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Decalepis hamiltonii root fraction alleviates CCl_4 hepatotoxicity in a rat model

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ABSTRACT

Background: Decalepis hamiltonii (D. hamiltonii) is Indian folk medicine in herbal preparations, to reduce appetite, and cures dysentery, bronchitis, uterine hemorrhage, and other ailments.
Objective: The current investigation focused on the hepatoprotective effect of *D. hamiltonii* roots fractions against liver damage.
Materials and methods: The current research discussed the fraction from *D. hamiltonii root extracts* was used. Male Wistar rats (albino strain) were grouped into 4 distinct groups of six animals each. Group I: plain water and vehicle whereas Group II (CCl_4 control): CCl_4 (1 ml/kg, 20 % v/v in olive oil) over 7 days and vehicle; Over 7 days, Group III received Silymarin 100 mg/kg/day and tap water with 20 % v/v of CCl_4, whereas Group IV (treatment group) received DHE 50 mg/kg/day, 100 mg/kg/day, and water. Assessment of biochemical parameters, Mitochondrial modulation, gene expression analysis, and RT-PCR, was used to estimate the protective action of DHEF in CCl_4-intoxicated rats.
Results: The administration of CCl_4 increased levels of total bilirubin (0.63 ± 0.97 mg/dl) plasma amino transferases (110.36 ± 1.13 U/L, 86.56 ± 2.41 U/L and 1.51 ± 1.36 mg/dl respectively) which were mitigated by *D. hamiltonii* treatment. Activity like Lipid peroxidation and content of nitric oxide also augmented, while the antioxidant action measured by GSH (9.64 ± 0.18 U/mg protein), SOD (3.69 ± 0.22 U/mg protein), and CAT (1.47 ± 0.01 U/mg protein) was reduced. *Decalepis hamiltonii* root provided substantial restoration of GSH (14.92 ± 0.04 nmol/gm protein), SOD (4.20 ± 0.18 U/mg protein), and CAT (2.71 ± 0.04 U/mg protein) levels. In addition, the acute phase reactants stimulated by CCl_4 administration enhanced mRNA expressions of IL-6, IL-10, TNF-a, NF-κβ, and COX-2, which were enhanced by *D. hamiltonii* treatment.
Conclusions: In summary, DHEF protects the liver against CCl_4-induced damage, possibly by mitochondrial modulation mechanism. These findings indicate that *D. hamiltonii* significantly moderates oxidative stress of CCl_4-induced hepatotoxicity.

1. Introduction

In traditional system of medicine *Decalepis hamiltonii* has been used as traditional medicine as antioxidant, antihyperglycemic, anti-proliferative, antibacterial, anti-inflammatory properties [1]. Ellagic acid has anti-inflammatory and oxidative stress-reducing effects [2]. Cytochrome P-450 breaks down carbon tetrachloride (CCl_4) in hepatocytes, and the resulting free radical intermediates increase oxidative stress [3–5]. CCl_4 causes mitochondrial malfunction and causes cell death [6]. By producing hydroxyl radicals, CCl_4-mediated oxidative stress may cause DNA damage in cells [6,7]. In vivo model can be used to access the protective mechanism. The severity of damage and/or protection caused in experimental animals by known dose administration of distinct liver toxins is assessed by several biochemical reactions and metabolic indicators, as well as histological evaluations. In vivo models provide the high degrees of correlation between the human where all the histopathological and biochemical parameters can be measured. Only limitation for invivo studies are they require large amount of animals and long study time for animal activities.There is inter-individual variance, and despite the development of models that mimic several liver

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disorders, there are significant discrepancies in the pathogenesis of molecules between the model being studied and the human species. There is often gap between clinical trials and animal studies. Many putative hepatoprotective drugs that show promise in animal models do not show the same effectiveness or safety profile in human clinical trials [8]. NAPQI binding to cell proteins causes hepatocellular damage, which is followed by an unfolding series of intracellular processes including the formation of reactive oxidative ions, culminating oxidative stress in mitochondria and the activation of stress proteins. Aside from that, the liver's immune system may be stimulated, causing more harm to the hepatocellular system. The transcription of genes may also create such mediators, which may cause further harm. Under such conditions, destructive and protective pathways coexist, and the sort of balance between the two ultimately determines whether there is cell death or recovery [9,10].The discovery of novel medicines involves a number of processes, beginning with the identification of pharmacological side effects in cellular and animal models and ending with the demonstration of efficacy and safety in humans [11] (see Fig. 1).

2. Materials and methods

2.1. Plant identification and preparation

Plant roots were purchased from Naattu Marundhu Kadai evergreen Eco Farms Private Limited in Coimbatore, Tamil Nadu, India [12]. The roots were cleaned with distilled water, allowed to air dry for 7–10 days at room temperature, and then dried in an oven at 400 °C to remove any remaining moisture. The dried plant materials were ground into a powder and kept at 40 °C for later use. Methanol was used to do a soxhlation extraction on 50 g of powdered gum samples between 60 and 80 °C independently concentrated in a water bath at 40 °C and evaporated were the three filtrates. Bioguided fractionation was used [13]. Additionally, using the liquid-liquid separation butanol (F2), chloroform (F1) and water (F3), the prepared extracts were successively fractionated.

2.2. Preparation of crude extract and fractions from hamiltonii bark

D.hamiltonii was shade-dried to retain its chemicals. A sensitive digital balance (Denver (BT-224S)) weighed 100 g of root powder. The Powder was macerated in methanol and water for 24 h in a 1 L beaker. Following 24 h s of stirring, the extract was recovered from the marc using cloth and suction-filtered by Whatman No. 1 filter paper [14]. Three times maceration removed all plant components. In a rotary evaporator at 40 °C and reduced pressure, maceration filtrates were dried. The methanolic extract was dehydrated for 24 h. Brown crude methanolic extracts were stored at 4 °C [13,14]. 100 mL of chloroform was added to a separating funnel containing 5.3 g of hydro methanolic crude extract diluted in 100 mL of methanol-water to separate the chloroform sub-extract. While relieving pressure, the separating funnel was shaken. Then, two transparent layers developed. Draining the flask gathered the dark methanolic extract layer. Add 70 mL of n-butanol to 100 mL of methanolic extract in a separating funnel (brown). The mixture was mixed and left to stand until two clear layers developed. The separating funnel's n-butanol sub-extract was emptied. Repeating this method three times allowed chloroform to remove all moderately polar chemicals. After separating the n-butanol sub-extract, the methanolic subextract remained (M1). The aqueous subextract was emptied from the separating funnel into the methanolic subextract three times. The three sub-extracts were evaporated at 40 °C and kept at 4 °C until use [13,15].

2.3. Chemicals

Silymarin was obtained from Hi Media Laboratories Pvt. Ltd. Liver enzymes biomarkers kits were procured from Sigma Chemical Co., India. Double distilled water was used for extraction and fractionation. Every solvent utilized was of the analytical variety, and it was acquired from Sigma Aldrich.

2.4. Animals

Male Wistar rats (albino) weighing 150–200 g, 5 week of age were used for this study. Rats were procured from Lala Lajpat Rai Veterinary and Animal Sciences, Hisar, India. All experimental procedures were approved by Committee for Control and Supervision of Experimentation on Animals (CPCSEA) (BV/IAEC/78/2021). The animals were kept in an accredited animal room with a constant humidity (50 %), temperature (22 °C), and a regular light-dark cycle (lights on between 6:00 and 18:00). To eliminate bias from any diurnal variations in performance, animals from both groups were examined in alternating order. The animals were housed in clean polypropylene plastic cages with wood shavings as bedding. All animals had unrestricted access to water and were given normal conventional rat feed pellets (Vital feeds Ltd, Ibadan, Nigeria). Prior to the testing, the animals were allowed to grow acquainted to their surroundings and handled for a week. Additionally, efforts were taken to limit animal pain and sample size. Anaesthesia with xylazine (30 mg/kg) and ketamine (300 mg/kg) at high doses.

The rats were acclimatised for 7 days after being randomly assigned to different groups and before the experiment began, under regular conditions in the environment of relative humidity, temperature and dark/light cycle.

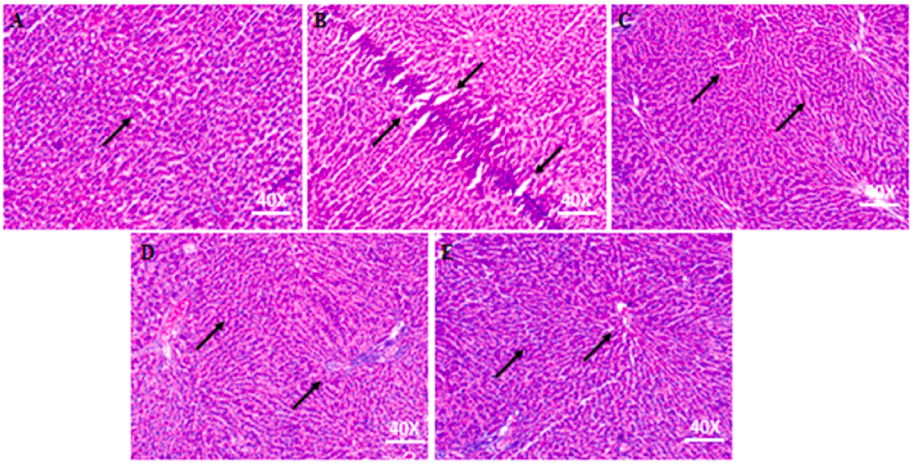

Fig. 1. Histopathological analysis of liver tissues; (A) control, (B) CCl_4 (C) Silymarin (100 mg/kg), (D) DHE (50 mg/kg), and (E) DHE (100 mg/kg).

2.5. Experimental plan for study

Group I (6 rats in each group) served as the standard control group and was only given distilled water (10 ml/kg). The other four groups received injections of CCl_4 (1 ml/kg, 20 % v/v in olive oil) twice a week for a total of 28 days. For 28 days, Groups III, IV, and V received silymarin (100 mg/kg, oral), DHE (50 mg/kg, oral), and DHE (100 mg/kg, oral) of the test substance. Blood was obtained from each rat's retro-orbital vein and placed in EDTA tubes after 12 h had passed since the last CCl_4 injection. Serum from additional blood was collected into separate Eppendorf tubes and stored at −20 °C for further analysis after centrifuging at 12,000 rpm for 20 min.

2.6. Acute toxicity studies

Acute toxicity studies of the plant fraction were performed as described in OECD guidelines 423 in Wistar Albino rats with doses range 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg. Acute toxicity was determined according to the method (Litchfield and Wilcoxon 1949). Albino rats were selected, weighed, grouped, and dosed orally with DHE respectively. Each animal was observed 5 min after dosing for signs of disgorge and kept in a polypropylene cage. Further, any sign of behavioural toxicity like changes in convulsion, excitation, lethargy, diarrhoea, sedation, salivation, tremors, motor activity, central nervous and autonomic systems, circulatory, and respiratory systems was observed for 15 min, 4 h, and 6 h. The animals were experiential for total of 2 weeks for the long term possible fatal outcomes [16].

2.7. Determination of aminotransferase activity alanine transaminase, aspartate transaminase and total bilirubin were estimated using the automatic analyzer

Biological samples were collected depending on the specific biomarker being measured. Samples were centrifuged to separate the liquid from cellular components and stored at appropriate temperature to prevent degradation of enzymes. The levels of liver enzymes were measured using commercial kits (Sigma Chemical Co., India). The plasma levels of alanine transaminase (ALT), aspartate transaminase (AST) and total bilirubin were estimated using the automatic analyzer [17].

2.8. Measurement of non-liver enzymatic biomarkers

The levels of cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were measured by using standard assay kits [18].

2.9. Isolation of liver mitochondria

Rats were assassinated using a high dose of ketamine and xylazine and liver tissues were collected for biochemical, gene expression, mitochondrial complex assays, Western blot, and histopathological analysis. For the assessment of various parameters including biochemical and histopathological analysis liver tissues were kept in phosphate buffer saline and 4 % formalin solution respectively. Assessment of gene expression, mitochondrial complex assays, and protein expression analysis of liver tissues was fixed in liquid nitrogen and stored at −80 °C. The liver tissues (1g) were homogenized with a peripheral blood smear (10 ml) at 4 °C before being centrifuged at 12000 rpm for 30 min (Thermo Scientific, USA). Successively, aliquot, enzymatic, and protein analyses were performed using this supernatant. The determination of Protein content in liver tissue was done according to the Bradford method. Albumin bovine serum was used as standard. By employing the previously described technique to measure the amount of malondialdehyde (MDA) in tissues, lipid peroxidation was assessed [19]. The activities of SOD, catalase, and GSH were also estimated using the tissue homogenates.

2.10. Mitochondrial complex activities assessment

The mitochondrial complexes were measured as described in previously published studies. In brief, liver mitochondria were isolated, and complex I activity was monitored at 340 nm for 2 min. The oxidation of NADH was measured in PBS (pH 7.5, 0.5 M) containing KCN; 10 mM, BSA; 50 mg/mL, and ubiquinone; 10 mM, added to start the reaction. The activity of complex II was observed at 600 nm for 2 min using PBS (pH 7.5, 0.5 M), decylubiquinol; 10 mM, BSA; 50 mg/ml, succinate; 400 mM, KCN; 10 mM, and Malonate; 1 mM which was added last to start the reaction. Complex IV was measured at 550 nm for 2 min in PBS (pH 7.0, 100 mM) using reduced cytochrome *c*; 10 mM and KCN; 10 mM using a spectrophotometer (EppendorfBioSpectrometer®) [20].

2.11. Gene expression analysis

Total RNA of liver tissue from normal control, CCl_4, silymarin (100 mg/kg), DH (50 mg/kg), and DH (100 mg/kg) groups (n = 6) was extracted using Trizol reagent. Based on absorbance measurements made using a Nanodrop™ 2000/2000c spectrophotometer (ThermoFisher Scientific, USA) at 260 and 280 nm, the quality and amount of the total RNA were assessed. The values of all the samples were between 1.8 and 2.0. Verso's c DNA synthesis kit was used to create new cDNA (ThermoFisher Scientific, USA). Using the Bio-Rad CFX96TM RT-PCR System, the RT-PCR for GAPDH, COX-2, IL-6, NF, SOD, and TNF was carried out (Bio-Rad, USA). The 2Cttechnique was utilized to measure gene expression, with GAPDH serving as the reference gene for normalization. A 20 l reaction mixture including 1 l of cDNA, 2 l of each primer [19], and 10 l of SYBR Green PCR Master Mix was used for each reaction [21]. The PCR protocol was started for 5 min at 98 °C, then 40 rotations of 10 s at 95 °C and 30 s at 72 °C were completed. For each PCR, a melting curve from 65 °C to 95 °C with intervals of 0.5 °C for 5 s was generated to ensure the quality of the amplified product.

2.12. Histopathology

For histopathological investigation, a portion of the liver samples was separated and kept in 10 % formalin for 48 h [18].

2.13. Statistical analysis

The GraphPad software Windows Version 8.0.2.0 was used to analyze the data, which were presented as a mean Standard Deviation for six rats in each group. Sidak's test was used after two-way and one-way ANOVA to calculate statistical differences. The significance level for the data was ****P > 0.0001, ***P > 0.001, **P > 0.01, and nsP> 0.05 (see Table 1).

3. Results

3.1. Hepatoprotective effect of DHEF on CCl_4-induced liver injury in rats

3.1.1. Measurement of liver enzymatic biomarkers

As shown in Table 2, CCl_4 –induced a noticeable increase in serum ALT(U/L) and AST(U/L) activities when equated to the normal level. Treatment with 50–100 mg/kg DHEF appreciably decreased the level of AST and ALT.

3.1.2. Measurement of non-liver enzymatic biomarkers

Administration of CCl_4 increased the level of cholesterol, LDL, triglycerides, and VLDL and decreased the level of HDL in the blood serum. Treatment with DHE reversed the effect of CCl4 significantly in a dose-dependent manner. At the dosing of 50 mg/kg, we found the VDL < HDL < cholesterol < triglyceride. Similarly, at the dose of 100 mg/kg, we

Table 1
Sequence of primer.

No.	Gene	Product Size (bp)	Accession number	Direction	Primer sequence (5′ to 3')	Annealing temperature
					Forward	
1	GAPDH	183 bp	NM_002046	Sense	CCCACTCCTCCACCTTTGAC	58.2
				Antisense	CCACCACCCTGTTCCTGTAG	
2	IL-6	244 bp	NM_000600	Sense	TCTATACCACTTCACAAGTCGGA	61.2
				Antisense	GAATTGCCATTGCACAACTCTTT	
3	NF-KB	194 bp	NM_021975	Sense	AAGTGATCCAGGCAGCCTTCC	62.0
				Antisense	TTCAGAGATAGCAGTGGGCCATC	
4	SOD	157 bp	NM_011434.2	Sense	CATGAATGGCTATGGCTCACA	57.6
				Antisense	TCCAACATGCCTCTCTTCATC	
5	COX-2	724 bp	HP101570	Sense	TACAAGCAGTGGCAAAGGCC	60.65
				Antisense	CAGTATTGAGGAGAACAGATGGG	
6	TNF-α	131 bp	NM_013693.3	Sense	5′-TTGACCTCAGCGCTGAGTTG-3′	61.6
				Antisense	5′-CCTGTAGCCCACGTCGTAGC-3′	

Table 2
Measurement of liver enzymatic biomarkers in CCl_4-induced hepatotoxicity in rats.

Groups	ALT (U/L)	AST (U/L)	Total bilirubin (mg/dl)
Control	35.45 ± 2.64	25.36 ± 2.45	0.41 ± 0.02
CCl_4 (1 ml/kg)	110.36 ± 1.13	86.56 ± 2.41	1.51 ± 1.36
Silymarin (100 mg/kg, oral)	45.41 ± 1.45	39.64 ± 1.57	0.63 ± 0.97
DHE F2 (50 mg/kg, oral)	65.17 ± 1.23	67.36 ± 1.91	0.89 ± 0.61
DHE F2 (100 mg/kg, oral)	51.89 ± 1.79	47.56 ± 2.12	0.59 ± 1.74

found the levels of VLDL < HDL < cholesterol < triglyceride. We also compared the results of DHEF2 with the standard compound silymarin (100 mg/kg) as shown in Table 3.

3.1.3. Assessment of biochemical (oxidative and antioxidative stress) parameters

Based on the results, CCl_4 administration increased the level of oxidative stress biomarkers including MDA (5.53 ± 0.12), NO (7.64 ± 0.08) and total protein content (26.81 ± 1.16) and decreased the level of antioxidant stress biomarkers i.e. catalase (1.47 ± 0.01), SOD (3.69 ± 0.22) and GSH (9.64 ± 0.18). Treatment with DHE F2 (50 and 100 mg/kg) reduced the level of oxidative stress biomarkers and elevated the level of antioxidant stress biomarkers in a dose-dependent manner. DHE F2 (50 mg/kg) reduced the MDA (1.65 ± 0.08), NO (4.40 ± 0.02) and total protein content (11.55 ± 0.67) and elevated the level of catalase (2.71 ± 0.04), SOD (4.20 ± 0.18) and GSH (14.92 ± 0.04). Interestingly DHE F2(100 mg/kg) significantly reduced the levels of MDA (2.58 ± 0.02), NO (2.85 ± 0.06) and total protein (15.93 ± 0.13) and markedly elevated the levels of catalase (3.21 ± 0.16), SOD (6.91 ± 0.12) and GSH (18.28 ± 0.99) as shown in Table 4. We also compared the results of DHE F2 with the standard compound silymarin (100 mg/kg) as shown in Table 4.

Table 3
Measurement of non-liver enzymatic biomarkers in CCl_4-induced hepatotoxicity in rats.

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	114.16 ± 6.59	127.72 ± 3.28	57.59 ± 2.95	31.02 ± 6.23	25.44 ± 0.65
CCl_4 (1 ml/kg)	258.49 ± 6.97	319.56 ± 4.15	19.46 ± 1.57	175.11 ± 6.96	19.46 ± 1.57
Silymarin (100 mg/kg, oral)	124.64 ± 4.76	133.66 ± 2.86	28.58 ± 1.48	69.32 ± 4.29	26.73 ± 0.57
DHE F2 (50 mg/kg, oral)	153.04 ± 2.22	164.31 ± 4.79	52.66 ± 2.61	67.51 ± 2.43	32.86 ± 0.95
DHE F2 (100 mg/kg, oral)	115.00 ± 5.39	127.00 ± 3.00	29.91 ± 2.94	59.68 ± 3.82	25.4 ± 0.60

3.1.4. Influence of DHEF on mitochondrial membrane potential

When equated to the control group, administration of CCl_4 dramatically reduced mitochondrial complex I > II-IV and produced pronounced mitochondrial enzyme complex malfunction. Rats treated with CCl_4 had their mitochondrial enzyme complex I > II > IV modification enhanced by DHE F2 (50 and 100 mg/kg) and silymarin consumption as shown in Table 5.

3.1.5. Gene expression analysis

To investigate the mechanism of the hepatoprotective effect of DHE against CCl_4-induced liver injury, we measured mRNA expression levels of COX-2, IL-6, NF-κβ, SOD, and TNF-α in liver tissues of all test groups. We observed that the mRNA expression of COX-2 (5.133 ± 1.32), IL-6 (3.40 ± 0.67), NF-κβ (12.69 ± 1.22), and TNF-α (7.40 ± 0.70) was-significantly up-regulated after the treatment with CCl_4.The mRNA expression of SOD (0.12 ± 0.24) in liver tissues was downregulated after the treatment with CCl_4.Treatment with DHE reduced the inflammation in the liver and reversed the effects of CCl_4in a dose-dependent manner. The dose of DHE (50 mg/kg) down-regulated the levels of inflammatory biomarkers including COX-2 (1.89 ± 1.17), IL-6 (1.20 ± 0.54), NF-κβ (1.60 ± 1.14), and TNF-α (1.43 ± 0.73) and up-regulated the level of

Table 4
Assessment of biochemical (oxidative and antioxidative stress) parameters in CCl_4 -induced hepatotoxicity in rats.

Groups	Catalase (μM/ml)	SOD (U/ml)	GSH (μM)	MDA (nM)	NO (mM)	Total protein (mg/ml)
Control	3.08 ± 0.30	6.04 ± 0.11	17.45 ± 0.44	2.30 ± 0.04	3.22 ± 0.05	15.12 ± 0.41
CCl_4 (1 ml/kg)	1.47 ± 0.01	3.69 ± 0.22	9.64 ± 0.18	5.53 ± 0.12	7.64 ± 0.08	26.81 ± 1.16
Silymarin (100 mg/kg, oral)	3.10 ± 0.0.04	5.81 ± 0.12	15.73 ± 0.25	2.11 ± 0.01	2.67 ± 0.08	13.86 ± 0.55
DHE F2 (50 mg/kg, oral)	2.71 ± 0.04	4.20 ± 0.18	14.92 ± 0.04	1.65 ± 0.08	4.40 ± 0.02	11.55 ± 0.67
DHE F2 (100 mg/kg, oral)	3.21 ± 0.16	6.91 ± 0.12	18.28 ± 0.99	2.58 ± 0.02	2.85 ± 0.06	15.93 ± 0.13

Table 5
Mitochondrial complex assay activities in CCl_4-induced hepatotoxicity in rats.

Groups	Mitochondrial complex activities		
	I	II	IV
Control	34.85 ± 1.95	3.98 ± 0.78	15.98 ± 1.84
CCl_4 (1 ml/kg)	10.98 ± 2.95	0.98 ± 0.85	4.98 ± 1.98
Silymarin (100 mg/kg, oral)	35.85 ± 1.98	3.69 ± 0.98	14.95 ± 0.78
DHE F2 (50 mg/kg, oral)	25.87 ± 1.78	2.45 ± 0.49	9.98 ± 1.36
DHE F2 (100 mg/kg, oral)	34.98 ± 1.85	3.96 ± 0.69	15.08 ± 1.89

SOD (0.79 ± 0.14). Similarly, at the dose of 100 mg/kg, DHE showed more significant effects on the mRNA expression and significantly downregulated the COX-2 (0.82 ± 0.46), IL-6 (1.03 ± 0.55), NF-κβ (0.83 ± 1.06) and TNF-α (0.93 ± 0.55) mRNA expression in liver tissues as compared to CCl_4 treated group. DHE at the dose of 100 mg/kg also elevated the mRNA expression of SOD (1.02 ± 0.18) markedly as compared to CCl_4 treated group. We also validated these results with the standard drug silymarin-treated group and found significant results as shown in Table 6.

3.1.6. Histopathology

The histopathological examination of the liver tissues of control, carbon tetrachloride, silymarin, and DHE (50 and 100 mg/kg) groups. The control's liver tissue showed a normal morphological structure with negligible lipogenesis and inflammation. In CCl4-treated rats liver showed the degeneration in hepatic cells with fat droplet deposition. Treatment with DHE decreased the sinusoidal dilation and accumulation of fat droplets in a dose-dependent manner **Fig: 1.**

4. Discussion

D. hamiltonii is conventional medicine widely used as a cure for fever, reducing inflammation 13,14], improving appetite, blood purifier, bronchial asthma, and other disorders [20]. As reported by Ashalatha, the major phytoconstituents found in the root part of *D. hamiltonii* were phenols and flavonoids [16,22]. The increased glutathione transferase–T activity may boost glutathione (GSH) synthesis to combat oxidative stress generated by CCl_4. It has been demonstrated that superoxide anions block catalase activity, hence decreased catalase activity in CCl_4-treated groups could be attributed to augmented superoxide anions [24]. The current study found that the n-butanol fraction of DHE has a hepatoprotective effect against liver damage caused by CCl_4 and that it may have effects on liver mitochondria and gene expression. The root extract's ability to prevent -induced hepatocyte metabolic alterations most likely results from the promotion of hepatic regeneration through increased protein synthesis, obstruction with CCl4's microsomal activation, and/or quicker CCl_4 detoxification and excretion. *D. hamiltonii* root extract is said to have a high antioxidant content [21,24]. Liver injury induced by CCl_4 is well known for acute hepatic failure and is frequently used to screen drugs for antihepatotoxic and hepatoprotective activities [25,26]. CCl_4 causes altered membrane integrity, and hepatocyte injury, and as a consequence enzymes in hepatocytes leak out [23]. Serum levels of enzymes released from cytoplasm and mitochondria determines the severity of hepatic damage [27,28]. The study revealed that ALT, AST, and total bilirubin levels significantly reduced in a dose-dependent manner when treated with DHEF as compared to the induced CCl_4 alone demonstrating the hepatoprotective effect for this root extract fraction. Significantly, the augmented serum concentrations of total cholesterol, LDL, decreased levels of HDL, and triglycerides, were restored to a normal level with DHEF co-treatment [29]. Further to explore the protective effect of DHEF gene expression analysis was carried out and it was observed that mRNA expression of COX-2 (5.133 ± 1.32), IL-6 (3.40 ± 0.67), NF-κβ (12.69 ± 1.22) and TNF-α (7.40 ± 0.70) was significantly up-regulated after the treatment with CCl_4.The mRNA expression of SOD (0.12 ± 0.24) in liver tissues was down regulated after the treatment with CCl_4 [30,31]. Treatment with DHE reduced the inflammation in the liver and reversed the effects of CCl_4 in a dose-dependent manner. The dose of DHE (50 mg/kg) down regulated the levels of inflammatory biomarkers including COX-2 (1.89 ± 1.17), IL-6 (1.20 ± 0.54), NF-κβ (1.60 ± 1.14), and TNF-α (1.43 ± 0.73) and up-regulated the level of SOD (0.79 ± 0.14). Similarly, at the dose of 100 mg/kg, DHE showed more significant effects on the mRNA expression and significantly down regulated the COX-2 (0.82 ± 0.46), IL-6 (1.03 ± 0.55), NF-κβ (0.83 ± 1.06) and TNF-α (0.93 ± 0.55) mRNA expression in liver tissues as compared to CCl_4 treated group. DHE at the dose of 100 mg/kg also elevated the mRNA expression of SOD (1.02 ± 0.18) markedly as compared to CCl_4 treated group. Administration of CCl_4 caused marled mitochondrial enzyme complex dysfunction and significantly decreased mitochondrial complex I, II, and IV (10.98 ± 2.95, 0.98 ± 0.85, 4.98 ± 1.98 respectively) as compared to the control group (34.85 ± 1.95, 3.98 ± 0.78, 15.98 ± 1.84 respectively). Ingestion of DHE (50 and 100 mg/kg) and silymarin improved mitochondrial enzyme complex I (25.87 ± 1.78, 34.98 ± 1.85, 35.85 ± 1.98 respectively), II (2.45 ± 0.49, 3.96 ± 0.69, 3.69 ± 0.98 respectively) and IV (9.98 ± 1.36, 15.08 ± 1.89, 14.95 ± 0.78 respectively) alteration in CCl_4 treated rats. In the present work, the mitochondrial membrane potential and gene expression analysis were detected as sensitive markers of mitochondrial function. The pretreatment of hepatoprotective medicines like DHEF significantly reduced m RNA levels in a dose-dependent manner, indicating that the hepatoprotective action of DHEF on liver mitochondria in rats may be connected to the regulate the mitochondrial modulation. In conclusion, the result of the current study suggested that DHEF has a hepatoprotective effect, and the mechanism underlying its protective effect may be correlated to mitochondrial protection and especially gene expression. Hepatocytes have a normal appearance; only a few cells have an increased number of vacuoles in the cytoplasm, but there is no pyknosis in the nucleus [32]. The findings of histopathological parameters and biochemical tests indicate that the root of *D. hamiltonii* is an excellent hepatoprotection. Our results thus confirm the utilization of *D. hamiltonii* in the treatment of hepatoprotective activity [31].

Table 6
Gene expression analysis in liver tissues of CCl_4-induced hepatotoxicity in rats.

Groups	COX-2	IL-6	NF-κβ	TNF-α	SOD
Control	1.00 ± 0.68	1.00 ± 0.63	1.00 ± 0.99	1.00 ± 0.34	1.00 ± 0.10
CCl_4 (1 ml/kg)	5.133 ± 1.32	3.40 ± 0.67	12.69 ± 1.22	7.40 ± 0.70	0.12 ± 0.24
Silymarin (100 mg/kg, oral)	0.86 ± 0.81	0.99 ± 0.14	0.61 ± 0.49	1.02 ± 0.54	0.92 ± 0.26
DHE F2 (50 mg/kg, oral)	1.89 ± 1.17	1.20 ± 0.54	1.60 ± 1.14	1.43 ± 0.73	1.02 ± 0.18
DHE F2 (100 mg/kg, oral)	0.82 ± 0.46	1.03 ± 0.55	0.83 ± 1.06	0.93 ± 0.55	1.02 ± 0.18

5. Limitations

Complicated complex phytoconstituents composition of herbal extract does not provide complete data on their synergistic effects [33,34]. There might be several components that impede with the desired and expected outcomes in certain concentrations for illustration in this research work, application of fraction in 50 and 100 mg/kg concentration effectively shows the hepatoprotective activity. According to the earlier studies isoflavonoids, flavonoids showed antioxidant activities, however other extract composition with synergistic and suppressing effect are yet to be defined. We recommend isolating the phytoconstituents by bio guided fractionation and further isolate obtained to be screened for its hepatoprotective activity.

6. Future study

Controlled prospective dual-blind multicenter trials on isolated active plant ingredients or related newly created compounds following structural alterations are proposed. This endeavour will result in the expansion of the currently restricted medications for the vast majority of liver illnesses.

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None.

Author contributions

The draft of the manuscript was written by Sonali. All authors participated in data collection, material and analysis. All authors read and approved the final manuscript. Swapnil, Sarvesh and Sohan were responsible for overall supervision. Swapnil and Sohan Contributed to conception and design. Smita, Sonali contributed to all experimental work, data and statistical analysis, and interpretation of data. All authors read and approved the final manuscript.

Data availability

Data will be made available on request.

Declaration of competing interest

We declare that we have no conflict of interest.

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