Int J Biochem Mol Biol 2025;16(1):1-15
www.ijbmb.org /ISSN:2152-4114/IJBMB0163412

Original Article

Hepatoprotective and antioxidant effects of *Celosia trigyna* and *Euphorbia hirta* in mitigating paracetamol-induced liver toxicity: bridging ethnomedicine and modern pharmacology

Tunbosun Emmanuel Akinboboye[1], Temitope Deborah Olaniyi[1], Gbadebo E Adeleke[1], Abiodun Bukunmi Aborisade[1,2], Adewale Adetutu[1]

[1]*Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria;* [2]*Department of Medical Biochemistry, Osun State University, Osun State, Nigeria*

Received January 18, 2025; Accepted March 16, 2025; Epub April 15, 2025; Published April 30, 2025

Abstract: Background: Paracetamol is a widely used over-the-counter drug for pain relief and fever management. However, its misuse through chronic overuse or acute overdose presents significant risks to human health, primarily causing hepatotoxicity and systemic oxidative stress. Methodology: This study evaluated the hepatoprotective, antioxidant, and anti-inflammatory effects of aqueous leaf extracts of *Celosia trigyna* and *Euphorbia hirta* in mitigating paracetamol-induced liver damage in male Wistar rats. Results: Paracetamol administration (150 mg/kg) significantly elevated liver function markers (ALT, AST, ALP, and bilirubin), oxidative stress parameters (MDA), and inflammatory cytokines (IL-6 and TNF-α), while depleting antioxidant defenses (SOD and GSH). Disrupted lipid profiles were also observed in the paracetamol-only group. Pretreatment with Celosia trigyna and Euphorbia hirta extracts (125 mg/kg and 250 mg/kg) effectively ameliorated these effects by normalizing liver function markers, reducing oxidative stress and inflammation, and restoring lipid profiles. Molecular docking identified bioactive compounds such as rutin, quercetin, and kaempferol as potent inhibitors of Glutathione-S-Transferase, Tumor Necrosis Factor-alpha, and Cytochrome P450, with binding affinities of -9.3, -7.2, and -8.3 kcal/mol, respectively. These interactions underpin the antioxidant and anti-inflammatory activities observed *in vivo*. Conclusion: These findings suggest that *Celosia trigyna* and *Euphorbia hirta* have the potential to serve as natural prophylactic or therapeutic agents for mitigating paracetamol toxicity. Further research is required to isolate their active compounds and explore their synergistic potential with conventional treatments. This study bridges traditional medicine and modern pharmacology, offering innovative approaches to managing drug-induced liver.

Keywords: Paracetamol-induced hepatotoxicity, oxidative stress, inflammation, *Celosia trigyna*, *Euphorbia hirta*, and hepatoprotective agents

Introduction

Paracetamol (acetaminophen) is one of the most widely used over-the-counter drugs globally, primarily for its analgesic and antipyretic properties. Despite its clinical efficacy, paracetamol abuse, whether through chronic overuse or acute overdose, poses a significant risk to human health, particularly liver function [1]. It is one of the leading causes of drug-induced liver injury (DILI) worldwide, contributing to acute liver failure and long-term hepatic complications [2].

Paracetamol is used as an analgesic and antipyretic and is one of the drugs that can cause liver damage [3, 4]. Paracetamol is generally sold freely on the market because its purchase does not require a doctor's prescription, so people can buy and consume it directly [5].

Paracetamol-induced hepatotoxicity is mediated by the excessive production of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). At therapeutic doses, NAPQI is efficiently detoxified by hepatic glutathione. However, in cases of overdose or prolonged misuse, gluta-

https://doi.org/10.62347/CLHF2294

thione stores are depleted, leading to oxidative stress, mitochondrial dysfunction, and hepatocyte necrosis [6].

The liver is susceptible to damage due to exposure to toxic substances. Factors that cause liver damage include viral infections, drug exposure, auto immune disorders and metabolite disorders [7-9].

Oxidative stress not only damages hepatocytes but also disrupts lipid metabolism and triggers inflammatory responses, further compounding liver injury [10]. For individuals with preexisting liver conditions or those exposed to chronic paracetamol misuse, the health risks extend beyond the liver to systemic complications such as cardiovascular and metabolic disorders [11].

The growing burden of paracetamol abuse underscores the need for effective prophylactic or therapeutic interventions. While synthetic hepatoprotective drugs such as N-acetylcysteine are available, they are not without limitations, including adverse effects and limited efficacy in chronic conditions [1]. This has spurred interest in exploring natural remedies derived from medicinal plants.

Celosia trigyna and Euphorbia hirta have been traditionally used in ethnomedicine for their therapeutic properties, including antioxidants, anti-inflammatory, and hepatoprotective effects [12]. *Celosia trigyna* is known for its high content of flavonoids, phenolics, and other bioactive compounds that exert protective effects against oxidative damage in liver tissues. Studies have shown that its aqueous extracts enhance antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase (CAT), which mitigate oxidative stress-induced hepatocellular damage. Moreover, *Celosia trigyna* has been reported to regulate inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), reducing hepatic inflammation and improving liver function parameters. *Euphorbia hirta*, widely used for its medicinal benefits, is rich in phytochemicals, including tannins, flavonoids, and polyphenols, which possess significant hepatoprotective properties. Its extracts have been found to attenuate paracetamol-induced liver damage by modulating oxidative stress, enhancing glutathione levels, and reducing lipid peroxidation.

Euphorbia hirta also exhibits anti-inflammatory effects by downregulating pro-inflammatory mediators and upregulating protective signaling pathways involved in hepatocyte regeneration. Additionally, it aids in stabilizing liver enzyme markers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), preventing hepatocellular degeneration and fibrosis. In the context of paracetamol abuse, *Celosia trigyna* and *Euphorbia hirta* offer promising hepatoprotective potential. Anti-inflammatory and pro-inflammatory processes are involved in developing liver disorders, suggesting that IL-6 may produce a noteworthy effect in the advance of liver diseases via immunosuppressive regulations [13, 14]. Chronic paracetamol exposure not only exacerbates oxidative stress but also leads to dysregulation of inflammatory cytokines such as TNF-α and IL-6, which contribute to the progression of liver injury [15]. Interventions that target these interconnected pathways could mitigate the risks associated with paracetamol misuse.

This study evaluates the hepatoprotective, antioxidant, and anti-inflammatory effects of aqueous extracts of *Celosia trigyna* and *Euphorbia hirta* in a rat model of paracetamol-induced liver injury. By assessing key biochemical markers, oxidative stress parameters, lipid profiles, inflammatory cytokines, and histological changes, this research aims to provide insight into the therapeutic potential of these plants. The findings may offer a foundation for developing plant-based prophylactic or therapeutic interventions to address the human health risks associated with paracetamol abuse and chronic drug exposure, bridging the gap between traditional medicine and modern pharmacology.

Materials and methods

Reagents and chemicals

Reagents including phosphate buffer, carbonate buffer, aqueous extracts, and laboratory kits for AST, ALT, ALP, and bilirubin determination were sourced from Sigma Chemical Co. and Bridge Biotech Ltd. Additional reagents like hydrogen peroxide, TCA, and ethanol were analytical grade to ensure result accuracy.

Table 1. Experimental protocol

Group	Treatment	Details
A	Normal Control	Feed and water only
B	Negative Control	Paracetamol-only (150 mg/kg)
C	Standard Control	Paracetamol + Silymarin (10 mg/100 g)
D	*Euphorbia hirta* (Low Dose)	Paracetamol + Euphorbia hirta (125 mg/kg)
E	*Euphorbia hirta* (High Dose)	Paracetamol + Euphorbia hirta (250 mg/kg)
F	*Celosia trigyna* (Low Dose)	Paracetamol + Celosia trigyna (125 mg/kg)
G	*Celosia trigyna* (High Dose)	Paracetamol + Celosia trigyna (250 mg/kg)

Plant material collection and identification

Celosia trigyna and Euphorbia hirta were collected from Odo Adi and the LAUTECH Anatomy Department, Ogbomosho, Nigeria. The plants were authenticated by a taxonomist in the Department of Pure and Applied Biology, LAUTECH. Leaves were air-dried, powdered, and prepared for extraction.

Preparation of aqueous extract

Air-dried *Celosia trigyna* and *Euphorbia hirta* leaves (250 g each) were powdered and soaked in 2,500 mL distilled water for three days. Filtration was performed using Whatman No. 1 paper, and the filtrates were concentrated using a rotary evaporator at 35°C. LD50 tests confirmed the non-toxic nature of both plants, as their values exceeded 5,000 mg/kg.

Experimental animals

Thirty-five male Wistar albino rats (150-180 g) were obtained from LAUTECH's Animal House. Animals were acclimatized for two weeks and divided into seven groups (n = 5). Ethical standards for animal use in research were adhered to.

Experimental design

The hepatoprotective effects of *Euphorbia hirta* and *Celosia trigyna* were evaluated using a paracetamol-induced hepatotoxicity model. The experimental groups and treatments are summarized in **Table 1**.

Administration protocol

Paracetamol (150 mg/kg) was administered via gavage for seven days. *Euphorbia hirta* and *Celosia trigyna* extracts were administered orally using a stomach tube. Silymarin served as standard control.

Animal sacrifice

Ethical standards for animal use in research were adhered to. At the end of the experiment, animals were humanely euthanized using cervical dislocation following appropriate ethical guidelines to minimize pain and distress.

Biochemical assays

Biochemical markers for liver function (ALT, AST, ALP, and bilirubin) were determined using commercial kits and spectrophotometric methods following the procedures outlined by Evans [16] and Schumann *et al*. [17]. Lipid profiles, including triglycerides, HDL, and total cholesterol, were analyzed as per Allain *et al*. [18]. Oxidative stress markers such as malondialdehyde (MDA) and reduced glutathione (GSH) were assessed using standard protocols described by Khoubnasabjafari *et al*. [19] and Ellman [20].

Alanine (ALT) and aspartate aminotransferases (AST)

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities were assessed using Sigma manufacturer's kit method. A 0.5 mL substrate mixture - DL-Aspartate (R1a) with α-ketoglutarate for ALT and DL-Alanine (R1b) with α-ketoglutarate for AST - was incubated at 37°C for 5 minutes. Then, 100 µL of serum was added, and the reaction was incubated for 30 minutes. Next, 125 µL of R2 (2,4-Dinitrophenylhydrazine) was introduced and left at room temperature for 20 minutes. The color was developed by adding 1.25 mL of 0.4 N NaOH, and absorbance was measured at 505 nm. Enzyme activities were expressed as IU/L using calibration curves generated with pyruvate calibrators (0.0-0.5 mL) following the same procedure but replacing the serum with the calibrator.

Determination of alkaline phosphatase (ALP) and bilirubin

Determination of biochemical parameter

Alkaline phosphatase (ALP) and bilirubin levels were measured in serum using commercial test kits from Randox Laboratories, UK, following the manufacturer's instructions. The procedures were conducted in accordance with the methods described by Sun et al. [21] and Kanu et al. [22].

Glutathione (GSH) assay

The spectrophotometric determination of GSH was based on Ellman's method [20]. In this method, 5,5'-dithiobis-(2-nitrobenzoic acid) is reduced by sulfhydryl (SH) groups, producing 2-nitro-5-mercaptobenzoic acid, which has an intense yellow color measurable at 412 nm.

Plasma/serum sample preparation: 50 µL of Protein Precipitation Reagent was added to 200 µL of plasma/serum in a centrifuge tube, mixed thoroughly, and centrifuged at 3000 rpm for 10 minutes. The clear supernatant was used for the assay.

Tissue homogenate preparation: 0.5 g of tissue was rinsed with PBS, homogenized in 2.5 mL of Protein Precipitation Reagent, and centrifuged at 3000 rpm for 10 minutes. The supernatant was used for the assay. A 2 mM GSH standard was used to prepare calibration standards immediately before use.

Superoxide dismutase (SOD) assay

The SOD activity was determined based on its ability to inhibit the autoxidation of pyrogallol in the presence of EDTA at pH 8.2. The method relies on the competition between pyrogallol autoxidation by superoxide ($O2^{\bullet-}$) and the dismutation of this radical by SOD.

The whole blood sample preparation: 0.5 mL of heparinized or EDTA-treated whole blood was centrifuged at 3500 rpm for 10 minutes, and the plasma was removed. The red blood cells were washed four times with 5 mL of 0.9% saline, centrifuging at 3500 rpm after each wash. The washed erythrocytes were resuspended in 2.0 mL of cold distilled water, mixed, and left at 4°C for 15 minutes. The supernatant was used for the assay.

Tissue sample preparation: Tissue samples were homogenized in 10 volumes of 50 mmol/L phosphate buffer (pH 7.4) (e.g., 0.5 g tissue in 5 mL buffer) and centrifuged at 3000 rpm for 20 minutes. The collected supernatant was used for the assay.

Calculation

Determine the change in absorbance of samples and blank using the following equation

$$\% \text{ inhibition of Pyrogallol autoxidation} = \frac{\Delta S \times 100\%}{\Delta C}$$

$$\text{SOD activity in (U/ml)} = \frac{\% \text{ inhibition of Pyrogallol autoxidation}}{50\%}$$

The malondialdehyde (MDA) assay is based on the reaction of MDA with 2-thiobarbituric acid at 25°C, forming a chromophore with a maximum absorbance at 532 nm via Knoevenagel-type condensation. For reagent preparation, the acid reagent was thawed at 4°C and left at room temperature for one hour before use. The indicator solution was prepared by dissolving the contents of one vial of indicator powder in 10 mL of the acid reagent and shaken until fully dissolved, which was sufficient for standards and 20 samples. The MDA standard stock solution (20 µM) was prepared immediately before use by diluting 20 µL of 10 mM MDA in 9.98 mL of distilled water.

For sample preparation, plasma samples were deproteinized using an acid, centrifuged, and filtered through a 0.2 µm syringe filter to remove cloudiness. In urine samples, interference from colored compounds was eliminated by running a sample blank for each test. Blood samples were collected and processed following standard procedures. A saturated ammonium sulfate solution was prepared, and 100 µL was added to 0.5 mL of serum or plasma. Subsequently, 35 mg of trichloroacetic acid (TCA) was added, and the mixture was vortexed to form a precipitate. The samples were then centrifuged, and the clear supernatant was collected for analysis without dilution.

Serum cholesterol levels were determined using a commercial kit according to the manufacturer's protocol. A 10 µL aliquot of serum was mixed with 1 mL of reagent containing buffer and enzymes, incubated at 37°C for 5 minutes, and absorbance was measured at 505 nm. Cholesterol concentration (mg/dL) was calculated using a standard curve based on the ab-

sorbance difference between the sample and standard. Similarly, triglyceride levels were measured using a commercial kit, where 10 µL of serum was incubated with reagent containing buffer and enzyme at room temperature for 10 minutes. Absorbance was read at 546 nm, and triglyceride concentration was determined using a standard curve.

High-density lipoprotein (HDL) cholesterol was assessed using a precipitation method with a commercial kit. Five microliters of serum were mixed with a precipitating reagent, incubated, and centrifuged. The supernatant was collected, and absorbance was measured at 700 nm. The concentration of HDL cholesterol (mg/dL) was calculated by comparing the absorbance of the sample with that of a known standard.

The determination of rat interleukin-6 (IL-6) levels was performed using a sandwich ELISA kit. The microplate wells were pre-coated with an antibody specific to rat IL-6. Standards or samples were added, followed by a biotinylated detection antibody and an avidin-horseradish peroxidase (HRP) conjugate. After washing to remove unbound components, a substrate solution was added. Wells containing IL-6 turned blue, and upon addition of a stop solution, the color changed to yellow. Optical density (OD) was measured at 450 nm (±2 nm), and IL-6 concentration was determined by comparing the OD of the samples with the standard curve.

Similarly, rat tumor necrosis factor-alpha (TNF-α) levels were quantified using a sandwich ELISA kit. Microplate wells were pre-coated with an antibody specific to TNF-α. Standards or samples were added, followed by a biotinylated detection antibody and an avidin-HRP conjugate. After washing, a substrate solution was introduced, turning wells containing TNF-α blue. The reaction stopped with a stop solution, changing the color to yellow. Optical density was measured at 450 nm (±2 nm), and TNF-α concentrations were determined by comparing the OD values of the samples with the standard curve.

Molecular docking study

Molecular docking simulations were performed to evaluate the binding affinities of bioactive compounds derived from *Celosia trigyna* and *Euphorbia hirta* against specific protein targets. The study focused on the interactions of compounds with Glutathione-S-Transferase (GST), Tumor Necrosis Factor-alpha (TNF-α), Bcl-2-associated X protein (BAX), Cytochrome P450 (CYP2E1), and Peroxisome Proliferator-Activated Receptor Alpha (PPAR-α).

Ligand preparation

Compounds were retrieved from the PubChem database in 3D SDF format and converted into PDB format using OpenBabel. Ligands were prepared by optimizing their geometry and minimizing energy using the MMFF94 force field in AutoDockTools.

Protein preparation

Target proteins were retrieved from the RCSB Protein Data Bank. Water molecules were removed, and polar hydrogens were added using AutoDockTools. The proteins were then converted into PDBQT format.

Docking protocol

AutoDock Vina was used to perform docking simulations. Grid boxes were centered on the active sites of the target proteins, with dimensions chosen to accommodate the ligands fully. Binding affinities (kcal/mol) were recorded for each ligand-target interaction. The number of hydrogen bonds and other interactions were analyzed using PyMOL and Discovery Studio Visualizer. Binding affinities, hydrogen bonding interactions, and molecular interactions were analyzed to identify the most potent bioactive compounds.

Statistical analysis

Data were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's post-hoc test. Statistical significance was set at $P < 0.05$. Analyses were performed using SPSS version 21.0 and GraphPad Prism.

Results

Monitoring of body and liver weight of rats

Table 2 presents the body weight and liver-to-body weight ratio changes in experimental animals following administration of aqueous leaf extracts of *Celosia trigyna* and *Euphorbia hirta*, and paracetamol. A significant increase ($P < 0.05$) in liver-to-body weight ratio was obser-

Table 2. Change in the body and liver relative weights of experimental rats treated with aqueous extracts of *Celosia trigyna*

Group	Weight gain (g)	Liver to body weight ratio
Group A: Normal Control	9.16±1.04[a]	3.99±0.05[a]
Group B: Paracetamol (150 mg/kg b.w)	12.89±0.19[b]	5.01±0.09[b]
Group C: Paracetamol (150 mg/kg b.w) + Sylimarin (2 mg/kg b.w)	9.49±0.33[a]	4.04±0.18[a]
Group D: Paracetamol (150 mg/kg b.w) + *Celosia trigyna* (125 mg/kg b.w)	10.15±0.26[a]	4.17±0.03[a]
Group E: Paracetamol (150 mg/kg b.w) + *Celosia trigyna* (250 mg/kg b.w)	9.82±0.44[a]	4.14±0.46[a]

Values are mean ± S.E.M (n = 5). a, b: Different alphabets superscripts denote significant difference at $P < 0.05$.

Table 3. Change in the body and liver relative weights of experimental rats treated with aqueous extracts of *Euphorbia hirta*

Group	Weight gain (g)	Liver to body weight ratio
Group A: Normal Control	9.16±1.04[a]	3.99±0.05[a]
Group B: Paracetamol (150 mg/kg b.w)	12.89±0.19[b]	5.01±0.09[b]
Group C: Paracetamol (150 mg/kg b.w) + Sylimarin (2 mg/kg b.w)	9.49±0.33[a]	4.04±0.18[a]
Group F: Paracetamol (150 mg/kg b.w) + *Euphorbia hirta* (125 mg/kg b.w)	2.65±0.57[a]	3.94±0.21[a]
Group G: Paracetamol (150 mg/kg b.w) + *Euphorbia hirta* (250 mg/kg b.w)	8.84±0.26[a]	3.79±0.17[a]

Values are mean ± S.E.M (n = 5). a, b: Different alphabets superscripts denote significant difference at $P < 0.05$.

ved in the paracetamol-only group (150 mg/kg body weight) compared to the normal control group. Pretreatment with extracts of *Celosia trigyna* and *Euphorbia hirta* at doses of 125 mg/kg and 250 mg/kg effectively mitigated this increase, demonstrating their protective effect. Specifically, the higher dose (250 mg/kg) showed a more pronounced effect in normalizing the relative liver weight compared to untreated controls (**Table 3**).

Effects on liver function parameters

The administration of paracetamol (150 mg/kg body weight) resulted in a significant increase ($P < 0.05$) in serum activities of ALT, AST, and ALP, along with total and direct bilirubin concentrations, compared to the normal control group (**Tables 4** and **5**). Pretreatment with aqueous extracts of *Celosia trigyna* and *Euphorbia hirta* dose-dependently reduced these elevated markers. Groups pre-administered 250 mg/kg extracts of *Celosia trigyna* or *Euphorbia hirta* demonstrated levels comparable to the normal control, indicating a dose-dependent hepatoprotective effect.

Lipid profile alterations

As shown in **Table 3**, paracetamol administration significantly ($P < 0.05$) increased serum concentrations of triglycerides (TAG) and VLDL while reducing HDL, LDL, and total cholesterol compared to the normal control group. Treatment with *Celosia trigyna* and *Euphorbia hirta* extracts at 250 mg/kg significantly restored lipid profiles, normalizing TAG and VLDL levels while improving HDL and LDL concentrations, consistent with the normal control group (**Tables 6** and **7**).

Oxidative stress markers

Table 8 summarizes the oxidative stress parameters. Paracetamol administration led to a significant decrease ($P < 0.05$) in hepatic SOD activity and GSH levels, alongside an increase in MDA concentrations, indicative of oxidative damage. Pretreatment with *Celosia trigyna* and *Euphorbia hirta* extracts significantly increased SOD and GSH levels while reducing MDA levels in a dose-dependent manner, reflecting their antioxidant properties (**Tables 8** and **9**).

Inflammatory markers

Figures 1 and **2** show the effects of the extracts on inflammatory markers IL-6 and TNF-α. The paracetamol-only group exhibited significantly elevated levels of these markers ($P < 0.05$), indicating inflammation. Pretreatment with *Celosia trigyna* and *Euphorbia hirta* extracts sig-

Table 4. Effects of Paracetamol on selected hepatic parameters of rats treated with aqueous extracts of *Celosia trigyna*

Group	AST (µ/l)	ALT (µ/l)	ALP (µ/l)	Total Bilirubin (mg/dl)	DirectBilirubin (mg/dl)
Group A: Normal Control	0.87±0.00[a]	1.16±0.29[a]	1.54±0.15[a]	0.27±0.18[a]	0.27±0.03[a]
Group B: Paracetamol (150 mg/kg b.w)	2.90±0.0.38[b]	3.20±0.29[b]	4.03±0.12[b]	5.23±0.51[b]	0.69±0.11[b]
Paracetamol (150 mg/kg b.w) + Sylimarin (2 mg/kg b.w)	0.87±0.00[a]	0.87±0.00[a]	1.84±0.46[a]	0.21±0.05[a]	0.25±0.01[a]
Group D: Paracetamol (150 mg/kg b.w) + *Celosia trigyna* (125 mg/kg b.w)	1.20±0.29[a]	0.8730±0.00[a]	1.84±0.46[a]	0.05±0.03[a]	0.11±0.01[a]
Group E: Paracetamol (150 mg/kg b.w) + *Celosia trigyna* (250 mg/kg b.w)	1.20±0.29[a]	1.16±0.30[a]	1.54±0.15[a]	0.17±0.12[a]	0.07±0.01[a]

Values were expressed as Mean ± SEM. a, b: Different alphabets superscripts denote significant difference at $P < 0.05$.

Table 5. Effects of Paracetamol on selected hepatic parameters of rats treated with aqueous extracts of *Euphorbia hirta*

Group	AST (µ/l)	ALT (µ/l)	ALP (µ/l)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Group A: Normal Control	0.87±0.00[a]	1.16±0.29[a]	1.54±0.15[a]	0.27±0.18[a]	0.27±0.03[a]
Group B: Paracetamol (150 mg/kg b.w)	2.90±0.0.38[b]	3.20±0.29[b]	4.03±0.12[b]	5.23±0.51[b]	0.69±0.11[b]
Group C: Paracetamol (150 mg/kg b.w) + Sylimarin (2 mg/kg b.w)	0.87±0.00[a]	0.87±0.00[a]	1.84±0.46[a]	0.21±0.05[a]	0.25±0.01[a]
Group F: Paracetamol (150 mg/kg b.w) + *Euphorbia hirta* (125 mg/kg b.w)	1.16±0.29[a]	1.16±0.30[a]	1.84±0.46[a]	0.22±0.11[a]	0.12±0.02[a]
Group G: Paracetamol (150 mg/kg b.w) + *Euphorbia hirta* (250 mg/kg b.w)	1.46±0.30[a]	1.16±0.29[a]	2.0±0.41[a]	0.24±0.06[a]	0.11±0.04[a]

Values were expressed as Mean ± SEM. a, b: Different alphabets superscripts denote significant difference at $P < 0.05$.

Table 6. Effects of paracetamol on lipid profile parameters in experimental rats treated with aqueous extracts of *Celosia trigyna*

Group	TAG (mg/dl)	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group A: Normal Control	118±5.26[a]	607±28.57[a]	0.81±0.01[a]	595±28.04[a]	23.64±1.05[a]
Group B: Paracetamol (150 mg/kg b.w)	657±126.6[b]	97.00±0.70[a]	0.72±0.01[a]	61.78±0.01[b]	131.4±18.01[b]
Group C: Paracetamol (150 mg/kg b.w) + Silymarin (2 mg/kg b.w)	203.50±47.48[a]	571.00±12.10[a]	0.82±0.02[a]	550.00±7.70[a]	40.7±9.49[a]
Group D: Paracetamol (150 mg/kg b.w) + *Celosia trigyna* (125 mg/kg b.w)	214.20±5.96[a]	599.00±10.39[a]	0.83±0.03[b]	577.00±9.17[a]	42.84±1.19
Group E: Paracetamol (150 mg/kg b.w) + *Celosia trigyna* (250 mg/kg b.w)	196.60±57.24[a]	622.00±17.38[a]	0.83±0.02[b]	602.00±23.3[a]	39.32±11.45[a]

a, b: Different alphabets superscripts denote significant difference at $P < 0.05$.

Table 7. Effects of paracetamol on lipid profile parameters in experimental rats treated with aqueous extracts of *Euphorbia hirta*

Group	TAG (mg/dl)	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal Control	118±5.26[a]	607±28.57[a]	0.81±0.01[a]	595±28.04[a]	23.64±1.05[a]
Paracetamol (150 mg/kg b.w)	657±126.6[b]	97.00±0.70[b]	0.72±0.01[b]	30.89±0.01[b]	131.4±18.01[b]
Paracetamol (150 mg/kg b.w) + Silymarin (2 mg/kg b.w)	203.50±47.48[a]	571.00±12.10[a]	0.82±0.02[a]	550.00±7.70[a]	40.7±9.49[a]
Paracetamol (150 mg/kg b.w) + *Euphorbia hirta* (125 mg/kg b.w)	226.10±28.30[a]	642.00±14.21[a]	0.83±0.02[a]	618.50±8.53[a]	45.22±5.66[a]
Paracetamol (150 mg/kg b.w) + *Euphorbia hirta* (250 mg/kg b.w)	193.90±0.87[a]	671.50±28.07[a]	0.83±0.01[a]	651.00±27.98[a]	38.78±0.18[a]

a, b: Different alphabets superscripts denote significant difference at $P < 0.05$.

Table 8. Effects of paracetamol on antioxidant indices in experimental animals pretreated with aqueous extract of *Celosia trigyna*

Group	MDA (μ/ML)	SOD (μ/ML)	GSH (μ/ML)
Normal Control	3.25±0.25[a]	1.51±0.34[a]	3.36±0.18[a]
Paracetamol (150 mg/kg b.w)	4.50±0.43[c]	1.35±0.05[b]	2.65±0.53[b]
Paracetamol (150 mg/kg b.w) + Silymarin (2 mg/kg b.w)	3.50±0.36[a]	1.35±0.05[b]	3.04±0.18[a]
Paracetamol (150 mg/kg b.w) + *Celosia trigyna* (125 mg/kg b.w)	4.00±0.25[b]	1.60±0.32[a]	2.73±0.03[b]
Paracetamol (150 mg/kg b.w) + *Celosia trigyna* (250 mg/kg b.w)	3.75±0.00[a]	1.25±0.26[b]	2.78±0.15[b]

Values were expressed as Mean ± SEM. a, b, c: Different alphabets superscripts denote significant difference at $P < 0.05$.

Table 9. Effects of paracetamol on antioxidant indices in experimental animals pretreated with aqueous extract of *Euphorbia hirta*

Group	MDA (μ/ML)	SOD (μ/ML)	GSH (μ/ML)
Group A: Normal Control	3.25±0.25[a]	1.51±0.34[a]	3.36±0.18[a]
Group B: Paracetamol (150 mg/kg b.w)	4.50±0.43[c]	1.35±0.05[b]	2.65±0.53[b]
Group C: Paracetamol (150 mg/kg b.w) + Silymarin (2 mg/kg b.w)	3.50±0.36[a]	1.35±0.05[b]	3.04±0.18[a]
Group D: Paracetamol (150 mg/kg b.w) + *Euphorbia hirta* (125 mg/kg b.w)	4.00±0.25[b]	1.60±0.32[a]	2.73±0.03[b]
Group E: Paracetamol (150 mg/kg b.w) + *Euphorbia hirta* (250 mg/kg b.w)	3.25±0.25[a]	1.51±0.34[a]	3.36±0.18[a]

Values are expressed as Mean ± SEM. a, b, c: Different alphabet superscripts denote significant differences at $P < 0.05$. MDA: Malondialdehyde; SOD: Superoxide Dismutase; GSH: Glutathione.

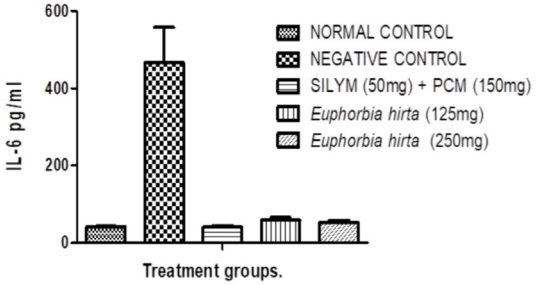

Figure 1. Effects of aqueous leaf extract of *Euphorbia hirta* on level of (A) IL-6 and (B) TNF-α of paracetamol-induced liver damage in male Wistar rats Values were expressed as mean ± SEM (n = 5). Different alphabet superscripts denote significant difference ($P < 0.05$). IL-6 = Interleukin-6, TNF-α = Tumor Necrosis Factor-Alpha.

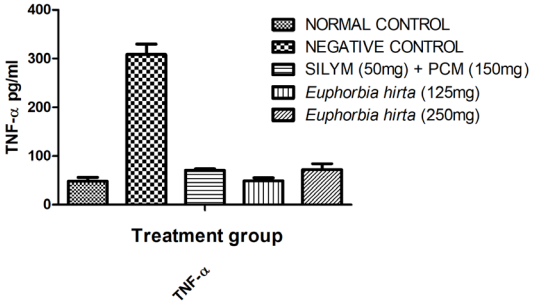

Figure 2. Effects of aqueous leaf extract of *Euphorbia hirta* on level of (A) IL-6 and (B) TNF-α of paracetamol-induced liver damage in male Wistar rats Values were expressed as mean ± SEM (n = 5). Different alphabet superscripts denote significant difference ($P < 0.05$). IL-6 = Interleukin-6, TNF-α = Tumor Necrosis Factor-Alpha.

nificantly reduced the levels of IL-6 and TNF-α, suggesting their anti-inflammatory potential.

Binding affinities and interactions

The docking results demonstrated varying binding affinities of bioactive compounds from *Celosia trigyna* and *Euphorbia hirta* against the target proteins. Compounds such as rutin, quercetin, and kaempferol exhibited the strongest binding affinities across multiple targets, suggesting their potential as significant bioactive molecules. **Table 10** summarizes the key docking results:

Celosia trigyna-derived compounds such as rutin and kaempferol showed superior binding affinities, particularly with GST and CYP2E1, indicating their potential antioxidative properties (**Figures 3**-**5**). *Euphorbia hirta* compounds, including chlorogenic acid and procyanidin, exhibited strong interactions with PPAR-α and BAX (**Figures 6** and **7**), suggesting their role in modulating apoptosis and inflammation. Hydrogen bonding interactions were prominent in rutin and chlorogenic acid, with up to five bonds observed, reinforcing their stability within the active sites of the target proteins.

Table 10. Binding affinities of bioactive compounds from *Celosia trigyna* and *Euphorbia hirta* against the target proteins

Ligand	Target Protein	Binding Affinity (kcal/mol)	Hydrogen Bonds
Rutin	GST	-9.3	5
Quercetin	TNF-α	-7.2	4
Kaempferol	CYP2E1	-8.3	3
Chlorogenic Acid	BAX	-7.6	3
Procyanidin	PPAR-α	-9.4	2

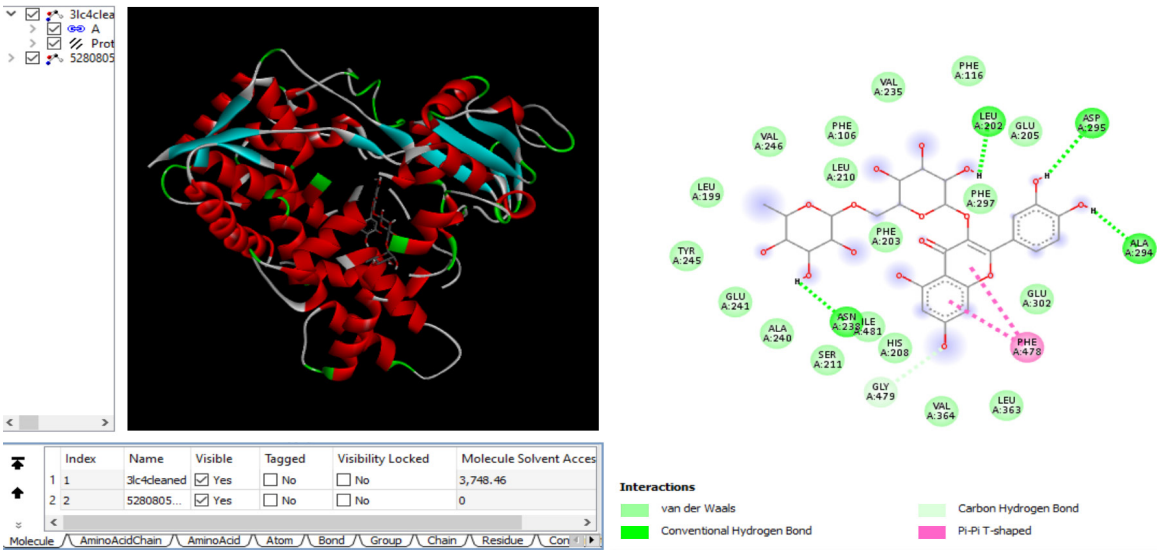

Figure 3. Interaction Rutin3D_COMPOUND_CID_5280805 with 3lc4 protein.

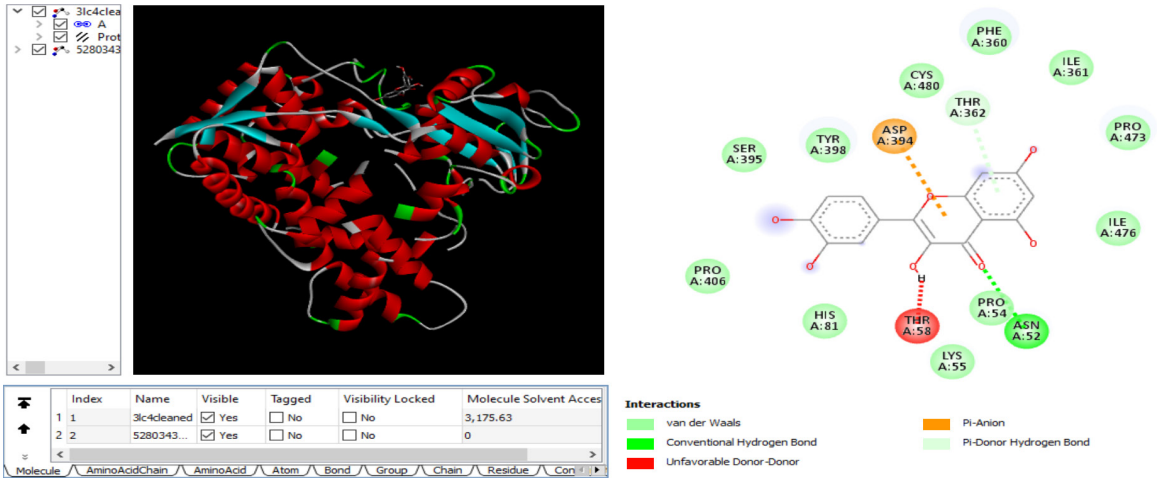

Figure 4. Interaction Quercetin3D_COMPOUND_CID_5280343 with 3lc4 protein.

Discussion

Paracetamol, widely used as an over-the-counter analgesic and antipyretic, is considered safe when used within therapeutic doses. However, both acute overdose and chronic misuse can lead to severe hepatotoxicity and systemic oxidative stress, posing significant human health risks [1]. The free radicals formed will cause necrosis and are secondary disorders as

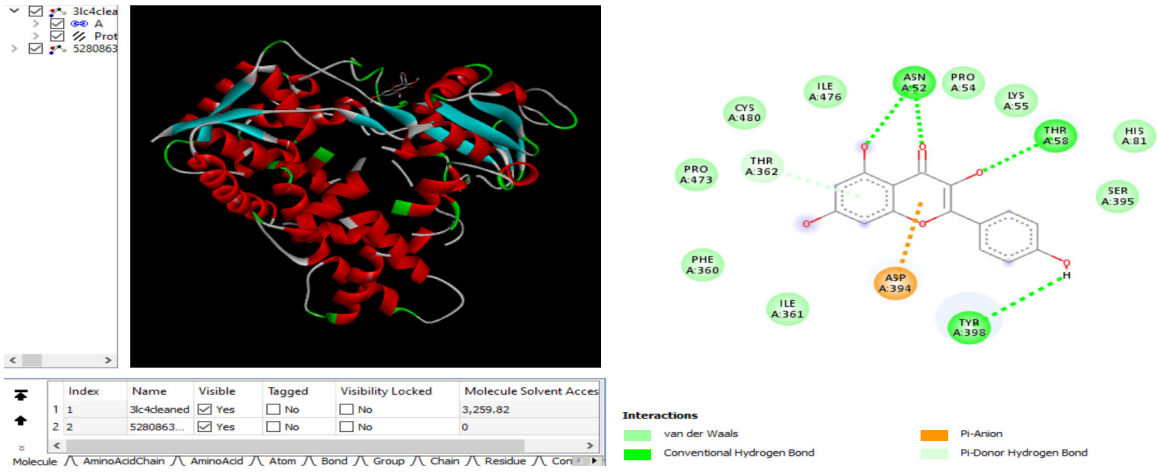

Figure 5. Interaction Kaempferol3D_COMPOUND_CID_5280863 with 3lc4 protein.

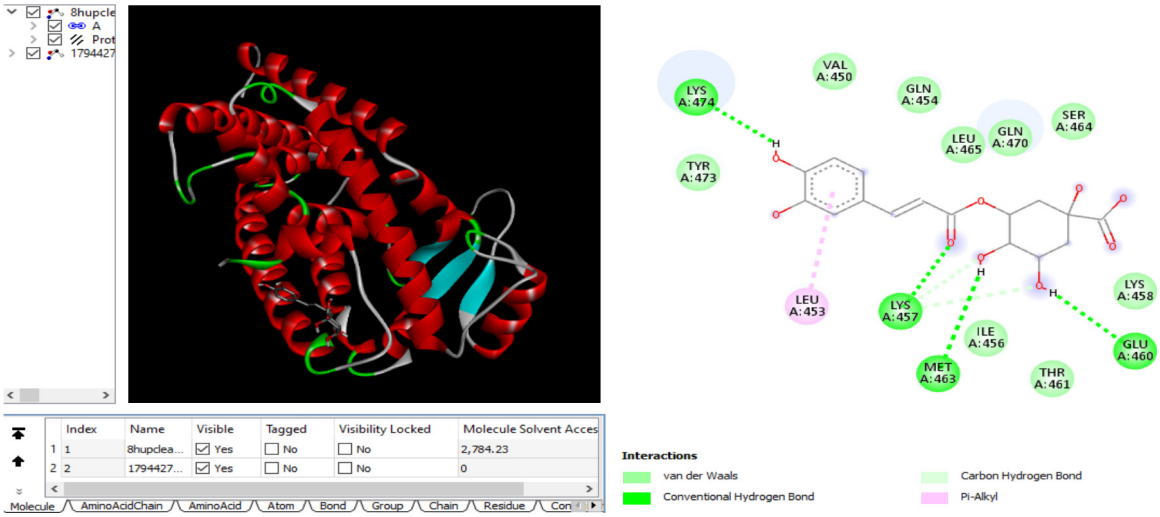

Figure 6. Interaction Chlorogenic Acid3D_COMPOUND_CID_1794427 with 8hup protein.

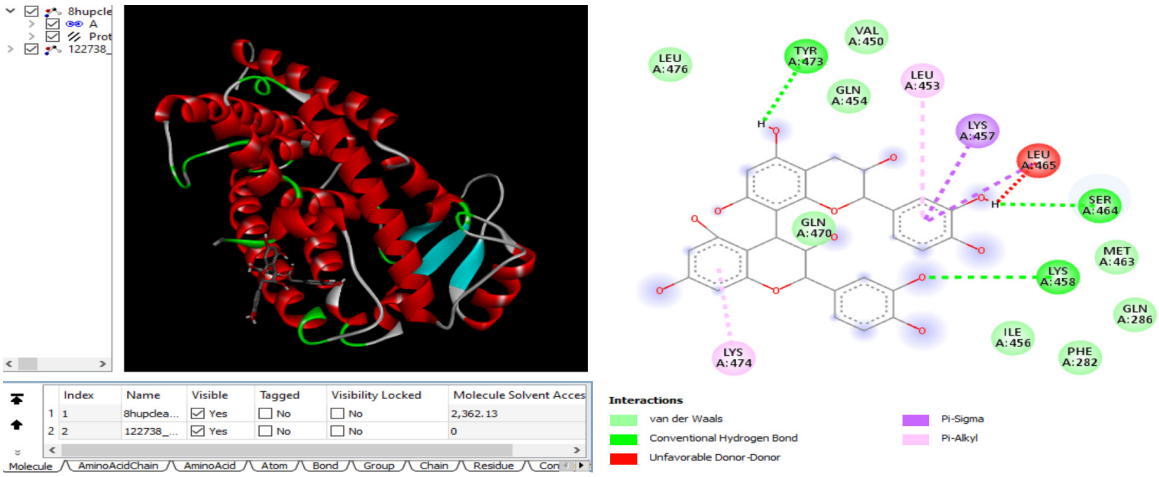

Figure 7. Interaction Procyanidin B23D_COMPOUND_CID_122738 with 8hup protein.

an effect of lipid peroxidation or injury conditions in cells that can cause premature death of cells and living tissues [23, 24].

This study evaluated the hepatoprotective and antioxidant properties of *Celosia trigyna* and *Euphorbia hirta* in mitigating paracetamol-induced liver damage, offering potential medicinal benefits for the prevention or treatment of liver dysfunction caused by drug toxicity.

The results demonstrate that both *Celosia trigyna* and *Euphorbia hirta* extracts provided significant protection against paracetamol-induced hepatotoxicity, as evidenced by the normalization of liver-to-body weight ratios and reductions in serum levels of liver function markers (ALT, AST, ALP, and bilirubin). Paracetamol administration caused a notable increase in liver-to-body weight ratios and elevated serum markers, indicating hepatocellular damage [2]. Hepatotoxicity occurs due to increased ROS (Reactive Oxygen Species) and oxidative stress, which causes damage to liver cell function [25]. This is consistent with the established mechanism of paracetamol-induced hepatotoxicity, which involves excessive production of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), leading to glutathione depletion, oxidative stress, and mitochondrial dysfunction [6].

Increased ALT, AST, and ALP levels in hepatotoxic-induced rats indicate damage to the integrity or function of liver cells due to paracetamol poisoning [26-28]. The administration of *Celosia trigyna* and *Euphorbia hirta* extracts, particularly at higher doses (250 mg/kg), reversed these alterations, like the effects of silymarin, a known hepatoprotective agent [29]. These findings suggest that the bioactive compounds in these plants, such as flavonoids, phenolics, and alkaloids, may act by scavenging free radicals, boosting endogenous antioxidant defenses, or modulating liver enzyme activities [12].

The antioxidant effects of *Celosia trigyna* and *Euphorbia hirta* were evident in their ability to normalize oxidative stress markers such as malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH). Given that SOD, CAT, and GSH play vital roles in safeguarding organisms against the detrimental effects of oxidative stress. They can prevent and scavenge excessive reactive oxygen species accumulation (ROS) [30]. Paracetamol-induced oxidative stress, as reflected by elevated MDA levels and depleted SOD and GSH, underscores the role of lipid peroxidation and reduced antioxidant capacity in liver injury [10]. Treatment with plant extracts effectively reduced MDA levels while restoring SOD and GSH, highlighting their potential to mitigate oxidative damage.

The observed dose-dependent improvements in oxidative stress parameters emphasize the plants' role in restoring redox homeostasis. This is critical because oxidative stress is a key mediator of liver damage and a driver of systemic complications such as inflammation and apoptosis [31]. It is well known that elevated oxidative stress, in the absence of proper antioxidant defense, might activate signaling pathways that cause inflammation and cell death, ultimately leading to tissue damage and liver failure [32].

Paracetamol administration significantly disrupted lipid metabolism, as seen in elevated triglycerides (TAG) and very low-density lipoprotein (VLDL) levels and reduced high-density lipoprotein (HDL) and total cholesterol (TC) concentrations. These changes reflect impaired hepatic lipid regulation and increased cardiovascular risks associated with chronic paracetamol misuse [11]. Treatment with *Celosia trigyna* and *Euphorbia hirta* extracts normalized lipid profiles, suggesting their potential to protect against paracetamol-induced dyslipidemia. The restoration of lipid parameters may be attributed to the plants' bioactive compounds, which likely modulate lipid metabolism pathways and reduce oxidative damage to lipoproteins. This is particularly relevant for individuals with underlying metabolic disorders or predisposing factors for cardiovascular diseases.

Elevated levels of inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), were observed following paracetamol administration. Chronic inflammation exacerbates liver damage and contributes to the progression of liver fibrosis and cirrhosis [15]. The significant reductions in IL-6 and TNF-α levels following treatment with *Celosia trigyna* and *Euphorbia hirta* indicate their anti-inflammatory potential, likely mediated by the inhibition of pro-inflammatory signaling path-

ways [33]. This anti-inflammatory activity is crucial for mitigating the systemic effects of paracetamol toxicity, which extend beyond the liver to other organs and tissues.

The docking simulations identified rutin, quercetin, and kaempferol as the most promising candidates based on their strong binding affinities and interaction profiles. These findings align with their known biological activities, including antioxidant, anti-inflammatory, and hepatoprotective effects. For instance, rutin's high affinity for GST (-9.3 kcal/mol) suggests it could modulate detoxification pathways, an essential function in combating oxidative stress.

The interactions of procyanidin with PPAR-α (-9.4 kcal/mol) highlight its potential role in lipid metabolism and inflammation regulation. Similarly, chlorogenic acid interactions with BAX (-7.6 kcal/mol) suggest a role in modulating apoptosis, which is crucial in protecting hepatocytes from paracetamol-induced toxicity. Bax-associated proteins can either stimulate or block apoptosis, and the interaction between proteins from competing factions determines whether the cell survives or undergoes programmed cell death [34]. These computational findings provide a molecular basis for the hepatoprotective effects observed in the *in vivo* experiments. The potent binding of compounds to GST and TNF-α correlates with the observed reduction in oxidative stress markers and inflammatory mediators in treated groups. Moreover, the docking results support the choice of *Celosia trigyna* and *Euphorbia hirta* as promising candidates for developing hepatoprotective therapies.

The interplay between paracetamol toxicity and the protective effects of *Celosia trigyna* and *Euphorbia hirta* has significant implications for human health. Chronic or acute misuse of paracetamol remains a major concern due to its widespread availability and potential for hepatotoxicity. This study highlights the need for prophylactic strategies and adjunctive therapies to mitigate these risks. The findings suggest that *Celosia trigyna* and *Euphorbia hirta* could serve as potential phytotherapeutic agents for managing liver injury associated with paracetamol abuse [35]. Their ability to enhance antioxidant defenses, reduce inflammation, and restore normal liver function markers underscores their therapeutic value. Moreover, these plants may offer a safer alternative to synthetic drugs, particularly for populations with limited access to conventional medical treatments [36].

Conclusion and future directions

The hepatoprotective and antioxidant properties of *Celosia trigyna* and *Euphorbia hirta* highlight their potential as natural remedies for paracetamol toxicity. Further research is needed to isolate active compounds, ensure safety, and evaluate efficacy. Exploring synergy with conventional treatments could enhance their application against liver damage. These findings bridge traditional medicine and modern pharmacology for liver health.

Acknowledgements

The authors would like to express our sincere gratitude to Dr Oladipo Kolawole for generously allowing us to utilize certain laboratory software essential to the completion of our research project.

Disclosure of conflict of interest

None.

Abbreviations

ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BAX, Bcl-2-associated X protein; CYP2E1, Cytochrome P450 2E1; GSH, Reduced Glutathione; GST, Glutathione-S-Transferase; HDL, High-Density Lipoprotein; IL-6, Interleukin-6; LD50, Median Lethal Dose; LDL, Low-Density Lipoprotein; MDA, Malondialdehyde; NAPQI, N-acetyl-p-benzoquinone imine; PPAR-α, Peroxisome Proliferator-Activated Receptor Alpha; ROS, Reactive Oxygen Species; SOD, Superoxide Dismutase; TAG, Triglycerides; TNF-α, Tumor Necrosis Factor-Alpha; VLDL, Very Low-Density Lipoprotein.

Address correspondence to: Adewale Adetutu, Department of Biochemistry, Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso 210214, Oyo State, Nigeria. E-mail: aadetutu@lautech.edu.ng

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