Asian Journal of Research in Biochemistry

8(4): 40-55, 2021; Article no.AJRB.66666
ISSN: 2582-0516

Phytochemical Constituents of Carrot (*Daucus carota*) Fruit Juice and its Hepatoprotective Property in CCl_4-Induced Liver Cirrhotic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AN, MAA, FAK and AOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AN and MAA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2021/v8i430189
Editor(s):
(1) Dr. Mohamed Fawzy Ramadan Hassanien, Zagazig University, Egypt.
Reviewers:
(1) I Nengah Muliarta, Warmadewa University, Indonesia.
(2) Yousif Abd El-Aziz Elhassaneen, Minoufiya University. Egypt.
Complete Peer review History: https://www.sdiarticle4.com/review-history/66666

Original Research Article

Received 20 January 2021
Accepted 23 March 2021
Published 13 August 2021

ABSTRACT

Aim: This study evaluated the phytochemical constituents of Carrot fruit juice (CFJ) and its hepatoprotective property in CCl_4-induced liver cirrhotic rats.
Study Design: Sixty male rats of weight ranging from 150-180 g were completely randomized into six groups. All rats were administered 0.5 ml/kg CCl_4 subcutaneously thrice weekly except groups 1, 2, 3, and 4 while rats in groups 3 and 6 and groups 4 and 5 orally received 2.5 and 5.0 ml/kg of CFJ on daily basis for 12 weeks.

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Results: The preliminary qualitative phytochemical screening of extract revealed the presence of alkaloids, flavonoids, cardiac glycosides, carbohydrate, saponin, phenolic compound and tannins. The extract treated groups significantly revealed an increase in liver cirrhotic emaciated body weight and reduction in the liver index, a reversal of liver marker enzymes activities, an increase in enzymic and non-enzymic antioxidants with a decrease in malondialdehyde level reduction in C-reactive protein, interleukin-6, alpha-fetoprotein, and carcinoembryonic antigen. Exposure of animal to CCl_4 induces oxidative stress, increases the generation of reactive oxygen species and myeloperoxidase activity, and reduces cell viability but was reversed by the CFJ.
Conclusion: The result showed that CFJ is a promising therapeutic option for treating liver failure.

Keywords: Oxidative stress; reactive oxygen species; myeloperoxidase activity; cell viability; inflammation.

1. INTRODUCTION

The largest internal organ in vertebrates, including humans, is the liver and is a vital organ with lots of functions, as it is the key organ of metabolism and excretion, and supports every other organ. It is prone to many diseases due to the fact that it is often continuously exposed to xenobiotics [1]. Perpetual wound-healing in chronically damaged liver result in hepatic fibrosis, which leads to abnormal accumulation and production of connective tissue, ultimately leads to liver cirrhosis [2]. Usually, it occurs in response to liver damage and regenerates apoptotic cells after repeated injury [3]. The inflammatory response generated is accompanied by limited deposition of extra cellular matrix (ECM), so that if regeneration of dying cells fails during persistent liver injury, hepatocytes are replaced by abundant ECM, including fibrillar collagen, depending on the origin of injury. It has been reported that cause of liver disease, host, and environmental factors determine the rate of fibrosis progression [4]. Liver cirrhosis is the damage of liver cells, their gradual replacement with scar tissue, impairing blood flow through the liver thereby causing loss of liver function and liver cell death [5]. Liver cirrhosis has a high global prevalence and is considered as the end-stage of most liver pathological conditions. It leads to chronic liver dysfunction, altered metabolism, and eventually death [1]. Accumulated reactive oxygen species (ROS) production plays an important role in liver pathology, mostly in cases of alcohol-and xenobiotic toxicity-induced liver diseases [6]. Oxidative stress having been said to play a role in liver cirrhosis [7], as one of the treatments for cirrhosis [8]. Most conventional and synthetic drugs used to treat liver diseases often causes side effects, many people, even the developed countries, turn to complementary and alternative medicines (CAM). Medicinal plants including *Daucus carota* fruit has been shown to demonstrate hepatoprotection [4,7].

D. carota L., commonly known as 'wild carrot' or 'Queen Anne's-lace,' is an ecologically invasive erect biennial fruit naturally grown in Scotland. The ethnobotanical uses of this fruit include application in the treatment of cough, diarrhea, dysentery, cancer, malaria, and tumors among others [9]. The nutritional importance of carrot includes its possession of; pro-vitamin A (carotene content) which maintains good eye health, a good source of dietary fiber and trace mineral molybdenum, which is rarely found in many vegetables. Molybdenum, which helps in metabolism of fats and carbohydrates, is also important for absorption of iron. Carrot also contains magnesium, needed for bone forming, nerve and muscle relaxing, activating vitamin B, and clotting blood [10-12].

Carbon tetrachloride (CCl_4), a chemical hepatotoxin produces reactive free radicals trichloromethyl radical (CCl_3) and a proxy trichloromethyl radical (CCl_3O_2) when metabolized and has been used frequently in investigating the hepatoprotective effects of drugs and plants for the induction of hepatic damage in animal models. Single oral dosage of CCl_4 leads to centrizonal necrosis and steatosis [13], while sub-chronic/chronic administration leads to liver fibrosis, cirrhosis, and hepatocellular carcinoma [14]. CCl_4 impairs liver cells directly by distorting the permeability of the plasma, lysosomal, and mitochondrial membranes. Severe centrilobular necrosis occurs from highly reactive free radical metabolites and is also formed by the mixed function oxidase system in hepatocytes via CYP2E [15,16]. CCl_4 augments lipid peroxidation and protein oxidation in hepatic cells, induces hepatic damage as well as apoptosis [17], induces necrosis, stimulates inflammation

response, and causes fibrosis, which spreads to link the vascular structures that feed into and drain the hepatic sinusoid [18,19]. It activates the hepatic stellate cell (HSC) inducing hepatocyte apoptosis and necrosis [20].

In the present study, we screened for the phytochemical constituents of CFJ extract and explored its hepatoprotective property in CCl_4-induced liver cirrhotic rats. Also, investigating the myeloperoxidase activity and anti-inflammatory of CFJ of the cirrhotic rats will be in the scope of this study.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Carbon tetrachloride (CCl_4) was purchased from Merck, Germany), lactate dehydrogenase (LDH) assay kit, Fortress Diagnostics Limited, Antrim, (UK); c-reactive protein (CRP), α-feto protein (AFP), and carcinoembryonic antigen (CEA) assay kits, Calbiotech, Spring Valley City, California, USA; interleukin-6 (IL-6) and caspase-3 assay kits, Elabscience Biotechnology Co. Ltd., Wuhan, Hubei, China; malondialdehyde (MDA) (Prod. No. FR40) was procured from Oxford Biomedical Research Inc., Rochester Hills, Michighan; methyl tetrazolium (MTT), myeloperoxidase (MPO), alanine amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) are product of Randox Laboratories Ltd. (Antrim, UK). All other chemicals and reagents used were procured from Sigma-Aldrich Inc., St. Loius, Missouri.

2.2 Plant Material and Authentication

Carrot fruit was procured from Mandate market, Ilorin, Kwara State, Nigeria in December 2016. The plant was identified and authenticated at Herbarium Unit of the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria, and a voucher sample (voucher No. UILH802/1075) was deposited at the University Herbarium.

2.3 Preparation of CFJ

Fresh ripe carrot fruit were washed off any excess dirt with water and placed in a juice extractor (W-868, 7 in 1 Ziad juicer/blender, China) to extract the juice. The collected juice was filtered through 8-fold muslin cloth and the pulp free juice was collected in clean container.

2.4 Phytochemical Screening

A preliminary study was done to investigate the presence of alkaloids, flavonoids, cardiac glycosides, reducing sugar, saponins, phenolics, tannins and carbohydrates on carrot juice using the standard laboratory procedures previously described [21-22].

2.5 Phytochemical Analysis

2.5.1 Phenolic content

The total phenolics content of the CFJ was determined with the Folin-Ciocalteu's reagent (FCR). In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added and the final volume of the tubes were made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using gallic acid solutions as standard. The total phenolic content of the extract was expressed in terms of milligrams of gallic acid equivalent [21].

2.5.2 Flavonoids content

Total flavonoid content was determined by aluminium chloride method using quercetin as a standard. 1 ml of test sample and 4 ml of distilled water were added to a volumetric flask (10 ml volume). After 5 min, 0.3 ml of 5 % sodium nitrite, 0.3 ml of 10 % aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M sodium hydroxide was added to the reaction mixture and the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as milligram quercetin equivalent [21].

2.5.3 Total antioxidant capacity

Total antioxidant capacity was determined spectrophotometrically by the phosphor molybdenum method. 1 ml of the CFJ was mixed with 3 ml reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The blank solution contained 4 ml reagent solution only. The mixture was incubated at 95°C for 150 min and was cooled to room temperature. Absorbance was measured at 695

nm. Total antioxidant capacity (TAC) was expressed as µg/ml ascorbic acid equivalent [22].

2.6 Biological Experiment

2.6.1 Experimental rats

Healthy male albino rats of Wistar strain with body weight ranging from 150-180 g were obtained from the Animal holding unit of the Department of Biochemistry, University of Ilorin, Kwara State, Nigeria. The animals were housed in wire-floored cages under standard laboratory conditions with a 12 h light and 12 h dark cycle at room temperature and fed pellet diet and water *ad libitum*. This study was carried out according to the guidelines of National Research Council Guide [23] and in accordance with the principles of Good Laboratory procedure (GLP) following approval of the Institutional Ethical Committee on the Use and Care of Animals.

2.6.2 Experimental design

A completely randomized experimental design was adopted for the study. Sixty (60) male albino rats were randomized into six groups of ten rats each after acclimatization for a week. They were fed with standard diet and water *ad libitum*.

CCl_4 was administered thrice weekly as a subcutaneous injection (0.5 ml/kg body weight) diluted 1:1 in paraffin oil for 12 weeks. The CFJ was administered orally at a dose of 2.5 and 5.0 ml/kg body weight. The experimental design was as follows:

Group i: Received distilled water (0.5 ml/kg body weight subcutaneously) thrice weekly for 12 weeks.
Group ii: Received CCl_4 (0.5 ml/kg body weight subcutaneously) thrice weekly for 12 weeks.
Group iii: Received CCl_4 (0.5 ml/kg body weight subcutaneously) + CFJ (2.5 ml/kg body weight orally) thrice weekly for 12 weeks.
Group iv: Received CCl_4 (0.5 ml/kg body weight subcutaneously) + CFJ (5.0 ml/kg body weight orally) thrice weekly for 12 weeks.
Group v: Received CFJ (2.5 ml/kg body weight orally) thrice weekly for 12 weeks.
Group vi: Received CFJ (5.0 ml/kg body weight orally) thrice weekly for 12 weeks.

Daily observation of clinical symptoms was conducted throughout the study. Body weights were measured twice a week. After the treatment period of 12 weeks, the animals were sacrificed through slight anesthesia and blood was collected through jugular puncture.

2.6.3 Liver index measurement

The liver index of rats was measured by the Zou's methods [24]. The liver index was gained via the following calculation:

$$\text{Liver index} = \frac{\text{Wet weight of liver}}{\text{Body weight}} \times 100\%$$

2.6.4 Preparation of serum and tissue homogenates

Serum and liver homogenate were prepared after the rats had been culled. Rats were sacrificed on the 12th week and blood samples were collected and allowed to clot for 15 min and then centrifuged at 300 g for 5 min for serum preparation. Liver was homogenized in sucrose–Tris buffer (0.25 mol/l sucrose, 10 mmol/l Tris-HCl, pH 7.4) [25].

2.6.5 Biochemical estimation

Serum Biochemical Assays: After blood collection, the serum was separated by centrifugation at 3000 g for 15 min at room temperature. Serum biochemical parameters such as AST, ALT. GGT and ALP in rats were analyzed using RANDOX kits in accordance with the manufacturer's protocols.

Antioxidant (Enzymatic and Non-Enzymatic) Assessment: The liver tissue sample was homogenized with Tris-HCl buffer (pH 7.0) and centrifuged at 2600 rpm for 20 min at 4 °C. The supernatant was used to determine superoxide dismutase (SOD) [24], catalase (CAT) [26], glutathione peroxidase (GPx) [27], glutathione-s-transferase (GST) was estimated by using Sigma GST assay kit (Sigma-Aldrich Corp., Saint Louis Missouri, USA) and reduced glutathione (GSH) was estimated according to the protocol of Cayman Glutathione Assay kit (Cayman Chemical Company, Ellsworth Rd. Ann Arbor, Missouri, USA).

Assay for Hepatic Malondialdehyde (MDA): Hepatic MDA levels were estimated using colorimetric TBARS microplate assay kit (Oxford Biomedical research Inc., Rochester Hills, Michighan).

Assessment of Cytokine: Interleukin 6 (IL-6) serum levels were measured using enzyme

linked-immunosorbent assay (ELISA) according to the manufacturer's instructions (Elabscience Biotechnology Co., Ltd, Wuhan, Hubei China). In determining the biomarker, standard was prepared in different concentrations to determine the accuracy of test, and standard curve was used to determine results after reading the absorbance at 450 ± 2 nm. All samples and standards were assayed in duplicate. Results are expressed as picogram per millimeter (ρg/ml).

Inflammatory Assessment: C-reactive protein (CRP): Serum measurement was measured using enzyme linked-immunosorbent assay (ELISA). Myeloperoxidase (MPO) activity*:* The MPO enzyme activity was determined in liver S1 according to the method described by Grisham et al. [28], with slight modifications [29]. Briefly, a sample of the liver S1 preparation (20 ml) was added to a medium containing potassium phosphate buffer (50 mM; pH 6.0), hexadecyltrimethylammonium bromide (0.5 %), and N, N, N', N'-tetramethylbenzidine (1.5 mM). The kinetic analysis of MPO was started after H_2O_2 (0.01 %) addition, and the colour reaction was measured at 655 nm at 37°C.

Tumor Marker Assessment: Alpha-Fetoprotein (AFP) and Carcinoembryonic Antigen (CEA) serum levels were measured using ELISA (Calbiotech Inc., California) and results were given in ng/ml.

Assessment of Metabolic activity [Methyl-Tetrazolium (MTT) Reduction Levels]: MTT reduction levels were determined as an index of dehydrogenase enzymes functions involved in cellular viability [30]. Aliquots of liver S1 (200 ml) were added to a medium containing 0.5 mg/ml of MTT and were incubated in the dark for 1 h at 37 °C. The MTT reduction reaction was quenched by the addition of 1 ml of dimethylsulfoxide (DMSO). The formed formazan levels were determined spectrophotometrically at 570 nm, and the results were corrected by the protein content [31].

Lactate Dehydrogenase: Lactate dehydrogenase activity was measured using LDH colorimetric assay kit, (Abcam, Cambridge, Massachusetts).

Intracellular Reactive Oxygen Species Production: 2'-7'-Dichlorofluorescein (DCF) levels were determined as an index of the reactive species production by the cellular components [32]. Aliquots (20 ml) of liver S1 was added to a medium containing Tris–HCl buffer (10 mM; pH 7.4) and 2'-7'-dichlorofluorescein diacetate DCFH-DA (1 mM). After DCFH-DA addition, the medium was incubated in the dark for 1 h until fluorescence measurement procedure (excitation at 488 nm and emission at 525 nm, and both slit widths used were at 1.5 nm). DCF levels were determined using a standard curve of DCF, and results were corrected by the protein content.

Histological Analysis: Liver tissues were fixed in 10% buffered formaldehyde and processed for histological examination by conventional methods and stained with hematoxylin and eosin (H&E).

2.7 Statistical Analysis

All data were expressed as mean and standard deviation (S.D.). The statistical significance was performed using one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) statistical software for Windows, Version 16.0 (Chicago, IL, USA). Bonferroni post-test was used to compare the mean. $P \leq 0.05$ suggested the statistical significance of difference.

3. RESULTS

3.1 Phytochemical Analysis of CFJ

The total phenolic content, total flavonoid content, and total antioxidant capacity of CFJ (Table 1).

Table 1. Phytochemical analysis of CFJ

Phytochemicals	Quantity
Total Phenolic Content (mg GAE/ g)	12.96 ± 0.07
Total Flavonoid Content (mg QE/ g)	7.12 ± 0.05
Total Antioxidant capacity (µg/ml AAE/ g)	1154 ± 0.18

Values represent means ± SD of triplicate readings. GAE (gallic acid equivalent); QE (quercetin equivalent); AAE (ascorbic acid equivalent)

3.2 Effect of CFJ on Body Weight Changes and Liver Index

During the course of the study, we performed regular check-up of signs in rats in the CFJ untreated and treated groups. During the first 6 weeks in CFJ untreated group, body weight kept on decreasing with poor appetite and agility.

From the seventh week, their weight slightly increased (Table 2). The mean body weight gain of CCl_4 group was statistically reduced when compared with the control group. Treatment with either 2.5 or 5.0 ml/kg b. wt. CFJ increases the final body weights of rats, respectively when compared with CCl_4 group (Fig. 1a). Liver index was calculated as the percentage of body weight. The results showed that the liver index in CFJ untreated group were significantly higher than the control group and when treated with the CFJ, these indices were significantly lower than the untreated group (Fig. 3).

3.3 Liver Marker Enzymes

Biochemical parameters like AST, ALT, ALP and GGT activity was significantly elevated in CCl_4-treated groups when compared with the control. Upon treatment with CFJ at 2.5 and 5.0 ml/kg dose, there was a significantly decrease in the liver marker enzymes (Fig. 2).

3.4 Enzymatic and Non-Enzymatic Antioxidants

Enzymatic antioxidants like SOD, CAT, GPX and GST and non-enzymatic antioxidant, GSH activity in liver was found significantly reduced in CCl_4-treated group in comparison to the control. Treatment with 2.5 and 5.0 ml/kg body weight. CFJ significantly increased the level of enzymatic and non-enzymatic antioxidants. LPO activity in liver was found significantly increased in CCl_4 treated group when compared to control and upon treatment with 2.5 and 5.0 ml/kg CFJ treated group significantly decreased the level of LPO when compare with CCl_4 group (Fig. 3).

Table 2. Effect of CFJ on body weight changes and liver index on CCl_4-induced liver cirrhotic rats

Groups	Body weight Gain (g)
Control	110.24±11.49
CCl_4	45.80±8.33*
CCl_4 + *D. carota* fruit juice (2.5 ml/kg b. wt.)	70.37±8.45**
CCl_4 + *D. carota* fruit juice (5.0 ml/kg b. wt.)	93.07±8.00**
D. carota fruit juice (2.5 ml/kg b. wt.)	145.60±6.31**
D. carota fruit juice (5.0 ml/kg b. wt.)	159.09±10.44**

*All values are expressed as the mean ± S.D. (n = 10). *compared with the control group, **compared with the CCl_4 group*

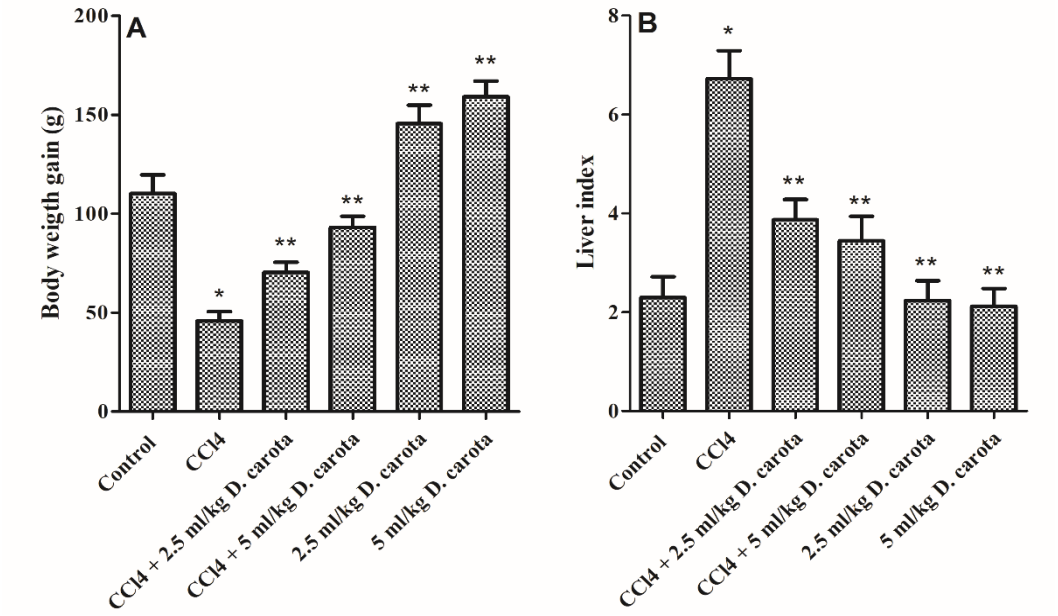


Fig. 1. Effect of CFJ on body weight gain and liver index of CCl_4-induced liver cirrhotic rats
*All values are expressed as the mean ± S.D. (n = 10). *compared with the control group, **compared with the CCl_4 group*

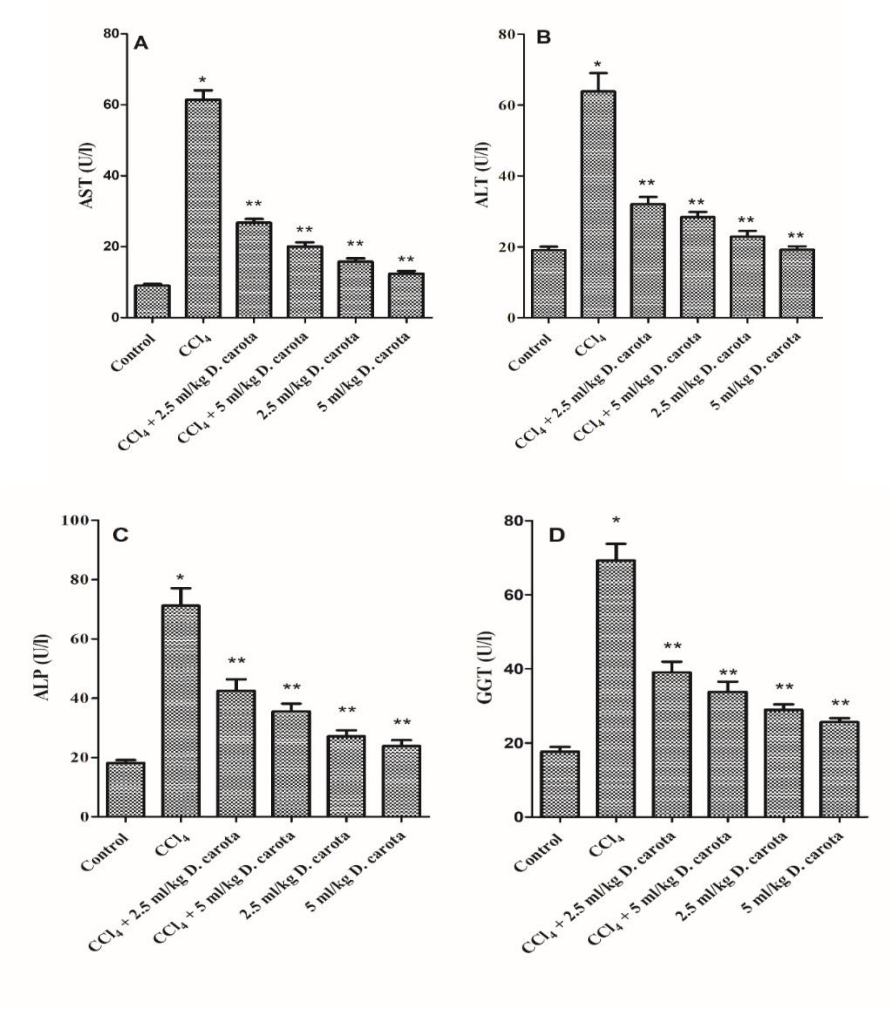
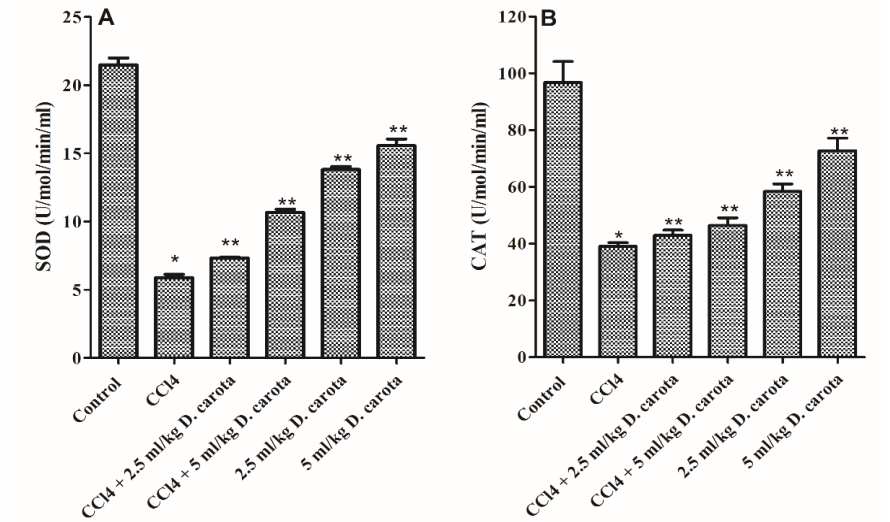


Fig. 2. Effect of CFJ on serum liver functions of CCl_4-induced liver cirrhotic rats. (A) Aspartate aminotransferase activity (B) Alanine aminotransferase activity (C) Alkaline phosphatase activity (D) Gamma glutamyl phosphatase activity

*All values are expressed as the mean ± S.D. (n = 10). *compared with the control group, **compared with the CCl_4 group*



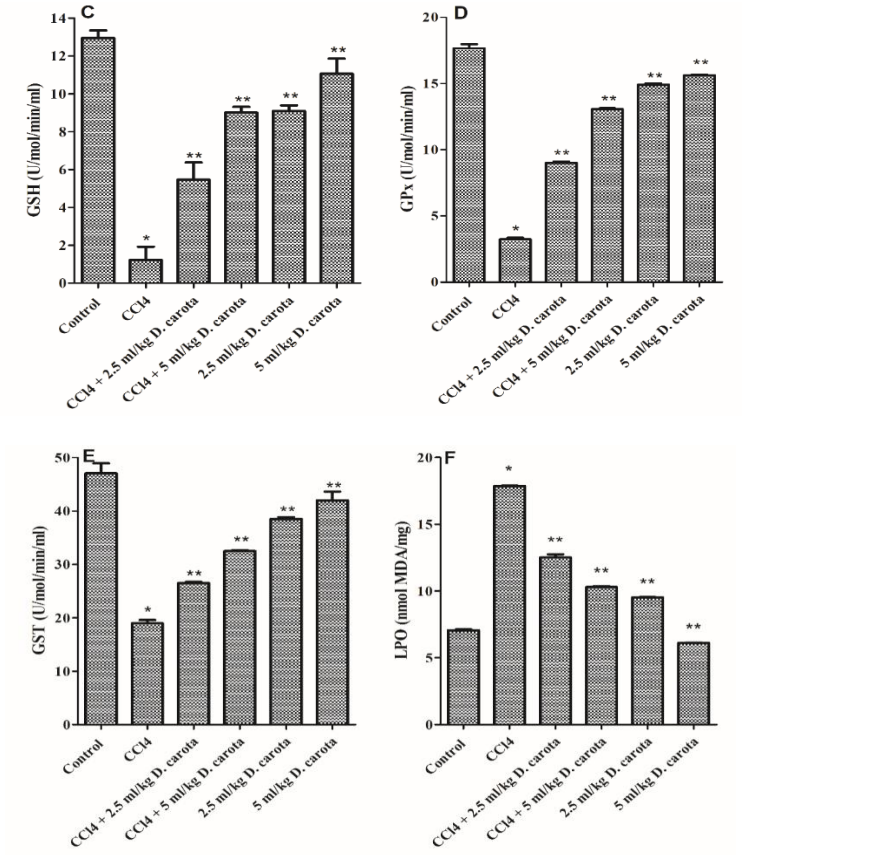


Fig. 3. Effect of CFJ on enzymic and non-enzymic antioxidant. (A) Superoxide dismutase (B) Catalase (C) Reduced glutathione level (D) Glutathione peroxidase activity (E) Glutathione-s-transferase activity (F) Lipid peroxidation level

*All values are expressed as the mean ± S.D. (n = 10). *compared with the control group, **compared with the CCl_4 group*

3.5 Inflammatory Biomarkers

There was a significant increase in the concentration of serum inflammatory biomarker IL-6 and CRP in CCl_4-treated rats. Treatment with CFJ significantly reduces IL-6 and CRP in a dose dependent manner when compared with CCl_4-treated rats (Fig. 4).

3.6 Tumor Marker Enzymes

The result revealed a significant increase in the concentration of serum tumor markers, CEA, and AFP in CCl_4-treated group. Treatment with CFJ significantly reduces CEA and AFP in a dose dependent manner when compared with CCl_4-treated rats (Fig. 5).

3.7 Dehydrogenases Activity Reduction Levels

The MTT reduction assay showed that CCl_4 caused severe liver injury, reducing cell viability by 30% compared to that of the control group (Fig. 6a). When treated with CFJ, we observed that the treatment minimized the toxic effects of CCl_4, which significantly increase the cell viability when compared with the CCl_4-treated group. This may suggest that the treatment was able to protect against the damage caused by CCl_4 (Fig. 6a).

3.8 Reactive Oxygen Species Production Analysis

The intoxication with CCl_4 caused a significant increase in the intracellular levels of reactive oxygen species in CCl_4-treated rats, as evidenced by the increase in the production of DCF when compared to the control group (Fig. 6b). Treatment with CFJ effectively reduced the formation of intracellular reactive oxygen species when compared with CCl_4-treated group, maintaining the formation of DFC at basal level, and reducing the damage caused by reactive oxygen species (Fig. 6b).

3.9 Myeloperoxidase Activity

Chronic toxic doses of CCl_4-initiated an inflammatory response, as evidenced by the marked increase in MPO activity when compared with the control group (Fig. 7a). *D. carota* treated group showed a significant reduction in MPO activity when compared with CCl_4-treated group, demonstrating a reduction in inflammatory processes (Fig. 7a).

3.10 Lactate Dehydrogenase Activity

Chronic toxic doses of CCl_4 indicated cellular leakage and loss of functional integrity, as evidenced by the marked increase in LDH activity when compared with the control group (Fig. 7b). *D. carota* treated group showed a significant reduction in LPO activity when compared with CCl_4-treated group, demonstrating a reduction in inflammatory processes (Fig. 7b).

3.11 Histopathology

Independent of the treatment received, all animals treated with CCl_4 exhibited hepatocyte ballooning degeneration and microvesicular steatosis without necrosis (Fig. 8). The untreated group revealed massive and confluent necrosis with mixed inflammatory infiltration as well as massive lobules with fatty changes.

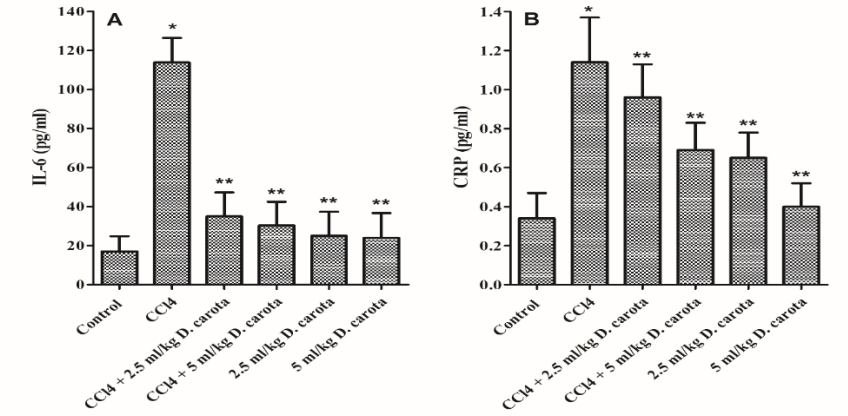


Fig. 4. Effect of CFJ on IL-6 and CRP in CCl_4-induced liver cirrhosis. (A) Interleukin-6 level (B) C-reactive protein level

All values are expressed as the mean ± S.D. (n = 10). *compared with the control group, **compared with the CCl_4 group

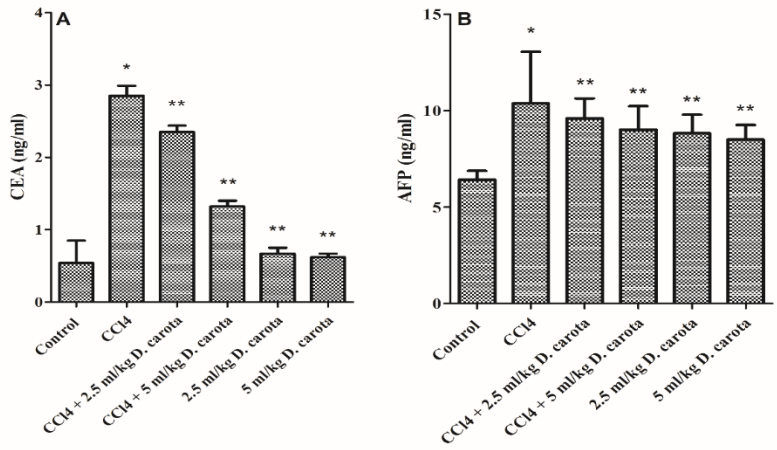


Fig. 5. Effect of CFJ on CEA and AFP in CCl_4-induced liver cirrhosis. (A) Carcinoembryonic antigen level (B) α-feto protein level

*All values are expressed as the mean ± S.D. (n = 10). *compared with the control group, **compared with the CCl_4 group*

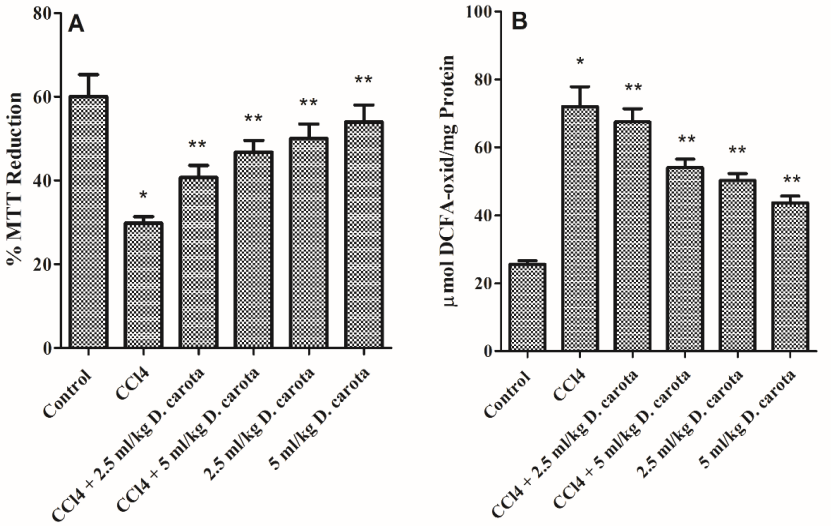


Fig. 6. Effect of CFJ on % MTT reduction and production of DCFA-DA in CCl_4-induced liver cirrhosis in rat. (A) Mitochondrial dehydrogenase activity (B) Intracellular level of reactive oxygen species

*All values are expressed as the mean ± S.D. (n = 10). *compared with the control group, **compared with the CCl_4 group*

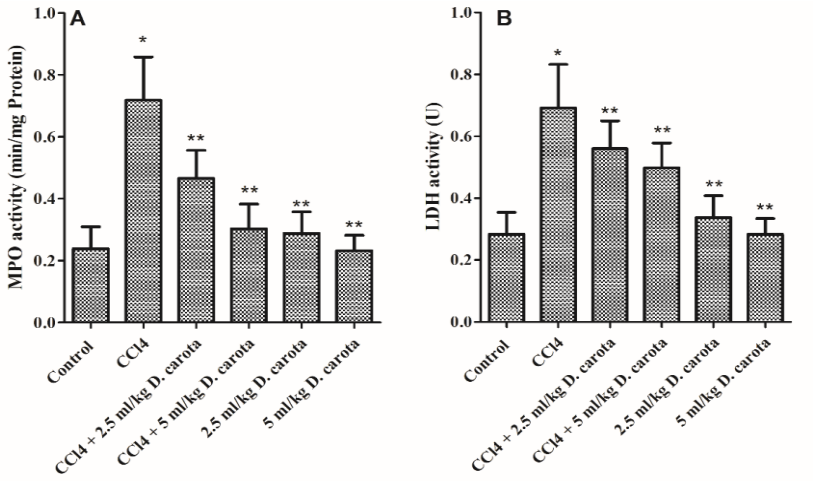
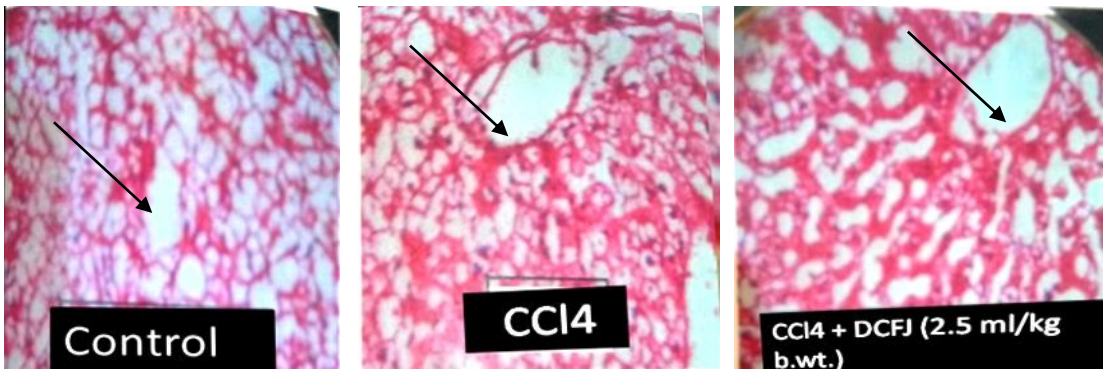


Fig. 7. Effect of CFJ on MPO and LDH activities in CCl_4-induced liver cirrhosis in rat. (A) Myeloperoxidase activity (B) Lactate dehydrogenase activity

*All values are expressed as the mean ± S.D. (n = 10). *compared with the control group, **compared with the CCl_4 group*



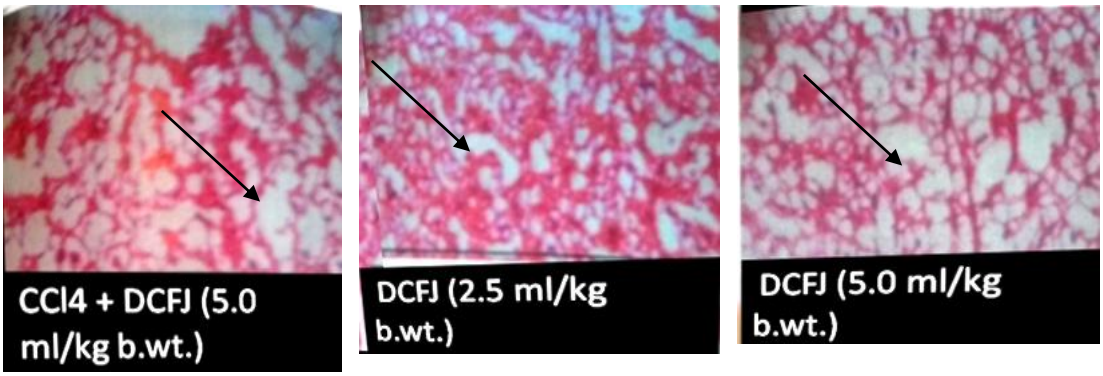


Fig. 8. Micrograph of the liver of rats induced CCl_4 liver cirrhosis (X 400 magnification)

4. DISCUSSION

CCl_4 administration leads to liver fibrosis via infiltration of cells and liver damage. The pathological progression of liver fibrosis is highly connected to the fibrotic response and the proliferation of connective tissue [32]. Exposure of liver tissues to CCl_4 result in over production of free radicals [33], which triggers oxidative stress and has been considered as a conjoint pathological mechanism, and therefore contributes to initiation and progression of liver injury [34]. Oxidative stress further stimulates the production of inflammatory cytokines [35], causes necrosis of hepatocytes, induces inflammation, and further promotes progression of hepatic fibrogenesis [36], which was demonstrated by CCl_4.

Phenolics and flavonoids have been reported to contribute significantly to antioxidative nature of fruits and vegetables. Flavonoids especially have been reported to have anticancer, anti-inflammatory, antifungal, antimicrobial, antibacterial and antiviral activities [37]. Our result revealed an appreciable amount of both phenolic and flavonoid contents and a high antioxidant activity.

The increase in the serum activity of AST, ALT, ALP and GGT which is released into the circulation after tissue damage is reflected by the damage to the cellular integrity of the liver cell by CCl_4. Their measurements can be used to make assessment of liver function [38-40]. The liver microsomal oxidizing systems produce reactive metabolites of CCl_4 such as trichloromethyl radical (CCl_3) or trichloroperoxyl radical (CCl_3O_3) and these free radicals increases lipid peroxidation which produces hepatocellular damage and enhances production of fibrotic tissue [41-43]. Damage to the hepatocyte and alteration of permeability of the membrane due to hepatic injury from CCl_4 causes release of enzymes normally located in the cytosol into the blood stream. The increase in activities of hepatospecific enzymes, ALT, AST, ALP and GGT observed in CCl_4-treated rats corresponds to the extensive hepatic damage induced by CCl_4. The tendency of these enzymes to return towards a near normalcy in groups treated with the CFJ is a clear indication of antihepatotoxic and antioxidant effect of CFJ. Based on the MTT assay, a significant reduction in hepatic cell viability was observed, indicating severe tissue damage. Upon treatment with CFJ, the result showed that the extract was able to effectively reduce the severity of the CCl_4-induced tissue insult, as evidenced by the low levels of liver marker enzymes and the recovery of the cell viability, which indicated less hepatic damage.

Antioxidant defense systems either enzymatic or non-enzymatic are also affected by free radicals. They reduce the intracellular concentration of GSH, GPx, GST and decrease the activity of SOD and CAT. Lipid peroxidation induced by free radicals, is a chain reaction that involves the oxidation of polyunsaturated fatty acids in membranes and is an indicator of oxidative cell damage. Measurement of oxidative stress in humans directly may be difficult to evaluate since the active oxygen species and free radicals are extremely short-lived [44]. Instead, products of the oxidative process are measured. The elevation of MDA levels, a biomarker of oxidative harm and one of the end products of lipid peroxidation in the liver, and the reduction of hepatic GSH, SOD, CAT and GST activity are important indicators in CCl_4-induced liver cirrhosis in rats. The reduction in MDA levels and elevation seen in hepatic GSH, SOD, CAT and GST activity may suggest the protection of the liver through antioxidant defense system and inhibiting lipid peroxidation leading to restoration

of oxidant/antioxidant balance of the liver and preservation of membrane integrity.

Hepatic damage usually leads to inflammatory response which is often caused by over production of free radicals generated by various endo- and exogenous compounds processed in the liver. Upon persistence of oxidative insults in the liver, the damage done by free radicals increases resulting in inflammation, activation of HSCs, and the formation of scar tissue [45]. IL-6 has long been recognized as an important proinflammatory cytokine whose expression is associated with many inflammatory disorders. Serum levels of IL-6 increases rapidly after an infection or organ inflammation and subsides when the infection disappears and is therefore used in clinical practice as a diagnostic marker to detect inflammatory conditions, especially sepsis. The increase in the level of IL-6 by CCl_4 may be related to the stimulation of nuclear factor-kB which contributes to the production of inflammatory cytokines [46]. The up-regulation of IL-6, being the chief stimulator of the production of CRP, induces systemic inflammatory response [47]. CCl_4 stimulated IL-6 protein synthesis, which triggers the activation of transcription factors that bind to DNA elements and stimulate increased transcription of CRP, resulting in a rise in its level [48].

CCl_4 overdose can also lead to an inflammatory response [49-50]. MPO is an enzyme present in neutrophils and involved in the formation of ROS and inflammatory processes [29]. Myeloperoxidase often react with hydrogen peroxide and chloride anions to form free radicals and oxidizing agents [51-52]. The result showed that animals that received an overdose of CCl_4 showed a marked elevation in MPO activity, indicating the progression of an inflammatory response. The animals treated with CFJ showed a significant reduction in MPO activity, demonstrating reduced inflammation induced by CCl_4.

The increased serum level of AFP and CEA in the animals treated with CCl_4 indicated that it is a potent hepatotoxin, enhances reactive oxygen species (ROS) production, and causes oxidative DNA damage, which play a key role in liver cirrhosis [53-56]. AFP has been known to have high specificity for hepatocarcinoma [57], and its serum concentration can be used in diagnosis of hepatocarcinoma. CEA on the other hand is said to be elevated under conditions such as smoking, pancreatitis, inflammatory bowel disease, and cirrhosis of liver [58]. Therefore, the study affirmed that CCl_4 can induce liver cirrhosis and degeneration in liver cells in rats as indicated by the elevation of AFP and CEA level in serum. Similarly, Engelhardt et al. [55] reported that AFP was much higher after the exposure to CCl_4 for 3-4 days. Treatment with CFJ decreases the levels of AFP and CEA which might be due to response to therapy.

Estimation of ROS production using DCF showed that chronic administration of CCl_4 promoted an increase in the generation of ROS and treatment with CFJ resulted in a significant reduction in the generation of ROS and subsequently reduced the damaging effects of ROS in the liver.

CCl_4 administration in rats caused increased in serum LDH activity which is also in line with the work of Wills and Asha [56]. We observed that pretreatment of CCl_4 intoxicated rats with CFJ decreased serum LDH activity which may suggest that the treatment may affect the damage tissue so as to cause accelerated regeneration of the damage tissue and therefore leads to reduction of LDH leakage from liver.

The histopathological analysis of CCl_4-induced group revealed necrosis along with lobules with massive fatty changes. Treatment with the extract compared well with the control.

5. CONCLUSION

The report from this study revealed a significant reduction in chronic hepatic failure induced by CCl_4, reducing the level of damage caused by oxidative stress and inflammation and restoring histopathological changes to some extent. These results suggest that carrot is a promising and potential therapeutic agent for hepatic cirrhosis. However, more studies are needed to better understand the mechanisms by which carrot reverses the effects of CCl_4.

ETHICAL APPROVAL

This study was carried out according to the guidelines of National Research Council Guide and in accordance with the principles of Good Laboratory procedure (GLP) following approval of the Institutional Ethical Committee on the Use and Care of Animals.

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this

research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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