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ORIGINAL ARTICLE

Carbon Tetrachloride-Induced Hepatocellular Damage in Balb C Mice and Pharmacological Intervention by Extract of *Daucus Carota*

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Authors' Contributions
1 Conception & Study Design, Data Collection & Processing, Data Analysis and/or Interpretation.
2 Conception & Study Design, Drafting of Manuscript, Critical Review.
3 Data Collection & Processing, Data Analysis and/or Interpretation.
4 Drafting of Manuscript, Critical Review.
5 Data Analysis and/or Interpretation, Drafting of Manuscript.
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ABSTRACT

Background: Present research was planned to examine the shielding effect of *Daucus carota* root extract pre-treatment on the carbon tetrachloride-induced hepatotoxicity in Balb C mice.

Objective: To cover the assessment of the enzymatic activity such as Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) and biochemical constituents like cholesterol, protein, lipids, glucose and urea contents in both the blood and liver while RNA and DNA substances exclusively in the liver.

Setting: The induction of carbon tetrachloride (CCl_4)-induced hepatotoxicity caused a rise in ALT of plasma and a decline in LDH.

Method: The mice were treated with CCl_4 and *Daucus carota* extract to check their effect on the level of biochemical constituents.

Main Outcome Measure: *Daucus carota* extract pre-treatment obliterated CCl_4-induced variations in the actions of enzymes.

Results: cholesterol content decreased while glucose level increased in the blood. Glucose, lipids, cholesterol and urea in the liver were reduced while total protein level rose. *Daucus carota* extract pre-treatment also prohibited CCl_4-induced variations in total protein contents, urea, glucose and lipids in the liver. CCl_4 action caused huge hepatic damage. This was averted by *Daucus carota* extract.

Conclusion: These outcomes reveal that *Daucus carota* root extract pre-treatment inhibited hepatic damage induced by CCl_4 in the mice model, which corroborates its defensive effects against hepatic damage produced by both non-oxidative and oxidative mechanisms.

Keywords: Hepatotoxicity, Carbon tetrachloride, *Daucus carota* root extract, Balb C mice, Pre-treatment.

INTRODUCTION

The liver is the major detoxifying organ present in our body [1] that's why most of the toxicological problems are related to it [2]. Liver cells are harmed by oxidative damage through hepatotoxicants [3].

Carbon tetrachloride a xenobiotic liberated in water from numerous industries and causes hepatotoxicity in animals exposed to it [4]. Production of free radicals also takes place during normal physiological courses [5] but, when the balance between free radical scavenging and reactive oxygen species (ROS) is disturbed, it leads to tissue damage and necrosis [6]. CCl_4 is an eminent hepatotoxin widely used in experimental animals to test liver damage. The basic causes behind CCl_4-induced hepatic damage are free radical generation, reduced antioxidant enzyme activity and lipid peroxidation [7-9]. Hepatotoxins bout on the fatty acids of cell membranes which causes a rise of lipid peroxides which lose functional integrity of liver mitochondria leading to hepatic damage [10]. Hepatopathies are treated by producing an antioxidant effect or by the lessening of free radicals production [11]. Synthetic and as well as natural drugs are obtainable for the cure of liver-related diseases [12] but plant-based medicines got the utmost importance against drug-induced hepatotoxicity [13]. Therefore plant-based medicines, rich in antioxidants, are the need of the period due to toxicity problems related to certain drugs [14,15]. In this regard, many plants are used to treat liver diseases. Present research deals with the study of the antioxidant potential of *Daucus carota*.

AIM OF THE STUDY

The goal of the experiment was to cover the assessment of the enzymatic activity such as alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) and biochemical constituents like protein contents, cholesterol, urea, glucose and lipids in both the blood and liver while RNA and DNA contents only in the liver.

Ethical Approval

Animal trials were directed in accord with indigenous (law of Government College University, Lahore, Pakistan) and international law (Wet op de dierproeven, Wod, Article 9 of Dutch Law). This article was written without any experimentation on human beings.

MATERIALS AND METHODS

Preparations of the Aqueous Extract of *Daucus Carota* Root

The *Daucus carota* root was acquired from the local market and carried to the department of botany for confirmation. The roots were freed of superfluous materials, dried in the shade at room temperature, and ground into a uniform powder. 20 g of the powder was added to a 250 ml conical flask which contains 150 ml distilled water. The solution was stirred and allowed to soak for 24 hrs. The plant extract was filtered through cotton wool implanted in a glass funnel. Then the filtrate was filtered through filter paper again. The filtrate was concentrated by evaporation and poured into a 100 ml flask.

Chemical Used

Carbon tetrachloride was obtained from Sigma, Aldrich (USA).

Experimental Animals and Dose Preparation

Twenty-four Balb C mice were acquired having an average weight of 50 g from GC University Lahore, Pakistan. The mice were treated kindly, kept in cages placed in the aired and hygienic animal house under proper settings of temperature and wetness. Mice were kept in an animal house at 20°C ± 2°C and exposed to ordinary photoperiod of 12 hrs dark and 12 hrs light cycle. The purified drinking water and standard laboratory pellet feed were provided to animals ad libitum. The lethal dose (LD50) of CCl_4 was taken as 1 ml/kg b. w. The mice were distributed into four groups of six mice each namely I, II, III and IV. The Control group was Group I. Group II was provided with *Daucus carota* extract (100 mg/kg body weight), Group III was administered with CCl_4 (0.4 ml/kg b. w) and Group IV was provided with *Daucus carota* extract plus CCl_4 (0.4 ml/kg body weight). The injections were administered intra-peritoneal. Animals were anesthetized after 24 h of the last dosing, dissected and blood was collected. Blood was saved in heparinized (20 µl heparin/1 ml of blood) tubes and was centrifuged at 3000 rpm for 30 mins for the isolation of plasma for the assessment of biochemical components and enzymatic activities. The liver was separated from the body and divided into two portions, one for preparation of total nucleic

acid, cholesterol, protein, lipids extract and the other for saline extraction (See Figure **1**).

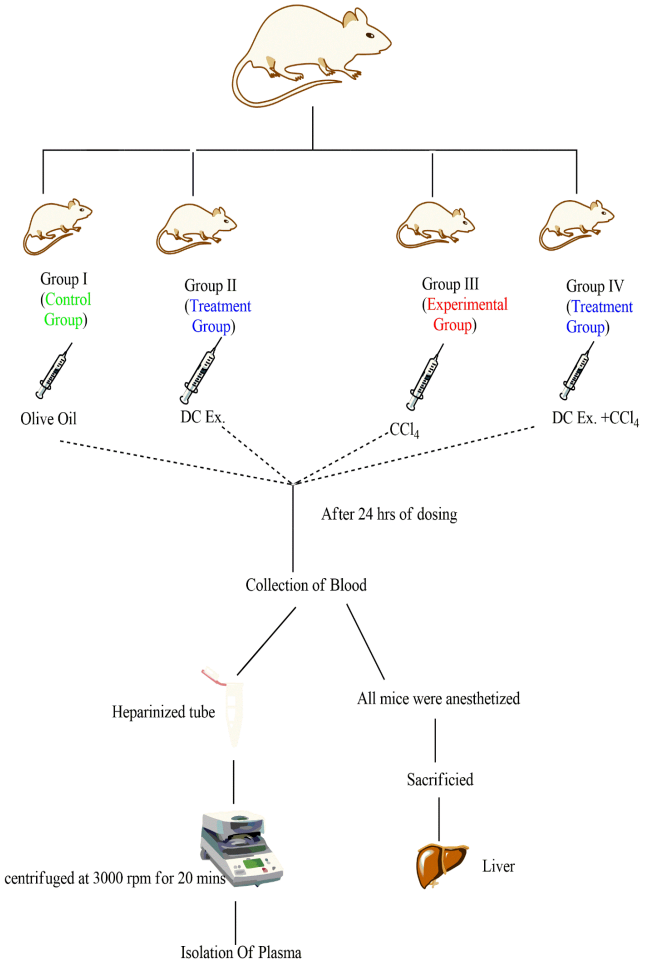

Figure 1. A scheme showing grouping, treatment, biochemical and hepatic analysis of animals.

For the valuation of activities of LDH, AST, ALT and hepatic soluble proteins, urea and glucose, 5 ml of 0.9% saline solution was used to homogenize the 250 mg liver part.

Lipids, Nucleic Acid and Protein Extraction

A separate part of the liver was pulverized in boiling ethanol and allowed to centrifuge at 3000 rpm for 20 minutes for obtaining lipids, nucleic acid and proteins. The supernatant was stored in another tube and normal ethanol was mixed with pellet for the second time. It was centrifuged at 3000 rpm after being kept overnight. After the collection of supernatant from the upper portion, a mixture of methanol and ether (3:1) was used to mix the pellet. After preserving it for 24 h, this was again centrifuged at 3000 rpm for 20 mins. The estimation of total lipids and cholesterol contents was done with the already mixed supernatant. The pellet was dried for 24 h in vacuum desiccators after the extraction of lipid. Nucleic acid and protein contents were extracted and estimated by a method mentioned by Mughal *et al*. [16].

Estimation of Biochemical Components in Liver and Plasma

The level of glucose, total lipids, cholesterol, urea, total protein, soluble protein, AST, ALT and LDH in plasma and the saline extract and DNA and RNA only in the saline extract were estimated by the method described by Mughal *et al*. [16].

Statistical Analysis

All data was stated as mean±SEM. To calculate variances among the studied groups, statistical analysis was done using the one-way analysis of variance (ANOVA). The significant value was taken as $P<0.05$.

RESULTS

Effects on Biochemical Components

Effects on Liver

Effect on Total Lipid

High significant reduction in total lipid level (CCl_4: 47.2±2.7 mg/g) as compared to control (64.8±2.3 mg/g) was caused by the CCl_4 (0.4 ml/kg) Intraperitoneal administration. When the combination of Daucus carota extract and $CCl_4$was given it resulted in a significant rise in the total lipid level (65.0±1.5 mg/g) in comparison to the CCl_4 treated group (Figure **2**).

Effect on Urea

A highly significant reduction in urea level (CCl4: 5.8±0.3 mg/g) as compared to control (14.8±1.5 mg/g) was caused by CCl_4 intraperitoneal administration. When the combination of *Daucus carota* extract and CCl_4 was given, it produced a highly significant rise in its level (10.5±0.9 mg/g) as compared to CCl_4 (Figure **2**).

Effect on Glucose

A high significant drop in glucose level in comparison to control (control: 6.3±1.1 mg/g, CCl_4: 1.1±0.1 mg/g) was caused by CCl_4 Intraperitoneal administration. When the combination of *Daucus carota* extract and CCl_4 was given, it caused a highly significant increase (6.0±0.7 mg/g) in its level as compared to CCl_4. (Figure **2)**.

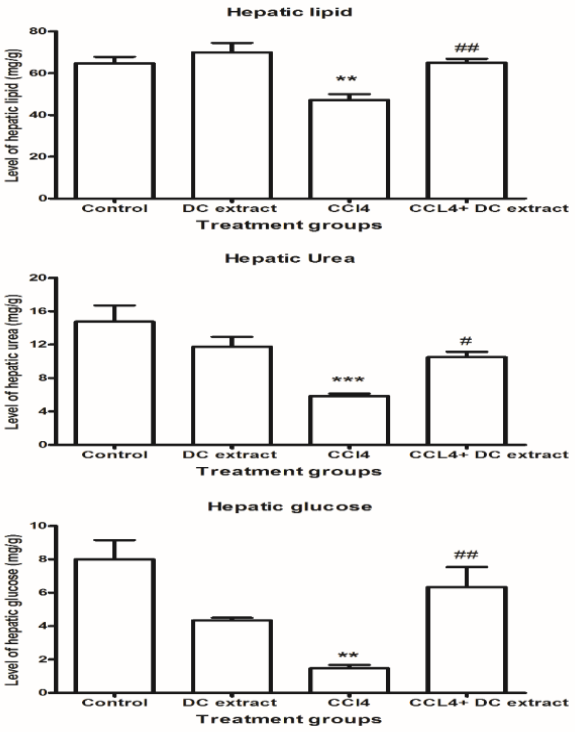

Figure 2. Assessment of biochemical components (lipid, urea and glucose) in the liver of Balb C mice.

(*) indicates the significant difference between control and CCl_4. (#) indicates significant difference between CCl_4 and CCl_4 + DC extract. Each bar represents the mean value of six replicates and SEM. Statistical icons: #= p ≤ 0.05, **, ## = p ≤ 0.01, ***, = p ≤ 0.001

Effect on Cholesterol

A highly significant drop in cholesterol level (6.3±0.4 mg/g) as juxtaposed to control (10.25±0.64 mg/g) was caused by CCl_4 Intraperitoneal administration. When the combination of *Daucus carota* extract and CCl_4 was given, it caused no significant rise in its level as compared to CCl_4. Figure **3**.

Effect on Total Protein

Intraperitoneal delivery of CCl_4 resulted in a significant rise in total protein level (185.0±1.9 mg/g) as compared to control (164.0±4.1 mg/g). When the combination of *Daucus carota* extract and CCl_4 was given, it resulted in a highly significant reduction in its level (157.0±3.4 mg/g) when matched to CCl_4.

Figure **3**.

Effect on Soluble Protein

Intraperitoneal delivery of CCl_4 instigated a significant rise in soluble protein level (79.8±1.5 mg/g) as compared to control (50.5±1.5 mg/g). When the combination of *Daucus carota* extract and CCl_4 was given, it resulted in a highly significant drop in soluble protein level (59.5±0.95 mg/g) as compared to CCl_4. Figure **3**.

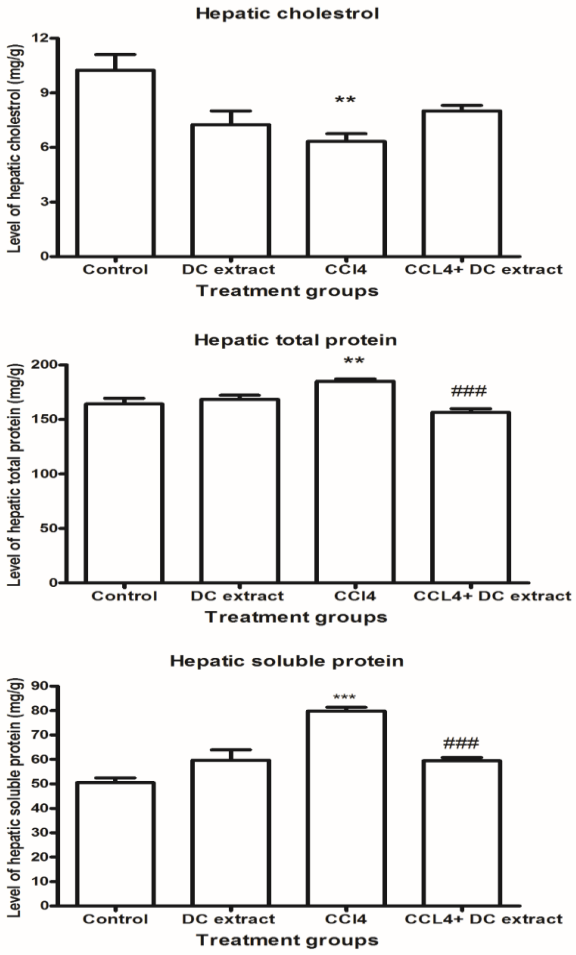

Figure 3. Assessment of biochemical components (cholesterol, total protein and soluble protein) in the liver of Balb C mice.

Effect on DNA

Intraperitoneal organization of CCl_4 resulted in a significant decline in DNA level (6.2±0.9 mg/g) as compared to control (10.0±0.6 mg/g). When *Daucus carota* extract was given with CCl_4 it resulted in a significant rise in its level (9.2±0.3 mg/g) as compared to CCl_4. Figure **4**.

Effect on RNA

CCl_4 intraperitoneal administration resulted in a highly significant decrease in hepatic RNA level (5.5±0.6 mg/g) as compared to control (11±0.8 mg/g) was observed by CCl_4 Intraperitoneal administration. When the combination of *Daucus carota* extract and CCl_4 was given, it resulted in a significant rise in its level (9.5±0.7 mg/g) as compared to CCl_4. Figure **4**.

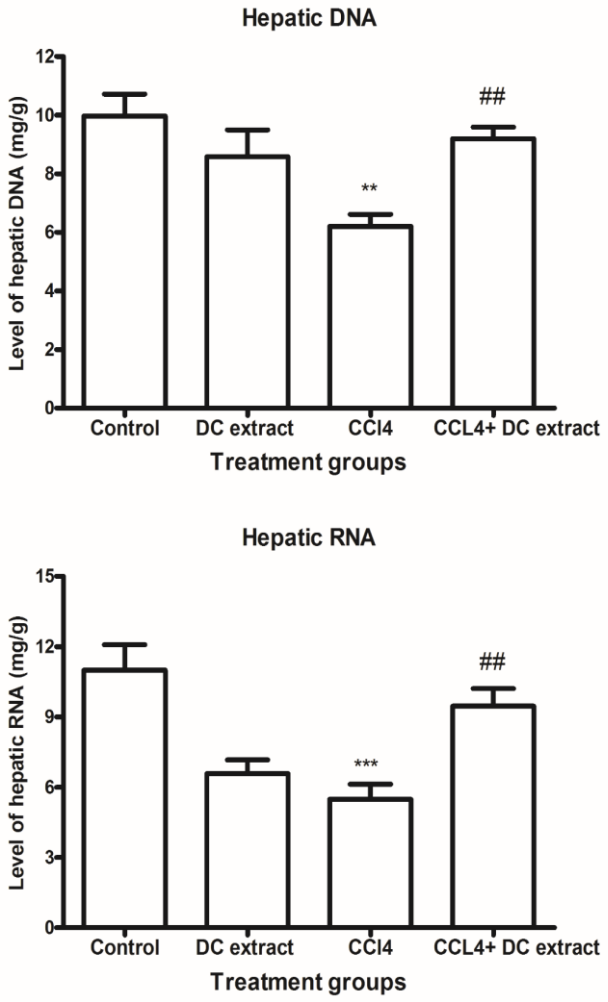

Figure 4. Assessment of biochemical components (DNA and RNA) in the liver of Balb C mice.

Each bar signifies the mean value of six replicates and SEM. Statistical icons: **, ## = p ≤ 0.01, ***= p ≤ 0.001.

Effects on Enzymatic Activities

CCl_4 intraperitoneal administration and CCl_4+ *Daucus carota* extract mixture produced no significant change in AST and ALT. ALT (control: 0.17±0.038 IU/g, CCl_4: 0.06±0.01 IU/g, *Daucus carota* extract: 0.097±0.002 IU/g, *Daucus carota* extract + CCl_4: 0.005±0.004 IU/g), AST (control: 38.15±4.53 IU/g, CCl_4: 27.83±6.13 IU/g, *Daucus carota* extract: 42.25±2.23 IU/g, *Daucus carota* extract + CCl_4: 31.5±2.9 IU/g). CCl_4 intraperitoneal administration produced no significant variation in LDH level (1.96±0.06 IU/g) as matched to control (1.99±0.22 IU/g) and caused a highly significant decrease (0.88±0.02 IU/g) when CCl_4 was given in combination with *Daucus carota* extract Figure **5**.

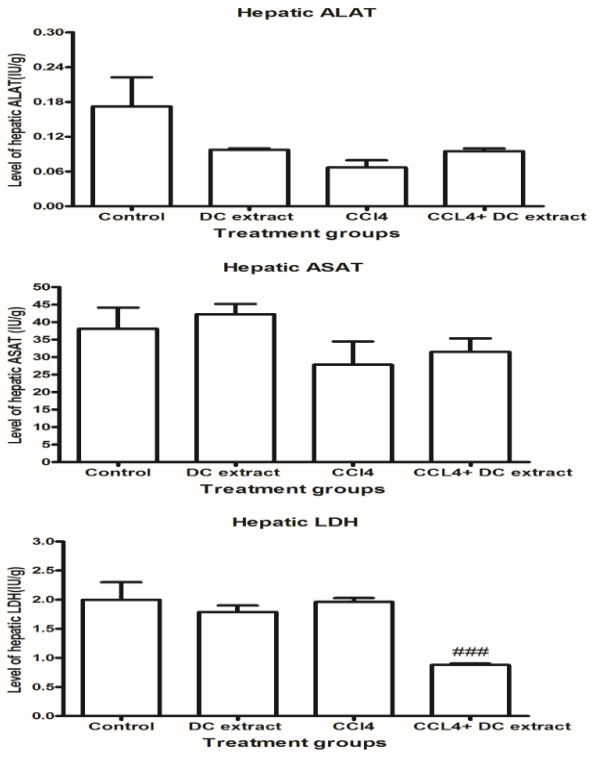

Figure 5. Analysis of enzymes (ALT, AST and LDH) in the liver of Balb C mice.

Each bar embodies the mean value of six replicates and SEM. Statistical icon: ###= p ≤ 0.001.

Effects on Blood

Effects on Biochemical Components

Effect on Total Lipids

CCl_4 intraperitoneal administration produced no significant variation (240.8±42.4 mg/100 ml) as compared to control (215.3±31.9 mg/100 ml). When *Daucus carota* extract + CCl_4 were given in combination it again showed no significant change (200.0±5.3 mg/100 ml). Figure **6**.

Effect on Urea

CCl_4 intraperitoneal administration produced no significant change in urea level (29.7±2.5 mg/100 ml) as compared to control (35.3±3.5 mg/100 ml). When the combinations of *Daucus carota* extract and CCl_4 was given, it resulted in a highly significant rise in its level (45.6±1.5 mg/100 ml) as compared to CCl_4. Figure **6**.

Effect on Glucose

CCl_4 intraperitoneal administration produced an extremely significant rise in the level of glucose (214.0±18 mg/100 ml) as compared to control (85.0±4.6 mg/100 ml). When *Daucus carota* extract

was given with CCl_4 it caused a significant decline (138.0±14.8 mg/100 ml) in its level as compared to CCl_4. Figure **6**.

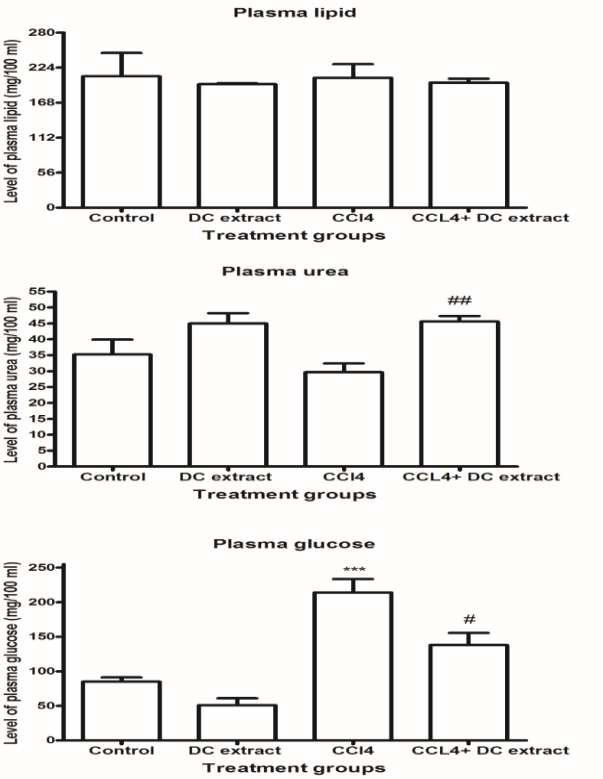

Figure 6. Assessment of biochemical components (lipid, urea and glucose) in plasma of Balb C mice

Each bar embodies the mean value of six replicates and SEM. #= p ≤ 0.05, ## = p ≤ 0.01, ***, = p ≤ 0.001.

Effect on Cholesterol

CCl_4 intraperitoneal administration produced a highly significant drop in cholesterol level (150.3±16.1 mg/100 ml) as compared to control (309.0±31.30 mg/100 ml). When the combination of CCl_4 and *Daucus carota* extract was given, it produced no significant variation in its level (124.8±6.7 mg/100 ml) as compared to CCl_4. Figure **7**.

Effect on Cholesterol

CCl_4 intraperitoneal administration and CCl_4+ *Daucus carota* extract produced no significant variation in total protein level in comparison to control (control: 4107.8±204.9 mg/100 ml, CCl_4: 3970.2±158 mg/100 ml, *Daucus carota* extract + CCl_4: 4631.8±238.8 mg/100 ml). Figure **7**.

Effect on Total Protein

CCl_4 intraperitoneal administration and CCl_4+ *Daucus carota* extract produced no significant variation in total protein level in comparison to control (control: 4107.8±204.9 mg/100 ml, CCl_4: 3970.2±158 mg/100 ml, *Daucus carota* extract + CCl_4: 4631.8±238.8 mg/100 ml). Figure **7**.

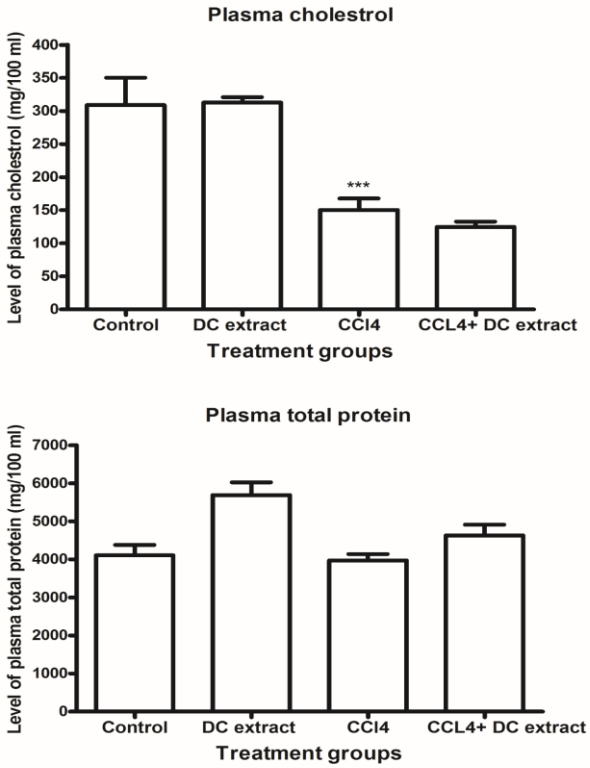

Figure 7. Assessment of biochemical components (cholesterol and total protein) in plasma of Balb C mice

Each bar signifies the mean value of six replicates and SEM. ***, = p ≤ 0.001.

Effects on Enzymatic Activities

Effect on ALT

Intraperitoneal delivery of CCl_4 produced a vastly significant rise in the level of ALT (597.8±66.2 mg/100 ml) as compared to control (99.7±13.8 mg/100 ml). When the combination of CCl_4 and *Daucus carota* extract was given it resulted in a highly significant reduction in its level (55±3.3 mg/100 ml) as compared to CCl_4. Figure **8**.

Effect on AST

Intraperitoneal delivery of CCl_4 resulted in no significant alteration in the AST level (452.5±56.2 mg/100 ml) as compared to control (293.8±15.7 mg/100 ml). When the combination of CCl_4 and *Daucus carota* extract was given, it resulted in a highly significant reduction in its level (212.6±19.4 mg/100 ml) as compared to CCl_4 (452.5±56.2 mg/100 ml. Figure **8**.

Effect on LDH

CCl_4 intraperitoneal administration produced a highly significant decline in the level of Plasma LDH (2.0±0 mg/100 ml) as compared to control (87.3±3.5 mg/100 ml). When *Daucus carota* extract + CCl_4 were given in combination a significant increase in its level (22.2±1.7 mg/100 ml) was seen as compared to CCl_4. Figure **8**.

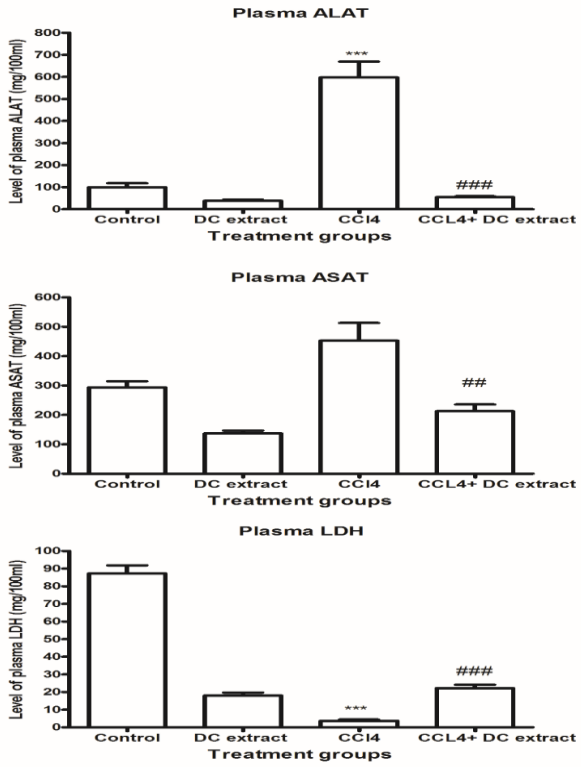

Figure 8 Analysis of enzymes (ALT, AST and LDH) in plasma of Balb C mice

Each bar denotes the mean value of six replicates and SEM. ## = $p \leq 0.01$, ***, ### = $p \leq 0.001$.

DISCUSSION

CCl_4 is used as a dry cleaning agent, refrigerant and, solvent for oils and fats. Its inhalation causes damage to the kidneys and liver. CCl_4 is also a human carcinogen [17]. The major detoxifying organ present in our body is the liver [1] that's why most of the toxicological problems are associated with it [2]. Liver cells are harmed by oxidative damage through hepatotoxicants [3]. This indicates the necessity of studying the hepatotoxicity induced by CCl_4 and possible preventive opportunities.

CCl_4 intraperitoneal administration produced a highly significant drop in hepatic total Urea, lipid, cholesterol and glucose level whereas a highly significant surge in total proteins was related to control. The same pattern was in the study of Benahmed et al. [18]. When the combination of CCl_4 and *Daucus carota* extract was given, it abolished all the changes significantly except cholesterol contents which remained significantly unchanged in comparison to CCl_4 treated group. Increased consumption of glucose could be resulted in hepatic glucose contents reduction due to CCl_4 administration during toxic insult [19]. Toxic constituents are exposed to P-450 metabolism (Phase I biotransformation). The products of phase I then conjugate with the phase II enzymes. Glucoronidase and similar conjugates are eliminated from the body.

This use of glucose in process of glucuronidation may have resulted in a low level of glucose. The energy produced from glucose also helps to battle toxins produced in stress conditions. Increased level of protein contents while the decreased level of hepatic cholesterol, glucose, total lipids and urea has also been observed in Wister Albino rats [20] and Balb C mice after CCl_4 treatment [16]. Pre-treatment of *Daucus carota* extract prohibited CCl_4 induced variations in cholesterol, lipids and glucose contents to some level. The increased level of protein production during the regeneration of damaged tissues could have resulted in this increase in total protein. Loss of functioning of liver tissue might be the reason for the decreased level of hepatic urea contents.

Administration of CCl_4 single dose to Balb C mice produced an extremely significant rise in ALT level of plasma, very significant decline in LDH level of Plasma though no significant alteration in AST level of Plasma in comparison to control and *Daucus carota* extract. When the combination of CCl_4 and *Daucus carota* extract was given, it caused a highly significant fall in the level of ALT and a highly significant surge in LDH level in comparison to CCl_4. An increase in plasma LDH, AST and ALT were also observed in rats [21] and Balb C mice [16] after CCl_4 administration. Also in the previous experiment conducted by Zhang *et al*. [22] the rise in ALT and AST level was observed. Treatment with glucagon-like peptide-1 analogue liraglutide resulted in decrease in the level of ALT and AST. Administration of CCl_4 in rats for 24 h induced surge in plasma ALT and AST activities, exhausted sulfhydryl contents, reduced total antioxidant abilities, induced

genotoxicity and major liver lipid peroxidation. CCl_4 raises AST and ALT levels along with lipid peroxidative enzymes, for example, catalase and superoxide dismutase in the liver [23]. These may indicate injury to the liver tissues caused the escape of these enzymes into the blood. A vastly significant fall in cholesterol contents and rise in the glucose of plasma \was detected after administration of CCl_4. When the combination of CCl_4 and *Daucus c*arota extract was given, it resulted in a highly significant rise in cholesterol contents and a highly significant decline in the level of glucose as matched to CCl_4. The liver injury could have resulted in a reduced level of cholesterol because the liver crops 80% cholesterol in the body [24]. CCl_4 induction caused an extremely significant drop in urea level of plasma while total proteins of plasma and total lipids level showed no significant change. *Daucus carota* extract pretreatment eliminated the variations in plasma contents of urea. These results were in agreement with the study of Afzal *et al*. [25] *Daucus carota* extracts in petroleum ether solvent reduced the serum urea, uric acid and creatinine. A decreased level of plasma urea may represent liver injury as the production of urea is the purpose of the liver through amelioration of these substances by *Daucus carota* extract pre-treatment prove its hepatic protective effects. Kula *et al*. [26] isolated the bioactive compounds present in Daucus carota. The oils were dominated by monoterpene hydrocarbons (66–85%) and myrcene (12–24%). The most abundant sesquiterpene constituents were β-caryrophyllene (4.6–13.2%) and carotol. These components might be responsible for its role in protection of liver effect. Shebaby et al. [27] reported that Daucus carota extract are composed of β-himachalene, α-selinene, β-selinene, β-caryophyllene, α-humelene, γ-selinene, α-longipinene. The F2 fraction was markedly dominated by the sesquiterpene 2-himachalen-6-ol and noticeable amounts of three phenylpropanoids:elemicin, (E)-methyl isoeugenol, and methyl eugenol. The results of their study showed that *Daucus carota* extract is effective in CCl_4 induced hepatotoxicity.

CONCLUSION

It was determined that CCl_4 at a given dose in mice is a potential hepatotoxicant. *Daucus carota* extract can stop the injury produced by CCl_4.

Conflicts of interest

All authors declare there are no conflicts of interest.

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