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EXPLORING THE HEPATOPROTECTIVE POTENTIAL OF *RICINUS COMMUNIS* FRACTIONS: INSIGHTS FROM BIOCHEMICAL, HISTOPATHOLOGICAL AND MOLECULAR DOCKING ANALYSIS

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Abstract

Liver cancer is a prevalent and serious global malignancy due to its rapid growth and poor prognosis, often stemming from liver damage progressing to fibrosis, cirrhosis, and ultimately cancer. In this context, a hepatoprotective study was conducted on crude methanol extracts of root, stem, leaves, flowers and seeds of *Ricinus communis*. This treatment regimen was consistently implemented over 64 consecutive days at various doses. Carbon tetrachloride (CCl_4) was used as the inducing agent while silymarin was the standard control. This study investigated the hepatoprotective effects of various fractions of *R. communis* using biochemical, histopathological, and *In silico* molecular docking analysis. Hepatic injury was induced in rats via CCl_4 administration, followed by treatment with different fractions. Biochemical analysis revealed significant changes in liver function markers upon CCl_4 exposure, including elevated levels of serum ALT, AST, and ALP, distinctly attenuated by treatment with *Ricinus communis* fractions. Notably, leaf fractions demonstrated superior hepatoprotective effects than root and flower fractions. The histopathological examination further supported these findings, showing remarkable recovery in liver structure with minimal inflammation and fatty changes/lipid alterations, particularly with leaf fractions. Additionally, *in silico* molecular docking analysis elucidated potential interactions between *R. communis* fractions and specific enzymes implicated in liver injury, further supporting the observed hepatoprotective effects. Ligands L1, L2, and L7 showed the strongest potential for enzyme inhibition. Overall, this study underscores the promising hepatoprotective properties of *R. communis* fractions, especially those derived from the leaves. These findings suggest the potential therapeutic utility of *R. communis* in the management of hepatic disorders.

Keywords: *Ricinus communis*, Liver cancer, Hepatoprotective, Biochemical analysis, Histopathology, CCl_4, Hepatic disorders, Molecular docking analysis.

Introduction

The liver plays a crucial role in numerous metabolic functions within the body, making it a vital organ. The treatment of both acute and chronic liver diseases remains a significant challenge globally due to limited drug availability and effectiveness (ChanHo *et al.,* 2007; Jain *et al.,* 2023). These diseases, which pose significant public health challenges, have spurred considerable interest in finding effective treatments (Al-Snafi *et al*., 2019; Le, *et al.,* 2022; Motwani *et al.,* 2021). The stability between oxidative processes and antioxidant generation in the body regulates oxidative stress levels. Intracellular processes can generate various reactive nitrogen and oxygen species, damaging DNA, proteins, and lipids (Taamalli *et al.,* 2020). Common hepatotoxins such as carbon tetrachloride (CCl_4) have been widely utilized in animal models to induce liver damage, resulting in cellular necrosis (Alirezaei *et al*., 2012). CCl_4 is converted into toxic intermediates by hepatic microsomal cytochrome P_{450}, initiating lipid membrane peroxidation (Taamalli *et al.,* 2020; Narendra & Manasi, 2021; Yadav *et al.,* 2021). Natural compounds have emerged as promising options for treating liver diseases due to their lower toxicity and greater bioavailability (Ahsan *et al.,* 2009; Bansal *et al.,* 2014). Herbal remedies, derived from various plant formulations, have shown efficacy in liver disease treatment. Over a hundred plants containing approximately more than a hundred phytocomponents have been reported to possess hepatoprotective properties (Ali *et al.,* 2019; Chavan & Kuvalekar, 2019; Khatri & Anju, 2023).

Ricinus communis commonly known as the castor oil plant, is an evergreen shrub primarily found in tropical regions and belongs to the Euphorbiaceae family. This plant is renowned for its therapeutic properties, including antiulcer, antidiabetic, and antioxidant activities (Kumar, 2017). Phytochemical screening has revealed the presence of glycosides, alkaloids, anthraquinones, flavonoids, saponins, phlobatannins, and steroids (Babu *et al*., 2017; Ojezele, 2020). Considering the significance of *R. communis* L in the field of medicine, this study aimed to evaluate the hepatoprotective activity of extracts from different parts of the plant, such as the roots, leaves, and flowers, against CCl_4-induced liver damage in rats. Additionally, *In silico* molecular docking analysis supported this investigation.

Material and Methods

Plant collection and authentication: *Ricinus communis* was collected from Khar district Malakand, Khyber Pakhtunkhwa, located at 34°49'N, 71°84'E. The samples were identified and submitted to the herbarium of the Department of Botany, University of Peshawar, Khyber Pakhtunkhwa, Pakistan having voucher specimen number (Nawab-13115/PESH-UNI).

Extraction and fractionation: The seeds, flower, stem and root were ground. The grinded parts each weighing 200 g were soaked in methanol for a week (6-7 days). The extraction resulted in methanolic extracts of seeds (22 g),

flower (26 g), stem (33 g) and root (34 g) by using a rotary evaporator (Buchi R-300, Vacuum Pump V-600, Switzerland) *in vacuo* at a temperature of 45°C. The respective crude extracts were suspended in distilled water and kept for a night. The sequential separation of each crude methanolic extract was performed in four different solvents (n-hexane, ethyl acetate, dichloromethane and n-butanol).

Ethical consideration: The experimental protocol for the *In vivo* animal study received approval from the Ethics Committee and the Animal Care & Use Committee (approval no. 5120/RCK/AUP) at the University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan. This approval was granted following the guidelines outlined in the UK Animal Scientific Protocol Act 1986 (Holland, 1986), ensuring adherence to ethical principles and the safe use of laboratory animals.

Animals study: The present study utilized a total of thirty-six adult Wistar rats, encompassing both male and female individuals, with weights ranging from 226g to 340g for males and 290g to 370g for females. These rats were procured from the Veterinary Research Institute (VRI) located in Peshawar, Khyber Pakhtunkhwa, Pakistan. The animals were maintained under controlled environmental conditions, including a temperature of 23±2°C and a light-dark cycle of 12 hours. They were provided with standard laboratory-grade nourishment and access to tap water. A fasting period of 2 hours before and after drug administration was observed. It is important to note that the ethical considerations governing the use of animals, such as rats and mice, in research studies are imperative and regulated by the policies and guidelines of The University of Agriculture, Peshawar, KP, Pakistan. Consequently, ethical approval for conducting this doctoral experiment was diligently obtained from the Ethics Committee at the University of Agriculture, Peshawar. The animals were housed in a standard laboratory environment maintained at a temperature of 25±2°C, accompanied by controlled humidity and a 12-hour cycle of daylight. Throughout the study, all animals were provided unrestricted access to both food and water (Zhang *et al.,* 2022).

Hepatoprotective (Hepatocurative activities): The subjects under investigation were allocated into six distinct groups. One group served as the control, while another group was subjected to CCl_4 treatment. A third group was administered the established therapeutic agent silymarin (standard control). The remaining three groups received treatment with extract fractions derived from the roots, flowers, and leaves of the plant. The administration of these fractions occurred twice weekly, ensuring the entirety of the dosage regimen was completed over 60 days. To induce a reversible hepatic condition, a volume of 0.5ml of CCl_4 was employed (Lam *et al.,* 2016; Idris *et al.,* 2009; Okaiyeto *et al.,* 2018). Throughout the entire dosing period, adjustments were made based on the animals' body weights. A concise overview of the experimental groups is as follows:

Group 1: The subjects were administered intraperitoneal injections (i.p.) of olive oil at a dosage of 50 mL/kg (v/v), twice a week, serving as the vehicle. Additionally, normal saline was orally administered at a volume of 0.5 mL daily, consistently for 64 consecutive days.

Group II: The subjects were administered intraperitoneal injections (i.p.) of CCl_4, with a dose of 3 ml/kg, as a solution comprising 50% CCl_4 and 50% olive oil. These injections were administered twice a week, consistently over 64 consecutive days.

Group III: The subjects underwent intraperitoneal injections (i.p.) of CCl_4, with a dosage of 3 ml/kg, administered as a solution containing 50% CCl_4 and 50% olive oil. These injections were conducted twice a week. Additionally, throughout a continuous period of 64 days, the animals were concurrently administered the standard control silymarin at a dosage of 50 mg/kg.

Group IV: The subjects were subjected to intraperitoneal injections (i.p.) of CCl_4, with a dose of 3 ml/kg, administered as a solution comprising 50% CCl_4 and 50% olive oil. These injections were administered on the 1st, 21st, and 64th days of the experimental cycle. Concurrently, the animals were treated with various plant fraction extracts, administered at a dosage of 200 mg/kg, twice a week. This combined treatment regimen was consistently applied over 64 consecutive days.

Group V: The subjects were subjected to intraperitoneal injections (i.p.) of CCl_4, administered as a solution containing 50% CCl_4 and 50% olive oil, at a dosage of 50 ml/kg (v/v). These injections were administered on the 1st, 21st, and 64th days of the experimental cycle. Concurrently, the animals were treated with different plant fraction extracts, administered at a dosage of 200 mg/kg, twice a week. This combined treatment protocol was consistently applied for 64 consecutive days.

Group VI: The subjects underwent intraperitoneal injections (i.p.) of CCl_4, administered as a solution comprising 50% CCl_4 and 50% olive oil, at a dosage of 50 ml/kg (v/v). These injections were administered on the 1st, 21st, and 64th days of the experimental cycle. Concurrently, the animals were administered various plant fraction extracts at a dosage of 200 mg/kg, twice a week. This combined treatment regimen was consistently implemented for 64 consecutive days.

Measurement of serum biochemical parameters: On the 60^{th} day of the ongoing experiment, both the control and treated animals were humanely euthanized under mild ether anaesthesia. Blood samples of approximately 3 ml were collected via cardiac puncture and placed in plain tubes. The collected blood samples were subsequently subjected to centrifugation at 2500 rpm for 15 minutes. This procedure facilitated the separation of serum from the coagulated blood, which was then stored by freezing at a temperature of -40°C until the analysis phase. Biochemical analyses were conducted utilizing

colourimetric techniques, employing commercially available kits of American origin. These assessments encompassed a range of measurements, including Total Protein, Globulin, Albumin, Bilirubin, alkaline phosphatase (AP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) enzyme levels. The objective of these analyses was to discern and evaluate liver function (Rašković *et al.,* 2014).

Histopathology Previously, both the control and treated animals underwent humane sacrifice under the influence of mild ether anaesthesia. Following this, liver tissues were carefully obtained and sectioned into smaller fragments. These fragments were subsequently immersed in a 10% neutral buffered formalin solution for one week, facilitating the preparation of the tissues for histopathological examination. Subsequently, paraffin sections were generated using an Automatic tissue processor (Auto technique), and these sections were sliced into 3-4 mm thick segments using a rotary microtome. The extent of necrosis induced by CCl_4 was evaluated through the analysis of morphological alterations within the liver sections. These sections were stained with Hematoxylin-Eosin dye and subjected to histopathological scrutiny employing established protocols. The objective of this examination was to ascertain and document any observable histopathological changes within the liver tissue (El-Demerdash *et al.,* 2021).

Molecular docking study: The molecular study involved evaluating the ligands (L1-L7) against cyclooxygenase-2 (3PGH) and CYP2C9 (4NZ2) enzymes to support their activities. The three-dimensional structures of the ligands were obtained from the PubChem database (https://pubchem) and saved in PDB format (Palleti *et al*., 2011; Saravanan *et al*., 2022). Similarly, the structures of cyclooxygenase-2 (3PGH) and CYP2C9 (4NZ2) enzymes were retrieved from the Protein Data Bank (http://www.rcsb.org/pdb). Molecular Operating Environment (MOE) software was utilized to process the structures of enzymes 3PGH and 4NZ2, with water molecules and heteroatoms removed to maintain cellular pH. Subsequently, the selected ligands (L1-L7) were docked into the active pockets of 3PGH and 4NZ2 enzymes using a docking program. Energy minimization was performed, and the amino acid chains, along with dummy atoms, were isolated. A ligand library (L1-L7) was prepared, and their structures were energy-minimized. Three poses were selected, and their affinity was calculated using the London Dock Score method with an induced fit system (Ahmad *et al*., 2021; Shah *et al.,* 2022).

Results

Assessment of hepatocurative and histopathology selected fractions of *R. communis*: Carbon tetrachloride (CCl4) administration caused notable changes in the assessment of liver function, as demonstrated by distinguishable shifts in markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase, bilirubin and total protein levels. The liver histology microscopic examination of the control sample revealed well-defined central veins and hepatocytes reflecting normal architecture. The histopathological analysis of normal liver architecture in control rats, with intact hepatocytes around the central vein and no signs of damage including inflammation or steatosis were observed. In contrast, CCl_4 exposure caused severe liver injury, including steatosis, necrosis, inflammation, and congestion, however, Silymarin treatment significantly reduced inflammation and fatty changes while promoting hepatocyte regeneration. The present study validated the effectiveness of the examined plant material as compared to Silymarin. Conversely, subjects exposed to CCl_4 showed varying degrees of aberration characterized by central lobular necrosis in hepatocytes. The CCl_4 treatment exposed significant liver damage, including hepatocyte necrosis, steatosis, inflammation around the portal and venous areas, and congestion in the sinusoids. The hepatocyte's confirmation was noticed as an organized arrangement around the central vein. The prominent intracellular vacuoles indicating fatty changes and periportal hepatocyte necrosis were observed. In addition, the presence of perivenular and portal inflammation and sinusoidal congestion was detected as well. Treatment of CCl_4-injured livers with the selected fractions of *Ricinus communis* elicited a degree of recovery, manifesting as diminished inflammation, fatty alteration and hepatocyte regeneration (Figs. 1-3). Leaf fractions compared to other fractions (Water, Ethyl acetate, Butanol, and n-hexane) achieved the highest recovery (>95%), restoring hepatocellular architecture, resolving fatty changes, and alleviating inflammation [Fig. 1(A-D)]. The Butanol fraction of the flower part provided 65–70% recovery, with improved architecture and reduced but persistent fatty changes and inflammation. Root fractions showed 80–85% recovery, with marked hepatocyte regeneration and reduced inflammation [Fig. 3(H-J). The outcome confirmed that Leaf fractions were most effective, followed by root and flower extracts. The histopathological examination divulges (Fig. 1) an almost complete restoration of hepatocellular lobular architecture, in addition, a mild perivenular inflammation is discernible (indicated by the arrow), while no evidence of fatty change was observed. Likewise, the study as shown in Figures 1, 2, and 3 communally depicts the comprehensive resolution of CCl_4-induced injury, exemplified by the restoration of hepatocellular architecture encircling the central vein, coupled with an absence of inflammation and fatty changes. Mild sinusoidal congestion is also apparent (indicated by arrows). The root fractions of the *R. communis* yielded hepatocyte regeneration, mild lobular inflammation, and a total absence of fatty alterations [Fig. 3]. The hepatocellular architecture around the central vein is reinstated in the flower fraction (as indicated in Fig. 2 by the star symbol). Similar benefits were observed in rats treated with *R. communis* flower extracts, wherein restoration of hepatocellular architecture around the central vein and reduction in steatosis and inflammation were evident (Fig. 2).

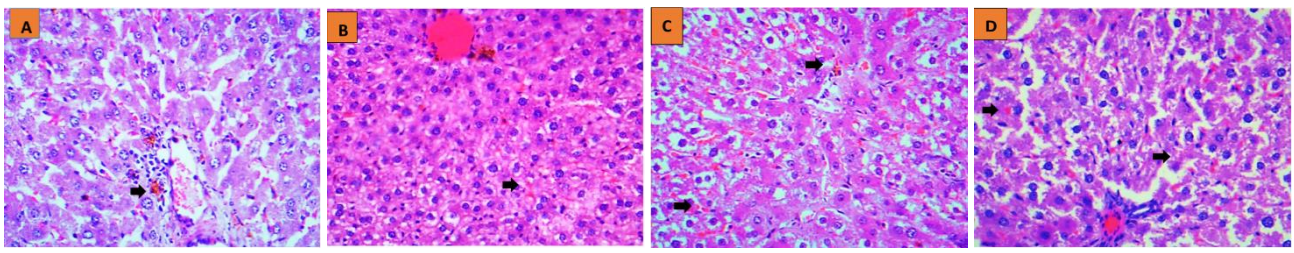

Fig. 1. Effect of leaf extract on rat's liver, [A] butanol fraction, [B] water fraction, [C] *n*-hexane fraction, [D] Ethyl acetate fraction. There is mild perivenular inflammation (arrow) and no fatty change. All Figures show complete resolution of CCl_4-induced injury by restoring hepatocyte architecture around the central vein with no inflammation and fatty change. Mild sinusoidal congestion is noted (arrows).

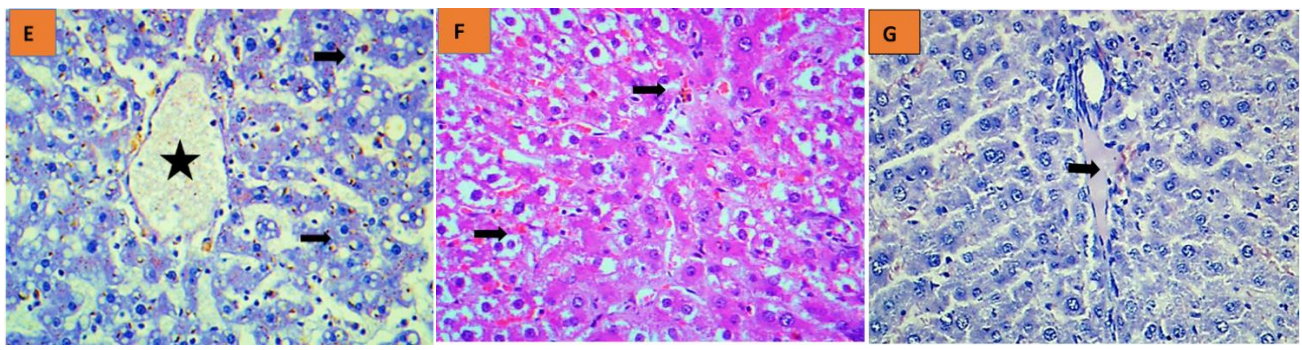

Fig. 2. Effect of flower extract on rat's liver, [E] Butanol fraction [F] Ethyl acetate fraction, [G] *n*-hexane fraction, show complete resolution of CCl_4-induced injury in the form of restoration of hepatocyte architecture around the central vein with no inflammation and fatty change.

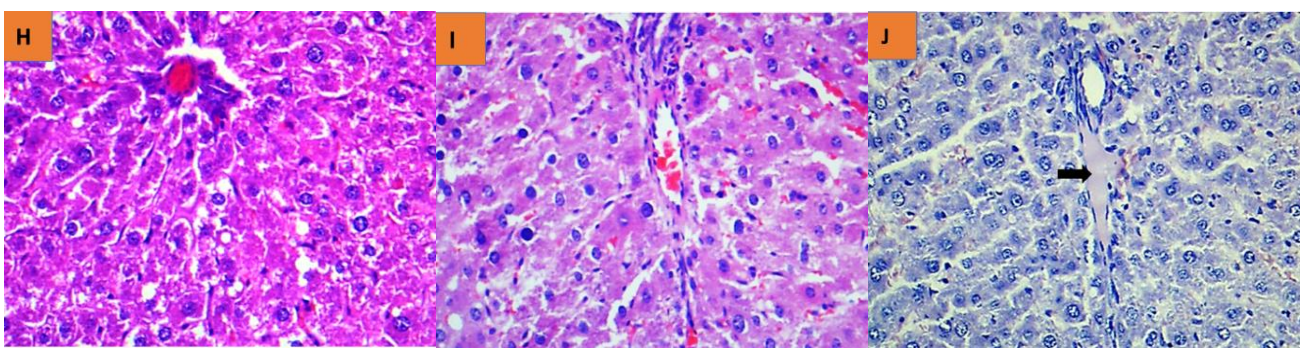

Fig. 3. Effect of Root extract on rat's liver, [H] Ethyl acetate fraction [I] Butanol fraction, [J] *n*-hexane fraction, shows complete recovery of hepatocytes from CCl_4-induced injury with no hepatocyte necrosis and absence of inflammation.

The biochemical analysis of selected fractions of Ricinus communis demonstrated in Table 1 using CCl_4-induced liver damage revealed significant alterations in plasma biomarkers. The study confirmed elevated levels of ALT, AST, and ALP in CCl_4 induced rats as compared to normal ($p<0.05$). Silymarin treatment partially normalized these disruptions, significantly reducing ALT and increasing globulin levels ($p<0.05$, $p<0.001$). Further, the findings confirmed that the Water Fraction of the leaf part of the examined plant (LH_2O) reduced AST and ALP ($p<0.05$) while bilirubin, albumin (ALB), and globulin levels were significantly increased ($p<0.05$). similarly, the Butanol Fraction (LB) significantly affect the AST, ALP, bilirubin, ALB, and globulin levels ($p<0.05$, $p<0.001$). The Ethyl Acetate Fraction (LEA) of the leaf part had an effect on ALT, AST, ALP, total protein (TP), and globulin, but had no significant effect on ALB ($p>0.05$). The data further confirm the n-Hexane Fraction (LNH) reduced ALT, AST, ALP, bilirubin, and globulin levels. The flower and root parts of the Ricinus communis also exhibited promising but comparatively lesser efficacy of liver biomarkers. It was observed that Flower Butanol Fraction (FB) improved ALT, AST, ALP, bilirubin, ALB, and globulin levels as compared to the CCl_4-treated group ($p<0.05$, $p<0.001$). Notably, *R. communis* L. extract at a dosage of 100 mg/kg/day also elicited a significant reduction in serum ALT and ALP levels ($p<0.05$). Elevated levels of total protein (TP) and albumin (ALB) ensuing from CCl_4 intoxication experienced a notable decline ($p<0.05$). Treatment with *R. communis* L. extracts from various plant parts (leaves, flowers, and roots) at dosages of 150 and 200 mg/kg/day contributed to the restoration of TP and ALB levels compared to the CCl_4-exposed group ($p<0.05$), while the 100 mg/kg/day dose did not demonstrate a substantial reduction in the elevated parameters. Remarkably, biochemical analysis of TP and ALB levels, along with ALT, AST, and ALP activities, failed to reveal statistically significant differences between the control cohort and subjects treated with various parts of the extract at the 200 mg/kg/day dosage ($p<0.05$). These results underscore the therapeutic potential of Ricinus communis-derived fractions in mitigating CCl_4-induced liver damage.

Table 1. Effect of CCl_4 in liver function tests (LFTs) like serum AST, ALT, Alkaline phosphate, bilirubin, and total protein levels.

Treatments	ALT (U/I)	AST (U/I)	ALP (U/I)	Bilirubin (mg/dL)	TP (g/dL)	ALB (g/dL)	Globulin (g/dL)
Normal	143.5 ± 4.24	145.55 ± 3.4	146.55 ± 3.19	0.555 ± 0.07	6.255 ± 0.35	3.75 ± 0.14	2.55 ± 0.14
CCl_4	193.5 ± 2.24***	494.5 ± 0.07***	155.5 ± 3.70***	0.455 ± 0.07*	7.655 ± 0.21*	2.16 ± 0.07*	4.05 ± 0.07*
Sylemarin	156.5 ± 2.12***	164.5 ± 3.36***	148.2 ± 2.5*	0.520 ± 0.05*	6.57 ± 0.15*	3.60 ± 0.04*	2.85 ± 0.11*
L-H_20	146.5 ± 1.41	160.5 ± 2.40*	113.5 ± 1.41*	0.525 ± 0.09*	7.15 ± 0.14	4.45 ± 0.07*	2.55 ± 0.07*
L-BoH	147.5 ± 1.41	173.55 ± 2.05**	242.55 ± 1.16***	0.455 ± 0.07*	6.155 ± 0.35	3.75 ± 0.42*	2.45 ± 0.07*
L-EA	152.55 ± 4.03*	152.55 ± 1.20*	138.5 ± 2.54*	0.455 ± 0.35*	7.155 ± 1.48*	3.85 ± 0.28	3.35 ± 1.0*6
L-nH	156.5 ± 5.65*	190.5 ± 2.12***	227.5 ± 2.12***	0.75 ± 0.07**	6.75 ± 0.98	3.95 ± 0.28	2.85 ± 0.70*
F-BoH	161.5 ± 1.41**	183.5 ± 4.94**	237.5 ± 0.70***	0.75 ± 0.07**	6.15 ± 0.07	3.85 ± 0.07	2.35 ± 0.01
F-EA	156 ± 5.61***	154.0 ± 1.22*	185 ± 3.41**	0.66 ± 0.08**	7.14 ± 1.49*	3.9 ± 0.29	2.4 ± 0.01
F-nH	161 ± 2.67***	179.5 ± 4.92**	179.5 ± 1.72**	0.65 ± 0.21*	6.17 ± 0.08	3.8 ± 0.29	2.3 ± 0.01
R-BoH	173.55 ± 1.34**	133.5 ± 5.65*	212.5 ± 2.40***	0.45 ± 0.14*	5.85 ± 0.07	4.65 ± 0.21*	1.25 ± 0.14*
R-EA	149.5 ± 3.19	182.55 ± 0.70**	188.55 ± 1.13***	0.755 ± 0.21*	6.85 ± 0.28	4.35 ± 0.56	2.55 ± 0.28
R-nH	164.5 ± 2.54*	201.5 ± 1.27***	178.5 ± 1.27**	0.255 ± 0.07*	5.855 ± 0.21	4.15 ± 0.84	1.85 ± 1.13

Table 2. Docking detail of ligands (L1-L7) against receptor CYP2C9 (3PGH).

Ligands	Biding energy (Kcalmol^{-1})	No. of interaction	Nature of interaction	Interaction distance (A^0)	Interaction residues
L1	-7.5095	02	2H-donor	3.00, 2.98	ASN367, ARG435,
L2	-7.9485	03	3H-donor	3.16, 3.03, 3.62	2ASN367, CYS437
L3	-5.6500	01	H-donor	3.13	ARG435
L4	-5.3304	01	H-acceptor	2.87	ARG 435
L5	-4.1090	---	---	---	---
L6	-5.7758	---	---	---	---
L7	-6.1940	01	H-donor	3.84	CYS 437

L1= 3,4,5-trihydroxy benzoic acid, L2= 3,4,5-trihydroxymethyl-benzoate, L3= p-hydroxycinnamic acid, L4=(E)-3-(4-hydroxyphenyl) acrylic acid, L5= (E)-3-(3-hydroxy-4-methoxyphenyl) acrylic acid, L6=(E)-nonyl-3-(3hydroxy-4-methoxyphenyl) acrylate.
Note: L= Ligands, and (---) represents no bonding interaction with ligands.

Table 3. Docking detail of ligands (L1-L7) against receptor cyclooxygenase-2 (4NZ2).

Ligands	Biding energy (Kcalmol^{-1})	No. of Interaction	Nature of Interaction	Interaction distance (A^0)	Interaction residues
L1	-6.8253	01	H-donor	3.38	GLY 416
L2	-6.8712	01	H-donor	3.06	GLY 109
L3	-5.3928	---	---	---	---
L4	-4.7764	---	---	---	---
L5	-6.0745	01	H-donor	2.94	ASN 289
L6	-5.3841	---	---	---	---
L7	-7.6661	01	pi-H	3.86	ARG 132

L1=3,4,5-trihydroxy benzoic acid, L2=3,4,5-trihydroxymethyl-benzoate, L3= p-hydroxycinnamic acid, L4=(E)-3-(4-hydroxyphenyl) acrylic acid, L5= (E)-3-(3-hydroxy-4-methoxyphenyl) acrylic acid, L6=(E)-nonyl-3-(3hydroxy-4-methoxyphenyl) acrylate.
Note: L= Ligands, and (---) represents no bonding interaction with ligands.

***In silico* molecular docking analysis:** *In silico* molecular docking is a crucial method in structural biology and computational drug design, wherein two molecules are assessed for their compatibility in both 2D and 3D spatial arrangements. In this research, the in silico molecular investigation was conducted using the Molecular Operating Environment software (MOE) to elucidate the binding mechanism of chosen ligands with specific enzymes such as 3PGH and 4NZ2. The ligand structures were generated using the builder tool within MOE for energy minimization, and the resulting ligands (L1-L7) were saved in an mdb file. These selected ligands (L1-L7) were then subjected to molecular docking against cyclooxygenase-2 (3PGH) and CYP2C9 (4NZ2) enzymes to validate their activities. Among all the ligands, the highest binding energy Kcalmol^{-1} was observed by L2 (-7.9485 Kcalmol^{-1}) with 3H-donor and interaction residues 2ASN367 and CYS437 against 3PGH. Similarly, L1 (-7.5095 Kcalmol^{-1}) with 2H-donor and interaction residues ASN367 & ARG435, also L7 were recognized (-6.1940 Kcalmol^{-1}) with nature of interaction H-donor and residues CYS 437. However, L3 was demonstrated -5.6500 Kcalmol^{-1} with H-donor and ARG435 residues, likewise, L4 were -5.3304 Kcalmol^{-1} with H-acceptor and ARG435 residues, respectively (Table 2 and Figs. 4 and 5). The molecular docking against 4NZ2, the highest binding energy, was recognized for ligand L7 (-7.6661 Kcalmol^{-1}) with Pi-H interaction and ARG132 residue. Similarly, the 2nd highest binding energy was observed by L1(-6.8253 Kcalmol^{-1}), L2 (-6.8712 Kcalmol^{-1}) and L5 (-6.0745 Kcalmol^{-1}) with H-donor interactions, and GLY416, GLY109 and ASN289 residues, respectively (Table 3). The *In silico* docking results demonstrated that the ligands (L1, L2 and L7) all showed the strongest potential for the inhibition of 3PGH and 4NZ2 supporting enzymes. However, other ligands also showed reasonable inhibitory effects.

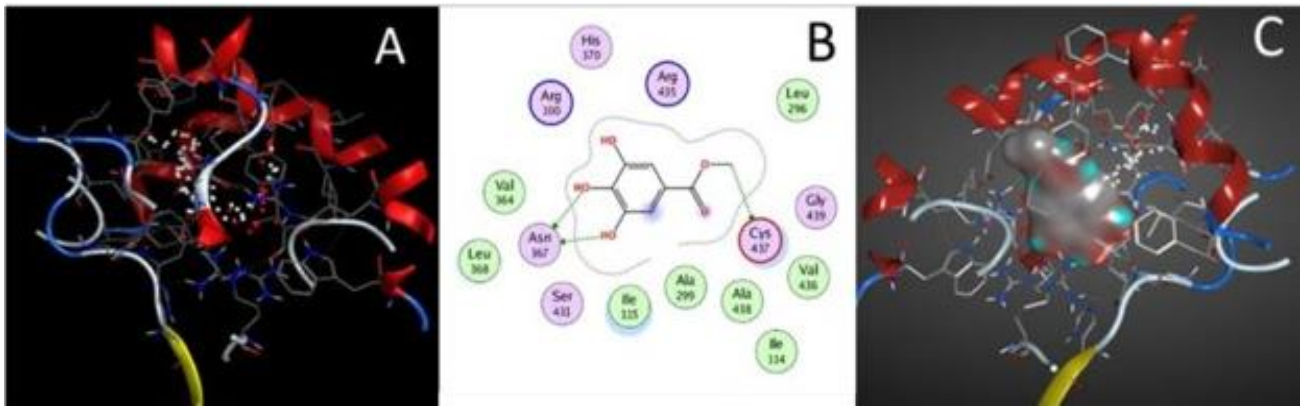

Fig. 4. (A) 3D structure of 3PGH (B) 2D and (C) 3D interaction of L2 with 3PGH.

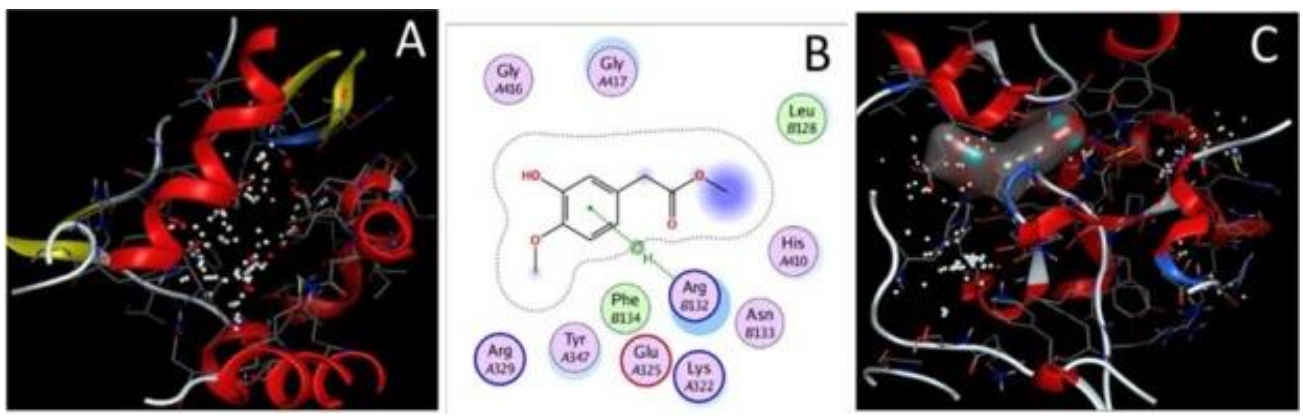

Fig. 5. (A) 3D structure of 4NZ2 (B) 2D and (C) 3D interaction of L7 with 4NZ2.

Discussion

CCl_4 is a widely used hepatotoxin in experimental animal models to understand liver diseases caused by xenobiotics, and this injury is due to trichloro methyl radical, a reactive oxygen species that produces toxic intermediates (Ali *et al.,* 2019). The toxicity of this radical is attributed to its covalent interactions with macromolecules. These interactions induce lipid peroxidation, which breaks down the lipid membrane of the endoplasmic reticulum membrane and triggers the release of serum enzymes. Such adverse effects of CCl_4 are observed through a decrease in the antioxidant potential of the enzyme (GSH) and an increase in TBARS. Eventually, an increase in serum bilirubin results in jaundice (Oyewole *et al.,* 2022).

Previous research studies have demonstrated the induction of CCl_4 reduces protein levels in Wistar rats which were significantly elevated by treating it with extracts of different parts of *Ricinus communis*, and validated the subject plant as having hepatoprotective potential (Naveen *et al*., 2016a and b). Previously, two leading compounds (ricinin, and N-dimethyl-ricinin) were isolated from the leaf part of *R. communis*, namely showed hepatoprotective activity (Visen *et al*., 1992). Correspondingly, galactosamine-induced liver damage barred significantly in an initial test using albino rats. Additionally, hepatocytes isolated from rats administered paracetamol demonstrated dose-dependent choleretic and anti-cholestatic effects as well as hepatoprotective activity. Important enzymes that link the metabolism of amino acids and carbohydrates are AST, ALT, ALP and LDH and are often used in the assessment of liver disease. An increase in their activity indicates inflammatory hepatocellular diseases and acute liver damage. Our results showed that CCl_4-treated rats had significantly higher AST, ALT, ALP and LDH activity. This is consistent with previous results that suggested that CCl_4 caused significant liver injury by altering its functional transition (BenHusna *et al*., 2019; Anis *et al*., 2022). These changes result in membrane permeability, allowing enzymes to escape into the extracellular environment. Pre-treatment with *Ricinus communis* extract before CCl_4 injection significantly reduced the increase in serum levels of ALT, AST ALP and LDH. This result demonstrated that *Ricinus communis* extract can reduce the elevated serum enzyme levels resulting from CCl_4 administration alone. This indicates the structural and functional integrity of the liver parenchyma cells. Similarly, the increased lipid and liver marker plasma levels in rats caused by CCl_4 were significantly inhibited by the leaf part of *Ricinus communis* pretreatment. Furthermore, this fraction improved biochemical and histological indices compared to the CCl_4-treated group. Based on our findings, it appears that *R. communis* has compounds that could effectively counteract the effects of CCl_4 poisoning and prevent sequelae of hepatotoxicity. These findings were also supported by molecular docking studies.

Conclusion

This study represents the hepatoprotective potential and molecular docking analysis of various fractions of *Ricinus communis*. The findings provide robust evidence of the therapeutic potential particularly leaf-derived fractions, in mitigating carbon tetrachloride-induced liver damage. Biochemical analyses demonstrated significant reductions in liver function markers, while histopathological evaluations confirmed substantial improvements in liver architecture, including reduced inflammation and fat accumulation. Furthermore, in silico molecular docking displayed key interactions and enzymes involved in liver injury, shedding light on the underlying mechanisms of action. These findings suggest *R. communis* may serve as a natural remedy for haptic disorders, necessitating further clinical investigation.

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