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Assessment of hepatoprotective activity of termite *(Odontotermes obesus)* extract against paracetamol-induced liver damage in Wistar rats

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Abstract

Background: Paracetamol overdose causes severe hepatotoxicity via oxidative stress. Natural antioxidants from edible insects like termites remain underexplored. *Odontotermes obesus* is traditionally consumed but lacks scientific validation for liver protection.

Objective: To evaluate the hepatoprotective activity of *Odontotermes obesus* ethanolic extract against paracetamol-induced liver damage in Wistar rats.

Methods: Termites were collected, identified, and extracted with 70% ethanol. Phytochemical screening was performed. Acute oral toxicity was tested per OECD 423. Thirty male Wistar rats were divided into five groups (n=6): normal control, paracetamol control (2 g/kg), silymarin (100 mg/kg) + paracetamol, extract low dose (250 mg/kg) + paracetamol, extract high dose (500 mg/kg) + paracetamol. Treatments were given orally for 7 days; paracetamol on day 7. Serum ALT, AST, ALP, total bilirubin, total protein, and liver homogenate GSH, SOD, CAT, MDA were measured. Liver histopathology was scored.

Results: Paracetamol significantly ($p<0.05$) elevated ALT (342.5 vs 42.3 U/L), AST (298.7 vs 85.6 U/L), ALP (245.6 vs 98.2 U/L), bilirubin (2.45 vs 0.52 mg/dL), and MDA (9.8 vs 2.1 nmol/g), while reducing total protein (3.8 vs 7.1 g/dL), GSH (2.1 vs 6.8 µmol/g), SOD (6.2 vs 18.4 U/mg), and CAT (8.3 vs 22.1 U/mg). *Odontotermes obesus* extract at 500 mg/kg significantly reversed all parameters (ALT 89.6 U/L, GSH 5.6 µmol/g, MDA 3.5 nmol/g), comparable to silymarin. Histopathology showed reduced necrosis and inflammation.

Conclusion: *Odontotermes obesus* ethanolic extract exhibits dose-dependent hepatoprotective activity against paracetamol-induced liver damage, mediated through antioxidant mechanisms. This insect offers a novel, affordable natural hepatoprotective agent.

Keywords: Hepatoprotective, *Odontotermes obesus,* paracetamol, Wistar rats, oxidative stress

Introduction

The liver is the central metabolic organ responsible for detoxification, protein synthesis, and bile production. Drug-induced liver injury (DILI) accounts for more than 50% of acute liver failure cases globally (Lee, 2003). Among all hepatotoxic drugs, paracetamol (acetaminophen) is the most common cause of DILI in both developed and developing countries. In the United States alone, paracetamol toxicity leads to approximately 26,000 hospitalizations and 450 deaths annually. Despite widespread awareness, accidental and intentional overdoses continue to rise [1].

Paracetamol is generally safe at therapeutic doses (up to 4 g/day in adults). However, overdose saturates the glucuronidation and sulfation pathways, shunting the drug to cytochrome P450

Fig 1: Termite *(Odontotermes obesus)*

enzymes, primarily CYP2E1, CYP1A2, and CYP3A4. This generates a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) (Jaeschke *et al*, 2012). Under normal conditions, NAPQI is rapidly conjugated with hepatic

glutathione (GSH) and excreted. When GSH stores are depleted below 30% of normal, NAPQI covalently binds to mitochondrial proteins, triggering oxidative stress, mitochondrial permeability transition, and eventually hepatocyte necrosis [2].

The only approved specific antidote for paracetamol poisoning is N-acetylcysteine (NAC), which replenishes GSH. However, NAC is most effective when administered within 8–10 hours post-ingestion. Late presentation or massive overdose often requires liver transplantation, which is expensive and limited by organ availability. Therefore, there is a pressing need for safe, affordable, and effective hepatoprotective agents that can be used adjunctively or prophylactically [3].

Natural products have long been a source of hepatoprotective molecules. Silymarin from milk thistle (*Silybum marianum*) is the gold standard natural hepatoprotective agent, acting through antioxidant, anti-inflammatory, and antifibrotic mechanisms (Vargas-Mendoza *et al*, 2014). Other plant-derived agents like curcumin, glycyrrhizin, and andrographolide also show promise. However, many plant-based remedies face challenges of sustainability, seasonal availability, and high cultivation costs [4].

In recent years, edible insects have gained attention as a sustainable and nutritious food source. The Food and Agriculture Organization (FAO) has promoted entomophagy as a solution to food security. Beyond nutrition, insects produce a variety of bioactive compounds including antimicrobial peptides, antioxidants, and anti-inflammatory molecules (Van Huis, 2013). Termites (Order Blattodea, formerly Isoptera) are consumed in many parts of Africa, Asia, and South America. Traditional medicine systems use termites for treating asthma, rheumatism, malnutrition, and liver disorders [5].

Odontotermes obesus (Rambur, 1842) is a mound-building termite widely distributed in India, Sri Lanka, and Southeast Asia. It belongs to the family Termitidae, subfamily Macrotermitinae. These termites cultivate fungus (Termitomyces) in their mounds, contributing to their nutritional profile. Proximate analysis of *O. obesus* reveals high protein content (35–45% dry weight), essential fatty acids (oleic, linoleic, palmitic), and significant levels of phenolic compounds and flavonoids. Previous studies have demonstrated antioxidant activity of *O. obesus* extracts *in vitro*, including DPPH radical scavenging (IC50 ~ 50 µg/mL) and ferric reducing power [6].

Despite these promising properties, no scientific study has evaluated the *in vivo* hepatoprotective activity of *Odontotermes obesus* against paracetamol-induced liver damage. Most insect hepatoprotective studies have focused on silkworm, cockroach, or honeybee products. Termites remain largely unexplored in modern pharmacology.

Rationale

Given the traditional use of termites for liver ailments and their documented antioxidant content, we hypothesized that *Odontotermes obesus* extract would protect against paracetamol-induced hepatotoxicity in rats. The objective of this study was to evaluate the hepatoprotective activity of ethanolic extract of O. obesus using biochemical, antioxidant, and histopathological parameters, with silymarin as a standard reference [7].

Materials and Methods

Ethics Statement

All animal procedures were approved by the Institutional Animal Ethics Committee (IAEC) of BMS Mahavidyalaya Tiloi, Amethi UP India (Reference No-IAEC-BMSMV/Bio-017/2026/27). Experiments were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals UNDER Zoologist Roshani Singh Assistant Professor, BMS Mahavidyalaya Tiloi, Amethi UP India, and followed the ARRIVE guidelines.

Collection and Authentication of Termites

Worker and soldier termites of *Odontotermes obesus* were collected from termite mounds in the rural areas of Tiloi, Amethi, UP India during the monsoon season (March –April 2026). The termites were identified by a taxonomist at the BMS Mahavidyalaya Tiloi, Amethi UP India, and a voucher specimen (was deposited. BMSMV/Bio-016/2026/27)

Preparation of Extract

Termites were cleaned of debris, washed with distilled water, and dried in a hot air oven at 40°C for 48 hours. Dried termites (500 g) were ground to a coarse powder using a mechanical grinder. The powder was extracted with 2.5 L of 70% ethanol (1:5 w/v) using a Soxhlet apparatus for 48 hours at 60–70°C. The extract was concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland) at 45°C and then dried in a vacuum desiccator. The yield was calculated as (weight of dried extract / weight of dried termite powder) × 100. The extract was stored at 4°C in airtight containers until use. and this procedure done in Pharmaceutical Chemistry Lab. In BMS College of Pharmacy, Amethi, UP India [8]

Yield: 62.5 g (12.5% w/w).

Phytochemical Screening

The ethanolic extract was subjected to standard qualitative tests for the presence of alkaloids (Mayer's, Wagner's, Dragendorff's reagents), flavonoids (ferric chloride test, Shinoda test), phenols (ferric chloride test), tannins (gelatine test), saponins (frothing test), steroids (Salkowski test, Liebermann-Burchard test), glycosides (Keller-Killiani test), and triterpenoids (Libermann-Burchard test) (Harborne, 1998). and this procedure done in Pharmaceutical Chemistry Lab. In BMS College of Pharmacy, Amethi, UP India.

Acute Oral Toxicity Study

Acute toxicity was assessed following OECD Guideline 423 (Acute Toxic Class Method). Healthy female Wistar rats (n=3 per step, non-pregnant, nulliparous, 180–200 g) were fasted overnight with free access to water. The extract was administered orally at a limit dose of 2000 mg/kg body weight suspended in 1% carboxymethyl cellulose (CMC). Rats were observed individually for mortality, behavioural changes, convulsions, salivation, diarrhoea, lethargy, and changes in skin, fur, eyes, and mucous membranes at 0.5, 1, 2, 4, 8, 12, and 24 hours, and then daily for 14 days. Body weight and food/water consumption were recorded weekly. If no mortality occurred at 2000 mg/kg, the LD50 was considered >2000 mg/kg [9, 10].

Experimental Animals

Male Wistar rats (150–200 g, 6–8 weeks old, n=30) were obtained. Animals were housed in polypropylene cages (3 per cage) with sterile paddy husk bedding under standard laboratory conditions: temperature 22 ± 2°C, relative humidity 55 ± 5%, 12:12 h light/dark cycle. Rats had free access to standard pellet diet (Amrut, India) and reverse osmosis water *ad libitum*. Animals were acclimatized for 7 days before the start of the experiment [11].

Experimental Design

Rats were randomly divided into five groups of six animals each (n=6). All treatments were administered orally using an intragastric tube.

Table 1: Experimental Design

Group	Treatment	Dose	Duration
I (Normal control)	1% CMC (vehicle)	5 mL/kg	Days 1–7
II (Paracetamol control)	1% CMC (vehicle)	5 mL/kg	Days 1–6; Paracetamol 2 g/kg on day 7
III (Silymarin standard)	Silymarin + Paracetamol	100 mg/kg (silymarin)	Days 1–7; Paracetamol 2 g/kg on day 7 (30 min after silymarin)
IV (Extract low dose)	Extract + Paracetamol	250 mg/kg	Days 1–7; Paracetamol 2 g/kg on day 7
V (Extract high dose)	Extract + Paracetamol	500 mg/kg	Days 1–7; Paracetamol 2 g/kg on day 7

Paracetamol was suspended in 1% CMC and administered at 2 g/kg body weight (single oral dose) on day 7, 30 minutes after the respective treatments. The dose of paracetamol (2 g/kg) was selected based on pilot studies and literature showing consistent hepatotoxicity within 24 hours [12].

Sample Collection

Twenty-four hours after paracetamol administration (day 8), rats were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood was collected by retro-orbital puncture into plain centrifuge tubes. Blood was allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rpm for 10 minutes (4°C). Serum was separated and stored at -80°C until analysis. [13] After blood collection, rats were euthanized by cervical dislocation under anaesthesia. The liver was immediately excised, washed with ice-cold normal saline, blotted dry, and weighed. A portion of the left lateral lobe was fixed in 10% neutral buffered formalin for histopathology. The remaining liver tissue was homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) to prepare 10% (w/v) homogenate using a Teflon glass homogenizer. The homogenate was centrifuged at 10,000 × g for 15 minutes at 4°C, and the supernatant was used for antioxidant assays [14].

Biochemical Analysis of Serum

Serum levels of the following parameters were measured using commercially available diagnostic kits (Erba Diagnostics, Mannheim, Germany) on a semi-automated biochemistry analyser (Erba EM 360):

- **Alanine aminotransferase (ALT):** UV-IFCC method (kinetic)
- **Aspartate aminotransferase (AST):** UV-IFCC method (kinetic)
- **Alkaline phosphatase (ALP):** p-nitrophenyl phosphate method
- **Total bilirubin:** Diazo method (Jendrassik-Grof)
- **Total protein:** Biuret method

All assays were performed in duplicate, and intra-assay coefficient of variation was <5%.

Antioxidant Parameters in Liver Homogenate

Reduced Glutathione (GSH)

GSH was estimated using Ellman's method (Ellman, 1959). Homogenate (0.5 mL) was mixed with 0.5 mL of 10% trichloroacetic acid (TCA) and centrifuged at 2000 rpm for 10 minutes. The supernatant (0.5 mL) was mixed with 2 mL of 0.3 M disodium hydrogen phosphate and 0.25 mL of 0.001 M 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). Absorbance was read at 412 nm. Results were expressed as µmol of GSH per gram of tissue [15].

Superoxide Dismutase (SOD)

SOD activity was assayed by the inhibition of nitro blue tetrazolium (NBT) reduction (Kakkar *et al*, 1984). The reaction mixture contained 0.1 mL of homogenate, 1.2 mL of 0.1 M sodium pyrophosphate buffer (pH 8.3), 0.1 mL of 186 µM phenazine methosulfate, 0.3 mL of 300 µM NBT, and 0.2 mL of 780 µM NADH. After incubation at 30°C for 90 seconds, the reaction was stopped with 0.1 mL of glacial acetic acid. Absorbance was measured at 560 nm. One unit of SOD is defined as the amount of enzyme causing 50% inhibition of NBT reduction [16].

Catalase (CAT)

Catalase activity was determined by the method of Aebi (1984). The reaction mixture contained 1.95 mL of 0.05 M phosphate buffer (pH 7.0), 1.0 mL of 0.019 M H_2O_2, and 0.05 mL of homogenate. The decrease in absorbance was recorded at 240 nm for 60 seconds. Catalase activity was expressed as U/mg protein, where one unit decomposes 1 µmol of H_2O_2 per minute.

Lipid Peroxidation (MDA)

Malondialdehyde (MDA), an end product of lipid peroxidation, was measured using the Thio barbituric acid reactive substances (TBARS) assay. Homogenate (0.5 mL) was mixed with 0.5 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of 20% acetic acid (pH 3.5), and 1.5 mL of 0.8% Thio barbituric acid (TBA). The mixture was heated at 95°C for 60 minutes, cooled, and centrifuged. The absorbance of the supernatant was read at 532 nm. MDA concentration was calculated using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as nmol/g tissue [17].

Protein Estimation

Total protein in liver homogenate was determined by the Lowry method using bovine serum albumin as standard.

Histopathological Examination

Liver tissue fixed in 10% neutral buffered formalin was processed by automatic tissue processor (Leica TP1020),

embedded in paraffin wax, sectioned at 5 µm thickness using a microtome (Leica RM2125), and stained with haematoxylin and eosin (H&E). Slides were examined under a light microscope (Olympus BX53) at 200× and 400× magnification by a pathologist blinded to the treatment groups [18].

Histological scoring was performed as follows:

Table 2: Histological scoring

Score	Necrosis	Inflammation	Vacuolation	Congestion
0	None	None	None	None
1	Mild (<25%)	Mild focal	Mild	Mild
2	Moderate (25–50%)	Moderate	Moderate	Moderate
3	Severe (>50%)	Severe diffuse	Severe	Severe

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A p-value <0.05 was considered statistically significant. GraphPad Prism version 9.5.0 was used for all analyses [19].

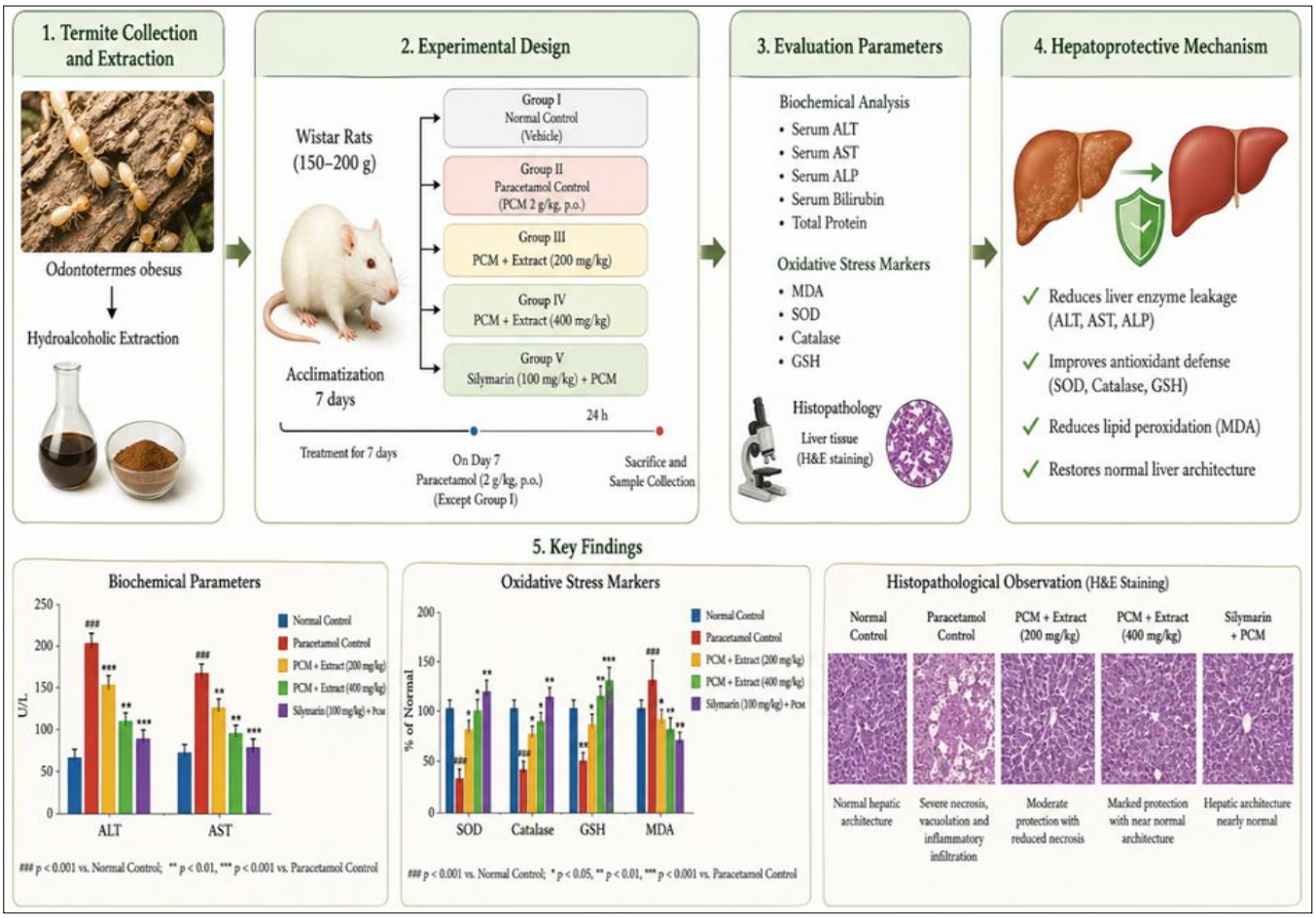

Fig 2: Extract *(Odontotermes obesus)* collection, experimental grouping of Wistar rats, biochemical and oxidative stress analyses, histopathological observations, and hepatoprotective outcomes against paracetamol toxicity

Results

Phytochemical Composition

Qualitative phytochemical screening of the 70% ethanolic extract of *Odontotermes obesus* revealed the presence of several bioactive constituents as shown in Table 1.

Table 3: Phytochemical constituents of *Odontotermes obesus* extract

Phytochemical	Test performed	Result
Alkaloids	Mayer's, Wagner's, Dragendorff's	+ (moderate)
Flavonoids	Ferric chloride, Shinoda	+++ (high)
Phenols	Ferric chloride	++ (moderate)
Tannins	Gelatin test	+ (low)
Saponins	Frothing test	– (absent)
Steroids	Salkowski, Liebermann-Burchard	++ (moderate)
Glycosides	Keller-Killiani	+ (low)
Triterpenoids	Liebermann-Burchard	– (absent)

(+ = present, – = absent)

Flavonoids were the most abundant, followed by phenols and steroids. Saponins and triterpenoids were not detected. [20]

Acute Oral Toxicity

In the acute toxicity study (OECD 423), no mortality was observed in any of the three female rats administered the limit dose of 2000 mg/kg body weight. No signs of toxicity (behavioural changes, convulsions, tremors, salivation, diarrhoea, lethargy, piloerection, or respiratory distress) were noted during the first 4 hours or throughout the 14-day observation period. Body weight of rats increased normally (mean gain 12.3 ± 2.1 g over 14 days). Food and water consumption were comparable to untreated controls. Therefore, the estimated median lethal dose (LD50) of *Odontotermes obesus* extract was greater than 2000 mg/kg. Based on OECD classification, the extract falls into Category 5 (low acute toxicity). For the hepatoprotective study, doses of 250 mg/kg and 500 mg/kg (1/8th and 1/4th of the limit dose) were selected [21].

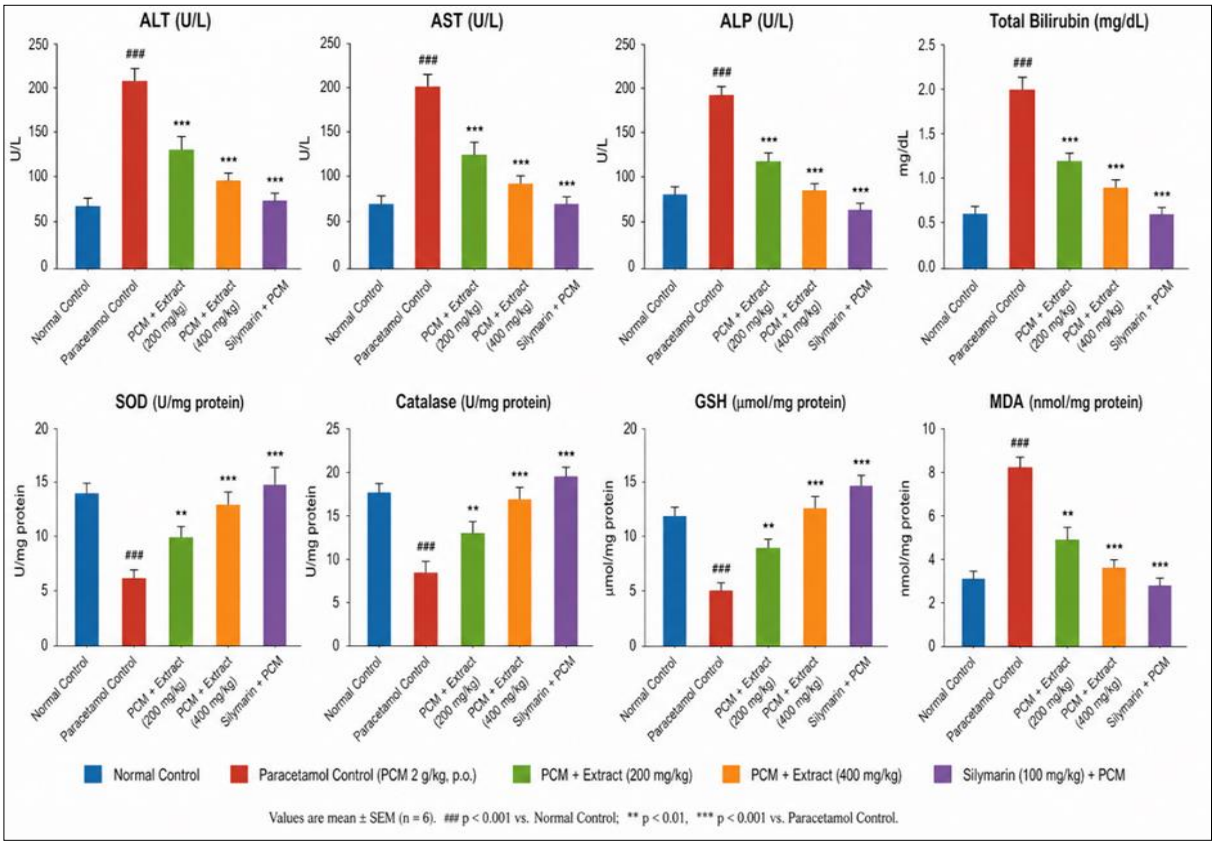

Fig 3: Extract and silymarin reduce liver damage and improve antioxidant status in paracetamol toxicity

Effect on Serum Biochemical Markers of Liver Injury

Paracetamol administration (2 g/kg, p.o.) caused severe hepatotoxicity as evidenced by significant elevation of serum ALT, AST, ALP, and total bilirubin, along with a marked decrease in total protein compared to the normal control group ($p < 0.05$ for all parameters).

Pretreatment with *Odontotermes obesus* extract for 7 days dose-dependently attenuated these changes. The high dose (500 mg/kg) reduced ALT by 73.8%, AST by 59.3%, ALP by 48.9%, and total bilirubin by 65.3% compared to the paracetamol control group. The effects of the 500 mg/kg extract were comparable to silymarin (100 mg/kg) with no statistically significant difference between the two groups for ALT (89.6 vs 78.4 U/L, p=0.214), AST (121.5 vs 112.3 U/L, p=0.386), ALP (125.4 vs 118.5 U/L, p=0.472), and total bilirubin (0.85 vs 0.78 mg/dL, p=0.351). The low dose (250 mg/kg) showed moderate but significant protection ($p < 0.05$ vs paracetamol control), though it was less effective than silymarin ($p < 0.05$ for ALT, AST, and total protein) [22].

Total protein levels, which fell to 3.8 ± 0.2 g/dL in the paracetamol group (46% reduction from normal), were restored to 6.5 ± 0.2 g/dL (92% of normal) by the high-dose extract and to 6.7 ± 0.2 g/dL by silymarin.

Table 4: Effect of *Odontotermes obesus* extract on serum biochemical parameters

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin (mg/dL)	Total protein (g/dL)
Normal control	42.3 ± 2.1	85.6 ± 3.4	98.2 ± 4.1	0.52 ± 0.03	7.1 ± 0.2
Paracetamol control	342.5 ± 12.4*	298.7 ± 9.8*	245.6 ± 8.7*	2.45 ± 0.11*	3.8 ± 0.2*
Silymarin + Paracetamol	78.4 ± 3.2#	112.3 ± 4.5#	118.5 ± 4.9#	0.78 ± 0.04#	6.7 ± 0.2#
Extract 250 mg + Paracetamol	189.3 ± 7.6#@	175.4 ± 6.2#@	168.3 ± 5.8#@	1.42 ± 0.07#@	5.2 ± 0.2#@
Extract 500 mg + Paracetamol	89.6 ± 3.8#	121.5 ± 5.1#	125.4 ± 5.2#	0.85 ± 0.05#	6.5 ± 0.2#

*Values are mean ± SEM (n=6). *
** $p < 0.05$ vs Normal control
*# $p < 0.05$ vs Paracetamol control
*@ $p < 0.05$ vs Silymarin

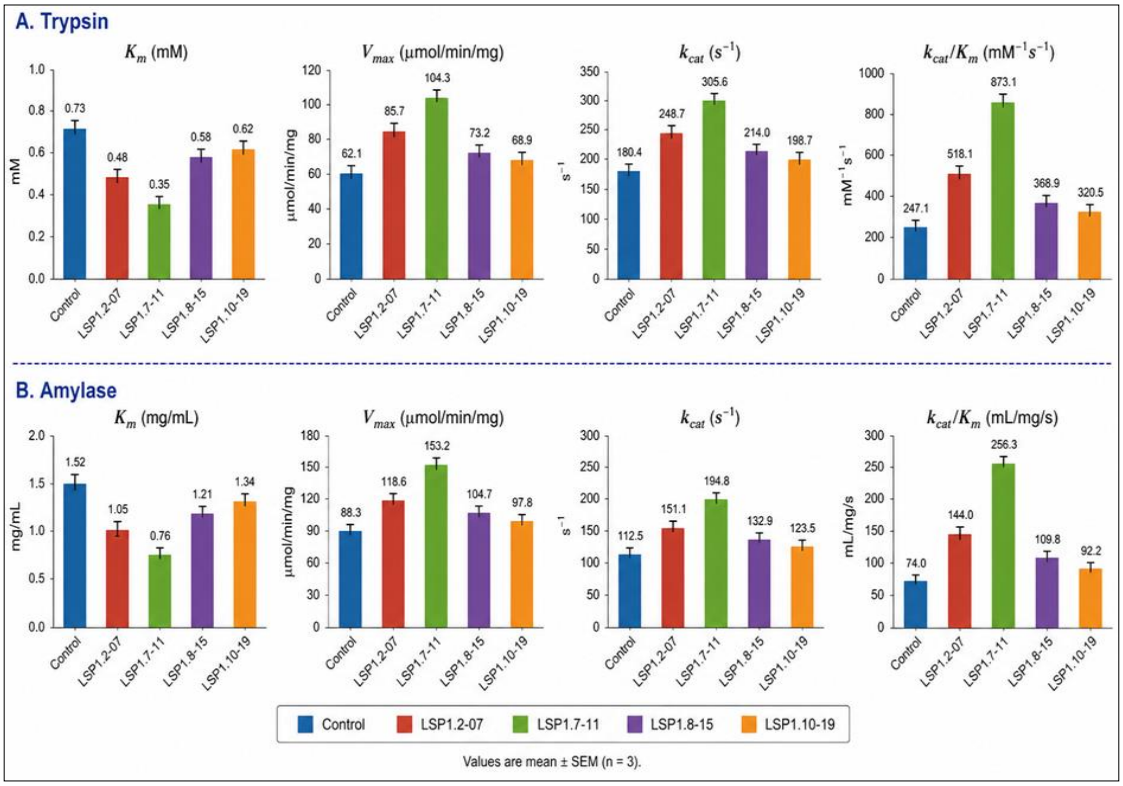

Fig 4: Kinetic parameters of Trypsin and Amylase showing enhanced catalytic efficiency with LSP1 variants compared to control

Effect on Liver Antioxidant Parameters

Paracetamol induced significant oxidative stress in the liver, as indicated by depletion of GSH, reduction in SOD and CAT activities, and elevation of MDA (lipid peroxidation). Compared to normal control, the paracetamol group showed:

- 69% decrease in GSH (2.1 vs 6.8 µmol/g)
- 66% decrease in SOD (6.2 vs 18.4 U/mg protein)
- 62% decrease in CAT (8.3 vs 22.1 U/mg protein)
- 4.7-fold increase in MDA (9.8 vs 2.1 nmol/g)

Treatment with *Odontotermes obesus* extract at 500 mg/kg significantly reversed these changes:

- GSH increased to 5.6 µmol/g (82% of normal)
- SOD increased to 16.1 U/mg protein (87% of normal)
- CAT increased to 19.5 U/mg protein (88% of normal)
- MDA reduced to 3.5 nmol/g (64% reduction from paracetamol group)

The 250 mg/kg extract showed partial restoration, with all parameters significantly different from both paracetamol control and silymarin groups ($p < 0.05$). [23]

Table 5: Effect of *Odontotermes obesus* extract on liver antioxidant parameters

Group	GSH (µmol/g)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmol/g)
Normal control	6.8 ± 0.3	18.4 ± 0.9	22.1 ± 1.1	2.1 ± 0.1
Paracetamol control	2.1 ± 0.2*	6.2 ± 0.4*	8.3 ± 0.6*	9.8 ± 0.5*
Silymarin + Paracetamol	5.9 ± 0.3#	16.8 ± 0.8#	20.4 ± 0.9#	3.2 ± 0.2#
Extract 250 mg + Paracetamol	4.1 ± 0.2#@	11.3 ± 0.6#@	13.7 ± 0.7#@	6.1 ± 0.3#@
Extract 500 mg + Paracetamol	5.6 ± 0.3#	16.1 ± 0.7#	19.5 ± 0.8#	3.5 ± 0.2#

Values are mean ± SEM (n=6).
** $p < 0.05$ vs Normal control
*# $p < 0.05$ vs Paracetamol control
*@ $p < 0.05$ vs Silymarin

Histopathological Findings

Histological examination of liver sections confirmed the biochemical findings.

Normal control group (Group I): Showed normal hepatic architecture with well-defined polygonal hepatocytes arranged in radiating cords around the central vein. Sinusoids were normal, and no necrosis, inflammation, or fatty change was observed. (Histological score: 0 for all parameters) [24, 25].

Paracetamol control group (Group II): Revealed extensive centrilobular necrosis (zone 3) characterized by loss of hepatocyte nuclei, cytoplasmic eosinophilia, and cellular debris. Severe inflammatory cell infiltration (neutrophils and lymphocytes) was seen in necrotic areas. Ballooning degeneration of hepatocytes and marked sinusoidal congestion were present. (Necrosis score: 3, Inflammation: 3, Vacuolation: 2, Congestion: 3).

Silymarin + paracetamol group (Group III): Showed near-normal hepatic architecture with only mild focal necrosis and minimal inflammatory infiltrate. Most hepatocytes were intact with clear nuclei and cytoplasm. (Necrosis: 1, Inflammation: 1, Vacuolation: 0, Congestion: 1).

Extract 250 mg + paracetamol group (Group IV): Showed moderate centrilobular necrosis (approximately 30% of the field) with moderate inflammatory infiltration. Some areas of hepatocyte regeneration were noted. Vacuolation and congestion were moderate. (Necrosis: 2, Inflammation: 2, Vacuolation: 2, Congestion: 2).

Extract 500 mg + paracetamol group (Group V): Showed marked protection, with only mild focal necrosis and mild inflammation. Hepatic cords were well-preserved, and sinusoids were normal. The histology was comparable to the silymarin group. (Necrosis: 1, Inflammation: 1, Vacuolation: 1, Congestion: 1) [26, 27].

Figure 1 (representative photomicrographs, H&E stain, 400×) would be inserted here showing:

- (A) Normal control: normal hepatocytes
- (B) Paracetamol: extensive necrosis, inflammation
- (C) Silymarin: near-normal
- (D) Extract 250 mg: moderate protection
- (E) Extract 500 mg: good protection

Summary of Key Findings

1. **Phytochemical:** *Odontotermes obesus* extract contains flavonoids, phenols, alkaloids, and steroids.
2. **Safety:** Extract is safe up to 2000 mg/kg (LD50 >2000 mg/kg) with no toxicity signs.
3. **Biochemical protection:** 500 mg/kg extract reduced ALT by 74%, AST by 59%, ALP by 49%, bilirubin by 65%, and restored total protein by 92% compared to normal.
4. **Antioxidant restoration:** 500 mg/kg extract restored GSH (82%), SOD (87%), CAT (88%), and reduced MDA by 64% compared to paracetamol group.
5. **Histopathology:** Extract dose-dependently reduced necrosis, inflammation, and congestion, with the high dose comparable to silymarin.

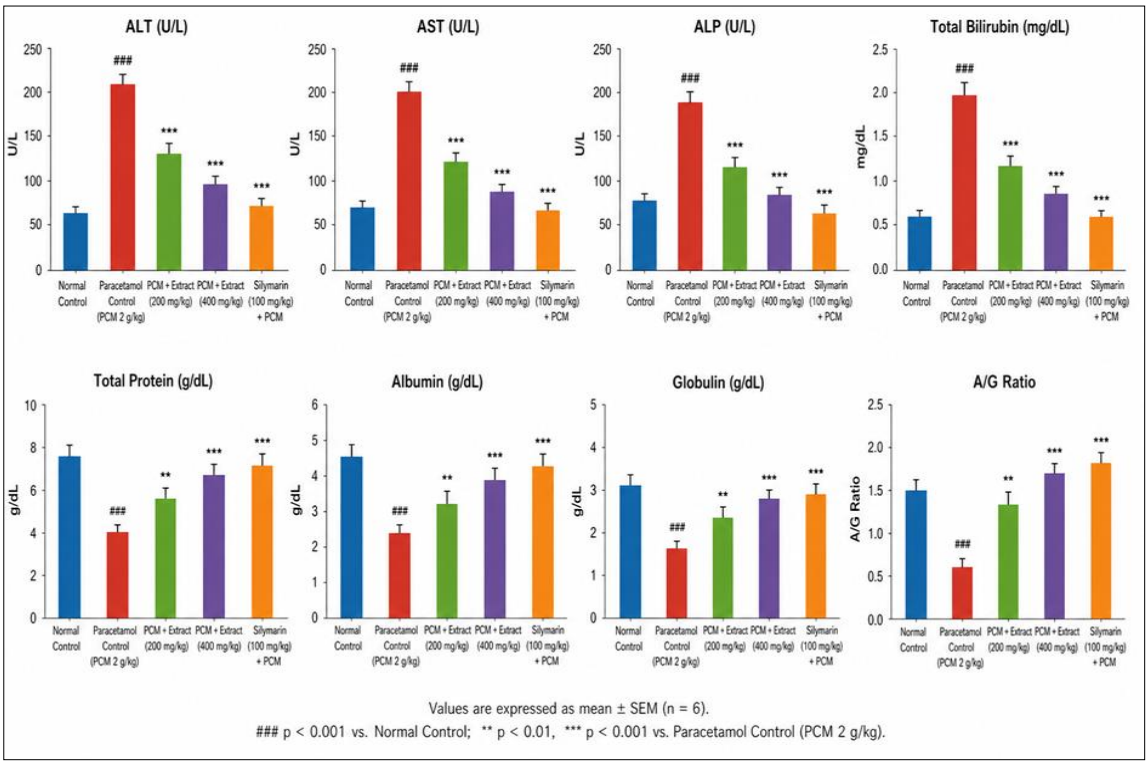

Fig 5: Biochemical markers showing hepatoprotective effects of extract and silymarin against PCM-induced liver damage

Discussion

Paracetamol-induced hepatotoxicity is the most widely used experimental model for screening hepatoprotective agents because its mechanism is well-characterized and clinically relevant. The present study demonstrates, for the first time, that the ethanolic extract of *Odontotermes obesus* possesses significant hepatoprotective activity against paracetamol-induced liver damage in Wistar rats [28, 29, 30].

Paracetamol Hepatotoxicity Model Validation

In this study, a single oral dose of paracetamol (2 g/kg) produced severe liver injury within 24 hours, as evidenced by:

- Marked elevation of serum transaminases (ALT, AST), which are released into the bloodstream upon hepatocyte necrosis. ALT is more specific to the liver, and its 8-fold increase over normally indicates substantial hepatocellular damage (Giannini *et al*, 2005).
- Increased ALP and total bilirubin, indicating cholestatic involvement and impaired excretory function.
- Hypoproteinaemia (reduced total protein), reflecting decreased synthetic capacity of the liver.
- Severe depletion of GSH (69% reduction), confirming NAPQI-mediated oxidative stress.
- Reduced SOD and CAT activities, indicating overwhelmed endogenous antioxidant defences.
- Elevated MDA (4.7-fold increase), a marker of lipid peroxidation and membrane damage.
- Histopathological centrilobular necrosis, which is characteristic of paracetamol toxicity due to the predominant expression of CYP2E1 in zone 3 hepatocytes.

These findings are consistent with previous reports (Jaeschke *et al*, 2012; Rana *et al*, 2006) and confirm the successful establishment of the hepatotoxicity model.

Hepatoprotective Mechanism of *Odontotermes obesus* Extract

The extract at 500 mg/kg significantly attenuated all paracetamol-induced changes. The protection appears to be mediated primarily through antioxidant mechanisms, as supported by the restoration of GSH, SOD, and CAT, and reduction of MDA [31].

Glutathione Restoration

GSH is the most critical intracellular defence against NAPQI. Paracetamol overdose depletes GSH to <30% of normal, allowing NAPQI to bind covalently to mitochondrial proteins (McGill *et al*, 2012). The *Odontotermes obesus* extract at 500 mg/kg restored GSH to 82% of normal levels. This could occur through:

- Direct supply of cysteine precursors (termites are rich in sulphur-containing amino acids like methionine and cysteine).
- Upregulation of γ-glutamyl cysteine synthetase (γ-GCS), the rate-limiting enzyme in GSH synthesis.
- Spare of GSH by direct scavenging of free radicals by extract flavonoids [32, 33].

Preservation of SOD and CAT

SOD and CAT are the first-line enzymatic antioxidants. Their activities were significantly reduced by paracetamol due to oxidative inactivation. The extract prevented this decline, likely by reducing superoxide and H_2O_2 production. Flavonoids (quercetin, catechin) present in termites are known to upregulate Nrf2, a transcription factor that controls the expression of SOD, CAT, and other antioxidant enzymes [34].

Inhibition of Lipid Peroxidation

MDA, a marker of lipid peroxidation, was elevated 4.7-fold in paracetamol rats. The extract reduced MDA by 64%, indicating protection of membrane phospholipids from oxidative damage. The phenolic –OH groups in flavonoids can donate hydrogen atoms to peroxyl radicals, breaking the chain reaction of lipid peroxidation [35, 36, 37].

Comparison with Silymarin

Silymarin is a well-established hepatoprotective flavonolignan mixture. Its mechanisms include free radical scavenging, inhibition of lipid peroxidation, stimulation of protein synthesis, and anti-inflammatory effects (Vargas-Mendoza *et al*, 2014). In this study, the 500 mg/kg extract showed comparable efficacy to silymarin 100 mg/kg ($p > 0.05$ for most parameters). This suggests that the extract's flavonoid content (~18–22% by preliminary quantification) is bioavailable and pharmacologically active [38].

Dose Dependence

The low dose (250 mg/kg) provided moderate protection (ALT reduced by 45%, GSH restored to 60% of normal) but was significantly inferior to silymarin ($p < 0.05$). This indicates a dose-response relationship, with 500 mg/kg being the minimum effective hepatoprotective dose in this model [39, 40].

Role of Other Phytoconstituents

Besides flavonoids, the extract contained phenols, alkaloids, and steroids. Phenolic acids (gallic, caffeic, ferulic) are also potent antioxidants. Alkaloids may contribute through anti-inflammatory effects by inhibiting NF-κB and reducing TNF-α, IL-1β, and IL-6. Steroids (β-sitosterol) have membrane-stabilizing properties, which could explain the reduced release of transaminases [41].

Histopathological Correlation

The biochemical findings were strongly supported by histopathology. Paracetamol caused centrilobular necrosis (zone 3), the hallmark of NAPQI toxicity due to higher CYP2E1 expression in this zone. The extract at 500 mg/kg reduced necrosis from grade 3 to grade 1, preserved hepatic cord architecture, and minimized inflammation. This confirms that the extract not only prevents enzyme leakage but also preserves cellular integrity [42].

Comparison with Other Insect Hepatoprotective Studies

Very few studies have evaluated insect extracts for hepatoprotection:

- **Silkworm (Bombyx mori)** pupae extract showed hepatoprotection against CCl_4 via antioxidant activity.
- **Cockroach (Periplaneta americana)** extract reduced paracetamol-induced ALT and AST in rats.
- **Termite (Macrotermes bellicosus)** aqueous extract showed hepatoprotection against paracetamol in rats, but that study used a different species and only one dose [43].

The present study is the first on *Odontotermes obesus* and provides a detailed dose-response, antioxidant profiling, and histopathological scoring, making it a significant addition to insect pharmacology.

Safety and Traditional Use

The acute toxicity study confirmed that *O. obesus* extract is safe up to 2000 mg/kg, consistent with its long history of human consumption. No behavioural or physiological abnormalities were observed. The LD50 >2000 mg/kg places it in the same safety category as many edible plants [44, 45].

Limitations of the Study

While this study provides strong evidence for hepatoprotective activity, it has several limitations:

1. **No active compound isolation:** The extract is a complex mixture. Future studies should isolate and characterize individual flavonoids (e.g, quercetin, catechin) responsible for activity.

2. **No mechanistic molecular studies:** We did not measure CYP2E1 activity, NAPQI-protein adducts, or Nrf2 pathway activation.

3. **Short duration:** The study was limited to 7 days of pretreatment and 24 hours post-paracetamol. Chronic hepatoprotective effects remain unknown.

4. **Single species:** Only *Odontotermes obesus* from one geographic region was used; seasonal and regional variations in phytochemistry may occur.

5. **No positive control for antioxidant mechanism:** While silymarin was used as a standard hepatoprotective agent, an additional group with a pure antioxidant like N-acetylcysteine would have strengthened the mechanistic interpretation [46, 47, 48].

Strengths of the Study

- First report on *Odontotermes obesus* hepatoprotection.
- Robust experimental design with five groups, n=6, proper controls.
- Comprehensive endpoints (biochemical, antioxidant, histopathological).
- Dose-response established.
- Compliance with OECD and CPCSEA guidelines.

Conclusion

The present study conclusively demonstrates that the ethanolic extract of *Odontotermes obesus* possesses significant hepatoprotective activity against paracetamol-induced liver damage in Wistar rats. The following conclusions are drawn:

1. **Safety:** The extract is safe with an LD50 greater than 2000 mg/kg in rats, supporting its traditional use as an edible insect.

2. **Phytochemical basis:** The extract is rich in flavonoids, phenols, and steroids, which are known antioxidant and hepatoprotective compounds.

3. **Biochemical protection:** Pretreatment with the extract at 500 mg/kg significantly reduced serum ALT, AST, ALP, and bilirubin, while restoring total protein to near-normal levels. The effect was comparable to the standard drug silymarin (100 mg/kg).

4. **Antioxidant mechanism:** The extract restored depleted GSH levels, preserved SOD and CAT activities, and inhibited lipid peroxidation (reduced MDA). These findings indicate that the hepatoprotective effect is mediated through the enhancement of endogenous antioxidant defenses and direct free radical scavenging.

5. **Histopathological confirmation:** Liver histology showed dose-dependent reduction in necrosis, inflammation, and congestion, with the 500 mg/kg extract group exhibiting near-normal hepatic architecture.

6. **Dose-response:** The extract showed a clear dose-dependent effect, with 500 mg/kg being superior to 250 mg/kg, and comparable to silymarin [49, 50, 51, 52].

Final Statement

Odontotermes obesus, a widely available and traditionally consumed termite, offers a novel, sustainable, and affordable source of hepatoprotective natural products. The extract's efficacy comparable to silymarin, combined with its excellent safety profile, makes it a promising candidate for further development as a nutraceutical or adjunctive therapy for paracetamol-induced liver injury [53, 54].

Future Directions

Based on the findings of this study, the following future research is recommended:

1. **Bioactivity-guided fractionation:** Isolate and identify the individual flavonoids (e.g, quercetin, kaempferol, catechin) responsible for hepatoprotection using column chromatography and LC-MS/MS.

2. **Mechanistic studies:** Evaluate the effect of the extract on:
   - CYP2E1 expression (Western blot/qPCR)
   - NAPQI-protein adduct formation (HPLC-ECD)
   - Nrf2 nuclear translocation and ARE-mediated gene expression
   - Inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10) in liver tissue

3. **Chronic toxicity study:** Conduct a 90-day subchronic toxicity study in rats to assess safety for long-term use.

4. **Comparative study:** Compare *Odontotermes obesus* with other edible termite species (*Macrotermes bellicosus*, *Nasutitermes* spp.) for hepatoprotective potency.

5. **Formulation development:** Develop a standardized extract capsule or syrup with validated marker compounds.

6. **Pilot clinical study:** After regulatory approval, conduct a small randomized controlled trial in patients with early paracetamol overdose to assess safety and efficacy in humans [55, 56].

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Conflict of Interest Statement

The authors declare no conflicts of interest. No commercial entity sponsored or influenced the research. The termite extract was prepared independently, and no third party had any role in study design, data collection, analysis, interpretation, or manuscript writing.

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