Asian Journal of Chemistry; Vol. 34, No. 4 (2022), 979-988

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23616

GC-MS Analysis, Molecular Docking and Hepatoprotective Effect of Ethanolic Extract of *Capparis zeylanica* on CCl_4-Induced Hepatotoxicity in Rats

R.N. Hota[1,*], B.K. Nanda[2], B.R. Behera[3], A. Bose[4] and D. Das[4]

[1]The Pharmaceutical College, Barpali, Bargarh-768029, India
[2]Institute of Pharmaceutical Sciences, Station Bazar, Jaleswar, Balasore-756032, India
[3]Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela-769015, India
[4]School of Pharmaceutical Sciences, Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar-751029, India

*Corresponding author: E-mail: raghunandan_hota@rediffmail.com

Received: 12 November 2021; *Accepted*: 29 January 2022; *Published online*: 10 March 2022; AJC-20743

Capparis zeylanica (Capparaceae) is a climbing shrub, commonly known as 'Asadua' in Oriya language. Folkloric it is used as anti-inflammatory, anti-rheumatic, hepatitis and liver tonics. The present work aimed to investigate the hepatoprotective activity of *Capparis zeylanica* root. The ethanolic extract was subjected to GC-MS analysis and quantification of rutin, quercetin and gallic acid by HPLC. *In vitro* antioxidant study was evaluated by DPPH, nitric oxide radical and hydrogen peroxide assay. Hepatoprotective activity was performed by CCl_4 (1.25 ml/kg, I.P) induced Wistar albino rats at dose of 200 and 300 mg/kg using silymarin (100 mg/kg) as standard. The liver tissue homogenate was examined for lipid peroxidation, SOD, CAT and GSH assay. Serum was taken for biochemical analysis and liver for histopathological study. *In silico* molecular docking study was performed by the indentified compounds. Gallic acid was quantified as highest in HPLC analysis. Fifty compounds were identified by GC-MS analysis. The extract exhibited significant free radical scavenging effect at highest concentration. The level of liver function enzymes (AST, ALT and ALP), bilirubin and protein were significantly improved after the administration of 200 and 300 mg/kg of extract. Alterations in SOD, CAT, GSH and lipid peroxidation levels were significantly checked by extract. Compounds propane 1,1-dipropoxy- (-5.2); *cis*-9-hexadecenal (-6.0); 17-octadecen-14-yn-1-ol (-6.0); 6-butyl-1,4-cycloheptadiene (-6.5) exhibited greater docking score as compared to standard silymarin (-5.1). The hepatoprotective potential of *C. zeylanica* could be due to its antioxidant effect and the synergistic effect of these compounds.

Keywords: *Capparis zeylanica*, Molecular docking, Hepatoprotective activity, GC-MS, Carbon tetrachloride.

INTRODUCTION

The liver is the most vital organ in the human body and plays a central role in the metabolism, transportation and breakdown of xenobiotics, making it vulnerable to chemical induced toxicity [1]. Exposure of the liver to drugs, toxic chemicals and environmental pollutants leads to the formation of reactive oxygen species (ROS), which are accountable for hepatitis, cirrhosis, liver cancer and many other diseases [2]. The hepatotoxicity induced by carbon tetrachloride leads to the formation of trichloromethyl and trichloromethyl peroxyl radicals, which bind to macromolecules such as DNA, lipids and proteins and lead to liver necrosis [3]. Oxidative stress, which occurs due to the imbalance between the antioxidant defence system and ROS production, is involved in the pathophysiological changes associated with various liver diseases such as hepatitis, hepatocellular carcinoma, and cirrhosis [4]. A lack of adequate therapy and severe adverse effects are the current two problems with synthetic drugs for the treatment of hepatic disorders [5,6]. Treatment options for liver diseases are mostly limited in their effectiveness, often have serious side effects and are too expensive, especially for developing countries [7,8]. There are some serious side effects associated with synthetic antioxidants.

Over the last decades, scientists have been actively searching for natural antioxidants that are cost-effective and free of/with minimal adverse effects [9]. It is imperative, therefore, to search for hepatoprotective medications that are both highly effective and low in risk of adverse effects. Such characteristics can be found in many medicinal herbs, which indicate that they may contain new active therapeutic substances. *Capparis*

zeylanica (Capparaceae), a many-branched thorny, sub-scandent climbing shrub, commonly known as 'Asadua' in oriya, find its distribution throughout the greater part of India as hedge plant [10,11]. Different parts are traditionally claimed to treat various ailments such as snake bite, small pox, swelling of testicles, anthelmintic, sedative, stomachic, antihidrotic, piles, cholera, anti-rheumatic and anti-inflammatory [12,13].

Folk medicine uses this remedy for treating stomach ulcers, hernias, swelling, itching, hepatitis, liver tonics and insect poisonings [14]. The root bark decoctions are prescribed in Unani medicine as an anthelmintic, anti-inflammatory and deobstruent to liver and spleen [15]. The plant has also been scientifically proven to have various pharmacological activities, including antibacterial [16], antimicrobial [17], anti-ulcer [18] and antibacterial [19]. Leaves and seeds are known to contain α-amyrin, fixed oil, glucocapparin and *n*-tricontane [12]. The plant is also known to possess an alkaloid, a phytosterol, a mucilaginous substance and water soluble acid, L-stachydrine, rutin and β-sitosterol [10]. β-Carotene was isolated from leaves [20]. The plant showed the presence of saponin, *p*-hydroxybenzoic, syringic, vanillic, ferulic and *p*-coumanic acid [21]. Literature review revealed that there is no scientific evidence on hepatoprotective activity of this plant. Taking into account its folkloric use and its established role in combating oxidative stress, the present study was examined the hepatoprotective potential of *C. zeylanica* root along with molecular docking analysis of compounds identified from GC-MS analysis.

EXPERIMENTAL

Plant material: *C. zeylanica* root was collected locally from Barpali city in the month of July-August 2022 and authenticated (TPC/COL/21/013) by Botanist Dr. Surya Kumar Barpanda, Shree Ram College, Sonepur, India. The prepared herbarium was kept in the Department of Pharmacology for future reference. The plant was allowed to dry under shade, followed by powdering and kept in air tight container and protected from light to prevent photo degradation of metabolites.

Extraction of plant material and phytochemical screening: The root was extracted by hot percolation method using petroleum ether (60-80 ºC) and ethanol in Soxhlet apparatus. The solvent was removed under reduced pressure using rotary evaporator. The obtained extract was calculated in terms of percentage, stored at cool place and subjected to phytochemical analysis [22]. The ethanolic extract of *C. zeylanica* (ECZ) was taken for experimental purpose.

Analysis by HPLC: The ethanolic extract of *C. zeylanica* (ECZ) was subjected to HPLC analysis coupled with auto sampler, photo diode array detector, Perkin-Elmer series 200, Waltham, USA for the quantification of gallic acid, quercetin and rutin following the method of Srivastava *et al.* [23]. Greater resolution of phenolic and flavonoid compounds were achieved by gradient elution of buffer concentration (solvent A) and acetonitrile (solvent B) with run time of 6 min (Table-1). Each of tests and reference sample (20 µL) were injected. Gallic acid was recorded at wavelength of 270 nm while quercetin and rutin at 370 nm.

TABLE-1
GRADIENT CONDITIONS OF SOLVENT FOR HPLC ANALYSIS

Time (min)	Buffer concentration (Solvent A)	Acetonitrile concentration (Solvent B)
0.01	95	5
18.00	70	30
25.00	55	45
28.00	15	85
35.00	15	85
40.00	55	45
45.00	95	5

GC-MS analysis: GC-MS analyses of ECZ were performed by Perkin-Elmer Clarus 680 system (Perkin-Elmer, Inc. USA) equipped with a fused silica column, packed with Elite-5MS capillary column (30 m in length × 250 µm in diameter × 0.25 µm in thickness). Constant flow rate of 1 mL/m was maintained using helium as carrier gas. Compounds were detected with a GC-MS spectral range of 40 to 600 *m/z* using ionization energy of 70 eV (electron volts) and a scan time of 0.2 s. At 250 ºC, the injector was kept at a constant injection volume of 1 µL. At first, the temperature of the column was maintained at 50 ºC for 3 m, and then it was increased by 10 ºC per minute until it reached 280 ºC. The final temperature was retained at 300 ºC for 10 min [24]. The phytometabolites were identified by comparing their retention time, peak area, peak height and mass spectral patterns with the spectral database of authentic compounds stored in the National Institute of Standards and Technology (NIST) library.

Estimation of total phenolic, flavonoid content and assay of *in vitro* antioxidant activity: Total phenolic [25] and flavonoid content [26] of ECZ was determined by following the standard methods with minor modifications. Total phenolic content was calculated by taking gallic acid (20-100 µg/mL) as standard and expressed as milligram gallic acid equivalents per gram (mg GAE/g). The absorbance was measured at 765 nm by spectrophotometer (Thermoscientific, USA) against blank. Flavonoid content was also quantified from the calibration curve drawn from concentration of 5-100 mg/L of quercitin as standard and the values were expressed as milligram of quercitin equivalent per gram (mg QE/g). The absorbance was taken at 506 nm by spectrophotometer against blank. The ECZ at concentration of 100, 200, 300, 400 and 500 µg/mL was investigated for free radical scavenging activity by DPPH [27], nitric oxide radical scavenging [28] and hydrogen peroxide assay [28] method using standard ascorbic acid (100-500 µg/mL). The percentage inhibition was calculated using the following equation.

$$\frac{\text{Control}_{Abs} - \text{Sample}_{Abs}}{\text{Control}_{Abs}} \times 100$$

where, Abs is the absorbance. All the assay methods were performed in triplicate. A linear regression curve obtained from percentage inhibition against concentration was used to calculate the IC_{50} values.

Animals: Wistar albino rats of either sex weighing about 160-190 g (3 months) were procured from authentic breeder, M/s Chakraborty Enterprises (Reg. no: 1443/PO/Bt/s/11/

CPCSEA, Kolkata, India). Prior to the experiment, animals were kept in animal cage and acclimatized to the laboratory condition by maintaining at 22 ± 30 ºC, followed by day and night cycle of 12 h for 7 days. During this, rats were provided with standard pellet diets and water *ad libitum*. The hygienic condition was maintained by cleaning the cages on every three days prior to avoid any infections. According to the ethical guideline of International standards proper human care was provided for the use of laboratory animals. The experimental protocol was approved by Animal Ethics Committee (1376/ac/10/CPCSEA).

Evaluation of hepatoprotective activity: Hepatoprotective activity of ECZ was performed according to the method of Navarro *et al.* [29]. Animals were divided into five groups each of six. Hepatotoxicity was induced to all groups except Group I by injecting intraperitoneally 1.25 ml/kg b. w. of CCl_4 dissolved in liquid paraffin in ratio 1:1 for 7 days. Group I received 1% carboxy methylcellulose (CMC), 2 mL/kg/day, orally, daily for 7 days and treated as normal control (CNT). Group II considered as CCl_4-induced hepatotoxicity (IND), received 1% CMC, 2 mL/kg/day, orally and treated as negative control. Group III received 100 mg/kg of silymarin (SLM) for 7 days and treated as positive control. Group IV & V received 200 & 300 mg/kg of ECZ for 7 days.

Biochemical investigations: Blood sample was collected from experimental rats by cardiac puncture after sacrificed by cervical dislocation. Biochemical investigation aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphate (ALP), total bilirubin (TB), total protein was carried out on serum after centrifugation of blood at 1000 rpm for 10 min by following established methods using commercially available enzymatic kits (Vitro scientific, Germany) [30-33].

Assessment of *in vivo* antioxidant activity: Tissue homogenate was prepared in ice-cold phosphate buffered (50 mM, pH 7.4) and subjected to centrifugation at 800 rpm for 10 min at 4 ºC. The resultant supernatant liquid was taken for estimation of lipid peroxidation (LPO), catalase activity (CAT), super oxide dismutase (SOD) and reduced glutathione (GSH). The lipid peroxide contents were measured at 532 nm in UV-visible spectrophotometer and expressed as moles malondialdehyde (MDA) per 100 mg of protein [34]. Catalase activity was performed following the established method of Beers &Sizer [35]. The activity was determined at absorbance of 240 nm in UV-visible spectrophotometer and presented as unit of H_2O_2/mg of tissue. SOD was estimated as per the protocol of Sagu *et al.* [36]. The values were measured at 480 nm against blank and reported as unit of SOD activity/mg of tissue. GSH was determined by the method of Ellman [37], where absorbance was read at 412 nm against a blank. The percentage of inhibition was calculated.

Histopathological examination of liver: The excised liver was fixed in neutral buffered formalin (10%) for 24 h, followed by dehydration in series of alcohol. Sections were exposed to xylene and embedded in paraffin wax box. Microtome sections were taken at thickness of 4 µm and stained with hematoxylin and eosin. Images were observed under light microscope using digital camera [38].

***In silico* molecular docking analysis:** Compounds identified from GC-MS analysis of ECZ were subjected to molecular docking studies by Autodock Vina software, version 1.1.2, using silymarin as standard. The binding affinity of the compounds with the targeted protein was expressed as Kcal/mol [39].

Statistical analysis: One-way ANOVA (Analysis of variance), posthoc Tukey HSD test was used for statistical analysis of the generated data and expressed as mean ± S.E.M (standard error mean). The '*p*' value less than 0.05 was considered as statistical significant.

RESULTS AND DISCUSSION

Phytochemical investigation: The results of the phytochemical investigation of *C. zeylanica* root revealed the presence of carbohydrates, alkaloids, phenols, flavonoids and steroids (Table-2).

TABLE-2
PRELIMINARY PHYTOCHEMICAL SCREENING OF *C. zeylanica* ROOT

Test	Petroleum ether	Ethanol
Test for alkaloid		
i) Mayer's test	–	+
ii) Wagner's test	–	+
iii) Hager's test	–	+
iv) Dragendorff's test	–	+
Test for carbohydrates & glycosides		
i) Molish's test	–	+
ii) Fehling's test	–	+
iii) Barfoed's test	–	+
iv) Benedict's test	–	+
v) Borntrager's test	–	+
Test for saponins		
Foam test	–	–
Test for proteins & amino acid		
Millon's test	–	+
Test for phenolic compounds & flavonoids		
i) Ferric chloride test	+	+
ii) Lead acetate test	+	+
iii) Alkaline test	–	+
Test for phytosterol		
Solkowski test	–	+

Analysis by HPLC: Polyphenols and flavonoids present in medicinal plants possess the ability to eliminate free radicals, increase antioxidant enzymes, modulate gene expression and therefore, can provide protection against destructive free radicals [40,41]. Rutin, quercetin and gallic acid were quantified as 0.02, 0.04 and 0.13% w/w in ECZ, respectively (Table-3). The chromatograms of standard rutin, quercetin and gallic acid were presented in Fig. 1 and ECZ in Fig. 2.

TABLE-3
QUANTITATIVE ESTIMATION OF PHENOLIC AND FLAVONOID COMPOUNDS IN ETHANOLIC EXTRACT OF *C. zeylanica* ROOT BY HPLC ANALYSIS

Tests	Percentage of yield (% w/w)
Gallic acid	0.13
Rutin	0.02
Quercetin	0.04

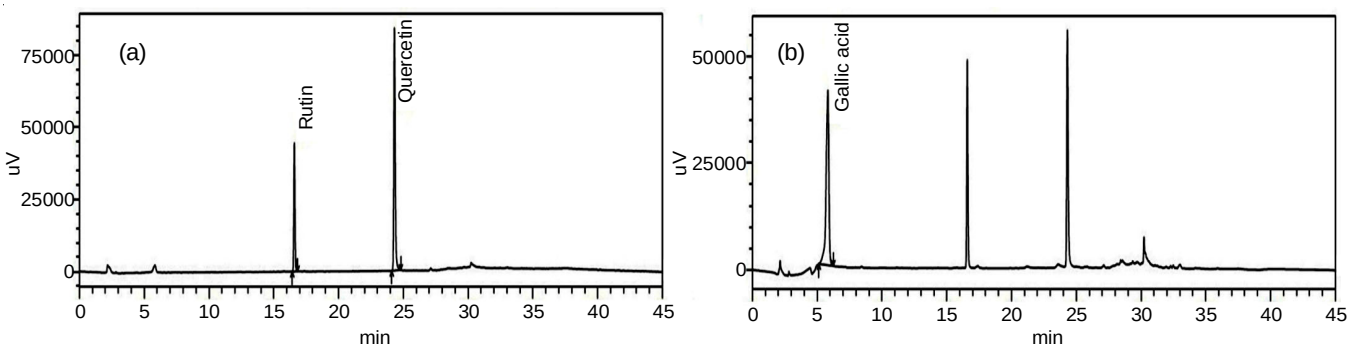


Fig. 1. HPLC chromatogram of (a) standard rutin and quercetin; (b) standard gallic acid

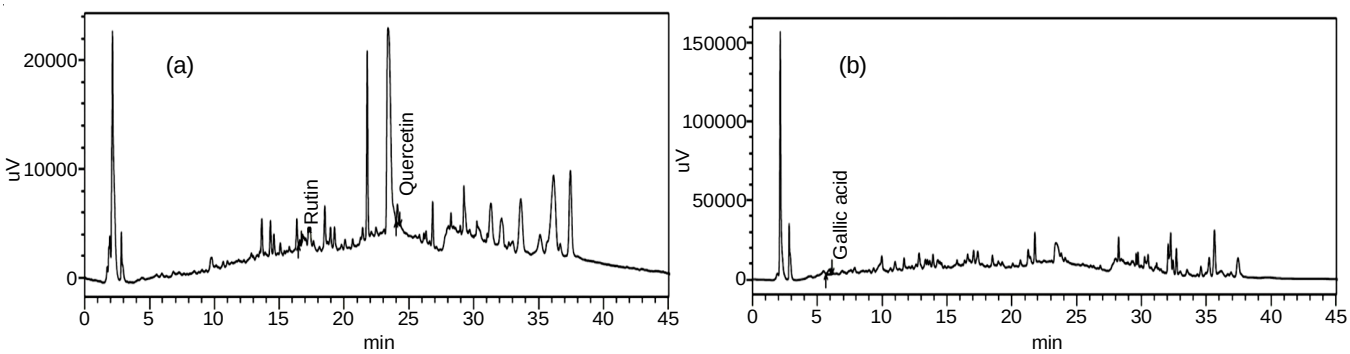


Fig. 2. HPLC chromatogram of (a) rutin and quercetin; (b) gallic acid in ethanolic extract of *C. zeylanica* root

Analysis by GC-MS: GC-MS chromatogram of ECZ was presented in Fig. 3. The functional groups, molecular formula, molecular weight, retention time and percentage peak areas of the respective compounds are depicted in Table-4. GC-MS analysis revealed 11 major compounds and 39 minor compounds. Major compounds were identified as 4-ethyl-2-hydroxy-cyclopent-2-en-1-one (2.54%); 1,3-difluoro-5-dimethyl-(isopropyl)silyloxybenzene (2.80%); 1-butanol, 3-methyl- (3.67%); α-tocopherol (3.70%); *n*-hexadecanoic acid (3.80%); methyl octadeca-9-yn-11-*trans*-enoate (5.23%); propane 1,1-dipropoxy- (6.68%); 17-octadecen-14-yn-1-ol (7.97%); *cis*-9-hexadecenal (8.86%); glycerol (10.07%) and 6-butyl-1,4-cycloheptadiene (19.26%).

The minor compounds were pentadecanoic acid, 14-methyl-, methyl ester (0.15%); hydrazinecarbothioamide (0.18%); ethanamine, 2,2-diethoxy (0.19%); 3-propylglutaric acid (0.19%); carbamic acid, phenyl ester (0.22%); propane, 2,2-dimethoxy- (0.24%); 5,8,11-heptadecatrien-1-ol (0.24%); dodecane, 2,7,10-trimethyl- (0.31%); 12-methyl-oxacyclododecan-2-one (0.31%); (*R*)-(-)-2,2-dimethyl-1,3-dioxolane-4-methanol (0.35%); 2-butanone, 3-methoxy-3-methyl- (0.38%); *o*-ethylhydroxylamine (0.38%); undecanoic acid (0.39%); erythritol (0.40%); D-alanine, N-propargyloxycarbonyl-, isohexyl ester (0.42%); Z-10-tetradecen-1-ol acetate (0.42%); 4-acetoxy-3-methoxystyrene (0.47%); 3,3,3-trifluoropropene (0.48%); *cis*-9-hexadecenoic acid (0.51%); 2-furancarboxaldehyde, 5-methyl- (0.52%); ditetradecyl ether (0.54%); *Z,Z,Z*-1,4,6,9-nonadecatetraene (0.56%); ethyl *trans*-4-decenoate (0.57%); octane, 2,4,6-trimethyl- (0.62%); cholesterol (0.63%); (1-methoxypentyl)cyclopropane (0.65%); undecanoic acid, ethyl ester

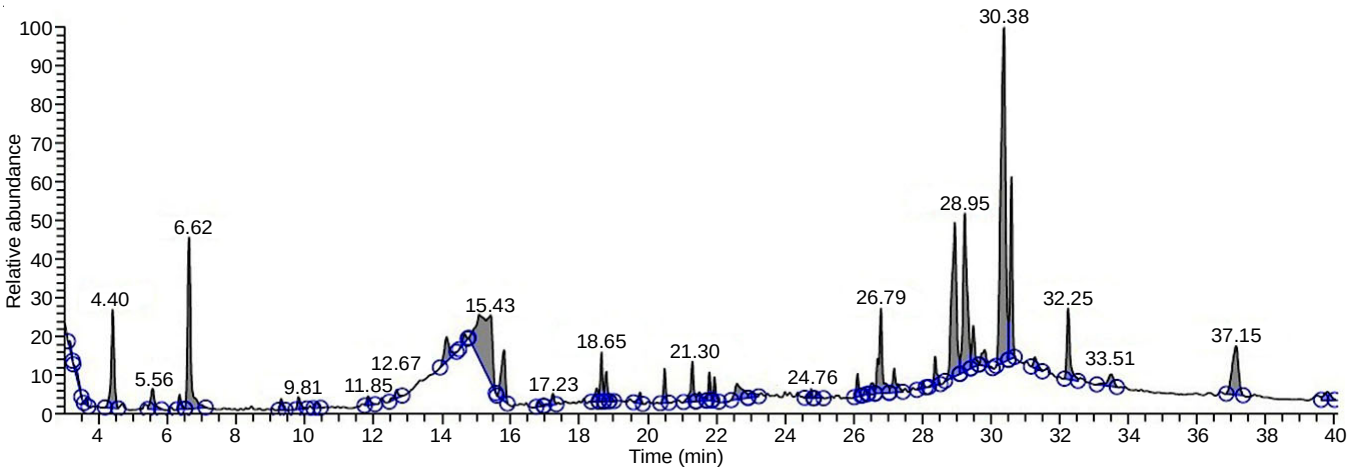


Fig. 3. GC-MS chromatogram of ethanolic extract of *C. zeylanica* root

TABLE-4
GC-MS ANALYSIS OF ETHANOLIC EXTRACT OF *C. zeylanica* ROOT

S. No.	Name of compounds	RT	m.f.	m.w.	Percentage of peak area	Chemical nature
1	Propane, 2,2-dimethoxy-	3.16	$C_5H_{12}O_2$	104.15	0.24	Ether
2	2-Butanone, 3-methoxy-3-methyl-	3.35	$C_6H_{12}O_2$	116.16	0.38	Ether
3	Hydrazinecarbothioamide	3.63	CH_5N_3S	91.13	0.18	Amine
4	1-Butanol, 3-methyl-	4.40	$C_5H_{12}O$	88.14	3.67	Alcohol
5	2,4-Azetidinedione, 3,3-diethyl-	5.56	$C_7H_{11}NO_2$	141.17	1.02	Imide
6	3,3,3-Trifluoropropene	6.34	$C_3H_3F_3$	96.05	0.48	Alkene
7	Propane, 1,1-dipropoxy-	6.62	$C_9H_{20}O_2$	160.25	6.68	Ether
8	(*R*)-(-)-2,2-Dimethyl-1,3-dioxolane-4-methanol	9.31	$C_6H_{12}O_3$	132.16	0.35	Alcohol
9	2-Furancarboxaldehyde, 5-methyl-	9.81	$C_6H_6O_2$	110.11	0.52	Aldehyde
10	Carbamic acid, phenyl ester	10.35	$C_7H_7NO_2$	137.14	0.22	Carbamate
11	Erythritol	11.85	$C_4H_{10}O_4$	122.11	0.40	Alcohol
12	D-Alanine, N-propargyloxycarbonyl-, isohexyl ester	12.67	$C_3H_8O_3$	255.31	0.42	Ester
13	Monoethanolamine	14.12	C_2H_7NO	61.08	1.54	Amine
14	O-Ethylhydroxylamine	14.65	C_2H_7NO	47.05	0.38	Amine
15	Glycerin	15.43	$C_3H_8O_3$	92.09	10.07	Alcohol
16	4-Ethyl-2-hydroxycyclopent-2-en-1-one	15.80	$C_7H_{10}O_2$	126.15	2.54	Ketone
17	Ethanamine, 2,2-diethoxy	16.82	$C_6H_{15}NO_2$	133.18	0.19	Ether
18	4-Acetoxy-3-methoxystyrene	17.23	$C_{11}H_{12}O_3$	192.21	0.47	Ether
19	Ethyl trans-4-decenoate	18.51	$C_{12}H_{22}O_2$	198.30	0.57	Ester
20	Decanoic acid, ethyl ester	18.65	$C_{12}H_{24}O_2$	200.31	1.38	Ester
21	Decane, 2,6,7-trimethyl-	18.79	$C_{13}H_{28}$	184.36	0.91	Alkane
22	3-Propylglutaric acid	18.94	$C_8H_{14}O_4$	174.19	0.19	Carboxylic acid
23	Undecanoic acid	19.77	$C_{11}H_{22}O_2$	186.29	0.39	Carboxylic acid
24	Pentanoic acid, 5-hydroxy-, 2,4-di-*t*-butylphenyl ester	20.49	$C_{19}H_{30}O_3$	306.40	0.92	Ester
25	Bicyclo[3.1.0]hexan-2-one, 1,5-*bis*(1,1-dimethylethyl)-3,3-dimethyl-	21.30	$C_{16}H_{28}O$	236.39	1.48	Aldehyde
26	Ditetradecyl ether	21.50	$C_{28}H_{58}O$	410.75	0.54	Ether
27	Undecanoic acid, ethyl ester	21.79	$C_{13}H_{26}O_2$	214.34	0.70	Ester
28	Octane, 2,4,6-trimethyl-	21.95	$C_{11}H_{24}$	156.30	0.62	Alkane
29	Azelaic acid	22.60	$C_9H_{16}O_4$	188.22	1.50	Carboxylic acid
30	Z-10-Tetradecen-1-ol acetate	23.05	$C_{16}H_{30}O_2$	254.41	0.42	Ester
31	Dodecane, 2,7,10-trimethyl-	24.76	$C_{15}H_{32}$	212.41	0.31	Alkane
32	12-Methyl-oxa-cyclododecan-2-one	24.93	$C_{12}H_{22}O_2$	198.30	0.31	Carbonyl
33	(1-Methoxy-pentyl)-cyclopropane	26.11	$C_9H_{18}O$	142.24	0.65	Ether
34	Pentadecanoic acid, 14-methyl-, methyl ester	26.33	$C_{17}H_{34}O_2$	270.45	0.15	Ester
35	*cis*-9-Hexadecenoic acid	26.55	$C_{16}H_{30}O_2$	254.40	0.51	Carboxylic acid
36	*n*-Hexadecanoic acid	26.79	$C_{16}H_{32}O_2$	256.42	3.80	Carboxylic acid
37	Eicosanoic acid, ethyl ester	27.18	$C_{22}H_{44}O_2$	340.58	1.19	Ester
38	5,8,11-Heptadecatrien-1-ol	28.02	$C_{17}H_{30}O$	250.40	0.24	Alcohol
39	9,12-Octadecadienoic acid, methyl ester	28.37	$C_{19}H_{34}O_2$	294.47	1.02	Ester
40	*cis*-9-Hexadecenal	28.95	$C_{16}H_{30}O$	238.40	8.86	Aldehyde
41	17-Octadecen-14-yn-1-ol	29.24	$C_{18}H_{32}O$	264.40	7.97	Alcohol
42	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	29.49	$C_{20}H_{34}O_2$	306.48	1.52	Carboxylic acid
43	*cis*,*cis*,*cis*-7,10,13-Hexadecatrienal	29.82	$C_{16}H_{26}O$	234.38	0.99	Aldehyde
44	6-Butyl-1,4-cycloheptadiene	30.38	$C_{11}H_{18}$	150.26	19.26	Alkene
45	Methyl octadeca-9-yn-11-*trans*-enoate	30.60	$C_{19}H_{32}O_2$	292.50	5.23	Ester
46	Z,Z,Z1,4,6,9-Nonadecatetraene	31.28	$C_{19}H_{32}$	260.50	0.56	Alkene
47	1,3-Difluoro-5-dimethyl-(isopropyl)-silyloxybenzene	32.25	$C_{11}H_{16}F_2OSi$	230.32	2.80	Aryl halide
48	γ-Tocopherol	33.51	$C_{28}H_{48}O_2$	416.70	0.94	Alcohol
49	α-Tocopherol	37.15	$C_{29}H_{50}O_2$	430.70	3.70	Alcohol
50	Cholesterol	39.81	$C_{27}H_{46}O$	386.65	0.63	Alcohol

(0.70%); decane, 2,6,7-trimethyl- (0.91%); pentanoic acid, 5-hydroxy-, 2,4-di-*t*-butylphenyl ester (0.92%); γ-tocopherol (0.94%); *cis*,*cis*,*cis*-7,10,13-hexadecatrienal (0.99%); 2,4-azetidinedione, 3,3-diethyl- (1.02%); 9,12-octadecadienoic acid, methyl ester (1.02%); eicosanoic acid, ethyl ester (1.19%); decanoic acid, ethyl ester (1.38%); bicyclo[3.1.0]hexan-2-one, 1,5-*bis*(1,1-dimethylethyl)-3,3-dimethyl- (1.48%); azelaic acid (1.50%); 8,11,14-eicosatrienoic acid, (*Z*,*Z*,*Z*)- (1.52%); and monoethanolamine (1.54%).

Total phenol and flavonoid contents: Total phenolic and flavonoid contents of ECZ were found as 55.29 ± 2.34 mg GAE/g and 39.15 ± 0.13 mg QE/g, respectively (Table-5).

***In vitro* antioxidant study:** Antioxidants from natural sources remove the free radicals generated by CCl_4 and other

TABLE-5
DETERMINATION OF TOTAL PHENOLIC, FLAVONOID CONTENT, *in vitro* ANTIOXIDANT ACTIVITY OF *C. zeylanica* ROOT WITH CORRESPONDING IC_{50} VALUE

	Total phenolics (mg GAE/g dE)	Total flavonoids (mg QE/g dE)	DPPH (%)	H_2O_2 (%)	Nitric oxide (%)
Ascorbic acid	–	–	91.66 ± 0.22	80.23 ± 0.47	76.44 ± 0.52
IC_{50} (µg/mL)	–	–	39.23 ± 0.17	45.36 ± 0.52	49.48 ± 0.58
C. zeylanica	55.29 ± 2.34	39.15 ± 0.13	45.27 ± 0.13*	44.32 ± 0.51*	65.13 ± 0.23*
IC_{50} (µg/mL)	–	–	85.18 ± 0.67	118.45 ± 0.51	89.32 ± 0.53

GAE = Gallic acid equivalent, QE = Quercetine equivalent; Values are expressed as mean ± SEM, n = 3 and was estimated by One-way ANOVA followed by Dunnet test. *$p < 0.05$ considered as significant.

hepatotoxic substances [42]. Because of their free radical termination powers, natural antioxidants help to prevent hepatotoxicity, carcinogenesis, mutagenesis, and aging associated with oxidative stress. The ECZ significantly scavenged the free radicals by DPPH (45.27 ± 0.13), hydrogen peroxide (44.32 ± 0.51) and nitric oxide assay (65.13 ± 0.23) method at highest concentration with corresponding IC_{50} values of 85.18 ± 0.67, 118.45 ± 0.51 and 89.32 ± 0.53, respectively in comparison to standard ascorbic acid (Table-5). In the current study, the free radicals scavenging activity of ECZ was well noticed against DPPH, H_2O_2 and nitric oxide at concentration 500 µg. This could be attributed to the presence of phenolic and flavonoid compound in root of *C. zeylanica*, which were quantified by HPLC.

Effect of ECZ on liver function enzymes, bilirubin and protein: The cytochrome p450 enzymes bioactivate carbon tetrachloride into free radicals that attack polyunsaturated fatty acids, resulting in the generation of peroxy and alkoxy radicals, which produce highly reactive lipid peroxides. Lipid peroxide production leads to the loss of integrity in the cell membrane, enzyme leakage, DNA damage, and necrosis of hepatocytes [43]. Liver biomarkers leak into the serum due to hepatocellular damage. Hepatocytes lack functional integrity when their ALT levels are elevated [44]. In addition, CCl_4 impairs bile flow, increasing both bilirubin content and ALP levels, which are excreted through bile. A significant increase in serum indicator enzymes (AST, ALT and ALP) and bilirubin was observed in the current study, which indicated that there was significant impairment of liver cellular function and structure. The ALP and bilirubin levels were significantly restored by ECZ and SLM, as a result of their ability to stabilize membranes and prevent biliary dysfunction. Furthermore, liver toxicity and oxidative stress are linked with the depletion of TP. Animals treated with CCl_4 showed a significant decline in TP, indicating hepatotoxicity in rats. It was seen that ECZ and SLM normalize the levels of TP in the liver, and thus reducing oxidative stress. The administration of ECZ at dose of 300 mg/kg was found more effective than 200 mg/kg against CCl_4-induced hepatotoxicity in rats. Group treated with SLM (100 mg/kg) significantly checked the altered enzyme levels (Table-6).

***In vivo* antioxidant study:** One of the serious health problems across the world is oxidative stress, which contributes to a number of hepatic disorders [45]. Carbon tetrachloride is frequently used to promote liver injury. Free radical reactions are primarily responsible for the hepatotoxicity induced by CCl_4 [46]. The toxicity of CCl_4 in liver tissue was associated with metabolic biotransformation by cytochrome P450 resulting in the formation of trichloromethyl ($CCl_3^{\bullet}$) and trichloromethylperoxy ($CCl_3OO^{\bullet}$) free radicals [47-49]. The resulted free radicals remove hydrogen atoms from the lipid membrane of hepatocytes and form lipid hydroperoxides, which leads to liver necrosis [50,51]. The elevated level of lipid hydroperoxides and free radicals caused decrease in antioxidant enzymes, DNA oxidative damage, genetic mutation, chromosomal changes, and reduced CYP2E1 activity, when they are present in the body at higher levels than normal. The CCl_4 increases hepatocyte MDA levels and causes lipid peroxidation in hepatocytes. In addition to lipid peroxidation, tissue damage is gauged by MDA, which is a secondary product of lipid peroxidation [52].

Catalase and SOD are key antioxidant enzymes responsible for protecting cells from oxidative injury caused by free radicals [53,54]. In present study, the administration of CCl_4 caused significant decrease in CAT and SOD activity as compared to normal control group. Ascorbic acid, tocopherol, glutathione system, catalase and superoxide dismutase are components of the hepatocyte's antioxidant defence system that protects it against free radical damage [55,56]. In present investigation, increase in MDA (Fig. 4a) and decrease on SOD,

TABLE-6
EFFECT OF ETHANOLIC EXTRACT *C. zeylanica* ROOT ON LIVER FUNCTION ENZYMES IN CCl_4 INDUCED HEPATOTOXICITY IN RATS

Treatment	Aspartate aminotransferase (AST)	Alanine aminotransferase (ALT)	Alkaline phosphatase (ALP)	Total bilirubin (TB)	Total protein (TP)
Group I	28.09 ± 0.65	45.77 ± 0.23	53.49 ± 0.21	14.55 ± 0.45	49.88 ± 0.09
Group II	134.09 ± 0.23**	129.66 ± 0.09**	134.99 ± 0.32**	82.66 ± 0.34**	14.77 ± 0.96***
Group III	32.65 ± 0.87**	52.76 ± 0.33***	60.88 ± 0.54***	28.99 ± 0.45**	46.98 ± 0.66***
Group IV	112.76 ± 0.22*	87.45 ± 0.82*	103.66 ± 0.53**	67.54 ± 0.65**	33.09 ± 0.45**
Group V	72.09 ± 0.33**	72.05 ± 0.11**	89.05 ± 0.56*	47.09 ± 0.88**	39.12 ± 0.87**

Values are expressed as mean ± SEM, n = 6 and was estimated using one-way ANOVA followed by posthoc Tukey HSD test. Comparisons are made between i) Group I *vs.* Group II, ii) Group II *vs.* Group III, IV and V. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ considered as significant.

CAT and GSH were noticed in IND rats (Fig. 4b-d). Administration of ECZ (200 & 300 mg/kg) significantly decre-ased the MDA content and increased the level of SOD, CAT and GSH indicated its hepatoprotection action. The ECZ at 300 mg/kg was found more effective than 200 mg/kg.

Effect of *C. zeylanica* on histopathological study: Animals of Group I revealed normal architecture of liver tissue (Fig. 5a). Histological images of negative control rats revealed crucial damage to the liver tissue as indicated by inflammation, deterioration of hepatic parenchyma, periportal necrosis, delation of sinusoidal capillary, central vein, periportal hypertrophy and ischemia (Fig. 5b). The SLM treated rats found improvement as marked with reduce in inflammation, steatosis and central vein (Fig. 5c). Group treated with ECZ 200 mg/kg showed mild decreased in hepatic injuries (Fig. 5d). However, significant reduction in hepatotoxicity was observed with ECZ 300 mg/kg as marked by histological character such as normal portal vein, central vein, and decrease in steatosis, periportal necrosis, periportal hypertrophy and leucocytary infiltration (Fig. 5e). This study strengthened the result of biochemical investigation.

***In silico* molecular docking analysis:** Molecular docking analysis of compounds *viz*. propane, 1,1-dipropoxy- (-5.2); *cis*-9-hexadecenal (-6.0); 17-octadecen-14-yn-1-ol (-6.0); 6-butyl-1,4-cycloheptadiene (-6.5) exhibited highest docking score than standard silymarin (-5.1) (Fig. 6 and Table-7).

TABLE-7
BINDING AFFINITY OF THE COMPOUNDS OF ETHANOLIC EXTRACT OF *C. zeylanica* ROOT FOR TGF-β1 (PDB ID: 1VJY)

Compounds	Best scores (Kcal/mol) TGF-β1 (PDB ID: 1VJY)
1,1-Dipropoxy-propane	-5.2
Glycerol	-4.6
cis-9-Hexadecenal	-6.0
17-Octadecen-14-yn-1-ol	-6.0
6-Butyl-1,4-cycloheptadiene	-6.5
Silymarin (standard)	-5.1

Many studies have established the link between anti-inflammatory, antioxidant and liver-protective mechanisms [57]. Thus, anti-inflammatory [19] and antioxidant [58-61] activities reported earlier could be responsible for hepatoprotective activity of root of *C. zeylanica*. Moreover, the greater docked score observed by propane, 1,1-dipropoxy-; *cis*-9-hexadecenal; 17-octadecen-14-yn-1-ol and 6-butyl-1,4-cycloheptadiene than SLM could also be attributed to the hepatoprotective potential of *C. zeylanica*. Furthermore, the histological examination of liver samples showed substantial evidence supporting the protective effects of ECZ.

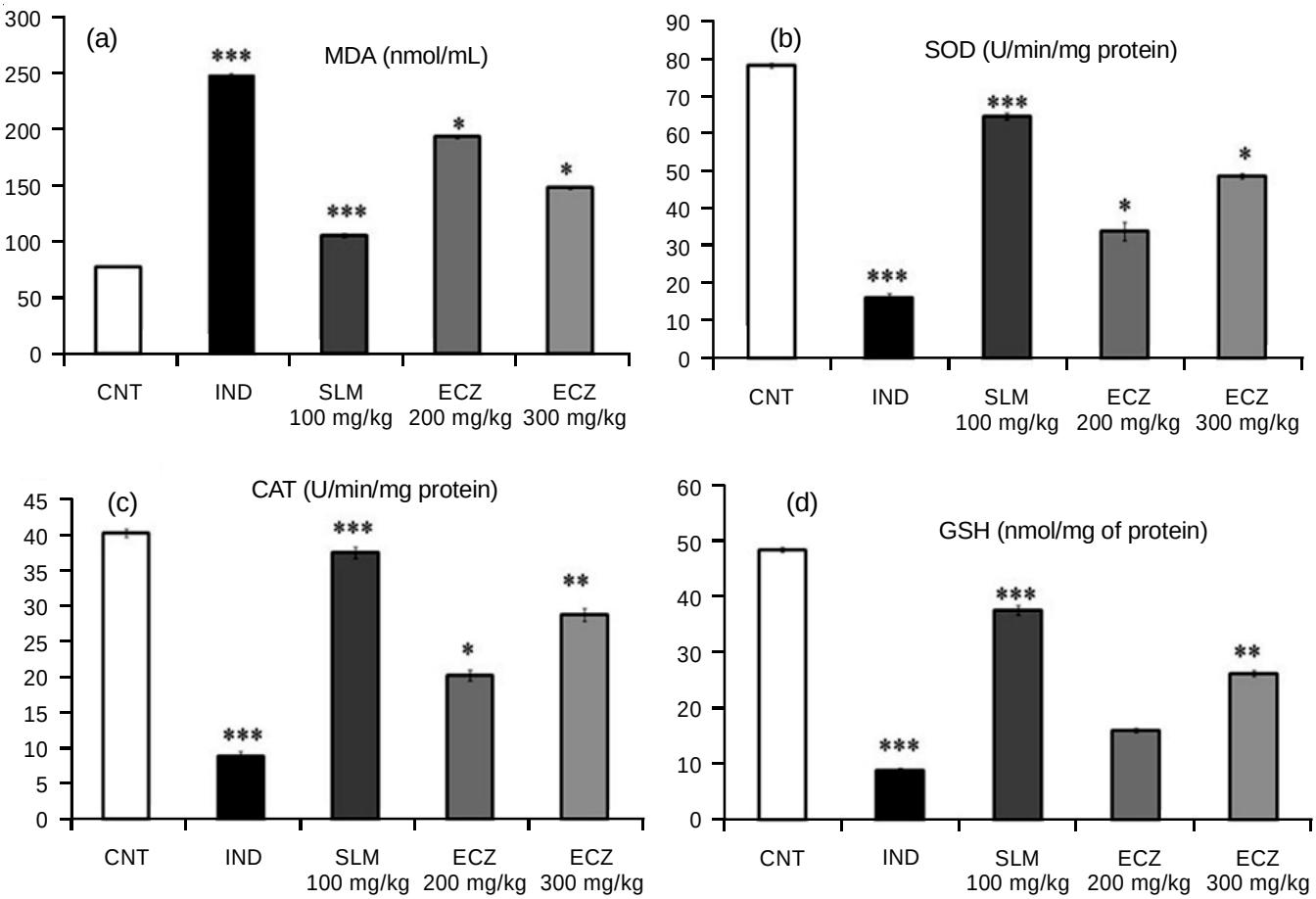


Fig. 4. Effects of ethanolic extract of *C. zeylanica* root on (a) MDA, (b) SOD, (c) CAT, (D) GSH in CCl_4 toxicity rats [values are expressed as mean ± SEM, n = 6 and was estimated using one-way ANOVA followed by posthoc Tukey HSD test. Comparisons are made between i) Group I *vs.* Group II, ii) Group II *vs.* Group III, IV and V. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ considered as significant

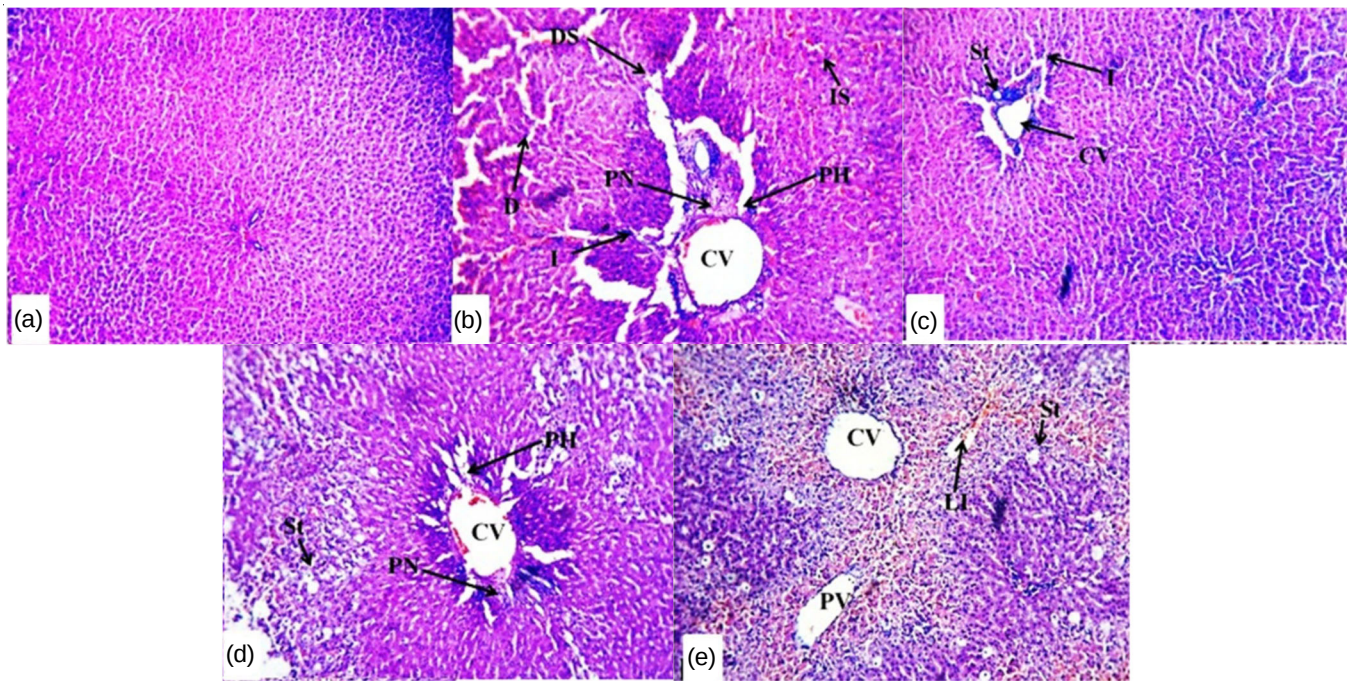
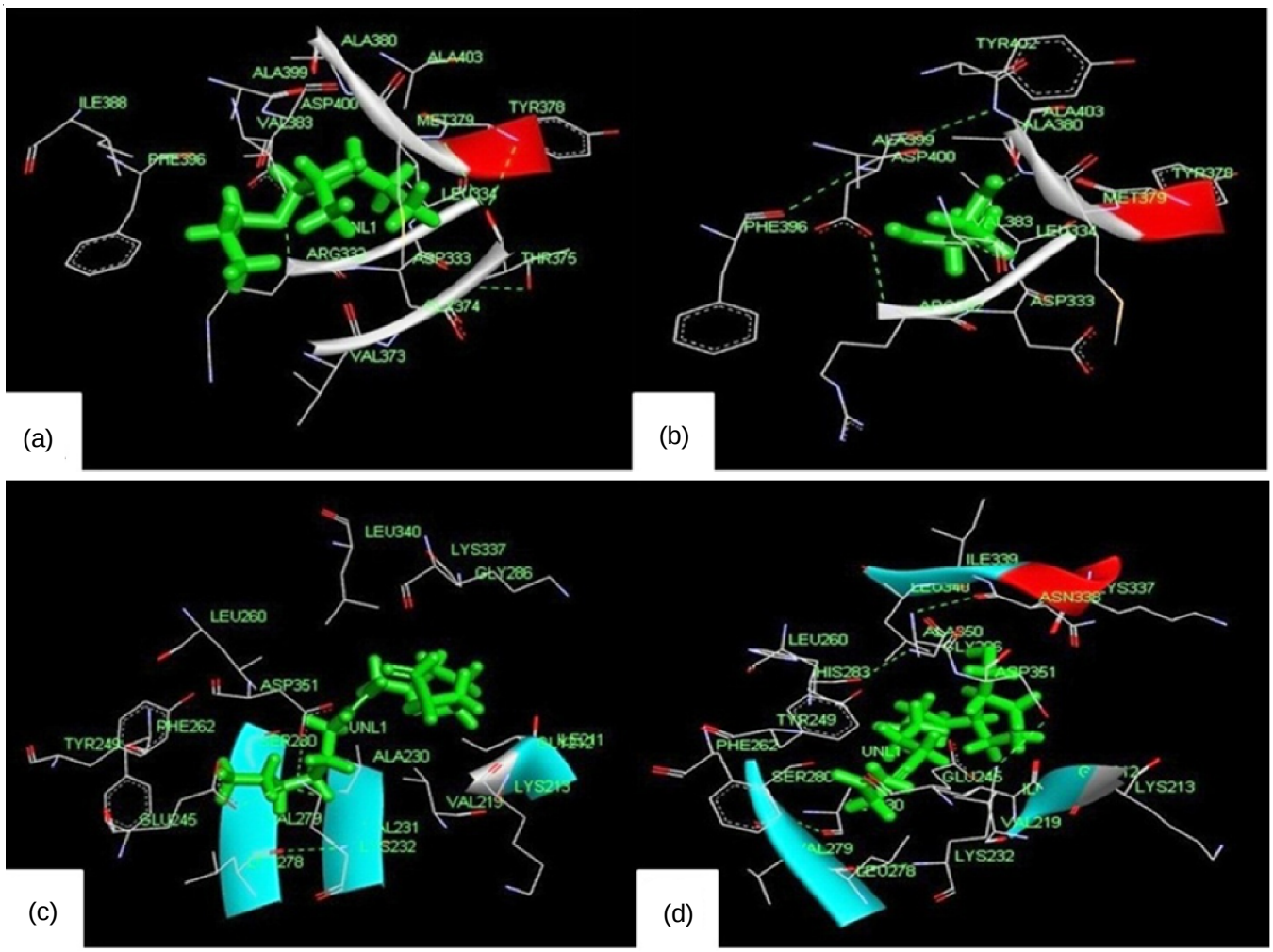


Fig. 5. Histopathological study of the liver; (a) Normal control; (b) CCl_4 hepatotoxic control; (c) CCl_4 hepatotoxic treated with silymarin; (d) CCl_4 hepatotoxic treated with 200 mg/kg b.w ECZ; (e) CCl_4 hepatotoxic treated with 300 mg/kg b.w ECZ [Abbreviations: PH = Periportal hypertrophy; D = Deterioration of hepatic parenchyma; PN = Periportal necrosis; DS = Delation of sinusoidal capillary; IS = Ischemia; LI = Leucocytary infiltration; CV = Central vein; PV = Portal vein; St = Steatosis



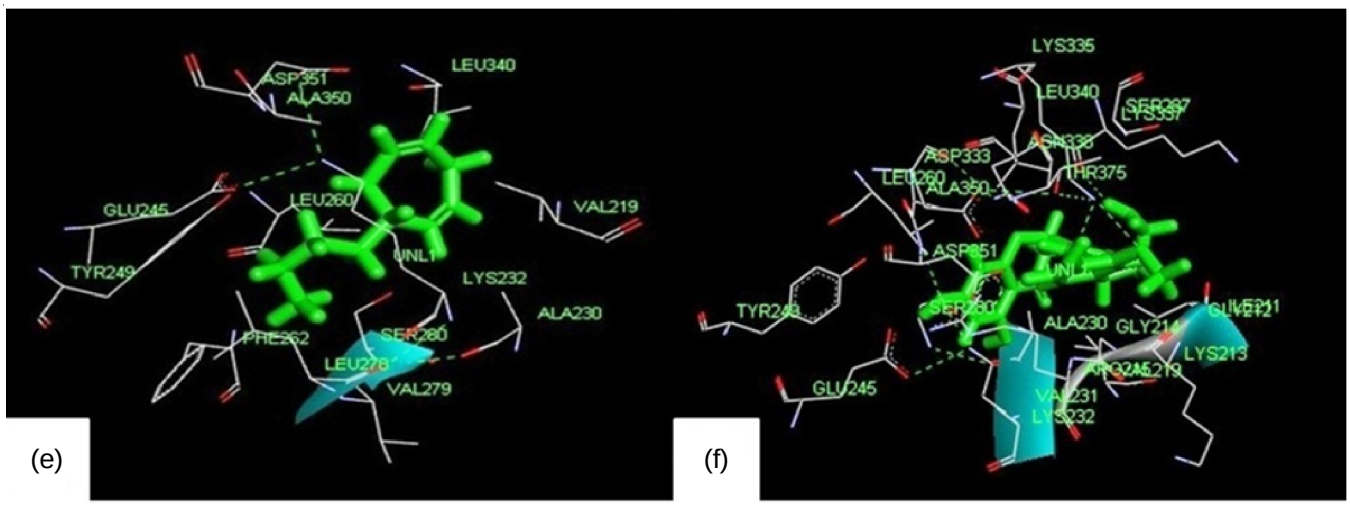


Fig. 6. Molecular docking structure of (a) propane, 1,1-dipropoxy-; (b) glycerin; (c) *cis*-9-hexadecenal; (d) 17-octadecen-14-yn-1-ol; (e) 6-butyl-1,4-cycloheptadiene; (f) Sylimarin for TGF-β1

Conclusion

The hepatoprotective activity of *Capparis zeylanica* root is probably due to the scavenging of free radicals and stabilization of membranes, as well as the protection of the endogenous antioxidant defence system by phenolic and flavonoid compounds. The appreciable quantity of gallic acid detected by HPLC could be responsible for free radical scavenging of *C. zeylanica*. Interestingly, the greater docking score achieved by propane, 1,1-dipropoxy-; *cis*-9-hexadecenal; 17-octadecen-14-yn-1-ol and 6-butyl-1, 4-cycloheptadiene than SLM could also be responsible for the hepatoprotective activity. To the best of our knowledge this study represents the first investigation that potentially ameliorates hepatoprotective activity of *C. zeylanica* against CCl_4-induced hepatotoxicity rats. Further investigation needs to be conducted to isolate and purify the active constituents responsible for hepatoprotection. The results of this research will be crucial in developing new and effective hepatoprotective treatments.

ACKNOWLEDGEMENTS

The authors are thankful to The Pharmaceutical College, Barpali and Central Instrumentation Laboratory, Panjab University, Chandigarh, India for conducting GC-MS analysis and Natural remedies Pvt. Ltd., Bengaluru, India for the HPLC analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. M.L. Nchouwet, N. Wansi, N. Kodjio, K. Poualeu, A. Nkeng-Efouet and A. Kamanyi, *Asian J Biomed. Pharm. Sci.*, **7**, 1 (2017).
2. H.M. Lin, H.C. Tseng, C.J. Wang, J.J. Lin, C.W. Lo and F.P. Chou, *Chem. Biol. Interact.*, **171**, 283 (2008); https://doi.org/10.1016/j.cbi.2007.08.008
3. D.A. Stoyanovsky and A.I. Cederbaum, *Biochemistry*, **35**, 15839 (1996); https://doi.org/10.1021/bi961295p
4. R. Zhu, Y. Wang, L. Zhang and Q. Guo, *Hepatol. Res.*, **42**, 741 (2012); https://doi.org/10.1111/j.1872-034X.2012.00996.x
5. C.R. Edwards, I.A. Bouchier and C. Haslett, Davidson's principles and practice of medicine, BPC Paulton books limited, Great Britain, (1996).
6. J.S. Au, V.J. Navarro and S. Rossi, *Aliment. Pharmacol. Ther.*, **34**, 11 (2011); https://doi.org/10.1111/j.1365-2036.2011.04674.x
7. A.S. Negi, J.K. Kumar, S. Luqman, K. Shanker, M.M. Gupta and S.P. Khanuja, *Med. Res. Rev.*, **28**, 746 (2008); https://doi.org/10.1002/med.20115
8. N. Ghosh, R. Ghosh, V. Mandal and S.C. Mandal, *Pharm. Biol.*, **49**, 970 (2011); https://doi.org/10.3109/13880209.2011.558515
9. S. Kwon, Y. Lee, H.-J. Park and D.-H. Hahm, *BMC Complement. Altern. Med.*, **17**, 1 (2017); https://doi.org/10.1186/s12906-016-1505-2
10. S.G. Joshi, Medicinal plants, Oxford and IBH Publishing: New Delhi (2003).
11. H.O. Saxena and M. Brahman, The Flora of Orissa, Regional Research Laboratory (Council of Scientific & Industrial Research), Bhubaneswar, India (1994).
12. D.N. Guha Bakshi, P. Sensarma and D.C. Pal, Lexicon of Medicinal Plants in India, Naya Prokash: Calcutta, India (1999).
13. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, International Book Distributor: Dehradun, India (2005).
14. P.N. Dhabale, V.N. Shrikhande and D.M. Sakharkar, *Int. J. Pharm. Sci. Res.*, **3**, 198 (2012).
15. B. Sundaramoorthy, D.K. Gopi, R. Andalil, S.K. Narayana and S.R. Parameswaran, *J. Ayur. Med. Sci.*, **2**, 174 (2017); https://doi.org/10.5530/jams.2017.2.9
16. R.S. Kumar, G. Anburaj, A. Subramanian, S. Vasantha and A.P. Selvam, *J. Pharmacogn. Phytochem.*, **8**, 1399 (2019).
17. V.V. Chopade, A.N. Tankar, R.O. Ganjiwale and P.G. Yeole, *Int. J. Green. Pharm.*, **2**, 28 (2008).
18. K.R. Sini, B.N. Sinha and A. Rajasekaran, *Avicenna J. Med. Biotechnol.*, **3**, 31 (2011).
19. N. Yaraksa, *J. Sci. Technol.*, **11**, 1 (2019).
20. R.P. Rastogi and B.N. Mehrotra, Compendium of Indian Medicinal Plants, Central Drug Research Institute Lucknow and National Institute of Science Communication and Information Resources: New Delhi (1990).
21. S.Y. Mendhekar, S.G. Padekar, S.H. Date, S.L. Jadhav and D.D. Gaikwad, *World J. Pharm. Res.*, **6**, 945 (2017).
22. J.B. Harborne, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman & Hall: London (1998).
23. S. Srivastava, R.K. Verma, M.M. Gupta, S.C. Singh and S. Kumar, *J. Liq. Chromatogr. Rel. Technol.*, **24**, 153 (2001); https://doi.org/10.1081/JLC-100001478)

24. N. Konappa, A.C. Udayashankar, S. Krishnamurthy, C.K. Pradeep, S. Chowdappa and S. Jogaiah, *Sci. Rep.*, **10**, 16438 (2020); https://doi.org/10.1038/s41598-020-73442-0
25. V.L. Singleton and J.A. Rossi, *Am. J. Enol. Viticult.*, **16**, 144 (1965).
26. Y.S. Park, S.T. Jung, S.G. Kang, B.G. Heo, P. Arancibia-Avila, F. Toledo, J. Drzewiecki, J. Namiesnik and S. Gorinstein, *Food Chem.*, **107**, 640 (2008); https://doi.org/10.1016/j.foodchem.2007.08.070
27. A. Braca, C. Sortino, M. Politi, I. Morelli and J. Mendez, *J. Ethnopharmacol.*, **79**, 379 (2002); https://doi.org/10.1016/S0378-8741(01)00413-5
28. L. Marcocci, J.J. Maguire, M.T. Droylefaix and L. Packer, *Biochem. Biophys. Res. Commun.*, **201**, 748 (1994); https://doi.org/10.1006/bbrc.1994.1764
29. M.C. Navarro, M.P. Montilla, A. Martin, J. Jiménez and M.P. Utrilla, *Planta Med.*, **59**, 312 (1993); https://doi.org/10.1055/s-2006-959688
30. S. Reitman and S. Frankel, *Am. J. Clin. Pathol.*, **28**, 56 (1957); https://doi.org/10.1093/ajcp/28.1.56
31. E.J. King and A.R. Armstrong, Eds.: H. Varley, Calcium, Phosphorus and Phosphate, In: Practical Clinical Biochemistry, CBS Publishers: New Delhi, p. 458 (1988).
32. S. Fiala, A.E. Fiala and B. Dixon, *J. Natl. Cancer Inst.*, **48**, 1393 (1972).
33. A. Stiehl, *Fortschr. Med.*, **100**, 842 (1982).
34. A. Sevanian and F. Ursini, *Free Radic. Biol. Med.*, **29**, 306 (2000); https://doi.org/10.1016/S0891-5849(00)00342-7
35. R.F. Beers Jr. and I.W. Sizer, *J. Biol. Chem.*, **195**, 133 (1952); https://doi.org/10.1016/S0021-9258(19)50881-X
36. H. Saggu, J. Cooksey, D. Dexter, F.R. Wells, A. Lees, P. Jenner and C.D. Marsden, *J. Neurochem.*, **53**, 692 (1989); https://doi.org/10.1111/j.1471-4159.1989.tb11759.x
37. G.E. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959); https://doi.org/10.1016/0003-9861(59)90090-6
38. D.E. Johnston and C. Kroening, *Pharmacol. Toxicol.*, **83**, 231 (1998); https://doi.org/10.1111/j.1600-0773.1998.tb01475.x
39. C. Das, G. Ghosh, A. Bose and D. Das, *S. Afr. J. Bot.*, **141**, 200 (2021); https://doi.org/10.1016/j.sajb.2021.04.033
40. J. Terao, *Forum Nutr.*, **61**, 87 (2009); https://doi.org/10.1159/000212741
41. M.A. Soobrattee, V.S. Neergheen, A. Luximon-Ramma, O.I. Aruoma and T. Bahorun, *Mutat. Res.*, **579**, 200 (2005); https://doi.org/10.1016/j.mrfmmm.2005.03.023
42. R. Sundararajan, N.A. Haja, K. Venkatesan, K. Mukherjee, B.P. Saha, A. Bandyopadhyay and P.K. Mukherjee, *BMC Complement. Altern. Med.*, **6**, 8 (2006); https://doi.org/10.1186/1472-6882-6-8
43. L.W. Weber, M. Boll and A. Stampfl, *Crit. Rev. Toxicol.*, **33**, 105 (2003); https://doi.org/10.1080/713611034
44. R.B. Drotman and G.T. Lawhorn, *Drug Chem. Toxicol.*, **1**, 163 (1978); https://doi.org/10.3109/01480547809034433
45. B. Huang, X. Ban, J. He, H. Zeng, P. Zhang and Y. Wang, *J. Ethnopharmacol.*, **131**, 276 (2010); https://doi.org/10.1016/j.jep.2010.06.029
46. R.O. Recknagel, E.A. Glende Jr., J.A. Dolak and R.L. Waller, *Pharmacol. Ther.*, **43**, 139 (1989); https://doi.org/10.1016/0163-7258(89)90050-8
47. R.S. Britton and B.R. Bacon, *Hepatogastroenterology*, **41**, 343 (1994).
48. A.V. Badarinath, K.M. Rao, C.M. Chetty, S.T. Ramkanth, T.V. Rajan and K. Gnanaprakash, *Int. J. Pharm. Technol. Res.*, **2**, 1276 (2010).
49. M. Al-Yahya, R. Mothana, M. Al-Said, M. Al-Dosari, N. Al-Musayeib, M. Al-Sohaibani, M.K. Parvez and S. Rafatullah, *Evid. Based. Complement. Altern Med.*, **2013**, 569037 (2013); https://doi.org/10.1155/2013/569037
50. H. Frank and B. Link, *Biochem. Pharmacol.*, **33**, 1127 (1984); https://doi.org/10.1016/0006-2952(84)90524-0
51. R.O. Recknagel and A.K. Ghoshal, *Exp. Mol. Pathol.*, **5**, 413 (1966); https://doi.org/10.1016/0014-4800(66)90023-2
52. H. Ohkawa, N. Ohishi and K. Yagi, *Anal. Biochem.*, **95**, 351 (1979); https://doi.org/10.1016/0003-2697(79)90738-3
53. M.R. Khan, W. Rizvi, G.N. Khan, R.A. Khan and S. Shaheen, *J. Ethnopharmacol.*, **122**, 91 (2009); https://doi.org/10.1016/j.jep.2008.12.006
54. M.R. Khan and D. Ahmed, *Food Chem. Toxicol.*, **47**, 1393 (2009); https://doi.org/10.1016/j.fct.2009.03.020
55. R.S. Rector, J.P. Thyfault, G.M. Uptergrove, E.M. Morris, S.P. Naples, S.J. Borengasser, C.R. Mikus, M.J. Laye, M.H. Laughlin, F.W. Booth and J.A. Ibdah, *J. Hepatol.*, **52**, 727 (2010); https://doi.org/10.1016/j.jhep.2009.11.030
56. N. Kaplowitz and H. Tsukamoto, *Prog. Liver Dis.*, **4**, 131 (1996).
57. F.H. Kamisan, F. Yahya, N.A. Ismail, S.S. Din, S.S. Mamat, Z. Zabidi, W.N.W. Zainulddin, N. Mohtarrudin, H. Husain, Z. Ahmad and Z.A. Zakaria, *J. Acupunct. Meridian Stud.*, **6**, 52 (2013); https://doi.org/10.1016/j.jams.2012.08.002
58. C.P. Macwan and M.A. Patel, *Int. J. Pharm. Pharm. Sci.*, **2**, 58 (2010).
59. K.R. Sini, B.N. Sinha and M. Karpagavalli, *Curr. Botany*, **1**, 13 (2010).
60. S.K. Mishra, P.N. Singh and S.D. Dubey, *Rev. Latinoam.*, **41**, 7 (2013).
61. S. Raj and Shivakumar, *J. Pharm. Innov.*, **8**, 176 (2019); https://doi.org/10.7897/2277-4572.085150