 mg/kg, respectively. Except for the normal control treatment, which received an additional dose of normal saline (10 mL/kg), the remaining animals were given an extra dose of 3000 mg/kg paracetamol (PCM) three hours after their last EAAS or NAC dose on the 7th day. After PCM

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administration, all animals underwent a 48-hour fasting period. Subsequently, blood samples were collected through retro-orbital puncture and divided into EDTA-containing tubes for hematological analysis and gel separator tubes for biochemical analysis. The animals were then humanely sacrificed using CO_2 inhalation,[14] and their livers were harvested for further examination.

Measurement of hematological and biochemical parameters
Upon the completion of the experimental phase, we proceeded to collect blood samples from the animals through retro-orbital puncture for hematological and biochemical analysis, following the methodology outlined by Anantha *et al.*[5] Blood specimens were drawn into EDTA-containing tubes and then analyzed for hematological parameters including red blood cell count (RBC), and white blood cell count (WBC) using an automated hematology analyzer (VET 2800, Mindray - Brazil). Blood preserved in gel separator tubes was subsequently centrifuged at 300 revolutions per minute for 10 minutes. The resultant serum was utilized to assess the activity of marker enzymes. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were quantified using the Reitman and Frankel method. Total bilirubin was assessed using the Malloy and Evelyn method.

Lipid peroxidation assay
The peroxidation of lipids was initiated by the ascorbate-iron (Fe^{2+}) system in the liver homogenate and quantified as reactive substances using the thiobarbituric acid method as outlined by Ilukho *et al*.[8] Freshly dissected mouse liver was homogenized to create a 10% solution in 150 mM cold KCl-Tris-HCl buffer. The reaction mix comprised liver homogenate, Tris-HCl buffer (20 mM, pH 7.0), $FeCl_2$ (2 mM), ascorbic acid (10 mM), and 0.5 mL of plant extract (25–100 µg/ml) in a final volume of 1 mL. Incubation of the reaction mixture occurred at 37°C for 1 hour. Lipid peroxidation was determined by quantifying malondialdehyde (MDA) equivalents via trichloroacetic acid (TCA), thiobarbituric acid (TBA), and HCl (TBA-TCA reagent: 0.375% w/v TBA; 15% w/v TCA; and 0.25 N HCl). The reaction mixture was combined with 2 mL of TBA-TCA reagent, heated in a boiling water bath for 15 minutes, and, after cooling, the resulting precipitate was removed by centrifugation at 10,000 × g for 5 minutes. Finally, the MDA concentration in the upper phase was determined spectrophotometrically at 535 nm.

Estimation of liver antioxidant activity
The *in-vivo* antioxidant activity was studied in the liver tissues of experimental mice. The tissue homogenates were prepared and subjected to estimation of antioxidants such as catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD) content according to the procedure detailed by Henneh *et al.* and Tukappa *et al*.[2,15]
Catalase (CAT): A 0.1 mL aliquot of liver homogenate was added to 1 mL of 4% ammonium molybdate and 2 mL of 0.03% H_2O_2 solution. The reaction mixture was measured using spectrophotometry at a wavelength of 410 nm.
Glutathione (GSH): The glutathione concentration was determined by precipitating proteins with 50% TCA. This mixture was then centrifuged for 15 minutes at a speed of 3000 revolutions per minute. The upper phase from the centrifugation process was mixed with 4.0 mL of 0.4 M Tris buffer (pH=8.0) and 0.1 mL of Ellman's reagent (DTNB). Subsequently, the new reaction mixture was incubated for 10 minutes. The addition of DTNB followed, and within 5 minutes, the absorbance was recorded at a wavelength of 412 nm.
Superoxide dismutase (SOD) activity was assessed in a solution of red blood cell lysate obtained from a 5% RBC suspension. The lysate was combined with 50 µL, containing 75 mM Tris–HCl buffer (pH 8.2), 30 mM EDTA, and 2 mM pyrogallol. The spectrophotometer recorded the rise in absorbance at 420 nm over 3-minute period. One unit of enzyme activity was defined as the quantity that inhibited 50% of the auto-oxidation rate of pyrogallol, expressed as the change in absorbance per minute at 420 nm.

Histopathological examination
Liver tissues were preserved in a 10% neutral buffered formaldehyde solution, gradually dehydrated with ethanol (ranging from 50% to 100%), cleared with xylene, and embedded in paraffin. Subsequently, 5-micron sections were prepared from the paraffin blocks. These sections underwent hematoxylin-eosin staining for histological assessment and were examined using an Olympus BX50 (Japan) binocular microscope to observe the liver's histological structure.

Statistical analysis
The data is expressed as mean values ± standard deviation (SD) and is presented in both tables and figures. Statistical analysis was carried out using one-way analysis of variance (ANOVA), with a significance level set at $p < 0.05$. The statistical analysis was executed using the Statgraphics Centurion IX software.

Results and Discussion

The phytochemical composition and the concentrations of phenolic and flavonoid content in the extract shallot
The qualitative analysis for phytochemical compounds in the black shallot extract indicated the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, cardiac glycosides, steroids, and phenolics, while anthraquinones and phlobatannins were not detected (Table 1). Quantitative analysis of plant chemicals revealed that the black shallot extract contained phenolics (22.74 ± 1.12 mg/100g) and flavonoids (38.3 ± 2.11 mg/100 g) (Table 2). Within the scope of the research project, we opted to employ varying doses of black shallot extract, specifically 200 mg/kg, 300 mg/kg, and 400 mg/kg, for the therapeutic intervention in cases of PCM-induced liver toxicity in mice.
Previous research has established the effectiveness of black garlic in mitigating liver damage. Tsai *et al.* administered black garlic extract at doses of 200 mg/kg and 500 mg/kg for seven consecutive days to address CCl_4-induced liver injury.[16] Tran *et al.* employed a branch extract of black garlic at a dosage of 200 mg/kg body weight to counteract CCl_4-induced liver damage in mice.[17] Shin *et al.* explored the prolonged use of black garlic extract at doses of 100, 200, or 400 mg/kg, administered five days a week for four weeks in rodent models to treat CCl_4-induced liver toxicity.[18] Building upon the positive outcomes reported by Tsai *et al*., Tran *et al.*, and Shin *et al.* in the context of black garlic's efficacy in liver injury treatment,[16-18] we have chosen comparable dosages of black garlic extract in our study for consistency and comparison with existing research. In the medical research field, varying doses can illuminate adverse effects and dose-dependent characteristics of a substance.

Table 1: Phytochemical of the extract of black shallot

Phytochemicals	EAAS	Phytochemicals	EAAS
Tannins	+	Alkaloids	+
Flavonoids	+	Saponins	+
Steroids	+	Phenolics	+
Terpenoids	+	Cardiac glycosides	+
Anthraquinones	-	Phlobatannins	-

(+): presence, (−): absence of tested phytochemicals.

Table 2: The content of total phenolic (TPC) and total flavonoid content (TFC) in the extract of black shallot

Sample	Total phenolic content (mg/100 g)	Total flavonoid content (mg/100 g)
EAAS	22.74 ± 1.12	38.3 ± 2.11

Concentration amount (mean ± SD) in mg per 100 g of the extract.

The selection of doses at 200 mg/kg, 300 mg/kg, and 400 mg/kg allows us to evaluate the dose-response relationship of black shallot. Employing multiple doses facilitates an examination of black shallot effects at different levels and offers insights into its versatility for

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treating liver toxicity. By specifically selecting these doses based on previous research, our goal is to produce clinically and practically meaningful results, enhancing our understanding of black shallots' therapeutic potential in PCM-induced liver toxicity.
Phytochemicals derived from plants, such as flavonoids, terpenoids, phenolics, alkaloids, and other compounds, are recognized for their potential to confer numerous benefits to the body, particularly in supporting liver function.[19-20] Key effects include minimizing liver damage, detoxification, and safeguarding the liver against harmful agents. Within black shallot extract, the presence of polyphenols and flavonoids allows for intricate interactions with other phytochemicals, creating a synergistic collaboration. This interaction has the potential to synergize their effects, enhancing the capacity to reduce liver damage and facilitate detoxification. The combination of these compounds not only targets the liver but also contributes to overall health benefits for the body.[19] Consequently, black shallot extract not only provides essential phytochemicals but also stimulates positive interactions among them, effectively protecting the liver and sustaining its functionality.

The effect of black shallot extract on hematology and biochemical parameters

The oral administration of PCM (3000 mg/kg) resulted in a significant increase ($p < 0.05$) in WBC, ALT, AST, ALP and TB levels, and a significant decrease ($p < 0.05$) in RBC amounts compared to the control treatments. However, mice treated with additional ESSA (200, 300, and 400 mg/kg) or NAC (50 mg/kg) exhibited a significant reduction ($p < 0.05$) in hematological and biochemical parameters (Table 3).
Paracetamol (PCM) induces liver cell damage, resulting in elevated serum levels of ALT and AST. The liver, as the organ with the highest enzyme content in the body, makes ALT an important biological marker for liver toxicity, while AST exhibits lower specificity for liver damage.[21] When the liver is under stress, these enzymes leak into the bloodstream in proportion to the extent of liver injury.[2] In the current study, the administration of 3000 mg/kg PCM led to a significant increase in the serum levels of ALT and AST. Additionally, ALP, an enzyme produced in the biliary and canalicular epithelium, is found at high concentrations in the blood, especially when there is damage to the liver. Liver toxicity is known to cause biliary obstruction, leading to the inability of the body to excrete ALP, resulting in elevated ALP levels as observed in the PCM treatment. The increase in white blood cell (WBC) count after PCM use also suggests a potential inflammatory response in the body.[2] However, pretreatment of mice with EAAS significantly reduced AST, ALT, ALP, and WBC levels. This demonstrates that EAAS can reduce transaminase enzyme activity, regenerate and stabilize liver cell membranes, reduce bile duct obstruction, lower inflammation, and protect the liver. The levels of red blood cells (RBC) and total bilirubin (TB) were increased and returned to normal levels. This reversal may be attributed to the EAAS's ability to mitigate PCM-induced liver damage. Once again, treatments pretreated with EAAS exhibited a significant increase in liver cell regeneration and a reduction in cell degeneration.

Black shallot extract affects the liver's antioxidant defense and lipid peroxidation systems

Table 4 presents the outcomes of treatment with EAAS on the mitigation of oxidative damage induced by PCM in hepatic tissues. PCM-induced intoxication resulted in a significant reduction ($p < 0.05$) in the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) when compared to the control treatment. However, administration of black shallot extract to the intoxicated mice led to a reversal of these effects ($p < 0.05$).
Figure 1 provides data concerning the impact of EAAS pre-treatment on PCM-induced damage to the lipid peroxidation system in hepatic tissues. The concentration of malondialdehyde (MDA) significantly increased ($p < 0.05$) in the PCM-intoxicated treatment in comparison to the control treatment. Nevertheless, the MDA levels in the treatments exhibited a significant improvement ($p < 0.05$) following the administration of various doses of EAAS.
Oxidative stress is the primary mechanism in the development of PCM-induced liver toxicity. This often occurs due to the high reactivity of N-acetyl-p-benzoquinamine (NAPQI) within cytochrome P450, ultimately weakening the antioxidant defense system. Antioxidants like SOD, CAT, and GSH are involved in directly scavenging various reactive oxygen species, such as superoxide and hydroperoxide produced during NAPQI breakdown. SOD represents the initial defense line responsible for removing singlet oxygen and dismutating superoxide radicals into H_2O_2. The removal of this compound is mainly carried out by liver CAT, which further facilitates the removal of MDA generated in the process, while GSH, through its catalytic activity on MDA, prevents the peroxidation reaction. The results concerning the antioxidants SOD, CAT, and GSH reflect the reduction in their levels in mice exposed to PCM-induced toxicity.

Table 3: Effects of black shallot extract on hematology and biochemistry of mice with paracetamol-induced hepatotoxicity

Parameters	Normal treatment	PCM treatment	PCM + NAC treatment	PCM + $EAAS_{200}$ treatment	PCM + $EAAS_{300}$ treatment	PCM + $EAAS_{400}$ treatment
RBC ($\times 10^6$ cells/mm^3)	6.88 ± 0.06^f	4.45 ± 0.07^a	5.78 ± 0.05^e	4.72 ± 0.07^b	5.02 ± 0.08^c	5.58 ± 0.05^d
WBC ($\times 10^3$ cells/mm^3)	3.37 ± 0.06^a	9.36 ± 0.08^f	3.83 ± 0.07^b	5.44 ± 0.07^e	4.88 ± 0.07^d	4.27 ± 0.06^c
TB (µmol/L)	0.27 ± 0.04^a	1.04 ± 0.03^e	0.42 ± 0.03^b	0.84 ± 0.04^d	0.69 ± 0.05^c	0.44 ± 0.02^b
AST (U/L)	16.99 ± 1.79^a	49.97 ± 3.47^d	19.99 ± 2.19^a	40.45 ± 3.35^c	33.31 ± 2.64^b	20.23 ± 2.21^a
ALT (U/L)	17.88 ± 1.63^a	54.21 ± 3.24^e	21.27 ± 2.13^b	43.63 ± 3.18^d	35.78 ± 2.72^c	22.89 ± 1.72^b
ALP (U/L)	128.19 ± 11.03^a	377.47 ± 21.95^e	152.61 ± 13.85^b	312.66 ± 18.86^d	261.61 ± 16.91^c	160.24 ± 14.99^b

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), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total bilirubin (TB)

Table 4: Effect of extract black shallot on antioxidants in the liver of mice with PCM-induced hepatotoxicity

Parameters	Normal treatment	PCM treatment	PCM + NAC treatment	PCM + $EAAS_{200}$ treatment	PCM + $EAAS_{300}$ treatment	PCM + $EAAS_{400}$ treatment
SOD (mM/min/mg tissue)	28.88 ± 8.86^b	14.52 ± 3.09^a	23.87 ± 6.96^{bc}	20.48 ± 4.98^{ab}	21.71 ± 4.52^{abc}	22.39 ± 4.27^{bc}
CAT (mM/min/g tissue)	17.72 ± 1.02^d	8.93 ± 0.99^a	14.63 ± 1.17^c	12.65 ± 1.11^b	13.32 ± 1.04^{bc}	13.73 ± 1.07^{bc}
GSH (nM/mg tissue)	4.85 ± 0.04^f	2.34 ± 0.06^a	4.33 ± 0.04^e	3.13 ± 0.05^b	3.71 ± 0.03^c	4.01 ± 0.04^d

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

This highlights the potential harm caused by PCM. This decline disrupts the liver's protective system, allowing for excessive H_2O_2 accumulation, ultimately weakening the system. Similarly, the level of lipid peroxidation in the liver (MDA) is related to superoxide radical

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generation. Therefore, the increase in MDA levels observed in this study indicates that PCM intoxication stimulates the free radical generation capability of mouse liver tissues.[22] Interestingly, pre-processing methods with black shallot significantly improved the disrupted antioxidant defense system, and these improvements were dose-dependent. This enhancement was achieved by improving the levels of diminished antioxidants caused by PCM exposure (CAT, SOD, and GSH) and reducing MDA production in the liver. These observed improvements are believed to be linked to the phenolic and flavonoid antioxidant components of EAAS. The results of this study offer profound insights into the potential use of black shallot as a dietary supplement for liver-related health issues.

Effect of black shallot extract on liver morphology and histology

Evaluation of mouse liver morphology revealed significant alterations (Figure 2A). In cases of PCM-induced damage, the livers displayed changes in color, a rough surface, tiny nodules on the surface, enlargement, and hardening (Figure 2Ab), in stark contrast to the normal liver macrostructure, which appeared healthy, with a smooth surface and soft texture (Figure 2Aa). Following treatment with EAAS and NAC, the livers were protected from PCM-induced damage, displaying a color similar to that of the control treatment, a smooth surface, and reduced dilation (Figure 2Ac and 2Ad, respectively).

Figure 2B illustrates microimages of liver sections depicting the toxicity caused by PCM in mice. The histological structure of the normal control treatment (Figure 2Ba) reveals a normal liver tissue structure with undisturbed sinusoidal spaces. However, in the negative control treatment (PCM treatment, Figure 2Bb), treated solely with PCM (3000 mg/kg), hepatocyte necrosis with characteristics such as condensed nuclei, darkened cytoplasm, and some vacuolization (bubble-like degeneration) was observed. Additionally, the sinusoidal dilation of hepatocytes was noticed in the negative control treatment. Nonetheless, hepatocyte damage was alleviated when observed in the treatments treated with EAAS 400 mg/kg (Figure 2Bc) as well as the treatment with NAC (Figure 2Bd), displaying uniform and normal sinusoidal spaces, with no alterations in hepatocyte structure.

Conclusion

The black shallot extract (EAAS) exhibits promising hepatoprotective effects against paracetamol-induced liver damage. EAAS treatment substantially improved hematological and biochemical parameters. Furthermore, EAAS treatment resulted in increased CAT, SOD, and GSH levels, coupled with decreased MDA levels in liver tissues, indicating the enhanced antioxidant defense mechanism and reduced lipid peroxidation associated with EAAS. The structural integrity of liver tissues was effectively maintained with EAAS treatment. These findings underscore EAAS as a robust shield against paracetamol-induced liver toxicity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

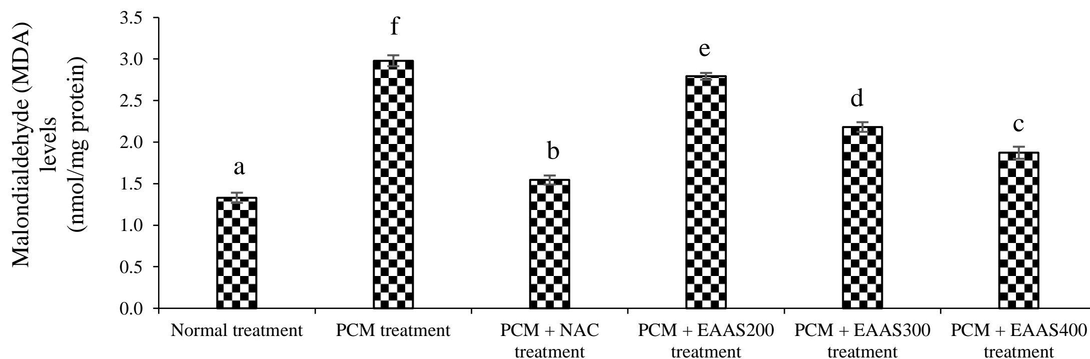

Figure 1: Effect of black shallot extract on malondialdehyde (MDA) levels of paracetamol-administered mice. Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatments ($p < 0.05$).

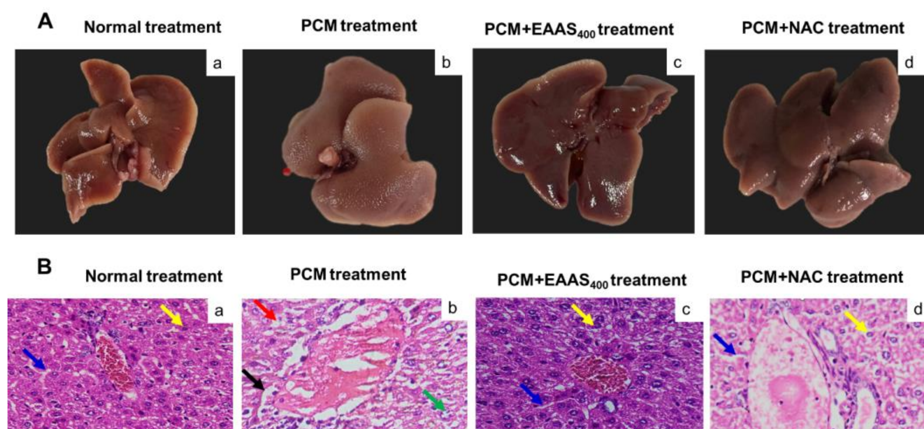

Figure 2: Display macroscopic (A) and microscopic (B) liver structure images (magnification ×200, H&E staining) of control s and those treated with EAAS and NAC. The images show dilated sinusoidal spaces (black arrows) and normal ones (blue arrows), hepatocyte necrosis (red arrows), swollen hepatocytes (green arrows), and normal hepatocytes (yellow arrows).

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