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Research Article

Roasted Chicory Root Preparation Attenuates Early Paracetamol-Induced Liver Injury in Rabbits: A Preliminary *In Vivo* Study With Supportive CYP2E1-Related Computational Analysis

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Abstract

Background: Paracetamol overdose causes acute liver injury partly through the cytochrome P450 2E1 (CYP2E1)-mediated formation of the reactive metabolite *N*-acetyl-*p*-benzoquinone imine. Chicory (*Cichorium intybus* L.) contains polyphenolic compounds with potential protective properties; however, the molecular interactions of these compounds with CYP2E1 and their relevance to biological responses observed after administration of roasted chicory root preparation remain to be experimentally validated. **Methods:** Ten polyphenolic compounds previously reported in chicory root were computationally evaluated against a homology-modelled rabbit CYP2E1 structure as a hypothesis-generating analysis using PyRx 0.9.8 with AutoDock Vina. Physicochemical, Lipinski, and ADMET properties were also predicted. Eighteen New Zealand rabbits were randomly allocated to control, paracetamol, and chicory plus paracetamol groups (n = 6/group). Paracetamol was administered orally at 1 g/kg body weight. The chicory plus paracetamol group received roasted chicory root preparation (150 mg starting-root equivalent per animal), followed by oral administration of paracetamol (1 g/kg body weight). After 4 h, serum hepatic, lipid-associated, and renal biochemical parameters were evaluated, and relative CYP2E1 gene expression was assessed by quantitative real-time PCR. Data were analyzed using one-way analysis of variance followed by Tukey's multiple-comparison test. **Results:** Caffeic acid and gallic acid showed the most favourable predicted CYP2E1 binding energies of −7.90 and −7.83 kcal/mol, respectively, with root mean square deviation (RMSD) values of 0.73 and 0.95 Å, respectively. Caffeic acid interacted with GLU-102, whereas gallic acid interacted with ASN-367 and ASN-220. Both compounds were predicted to have high gastrointestinal absorption. Paracetamol increased serum alanine aminotransferase from approximately 44 to 52 IU/L, whereas chicory co-administration reduced serum levels to approximately 35 IU/L ($p < 0.05$ versus paracetamol). Relative CYP2E1 expression increased from approximately 1.00-fold in controls to 1.75-fold following paracetamol administration and decreased to approximately 0.48-fold after chicory co-administration ($p < 0.05$ versus paracetamol). Changes in alkaline phosphatase, ferritin, lipid-associated parameters, and renal markers were variable and less conclusive. These findings indicate predicted interactions only and do not demonstrate confirmed CYP2E1 inhibition. **Conclusions:** Roasted chicory root preparation attenuated the early paracetamol-associated increase in serum ALT and was associated with reduced hepatic CYP2E1 gene expression in rabbits. Computational analysis identified caffeic acid and gallic acid as potential CYP2E1-interacting compounds; however, their presence in the administered preparation and their functional effects on CYP2E1 require further validation.

Keywords: acetaminophen; drug-induced liver injury; cytochrome P-450 CYP2E1; plant extracts; phenolic acids; cichorium; hepatoprotective agents; rabbits

1. Introduction

Drug-induced liver injury (DILI) remains a major concern in clinical pharmacology because of its diverse mechanisms, variable clinical presentation, and potential progression to acute liver failure [1]. Paracetamol, also known as acetaminophen, is widely used as an analgesic and antipyretic and is generally safe at recommended doses. However, overdose can produce severe hepatocellular injury and is an important cause of acute liver failure. A multicentre prospective study attributed 42% of acute liver failure cases in the evaluated cohort to paracetamol-associated liver injury, demonstrating its continuing clinical importance [2].

Paracetamol-induced hepatotoxicity involves cytochrome P450-mediated formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), followed by glutathione depletion, mitochondrial oxidative stress, and hepatocellular injury [3]. Under normal conditions, NAPQI is detoxified through conjugation with hepatic glutathione. Following overdose, excessive NAPQI formation depletes glutathione and promotes covalent protein binding, oxidative stress, mitochondrial dysfunction, nuclear DNA fragmentation, and hepatocellular necrosis [4]. Cytochrome P450 enzymes contribute to paracetamol bioactivation, with CYP2E1 representing a

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major component of this pathway. Experimental deletion of CYP1A2 and CYP2E1 markedly reduced NAPQI formation and paracetamol toxicity in mice, whereas restoration of functional human CYP2E1 in CYP2E1-null mice increased susceptibility to paracetamol-associated hepatotoxicity [5,6]. CYP2E1 is therefore a pharmacologically relevant molecular target for examining interventions that may influence the early response to paracetamol overdose.

Although *N*-acetylcysteine is the established antidote for paracetamol poisoning, its effectiveness is greatest when administered early, and additional approaches remain of interest for limiting oxidative and metabolic injury. Plant-derived phenolic compounds have received attention because they may influence antioxidant defence and cellular stress pathways. Caffeic acid has reduced paracetamol-induced liver injury in experimental models through activation of Keap1–Nrf2-regulated antioxidant responses and inhibition of ERK1/2-mediated early growth response-1 signalling [7,8]. Recent experimental findings have demonstrated that chlorogenic acid can promote hepatic recovery following acetaminophen-induced liver injury through activation of Nrf2-associated antioxidant responses and enhancement of fatty acid β-oxidation [9]. These findings suggest additional potential hepatoprotective mechanisms beyond CYP450-associated drug metabolism. These findings support investigation of phenolic-rich botanical preparations while also indicating that their effects may involve several pathways rather than direct CYP2E1 inhibition alone.

Plant-derived medicines may contribute to detoxification responses through several complementary mechanisms, including modulation of antioxidant defence systems, regulation of xenobiotic metabolism, reduction of oxidative stress, and support of endogenous cellular protective pathways [10,11]. However, the biological effects of botanical preparations depend on their chemical composition, extraction procedures, dose, and experimental context. Therefore, mechanistic interpretation of plant-derived interventions requires integration of phytochemical characterization with biological evaluation.

Chicory (*Cichorium intybus* L.) is a member of the Asteraceae family and is used as a food ingredient, medicinal plant, and roasted coffee substitute. The biological properties of Cichorium intybus have been associated with its diverse phytochemical constituents, including inulin, sesquiterpene lactones, hydroxycoumarins, and polyphenolic compounds, which have attracted increasing interest for their potential pharmacological applications [12]. Experimental studies have reported hepatoprotective effects of Cichorium intybus preparations in different liver injury models. More recently, Pathak et al. [13] demonstrated that C. intybus leaf extract attenuated experimentally induced hepatic injury, oxidative stress, and histopathological alterations, supporting further investigation of the plant's hepatoprotective potential. However, these studies used preparations, doses, treatment durations, and experimental models that differed from the acute roasted-root preparation evaluated in the present study.

Phytochemical investigations demonstrate that chicory contains several hydroxycinnamic-acid derivatives. Chlorogenic acid is distributed across chicory tissues, whereas 3,5-dicaffeoylquinic acid has been reported to accumulate predominantly in roots [14]. Recent phytochemical investigations have identified chlorogenic acid (5-O-caffeoylquinic acid), 3,5-dicaffeoylquinic acid, and other mono- and dicaffeoylquinic acid derivatives in chemically characterized extracts of Cichorium intybus roots [15,16]. Processing conditions are important when evaluating chicory-derived preparations because roasting can modify the composition and physicochemical characteristics of chicory roots. These considerations emphasize the importance of chemically characterizing roasted preparations before attributing biological effects to particular phytochemical constituents [17]. Consequently, compounds selected from published phytochemical studies should be regarded as literature-derived candidates unless their presence and concentration are analytically confirmed in the administered preparation.

Computational screening provides a practical approach for comparing the potential interactions of multiple natural compounds with a molecular target. AutoDock Vina predicts ligand-binding poses and provides scoring estimates that can be used for comparative ranking [18]. Physicochemical evaluation according to Lipinski-related criteria and prediction of absorption, distribution, metabolism, and excretion (ADME) properties can provide additional information regarding the drug-like behaviour of candidate molecules [19,20]. Nevertheless, docking scores and ADMET predictions are hypothesis-generating approaches and require experimental confirmation.

Accordingly, the present study combined computational screening and an animal model to explore potential CYP2E1-associated responses following administration of roasted chicory root preparation during acute paracetamol exposure. Ten candidate polyphenolic compounds selected from published literature on *C. intybus* were evaluated against a homology-modelled rabbit CYP2E1 structure using molecular docking, physicochemical characterization, Lipinski analysis, and ADMET prediction. The biological response to the complete roasted chicory root preparation was subsequently examined in a 4 h paracetamol-induced acute liver injury model in New Zealand rabbits through assessment of hepatic biochemical markers, lipid-associated parameters, renal biomarkers, and relative CYP2E1 gene expression.

Table 1. Protein sequence of rabbit CYP2E1 obtained from NCBI.

Sr.#	Protein	Length	Protein sequence
1	Cytochrome P450 2E1 of *Oryctolagus cuniculus* [P08682]	493 aa	MAVLGITVALLGWMVILLFISVWKQIHSSWNLPPGPFPLPI IGNLLQLDLKDIPKSFGRLAERFGPVFTVYLGSRRVVVLHG YKAVREMLLNHKNEFSGRGEIPAFREFKDKGIIFNNGPTWK DTRRFSLTTLRDYGMGKQGNEDRIQKEAHFLLEELRKTQG QPFDPTFVIGCTPFNVIAKILFNDRFDYKDKQALRLMSLFN ENFYLLSTPWLQVYNNFSNYLQYMPGSHRKVIKNVSEIKE YTLARVKEHHKSLDPSCPRDFIDSLLIEMEKDKHSTEPLYTL ENIAVTVADMFFAGTETTSTTLRYGLLILLKHPEIEEKLHEE IDRVIGPSRMPSVRDRVQMPYMDAVVHEIQRFIDLVPSNLP HEATRDTTFQGYVIPKGTVVIPTLDSLLYDKQEFPDPEKFKP EHFLNEEGKFKYSDYFKPFSAGKRVCVGEGLARMELFLLL SAILQHFNLKPLVDPEDIDLRNITVGFGRVPPRYKLCVIPRS

2. Materials and Methods

2.1 In Silico Study

2.1.1 Retrieval and Homology Modelling of Rabbit CYP2E1

The amino acid sequence of rabbit cytochrome P450 2E1 (CYP2E1; *Oryctolagus cuniculus*; accession number P08682), comprising 493 amino acids, was retrieved and used for structural modelling (Table 1). A three-dimensional model of rabbit CYP2E1 was generated using the SWISS-MODEL homology-modelling server [21]. The human CYP2E1 template P05181.1.A was selected for model construction. The generated model showed 96% query coverage, 79.11% sequence similarity, and a Global Model Quality Estimate (GMQE) value of 0.96 (Table 2). The stereochemical quality of the model was assessed using Ramachandran plot analysis, and additional structural validation was performed using the UCLA–DOE SAVES v6.0 server. The validated model was subsequently used as the receptor for molecular docking analysis.

Table 2. Structural descriptors for evaluating model quality.

Protein	Template ID	Query (%)	Similarities (%)	GMQE
Rabbit *CYP2E1*	P05181.1.A	96	79.11	0.96

2.1.2 Physicochemical Characterization of CYP2E1

The physicochemical properties of rabbit CYP2E1 were calculated using the ProtParam tool available through the ExPASy server [22]. The evaluated parameters included the number of amino acids, molecular weight, theoretical isoelectric point, negatively and positively charged residues, extinction coefficient, instability index, aliphatic index, and grand average of hydropathicity.

2.1.3 Selection and Preparation of Polyphenolic Ligands

Ten polyphenolic compounds previously reported in chicory (*Cichorium intybus* L.) root were selected from the published literature [14,23,24,25,26]. The selected compounds were caffeic acid, gallic acid, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, isochlorogenic acid, protocatechuic acid, *p*-coumaric acid, chicoric acid, and 3,5-dicaffeoylquinic acid. These compounds were evaluated as literature-derived candidate constituents because their presence and concentrations were not analytically confirmed in the roasted chicory preparation administered to the animals.

The three-dimensional chemical structures, PubChem compound identifiers, and isomeric SMILES of the selected compounds were retrieved from the PubChem database [27]. The ligand structures were imported into PyRx, energy-minimized, and converted into the docking-compatible PDBQT format. The possible influence of physiological pH-dependent ionization states and alternative protonation/tautomeric forms of phenolic compounds was not separately evaluated. Therefore, predicted binding energies should be interpreted with consideration of this limitation. Their PubChem identifiers and isomeric SMILES are provided in **Supplementary Table 1**.

2.1.4 Molecular Docking Analysis

Molecular docking of the selected polyphenolic compounds against rabbit CYP2E1 was performed using PyRx version 0.9.8 (https://pyrx.sourceforge.io/), which incorporates the AutoDock Vina docking algorithm [18,28]. The prepared receptor and ligand structures were imported into PyRx and converted into PDBQT format. The receptor active-site region was identified within PyRx, and the corresponding search space was selected for docking. The docking grid was defined around the predicted CYP2E1 binding region generated by PyRx; however, because the heme prosthetic group was not incorporated, the grid was not defined relative to the heme iron.

AutoDock Vina generated alternative ligand conformations and ranked the resulting receptor–ligand complexes according to their predicted binding energies. For each compound, the pose with the most favourable, or most negative, Vina score was selected as the representative docking configuration. Binding energies were expressed in kcal/mol.

The docking results were evaluated according to binding energy and root mean square deviation. Binding energy was used to compare the predicted interaction affinity of the compounds, whereas RMSD was used to assess variation among the generated docking poses. Compounds with favourable binding-energy and RMSD profiles were selected for further interaction analysis.

The selected receptor–ligand complexes were examined using BIOVIA Discovery Studio Visualizer 2025 (Dassault Systèmes, San Diego, CA, USA). Two-dimensional and three-dimensional interaction profiles were generated to identify interacting amino acid residues, hydrogen-bonding interactions, interaction types, and bond distances. Caffeic acid and gallic acid, which showed the most favourable docking profiles, were selected for detailed molecular-interaction analysis.

The docking workflow was performed using the generated CYP2E1 protein model. The incorporation of the heme prosthetic group and its spatial relationship with the docking grid were not experimentally reconstructed in the present computational workflow. Therefore, the docking results were interpreted as comparative ligand-interaction predictions rather than evidence of catalytic inhibition.

2.1.5 Drug-Likeness and ADMET Prediction

The drug-likeness properties of the selected polyphenols were evaluated according to Lipinski's rule of five [19]. The assessed parameters included molecular weight, number of hydrogen-bond acceptors, number of hydrogen-bond donors, and octanol–water partition coefficient.

Predicted absorption, distribution, metabolism, and excretion properties were evaluated using the SwissADME web server, accessed on 6 August 2026 [20]. The assessed parameters included gastrointestinal absorption, blood–brain barrier permeability, P-glycoprotein substrate status, skin permeation expressed as log Kp, and predicted inhibition of CYP1A2, CYP2C9, CYP2D6, CYP2C19, and CYP3A4.

Predicted acute oral toxicity classes were obtained using the ProTox-II web server, accessed on 6 August 2026 [29]. The predicted toxicity classes were used for comparative assessment of the selected compounds. SwissADME and ProTox-II findings were interpreted as computational predictions rather than experimentally confirmed pharmacokinetic or toxicological properties. ProTox-II is the current version of the platform and is supported by its 2024 methodology publication.

2.2 In Vivo Study

2.2.1 Experimental Animals

The experimental protocol was approved by the Institutional Ethics Committee of Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan, under approval number PSF/NSLP/P-CUVAS (923), and all approved animal-care and experimental procedures were followed.

Eighteen healthy New Zealand White rabbits (Oryctolagus cuniculus), comprising equal numbers of males and females (nine of each sex), approximately 2.5–3.5 months of age and weighing 0.5–1.8 kg, were included in the study. The animals were obtained from a local supplier in Bahawalpur, Pakistan, and maintained at the experimental animal facility of Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur. Before experimentation, the rabbits were acclimatized to laboratory conditions for seven days. During the experimental period, the animals were housed individually in standard stainless-steel cages under controlled environmental conditions, including a temperature of 25 ± 2 °C, relative humidity of 60–65%, and a 12 h light/12 h dark cycle. All rabbits were provided with standard rabbit chow and clean drinking water ad libitum. Following acclimatization, the rabbits were randomly allocated to three experimental groups containing six animals each.

2.2.2 Preparation of Roasted Chicory Root

Roasted chicory (*Cichorium intybus* L.) root material weighing 30 g was added to 1 L of distilled water and boiled for 2–3 min. The resulting aqueous preparation was allowed to cool, filtered, and stored at –20 °C until administration. No additional ingredients were added. Based on the amount of starting root material, the preparation had a nominal concentration of 30 mg/mL. Each 5 mL administration therefore contained the equivalent of 150 mg of starting roasted chicory root material per animal. Because the administered volume was fixed rather than adjusted according to body weight, the corresponding starting-root equivalent exposure varied among animals. Based on the recorded body-weight range (0.5–1.8 kg), the estimated exposure ranged approximately from 83 to 300 mg/kg body weight. Future studies should standardize botanical dosing according to body weight and chemically quantified constituents.

2.2.3 Experimental Design and Induction of Acute Liver Injury

The rabbits were randomly assigned to control, paracetamol, and chicory plus paracetamol groups. Animals in the control group received standard chow and drinking water without paracetamol or chicory treatment. Animals in the paracetamol group received a single oral dose of paracetamol at 1 g/kg body weight. Panadol® 500 mg tablets manufactured by GlaxoSmithKline, Pakistan, were used to induce acute liver injury. Animals in the chicory plus parac-

etamol group received 5 mL of roasted chicory root preparation, corresponding to 150 mg of starting-root equivalent per animal, followed by oral administration of paracetamol at 1 g/kg body weight. The treatments were administered sequentially, with chicory preparation given first and paracetamol administered afterward. The biochemical and molecular responses were evaluated 4 h after paracetamol administration. The 4 h time point was selected to evaluate early biochemical and transcriptional responses following acute paracetamol exposure before the development of extensive secondary hepatic injury.

2.2.4 Sample Collection and Serum Preparation

At the end of the experimental period, rabbits were anesthetized with ketamine hydrochloride (35 mg/kg body weight) and xylazine hydrochloride (5 mg/kg body weight) as a combined anesthetic regimen, administered intramuscularly into the longissimus dorsi muscle, following previously published rabbit anesthesia protocols [30,31,32]. Ketamine hydrochloride (KETALITE®, Elice Pharma, Pakistan; 50 mg/mL) and xylazine hydrochloride (Xylaject®, 2% solution; 20 mg/mL) were used before the euthanasia process to ensure the rabbits were fully unconscious, insentient, and free from pain during the procedure. After induction of deep anesthesia, rabbits were euthanized by exsanguination under deep anesthesia, and death was confirmed by the absence of respiration and cardiac activity, in accordance with the approved institutional animal-care procedure. Blood samples were collected under aseptic conditions, and serum was separated by centrifugation. Serum samples were stored at −20 °C until biochemical analysis. Liver samples were subsequently collected and preserved for RNA isolation and gene-expression analysis.

2.2.5 Biochemical Analysis

Serum alanine aminotransferase, alkaline phosphatase, and ferritin concentrations were measured to evaluate hepatic biochemical alterations. Lipid-associated parameters included total lipids, total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. Renal biochemical alterations were assessed by measuring serum urea and creatinine concentrations.

All biochemical parameters were determined using established clinical chemistry procedures and the respective diagnostic-assay instructions [33].

2.2.6 RNA Isolation and Complementary DNA Synthesis

Total RNA was isolated from liver samples using TRIzol reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically, and the isolated RNA was stored at −70 °C until further processing.

Complementary DNA was synthesized from 500 ng of total RNA using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The synthesized complementary DNA was diluted with RNase-free water and stored until quantitative real-time PCR analysis.

2.2.7 Quantitative Real-Time PCR Analysis

Relative CYP2E1 gene expression was determined using quantitative real-time PCR with SYBR Green chemistry. The reactions were performed using a 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). CYP2E1-specific primers were used to amplify the target gene, while glyceraldehyde-3-phosphate dehydrogenase served as the reference gene. The stability of GAPDH expression under all experimental conditions, primer amplification efficiency, and melt-curve characteristics were not independently evaluated in the present study and should be included in future validation studies. The CYP2E1 and GAPDH primer sequences are provided in **Supplementary Table 2**.

The thermal programme included an initial incubation at 50 °C for 2 min, followed by denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Relative CYP2E1 expression was calculated using the comparative $2^{-\Delta\Delta Ct}$ method, with GAPDH used for normalization and the untreated control group used as the calibrator [34].

2.3 Statistical Analysis

Experimental data were expressed as mean ± standard deviation. Differences among the control, paracetamol, and chicory plus paracetamol groups were evaluated using one-way analysis of variance followed by Tukey's multiple-comparison test. Statistical analysis was performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Structural Characterization and Validation of Rabbit CYP2E1

The rabbit CYP2E1 sequence consisted of 493 amino acids (Table 1). Homology modelling was performed using the SWISS-MODEL server based on the human CYP2E1 template (P05181.1.A). The generated three-dimensional model exhibited 96% query coverage, 79.11% sequence similarity, and a Global Model Quality Estimate (GMQE) value of 0.96, indicating the reliability of the predicted structure (Table 2). Fig. 1 presents the three-dimensional structure and validation analysis.

ProtParam analysis showed a calculated molecular weight of 56,945.02 Da and a theoretical isoelectric point of 8.41. The protein contained 60 negatively charged residues and 63 positively charged residues, indicating an overall

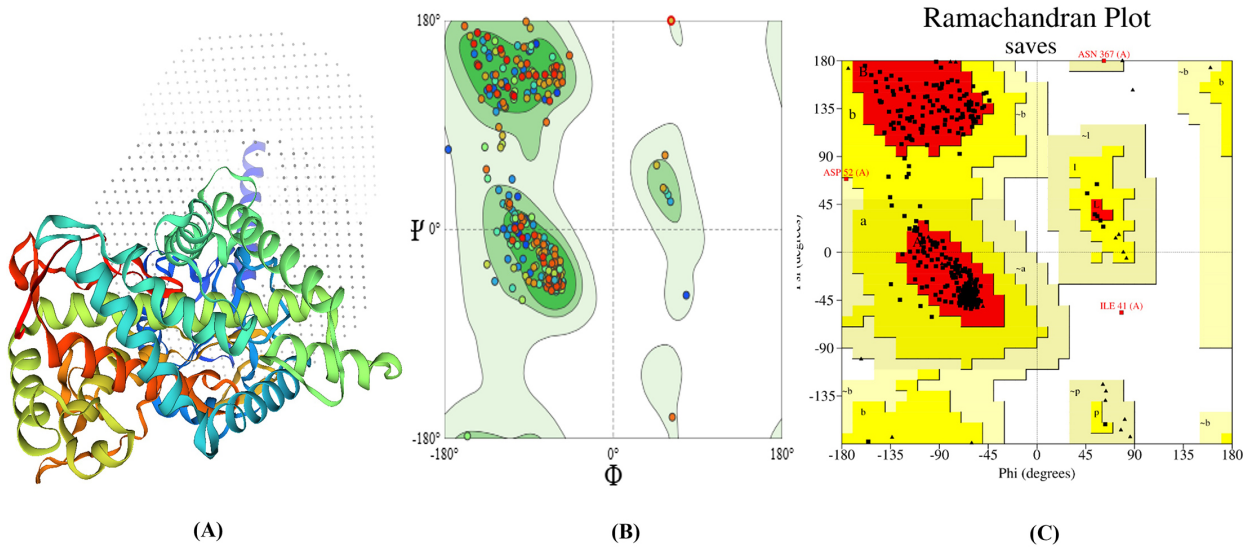


Fig. 1. Structural modelling and validation of rabbit CYP2E1. (A) Three-dimensional homology-modelled structure of rabbit CYP2E1 receptor. (B) Structural validation of the model using Ramachandran plot analysis, SWISS-MODEL quality assessment, and (C) UCLA–DOE SAVES v6.0 validation.

Table 3. Physicochemical parameters were calculated using the ProtParam tool on ExPASy.

S. No.	Physicochemical property	Rabbit *CYP2E1*
i	Number of amino acids (AAs)	493
ii	Molecular weight	56,945.02 Da
iii	Theoretical isoelectric point (pI)	8.41 (Basic)
iv	Negatively charged residues (Asp + Glu)	60
v	Positively charged residues (Arg + Lys)	63
vi	Overall charge	Positive (+)
vii	Extinction coefficient	53,080 M^{-1} cm^{-1}
viii	Instability index	41.47 (Unstable)
ix	Aliphatic index	92.47
x	Grand average of hydropathicity (GRAVY)	−0.238

positive charge. The extinction coefficient was 53,080 M^{-1} cm^{-1}. The instability index was 41.47, indicating predicted instability, whereas the aliphatic index and grand average of hydropathicity were 92.47 and −0.238, respectively (Table 3).

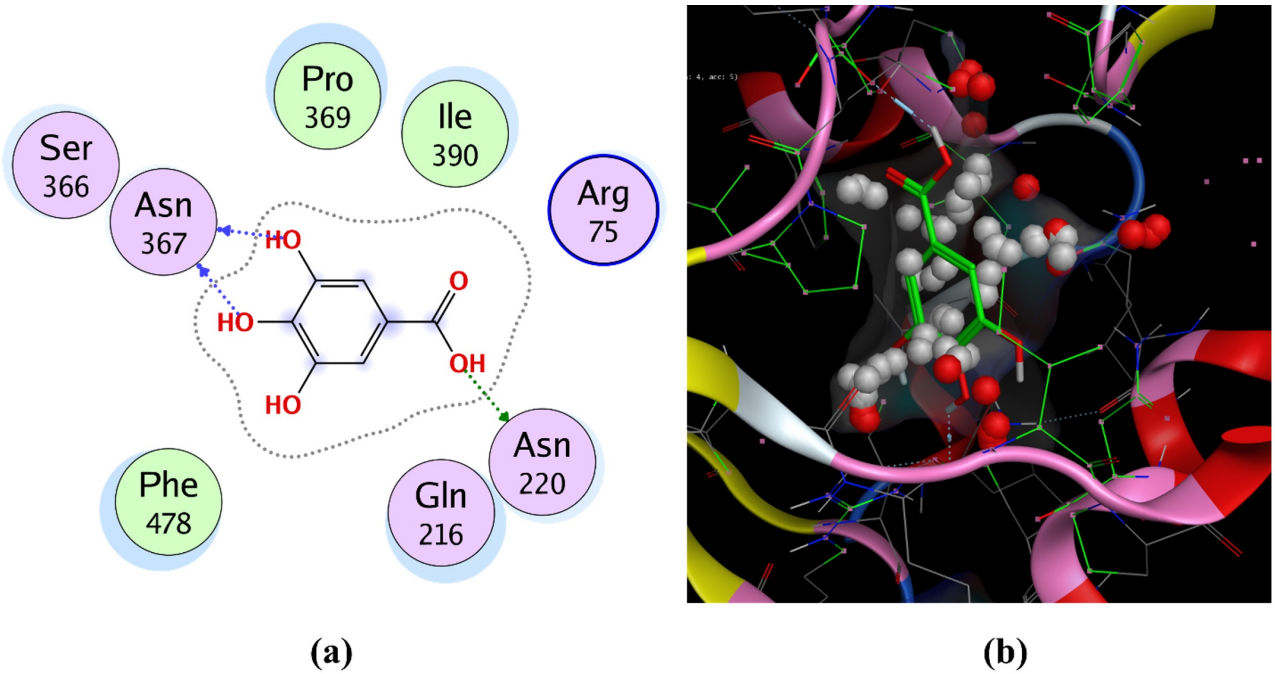
3.2 Computational Prediction of Interactions Between Reported Chicory Polyphenols and CYP2E1

The ten polyphenolic compounds previously reported in chicory root showed predicted CYP2E1 binding energies ranging from −7.90 to −4.30 kcal/mol. Caffeic acid exhibited the most favourable binding energy of −7.90 kcal/mol, with an RMSD value of 0.73 Å. It formed two hydrogen-donor interactions with GLU-102 at distances of 3.04 and 3.02 Å.

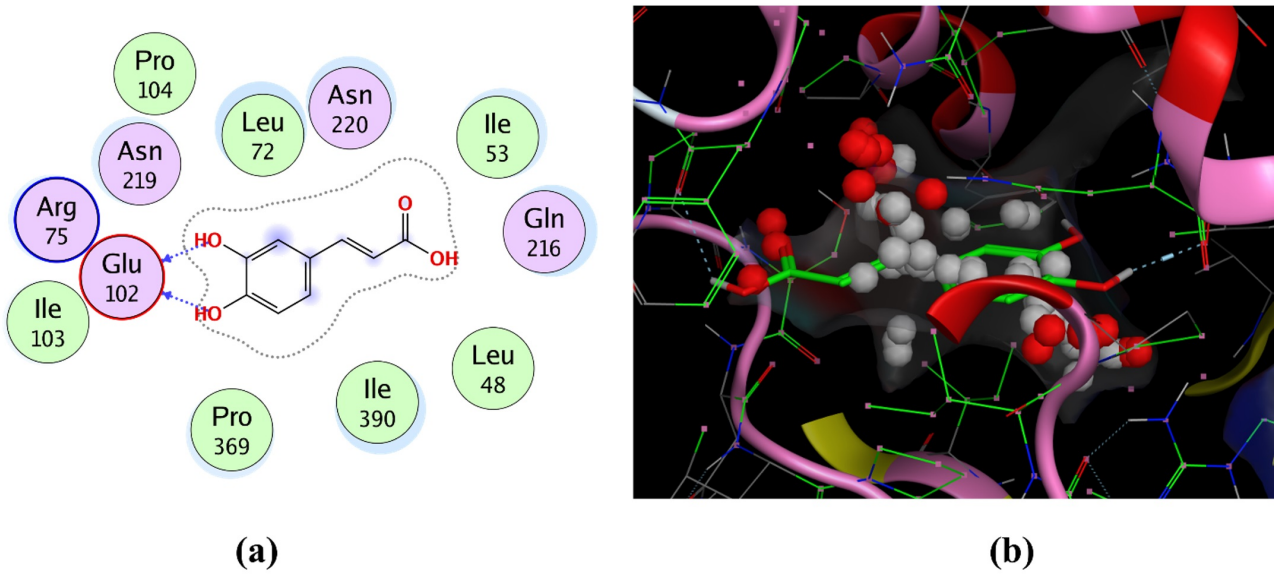
Gallic acid showed the second most favourable binding energy of −7.83 kcal/mol and an RMSD value of 0.95 Å. It formed hydrogen-donor interactions with ASN-367 at distances of 3.04 and 2.98 Å and with ASN-220 at a distance of 3.15 Å (Fig. 2).

Neochlorogenic acid showed a binding energy of −7.23 kcal/mol and interacted with SER-366, GLN-216, and ASN-219. Chlorogenic acid exhibited a binding energy of −7.11 kcal/mol and interacted with ASN-367 and GLN-216. Cryptochlorogenic acid showed a binding energy of −7.05 kcal/mol and formed interactions with ASN-223, ASN-220, and ILE-103. Isochlorogenic acid exhibited a binding energy of −7.02 kcal/mol and interacted with GLY-477, ASN-367, and ASN-219.

Protocatechuic acid showed a binding energy of −6.77 kcal/mol and interacted with ASN-367 and ASN-220. The binding energy of *p*-coumaric acid was −6.21 kcal/mol, with interactions involving ASN-220 and PRO-369. Chicoric acid exhibited a binding energy of −5.11 kcal/mol and interacted with GLU-102 and PHE-478. The least favourable docking result was obtained for 3,5-dicaffeoylquinic acid, which showed a binding energy of −4.30 kcal/mol, an RMSD value of 3.49 Å, and an interaction with GLN-216 (Table 4).



(A). Gallic acid



(B). Caffeic acid

Fig. 2. Molecular docking interactions of the highest-affinity chicory-derived polyphenolic compounds with rabbit CYP2E1. (A) Gallic acid showing predicted hydrogen-bond interactions with ASN-367 and ASN-220. (B) Caffeic acid showing predicted hydrogen-bond interactions with GLU-102.

3.3 Physicochemical, Drug-Likeness, and ADMET Profiles

Caffeic acid had a molecular weight of 180.16 g/mol, four hydrogen-bond acceptors, three hydrogen-bond donors, and a LogP value of 1.20. Gallic acid had a molecular weight of 170.12 g/mol, five hydrogen-bond acceptors, four hydrogen-bond donors, and a LogP value of 0.50.

Protocatechuic acid and *p*-coumaric acid had molecular weights of 154.12 and 164.16 g/mol, respectively. Their corresponding LogP values were 0.80 and 1.49. Caffeic acid, gallic acid, protocatechuic acid, and *p*-coumaric acid showed no violations among the four reported Lipinski-related parameters.

Table 4. Molecular docking analysis between CYP2E1 and the major polyphenolic compounds identified in chicory root.

PubChem ID	Compound	Binding energy (kcal/mol)	RMSD (Å)	Residue interactions	Interaction type	Distance (Å)
689043	Caffeic acid	−7.90	0.73	GLU-102	H-donor	3.04
				GLU-102	H-donor	3.02
370	Gallic acid	−7.83	0.95	ASN-367	H-donor	3.04
				ASN-367	H-donor	2.98
				ASN-220	H-donor	3.15
5280633	Neochlorogenic acid	−7.23	1.50	SER-366	H-donor	2.66
				GLN-216	H-donor	3.09
				ASN-219	H-acceptor	2.97
				GLN-216	H-acceptor	2.83
1794427	Chlorogenic acid	−7.11	1.60	ASN-367	H-donor	2.77
				GLN-216	H-acceptor	3.25
9798666	Cryptochlorogenic acid	−7.05	1.47	ASN-223	H-donor	2.99
				ASN-220	H-donor	2.68
				ASN-220	H-acceptor	3.03
				ILE-103	H-acceptor	3.08
5315832	Isochlorogenic acid	−7.02	1.82	GLY-477	H-donor	2.99
				ASN-367	H-donor	2.81
				ASN-219	H-acceptor	3.04
72	Protocatechuic acid	−6.77	1.23	ASN-367	H-donor	2.97
				ASN-220	H-donor	3.15
637542	p-Coumaric acid	−6.21	0.94	ASN-220	H-donor	2.95
				PRO-369	π–H	4.15
5281764	Chicoric acid	−5.11	1.42	GLU-102	H-donor	2.68
				PHE-478	H-donor	2.58
6474310	3,5-Dicaffeoylquinic acid	−4.30	3.49	GLN-216	H-acceptor	2.92

Chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, and isochlorogenic acid each had a molecular weight of 354.31 g/mol, nine hydrogen-bond acceptors, six hydrogen-bond donors, and a LogP value of −0.65. These compounds exceeded the recommended hydrogen-bond donor limit.

Chicoric acid had a molecular weight of 474.37 g/mol, 12 hydrogen-bond acceptors, seven hydrogen-bond donors, and a LogP value of 1.23. It exceeded the recommended hydrogen-bond acceptor and donor limits. The corresponding values for 3,5-dicaffeoylquinic acid were 516.41 g/mol, 12 hydrogen-bond acceptors, seven hydrogen-bond donors, and a LogP value of 1.03, resulting in violations of the molecular-weight, hydrogen-bond acceptor, and hydrogen-bond donor criteria (Table 5).

SwissADME predicted high gastrointestinal absorption for caffeic acid, gallic acid, *p*-coumaric acid, and protocatechuic acid, whereas low absorption was predicted for the remaining compounds. Only *p*-coumaric acid was predicted to cross the blood–brain barrier. Chicoric acid and 3,5-dicaffeoylquinic acid were predicted to be P-glycoprotein substrates.

The CYP450 analysis predicted that *p*-coumaric acid could inhibit CYP1A2, whereas protocatechuic acid could inhibit CYP2C9. None of the evaluated compounds was predicted to inhibit CYP2D6, CYP2C19, or CYP3A4. The predicted log Kp values ranged from −8.76 to −6.26.

Gallic acid and protocatechuic acid were assigned to predicted toxicity class 4. Caffeic acid, *p*-coumaric acid, the four chlorogenic acid derivatives, chicoric acid, and 3,5-dicaffeoylquinic acid were assigned to predicted toxicity class 5 (Table 6).

3.4 Effect of Roasted Chicory Root Preparation on Hepatic Biochemical Parameters

Paracetamol administration increased serum ALT levels from approximately 44 IU/L in the control group to approximately 52 IU/L in the PCM group. Co-administration of roasted chicory root preparation reduced ALT levels to approximately 35 IU/L. The chicory plus paracetamol group was marked as statistically different at $p < 0.05$ in Fig. 3.

Serum ALP levels were approximately 195 IU/L in the control group, 212 IU/L in the paracetamol group, and 200 IU/L in the chicory plus paracetamol group. No statistically significant differences were indicated for ALP (Fig. 3).

Serum ferritin decreased from approximately 134 ng/mL in the control group to approximately 80 ng/mL in

Table 5. ADMET-Tox analysis demonstrated drug-like properties of the major polyphenolic compounds identified in chicory root by using Lipinski rules.

Compound	PubChem IDs	MW (g/mol)	H-Bond acceptor	H-Bond donor	LogP (o/w)
Chlorogenic acid	1794427	354.31	9	6	–0.65
Neochlorogenic acid	5280633	354.31	9	6	–0.65
Caffeic acid	689043	180.16	4	3	1.2
Cryptochlorogenic acid	9798666	354.31	9	6	–0.65
Isochlorogenic acid	5315832	354.31	9	6	–0.65
Gallic acid	370	170.12	5	4	0.5
Protocatechuic acid	72	154.12	4	3	0.8
p-Coumaric acid	637542	164.16	3	2	1.49
Chicoric acid	5281764	474.37	12	7	1.23
3,5-Dicaffeoylquinic acid	6474310	516.41	12	7	1.03

Table 6. The ADMET profiling of the major polyphenolic compounds identified in chicory root.

ADMET profiling										
Parameters	689043	370	637542	72	5315832	9798666	1794427	5280633	5281764	6474310
Gastrointestinal Absorption	High	High	High	High	Low	Low	Low	Low	Low	Low
Blood-Brain Barrier	No	No	Yes	No	No	No	No	No	No	No
P-glycoprotein substrate	No	No	No	No	No	No	No	No	Yes	Yes
CYP450 1A2 Inhibitor	No	No	Yes	No	No	No	No	No	No	No
CYP450 2C9 Inhibitor	No	No	No	Yes	No	No	No	No	No	No
CYP450 2D6 Inhibitor	No	No	No	No	No	No	No	No	No	No
CYP450 2C19 Inhibitor	No	No	No	No	No	No	No	No	No	No
CYP450 3A4 Inhibitor	No	No	No	No	No	No	No	No	No	No
Log K_p (Kp in cm/s)	–6.58	–6.84	–6.26	–6.42	–8.76	–8.76	–8.76	–8.76	–7.77	–8.37
Predicted toxicity class	5	4	5	4	5	5	5	5	5	5

the paracetamol group and increased to approximately 135 ng/mL following chicory co-administration. However, no statistically significant differences were indicated for ferritin (Fig. 3).

3.5 Effect of Roasted Chicory Root Preparation on Lipid-Associated Parameters

Total lipid concentrations were approximately 700 mg/dL in the control group, 620 mg/dL in the paracetamol group, and 580 mg/dL in the chicory plus paracetamol group. The chicory plus paracetamol group was marked as statistically significant at $p < 0.05$ in Fig. 4.

HDL concentrations remained comparable among the groups, with approximate values of 39, 40, and 43 mg/dL in the control, paracetamol, and chicory plus paracetamol groups, respectively. LDL concentrations were approximately 113, 116, and 111 mg/dL in the corresponding groups.

Total cholesterol concentrations were approximately 160 mg/dL in the control group, 155 mg/dL in the paracetamol group, and 136 mg/dL in the chicory plus paracetamol group. Triglyceride concentrations were approximately 128, 98, and 96 mg/dL, respectively. Both paracetamol-treated groups were marked as statistically significant at $p < 0.05$ in the triglyceride panel (Fig. 4).

3.6 Effect of Roasted Chicory Root Preparation on Renal Biochemical Parameters

Serum urea concentrations were approximately 41 mg/dL in the control group, 35 mg/dL in the paracetamol (PCM) group, and 30 mg/dL in the chicory plus paracetamol group (Fig. 5a). The lowest serum urea concentration was observed in the PCM + Chicory group.

Serum creatinine concentrations were approximately 0.84 mg/dL in the control group, 0.82 mg/dL in the paracetamol (PCM) group, and 0.74 mg/dL in the PCM + Chicory group. Although creatinine concentrations were slightly lower in the PCM + Chicory group, no statistically significant differences were indicated among the experimental groups for either serum urea or creatinine (Fig. 5).

3.7 Effect of Roasted Chicory Root Preparation on CYP2E1 Gene Expression

Relative CYP2E1 gene expression was approximately 1.00-fold in the control group and increased to approximately 1.75-fold following paracetamol (PCM) administration. Co-administration of roasted chicory root preparation reduced CYP2E1 expression to approximately 0.48-fold. The PCM+chicory group was marked as statistically significant at $p < 0.05$ in Fig. 6.

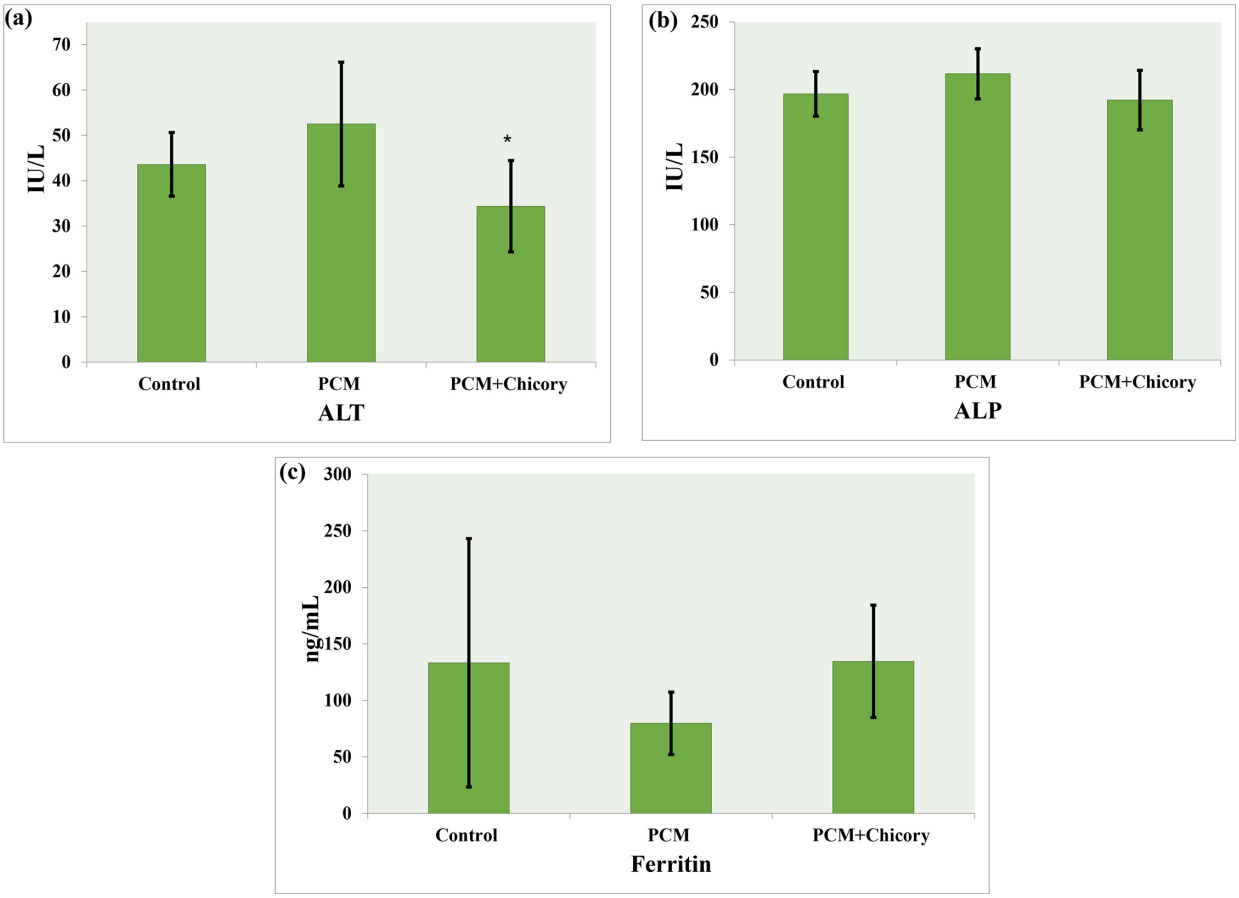


Fig. 3. Estimated levels of (a) alanine aminotransferase (ALT), (b) alkaline phosphatase (ALP), and (c) ferritin in control, PCM, and Chicory + PCM in the animal model. Each column represents the mean value, while each error bar represents the ± standard deviation. * shows results that were found statistically significant $p < 0.05$ following post hoc Tukey's test as compared to the PCM group.

4. Discussion

The present study investigated the response of roasted chicory root preparation during early paracetamol-induced acute liver injury using an integrated *in silico* and *in vivo* approach. The major findings were that roasted chicory root co-administration attenuated the paracetamol-associated increase in serum alanine aminotransferase (ALT), reduced hepatic CYP2E1 gene expression, and identified caffeic acid and gallic acid as computational CYP2E1-interacting candidates. However, these findings represent an early biological association and do not establish direct CYP2E1 inhibition or confirm that the identified compounds are responsible for the observed response. The computational and experimental components of this study should therefore be interpreted as complementary observations rather than direct mechanistic confirmation. The docking analysis was performed using compounds previously reported in chicory root, while the administered roasted preparation was not chemically characterized. Consequently, the compounds responsible for the observed biological effects cannot be conclusively identified.

Paracetamol hepatotoxicity is primarily associated with excessive formation of the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), depletion of hepatic glutathione, oxidative stress, mitochondrial dysfunction, and hepatocellular injury. CYP2E1 is an important contributor to paracetamol bioactivation, and modulation of CYP2E1-associated pathways has therefore been investigated as a potential approach for reducing susceptibility to drug-induced liver injury [4,5,6]. In the present study, paracetamol administration was associated with altered hepatic CYP2E1 mRNA expression, whereas co-administration of roasted chicory preparation reduced this response. These findings suggest that chicory treatment was associated with regulation of CYP2E1 transcription during early paracetamol exposure.

The observed reduction in serum ALT following roasted chicory administration indicates attenuation of early hepatocellular damage. ALT is a commonly used biochemical marker of hepatocyte injury because its release into circulation reflects cellular membrane disruption. The present observation is consistent with previous reports describing protective effects of *Cichorium intybus* prepa-

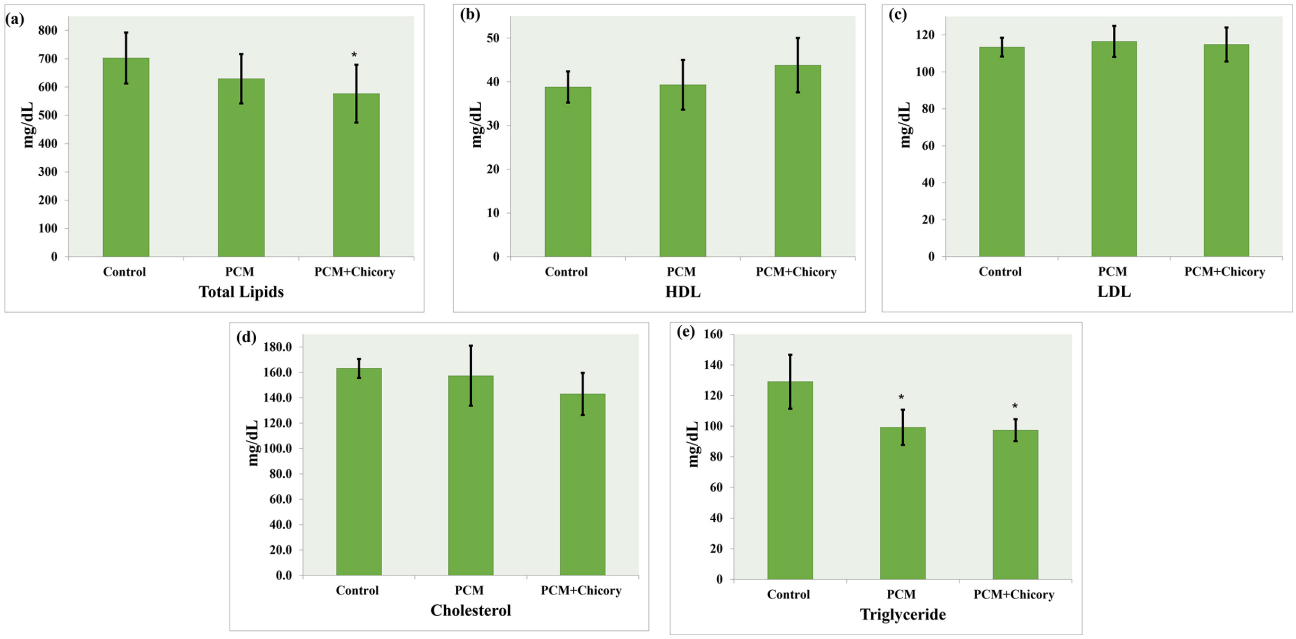


Fig. 4. Effects of chicory supplementation on lipid profile in paracetamol-treated groups. Lipid profile assessment including (a) total lipids (TL), (b) high-density lipoprotein cholesterol (HDL), (c) low-density lipoprotein cholesterol (LDL), (d) total cholesterol (TC), and (e) triglycerides (TG) levels in the control, paracetamol (PCM), and Chicory + PCM groups. Each column represents the mean value, while error bars indicate the standard deviation (SD). An asterisk (*) indicates a statistically significant difference ($p < 0.05$) compared with the control group.

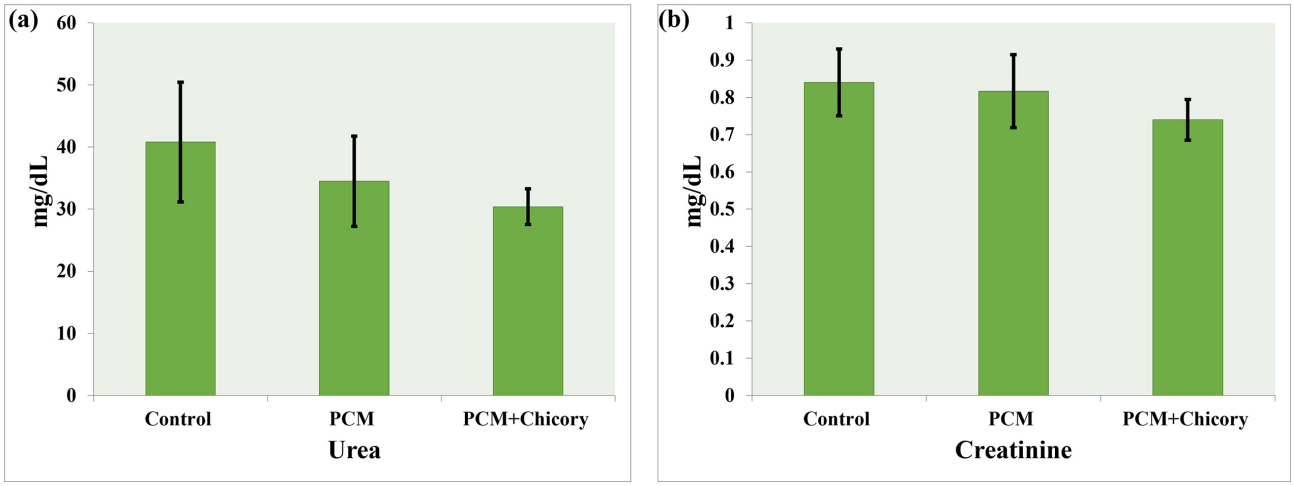


Fig. 5. Effects of roasted chicory root on renal biochemical parameters in paracetamol-treated rabbits. Serum concentrations of (a) urea and (b) creatinine in the control, paracetamol (PCM), and PCM + Chicory groups. Each column represents the mean value, while error bars indicate the standard deviation (SD).

rations in experimental models of liver injury, including acetaminophen-induced hepatotoxicity and alcohol-associated liver injury, where improvements in biochemical markers and hepatic histological alterations were observed [24,25]. However, differences in plant preparation, extraction method, dose, and experimental duration should be considered when comparing these findings with previous studies.

Chicory (*Cichorium intybus* L.) contains a diverse range of bioactive phytochemicals, particularly hydroxycinnamic acid derivatives and other phenolic compounds. Previous phytochemical investigations have identified chlorogenic acid, caffeoylquinic acid derivatives, including 3,5-dicaffeoylquinic acid, and several related phenolic constituents in chicory roots [14,23,24,25]. However, the composition and relative abundance of these compounds may vary depending on extraction conditions and ther-

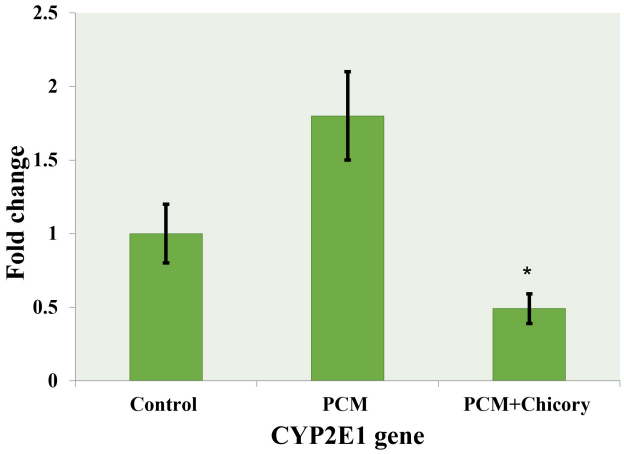


Fig. 6. Effect of roasted chicory root preparation on CYP2E1 gene expression in a 4 h acute liver injury animal model. Each column represents the mean value, while each error bar represents the ± standard deviation. * indicates statistical significance ($p < 0.05$) following Tukey's post hoc test compared with the paracetamol group.

mal processing. Elevated temperatures can influence the extraction, stability, and transformation of phenolic compounds, while roasting may further modify the phytochemical profile of chicory roots, particularly the concentrations of chlorogenic acid and related derivatives [15]. Accordingly, caffeic acid, gallic acid, and other candidate compounds were selected for the present computational investigation based on previously published phytochemical evidence [14,23,24,25]. Nevertheless, because the roasted chicory root preparation administered to the experimental animals was not subjected to compound-specific chemical characterization, the presence and concentrations of these individual compounds in the administered preparation could not be experimentally confirmed.

Molecular docking analysis identified caffeic acid and gallic acid as the compounds with the most favourable predicted interactions against the rabbit CYP2E1 model. Caffeic acid showed predicted hydrogen-bond interactions with GLU-102, whereas gallic acid interacted with ASN-367 and ASN-220. These findings suggest that these compounds may possess potential structural compatibility with the modeled CYP2E1 binding region; however, they should be interpreted as computational predictions rather than evidence of direct enzyme inhibition [18,35]. The predicted interacting residues (GLU-102, ASN-367, and ASN-220) represent docking-derived interaction sites within the modeled rabbit CYP2E1 structure, and their precise functional relevance within the canonical heme-containing catalytic region requires confirmation through experimentally validated structural and enzymatic studies [35]. Furthermore, the present docking workflow did not experimentally reconstruct the heme prosthetic group environment or assess catalytic effects on CYP2E1 activity. Therefore, the identified polyphenolic compounds should be considered candidate molecules for future mechanistic investigation rather than confirmed CYP2E1 inhibitors.

Phenolic compounds may influence hepatic responses through multiple mechanisms beyond CYP2E1 regulation. Caffeic acid has been reported to protect against paracetamol-induced liver injury through activation of Keap1–Nrf2 antioxidant signalling and inhibition of ERK1/2-mediated early growth response pathways [7,8]. Chlorogenic acid has also demonstrated protective effects through antioxidant mechanisms and regulation of hepatic stress responses [36]. Therefore, the biological effect observed in the current study may involve a combination of CYP2E1-associated transcriptional changes and other phytochemical-mediated pathways.

The reduction in CYP2E1 mRNA expression observed after chicory treatment should be interpreted cautiously. Gene-expression changes do not necessarily indicate corresponding alterations in protein abundance or enzymatic activity [37]. The present study did not measure CYP2E1 protein levels, catalytic activity, NAPQI-protein adduct formation, or hepatic glutathione depletion. Therefore, the contribution of CYP2E1-dependent mechanisms compared with CYP2E1-independent antioxidant or anti-inflammatory pathways remains unresolved.

The lipid-associated and renal biochemical parameters showed variable responses among experimental groups. Unlike ALT, these parameters did not demonstrate consistent alterations associated with paracetamol-induced injury or chicory treatment. These findings suggest that under the present experimental conditions, the most apparent biological response occurred at the hepatic biochemical and molecular levels during the early injury phase.

The computational analyses of Lipinski characteristics and ADMET properties provided additional comparative information regarding the selected polyphenolic candidates. However, these predictions represent theoretical assessments and should not be considered substitutes for experimental pharmacokinetic or toxicological evaluation [19,20,29].

Overall, this study provides preliminary evidence that roasted chicory root preparation may attenuate early paracetamol-associated hepatic alterations in rabbits. The combined computational and experimental approach identified CYP2E1-associated transcriptional regulation and candidate chicory polyphenols as areas requiring further investigation.

Limitations

Several limitations should be considered when interpreting the findings of the present study. First, the roasted chicory root preparation was not chemically characterized using analytical approaches such as LC–MS. Therefore, although caffeic acid, gallic acid, and other polyphenolic compounds previously reported in *Cichorium intybus* were

evaluated computationally, their actual presence and concentrations in the administered preparation were not experimentally confirmed. Accordingly, the compounds identified through molecular docking should be regarded as candidate molecules for future validation rather than confirmed mediators of the observed biological response.

Second, although reduced hepatic CYP2E1 gene expression was observed following chicory co-administration, the present study did not assess CYP2E1 protein abundance or catalytic activity. Furthermore, downstream indicators of paracetamol bioactivation, such as NAPQI–protein adduct formation and glutathione depletion, were not evaluated. Therefore, the involvement of CYP2E1-related pathways remains an associative finding and requires confirmation through functional mechanistic studies.

Third, the hepatoprotective response observed following roasted chicory administration may involve biological pathways beyond CYP2E1-associated responses. Recent experimental evidence indicates that chicory-derived sesquiterpene lactones can exert hepatoprotective and anti-inflammatory effects through modulation of NRF2 and NF-κB signaling pathways [38]. However, these potential mechanisms were not directly investigated in the present study.

Fourth, the computational docking analysis was performed as a comparative ligand-interaction prediction and did not include experimental validation of CYP2E1 binding or reconstruction of the complete enzymatic environment, including the heme-associated catalytic region. Therefore, docking results should be interpreted as hypothesis-generating observations rather than evidence of direct enzyme inhibition.

Finally, CYP2E1 gene expression analysis was normalized using GAPDH; however, independent validation of reference gene stability, primer efficiency, and melt-curve characteristics was not performed. Future studies incorporating chemically characterized preparations, CYP2E1 protein and activity measurements, and additional mechanistic biomarkers are warranted.

Taken together, these limitations indicate that the present findings provide preliminary evidence that roasted chicory root preparation is associated with attenuation of early paracetamol-induced hepatic alterations, while the precise molecular mechanisms underlying this response require further investigation.

5. Conclusion

Roasted chicory root preparation attenuated early paracetamol-associated ALT elevation and was associated with reduced hepatic CYP2E1 gene expression in rabbits. Molecular docking identified caffeic acid and gallic acid as potential CYP2E1-interacting candidates; however, their presence in the administered preparation and their direct effects on CYP2E1 catalytic activity remain unconfirmed. The observed response may involve CYP2E1-associated transcriptional regulation together with other phytochemical-mediated protective pathways. Further studies using chemically characterized chicory preparations, CYP2E1 protein and activity measurements, oxidative stress markers, histopathological evaluation, and longer experimental periods are required to establish the underlying mechanisms.

Abbreviations

ADMET, absorption, distribution, metabolism, excretion, and toxicity; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BBB, blood–brain barrier; cDNA, complementary DNA; CID, compound identifier; Ct, cycle threshold; CYP1A2, cytochrome P450 1A2; CYP2C9, cytochrome P450 2C9; CYP2C19, cytochrome P450 2C19; CYP2D6, cytochrome P450 2D6; CYP2E1, cytochrome P450 2E1; CYP3A4, cytochrome P450 3A4; DILI, drug-induced liver injury; EGR1, early growth response 1; ERK1/2, extracellular signal-regulated kinase 1/2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GMQE, Global Model Quality Estimate; GRAVY, grand average of hydropathicity; HBA, hydrogen-bond acceptor; HBD, hydrogen-bond donor; HDL-C, high-density lipoprotein cholesterol; Keap1, Kelch-like ECH-associated protein 1; LDL-C, low-density lipoprotein cholesterol; MW, molecular weight; NAPQI, N-acetyl-p-benzoquinone imine; Nrf2, nuclear factor erythroid 2-related factor 2; PDBQT, Protein Data Bank, partial charge and atom-type format; P-gp, P-glycoprotein; RMSD, root mean square deviation; RNA, ribonucleic acid; RT-qPCR, reverse-transcription quantitative polymerase chain reaction; SAVES, Structure Analysis and Validation Server; SD, standard deviation; SMILES, simplified molecular-input line-entry system; TC, total cholesterol; TG, triglycerides; TL, total lipids.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

YJ, MO, and MS carried out the experiments, analyzed and interpreted the data, and prepared the manuscript. All authors contributed to the revision and editorial changes of the manuscript. All authors read and approved the final version of the manuscript and have participated sufficiently in the work to take public responsibility for its content. All authors agree to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experimental protocol was approved by the Institutional Ethics Committee of Cholistan University of Vet-

erinary and Animal Sciences, Bahawalpur, Pakistan, under approval number PSF/NSLP/P-CUVAS (923). All animal care and experimental procedures were conducted in accordance with the approved ethical guidelines. The study was carried out in accordance with the ARRIVE guidelines 2.0 and the revised Animals (Scientific Procedures) Act 1986 in the UK.

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Conflicts of Interest

The authors declare no conflicts of interest. Given his role as the Editor-in-Chief, Mehmet Ozaslan had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Dalia O. Saleh.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/IJP56788.

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