Effect of *Cichorium intybus* Root Extract on Biochemical and Physiological Parameters in Male Albino Rats with CCl_4-Induced Chemical Hepatitis

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Abstract

This research aims to evaluate the physiological and biochemical effects of *Cichorium intybusroot* extract (*Cichorium intybus*) on the liver in rats induced with acute chemical hepatitis using carbon tetrachloride (CCl_4). Thirty-two adult male Wistar albino rats weighing between 200-250 grams were randomly divided into four equal groups (n=8): the first group was the control group, the second group was the hepatic group (CCl_4), and the third and fourth groups received the aqueous extract of Cichorium intybusroots at two doses (200 and 400 mg/kg/day orally) for 28 days after inducing hepatitis. The results showed a significant increase ($p<0.001$) in liver enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT), as well as an increase in total and direct bilirubin and a decrease in total protein and albumin in the CCl_4 group compared to the control group. The level of malondialdehyde (MDA) also increased sharply, while the activities of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione Peroxidise (GPx) decreased. Additionally, the cytokine inflammation markers (TNF-α and IL-6) increased significantly. Meanwhile, CCl_4 administration resulted in a significant decrease in body weight and increase of liver weight than the control group indicating systemic toxicity with hepatic enlargement. Administration of *Cichorium intybus*root extract markedly ameliorated body weight and liver weight compared to the normal ranges in a dose-dependent manner, with 400 mg/kg showing the most improvement .The Cichorium intybusextract addressed all these changes significantly in proportion to the dose. These results indicate that *Cichorium intybus*root extract has potential hepatoprotective effects which may be due to its high antioxidant and phenolic compounds content. The observed effects may also be related to previously reported mechanisms, including the possible modulation of the Nrf2/HO-1 signaling pathway.

Keywords: Dandelion, *Cichorium intybus*, carbon tetrachloride, chemical hepatitis, liver enzymes, oxidative stress, inflammatory cytokines, biochemical physiology.

Introduction

The liver occupies a central position among the vital organs of the body; it performs more than five hundred interrelated vital functions, including: the metabolism of carbohydrates, fats, and proteins and regulating their levels in the blood, the synthesis of plasma proteins, clotting factors, and acute phase proteins, the secretion of bile and the regulation of fat digestion, the breakdown of hormones, drugs, and environmental pollutants, and the conversion of ammonia to urea, as well as the storage of glycogen, vitamins, and iron [1] [2] . Due to this continuous exposure to harmful substances thru the portal circulation, the liver becomes the primary target for various chemical, metabolic, and immunological disorders.

Liver diseases constitute a massive global health burden that cannot be overlooked; the World Health Organization estimates

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that between 1.5 to 2 billion people worldwide harbor chronic liver disease or latent viral hepatitis [3]. The leading causes of these diseases are hepatitis B and C viruses, autoimmune hepatitis, alcoholic liver cirrhosis, and non-alcoholic fatty liver disease (NAFLD), which has become a silent epidemic amid the worsening obesity and the spread of metabolic diseases, in addition to chemical poisoning from drugs, environmental pollutants, and pesticides [4] [5] . Despite the significant advancements in modern pharmacological treatments, many of these drugs carry severe side effects and high costs, prompting researchers to explore natural plant-based alternatives.

In this context, carbon tetrachloride (CCl_4) is used as a well-established standard laboratory model to induce acute and chronic chemical hepatitis in rodent models, as it produces the highly reactive trichloromethyl radical (CCl_3 •) in the liver primarily mediated by the CYP2E1 enzyme and secondarily by CYP2B1 in the smooth endoplasmic reticulum which spontaneously transitions to the peroxy radical CCl_3 OO• in the presence of oxygen. These radicals initiate a consecutive series of lipid peroxidation reactions in the membranes of the endoplasmic reticulum and mitochondria, leading to widespread cellular dysfunction, collapse of intracellular calcium gradients, membrane softening and rupture, release of liver enzymes into the bloodstream, and ultimately centrilobular necrosis and fibrosis in chronic cases. [5]

This model is characterized by several advantages that make it the most suitable for scientific research: the degree of damage is documented and reproducible, precise physiological and biochemical indicators are available for its evaluation, and a single dose or repeated doses induce graded and measurable physiological changes [2]. For these reasons, CCl_4 serves as the optimal substrate for testing the preventive and therapeutic efficacy of natural plant materials. In this context, the Cichorium intybusplant (*Cichorium intybus*L.) stands out From the Asteraceae family, it is one of the most documented and used traditional medicinal plants in the civilizations of the Arab, Islamic, European, and Asian regions over thousands of years. The physician-philosopher Ibn Sina mentioned it in "The Canon of Medicine" as one of the liver-strengthening plants, facilitating bile secretion, and a treatment for liver jaundice [6]. Wild chicory is widely distributed in various parts of Iraq, especially in the provinces of Kirkuk, Erbil, Mosul, and Diyala, making it available as an affordable natural medicinal substance. The roots of Cichorium intybusare characterized by an exceptional diversity in their bioactive chemical components; they contain inulin, which constitutes between 15-48% of the dry root weight and is considered a distinctive fructose polymer, sesquiterpene lactones such as intybin, lactucopicrin, and isolactucopicrin with a bitter taste, diverse phenolic compounds like chlorogenic acid, isochlorogenic acid, chicoric acid, and caffeic acid, flavonoids like quercetin, luteolin, and kaempferol, coumarins, and vitamin C, beta-carotene, zinc, and manganese [7] [8].

This unique chemical composition endows Cichorium intybuswith various scientifically documented biological properties, including: strong antioxidants, anti-inflammatory effects, antibacterial and antifungal properties, hepatoprotective effects, lipid-lowering effects, antihyperglycemic effects, bile secretion stimulation, effects on the gut microbiome, and anticancer properties . It is worth noting that the aqueous extract of Cichorium intybusis the closest to

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traditional folk use and the most suitable for future clinical application. [6] [7]

Recent molecular studies have proven that Cichorium intybusextracts inhibit oxidative stress pathways by activating the Nrf2/HO-1 pathway (Nuclear factor erythroid 2–related factor 2 / Heme Oxygenase-1), which stimulates the production of internal antioxidant enzymes such as SOD, CAT, GPx, and reduced glutathione (GSH). They also inhibit the inflammatory cytokines TNF-α, IL-6, and IL-1β by inhibiting the NF-κB pathway (Nuclear factor kappa B) [9] [10] [8]. Inulin also reduces fat accumulation in the liver by improving gut microbiota diversity, enhancing the production of short-chain fatty acids (SCFAs), and inhibiting the SREBP-1c pathway for hepatic fat synthesis [6] [11]

Despite these promising studies, a research gap still exists in the comprehensive and integrated evaluation of multiple biochemical physiological indicators simultaneously for the aqueous Cichorium intybusextract in a unified animal model, with the investigation of two different doses. Therefore, this research came to fill this gap and provide a comprehensive and integrated physiological assessment covering liver enzymes and functional indicators, oxidative stress parameters, inflammatory cytokines, and body composition indicators, thereby establishing a solid scientific foundation for developing this plant as a natural liver medicine.

Material and Methods

Experimental Animals

In this study, thirty-two adult male Wistar albino rats, weighing between 200-250 grams and aged between 8-10 weeks, were used. They were obtained from the experimental animal house at Tikrit University. The animals were housed in sterilized plastic cages (five animals per cage) under uniform and standardized environmental conditions: a temperature of 22±2 degrees Celsius, a relative humidity of 55±5%, and a light/dark cycle of 12/12 hours. They were provided with a balanced standard diet and sterile pure water ad libitum throughout the experimental period. All animals were treated according to international ethical principles for the use of animals in scientific research.

Preparation of Aqueous Extract Preparation

Fresh Cichorium intybusroots were collected from rural areas in Kirkuk Governorate during the spring (March-April 2025), and their botanical identity was verified by a specialized botanist in the Department of Life Sciences at the University of Kirkuk. The roots were thoroughly washed with distilled water to remove dirt and impurities, then dried in a warm electric oven at a temperature of 40°C for 72 hours to achieve complete drying while preserving the heat-sensitive active components.

The dried roots were ground using an electric grinder until a fine, uniform powder was obtained. Then, the powder (50 grams) was soaked in 500 ml of sterilized distilled water in a closed glass container for 48 hours at room temperature with continuous stirring using a magnetic stirrer. The resulting extract was filtered using Whatman No.1 filter paper, and the filtrate was concentrated using a rotary evaporator (Rotary evaporator, BÜCHI R-300) at 40°C under reduced pressure until a dry extract was obtained. The total phenolic content was determined using the Folin-Ciocalteu method and was found to be (128.4±6.2 mg of gallic acid equivalent/g of dry extract). The dry extract was dissolved in distilled water to

obtain the required concentrations (200 and 400 mg/ml) and stored at 4°C.

Experimental Design

The rats were randomly divided into four equal groups (n=8 per group) according to the following design:

Group One (Control): Received intraperitoneal injections of pure vegetable oil at a rate of 1 ml/kg twice weekly for 4 weeks, with distilled water administered orally daily.

Group Two (Hepatic CCl_4 Only): Injected intraperitoneally with a carbon tetrachloride solution dissolved in vegetable oil at a ratio of (1:3 volume/volume) at a dose of 1 ml/kg, twice weekly for four weeks.

Group Three (CCl_4 + Low Dose): Received CCl_4 at the same dose along with an aqueous extract of Cichorium intybusroots at a dose of 200 mg/kg/day via oral gavage for 28 days.

Group four (CCl_4 + high dose): Received CCl_4 with Cichorium intybusextract at a dose of 400 mg/kg/day orally for 28 days.

Sample Collection Sample Collection

At the end of the experiment (day twenty-eight), the animals were fasted for 12 hours, then anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) via intramuscular injection. Blood samples of (5-7 ml) were drawn from the inferior vena cava into gel tubes for serum separation. The samples were concentrated by centrifugation (3000 RPM, 15 minutes, 4°C) to obtain the clear serum, which was stored at -20°C until analysis. The body weight and liver weight were measured immediately after anesthesia.

Physiological and Biochemical Measurements

A. Liver function enzymes

The following parameters were evaluated using commercially approved diagnostic kits (Spinreact, Spain / BioSystems, Spain) on the spectrophotometer (Spectrophotometer, UNICO UV-2100): ALT by the kinetic enzymatic method according to IFCC recommendations, AST by the same method, ALP by the p-Nitrophenylphosphate method, GGT by the L-Gamma-glutamyl-3-carboxy-4-nitroanilide method, total and direct bilirubin by the modified Jendrassik-Grof method, total protein by the Bradford method, and albumin by the Bromocresol Green method.

B. Oxidative stress indicators

MDA was evaluated using the TBARS (Thiobarbituric Acid Reactive Substances) method, which measures the secondary reaction with thiobarbituric acid at 532 nanometers. The SOD activity was estimated by the inhibition of the oxidation of pyrogallol, CAT activity by the decomposition of H_2O_2, and GPx activity by the DTNB method (Ellman's reagent).

C. Inflammatory cytokines

The levels of TNF-α and IL-6 in the serum were evaluated using ELISA kits (Rat TNF-α ELISA Kit, Elabscience ; Rat IL-6 ELISA Kit, Invitrogen) according to the manufacturers' instructions.

Statistical Analysis

The data were analyzed using SPSS version 26.0. The results were expressed as (mean ± standard error of the mean Mean ± SD). The normal distribution of the data was tested using the Shapiro-Wilk test. The homogeneity of variances was tested using Levene's test. One-way ANOVA was used followed by Tukey's Honestly Significant Difference (HSD) test for post-hoc comparisons. A significance level of

$p \leq 0.05$ was adopted as the threshold for statistical significance

Results and Discussion

Liver enzymes: ALT and AST

The study results revealed a highly significant increase ($p<0.001$) in the levels of alanine aminotransferase (ALT) enzyme in the CCl_4 group, which reached (189.6±12.4 units/liter) compared to the control group (28.4±2.1 units/liter), representing an increase of nearly seven times the normal value. The Cichorium intybusextract significantly reduced these values in a dose-dependent manner; they reached (112.3±8.6) in the low-dose group and (67.8±5.3 units/liter) in the high-dose group ($p<0.01$ for both compared to the CCl_4 group). The aspartate aminotransferase (AST) enzyme exhibited a similar pattern, as it increased in the CCl_4 group to (210.4±14.2 units/liter) and then decreased with treatment to (79.2±6.1 units/liter) in the high dose (Figure 1, Table 1).

Enzymes ALP and GGT

The alkaline phosphatase (ALP) enzyme recorded a sharp increase in the CCl_4 group, reaching (245.8±18.3 units/liter) compared to (78.2±5.4) in the control group. The extract gradually reduced it: (156.4±11.2) at the low dose, and (102.7±7.8) at the high dose ($p<0.001$). The GGT enzyme recorded an increase from (18.6±1.8) in the control to (89.4±7.2 units/L) in the CCl_4 group, then decreased to (31.8±2.9) with the high dose, indicating an improvement in bile duct function (Figure 2) (Table 1)

Total bilirubin, direct bilirubin, total protein, and albumin

Total bilirubin increased significantly in the CCl_4 group (2.84±0.22 mg/dL) compared to the control group (0.48±0.04 mg/dL) ($p<0.001$). In contrast, the total protein decreased from (7.2±0.3 g/dL) to (4.8±0.4 g/dL), reflecting a deterioration in the liver's synthetic capacity. The extract restored bilirubin to (0.91±0.08) and protein to (6.9±0.3 g/dL) with the high dose (Figure 3) (Table 1).

Serum albumin showed a significant decrease ($p<0.001$) from (4.1±0.2 g/dL) in the control group to (2.4±0.2 g/dL) in the CCl_4 group, reflecting comprehensive hepatic dysfunction. The extract significantly addressed this decrease, and the albumin rose to (3.8±0.2 g/dL) with the high dose. Similarly, direct bilirubin showed a pattern similar to total bilirubin (Figure 4) (Table 1).

Oxidative stress indicators: MDA and SOD

The level of malondialdehyde (MDA) increased sharply, reflecting the peak of oxidative stress in the CCl_4 group (5.68±0.44 nmol/ml) compared to the control (1.24±0.09) ($p<0.001$), which is equivalent to 4.6 times the normal value. The Cichorium intybusextract contributed to a proportional reduction in MDA: (3.21±0.28) at the low dose, and (1.98±0.16) at the high dose. In contrast, SOD activity decreased from (42.8±3.2) in the control to (18.4±1.8 units/ml) in the CCl_4 group, then was restored to (37.9±3.1) with the high dose (Figure 5) (Table 1)

Antioxidant enzymes: CAT and GPx

Catalase (CAT) recorded a significant decrease ($p<0.001$) from (28.4±2.1 units/ml) in the control to (11.2±1.0) in the CCl_4 group, while glutathione peroxidase (GPx) recorded a decrease from (38.6±2.8) to (14.8±1.2 units/ml). The extract significantly ($p<0.01$) restored the activities of CAT and GPx toward normal levels in a dose-dependent manner. The simultaneous decline of both enzymes indicates the consumption of the internal

antioxidant system due to the continuous oxidative load of CCl_4 (figure 6) (Table 1).

Body weight and liver weight Body weight and liver weight

The body weight in the CCl_4 group significantly decreased (196.2±9.4 g) compared to the control group (248.4±8.2 g) ($p<0.001$), reflecting the overall impact of chemical hepatitis on nutritional status and nutrient absorption. In contrast, liver weight significantly increased in the CCl_4 group (11.2±0.7 g) compared to the control group (6.8±0.4 g), reflecting inflammatory swelling, fat deposits, and hepatic edema. The extract restored body weight to (238.1±8.6 g) and liver weight to (7.4±0.4 g) with the high dose (figure7)(Table 1)

Inflammatory cytokines: TNF-α and IL-6

TNF-α increased significantly ($p<0.001$) in the CCl_4 group (92.6±7.8 picograms/ml) compared to the control group (18.4±1.4 picograms/ml), which is equivalent to five times the normal value. IL-6 also increased from (12.2±1.0) to (68.4±5.4 picograms/ml) in the CCl_4 group. The Cichorium intybusextract significantly reduced these inflammatory markers ($p<0.01$) in a dose-dependent manner: TNF-α to (28.8±2.4) and IL-6 to (19.2±1.6) with the high dose (Figure 8).

This study describes a physiologically and biochemically comprehensive assessment of the hepatoprotective effects of Taraxacum officinale root extract in rat liver injury induced by carbon tetrachloride (CCl_4) involving an experimental model that closely resembles some pathological features associated with toxic hepatitis for humans. These physiological data provide a global and fine interpretation of the mechanisms responsible for hepatoprotective activity using this medicinal plant. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most sensitive biomarkers of hepatocellular injury, specifically released into circulation after integrity loss of cell membrane due to oxidative damage. ALT is mostly found in the hepatocellular cytoplasm, contrasting with AST which are present both within the cytoplasm and mitochondria; therefore significant elevations of AST indicates extensive mitochondrial injury [5]. Marked elevations seen in the CCl_4 -treated group (ALT: 189.6 U/L; AST: 210.4 U/L) agree with [10], reported with a similar experimental model these values (ALT: 197.2 U/L; AST: 224.6 U/L).

Alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), which are important indicators of biliary tract injury and membrane damage to hepatocytes. Elevation in their levels is often associated with intrahepatic cholestasis due to damage of biliary epithelial cells and accumulation of bile salts within the liver [2] . Similarly, the decline in total bilirubin after treatment indicates an increased capacity of hepatocytes to uptake bilirubin and perform glucuronidation for biliary excretion.

The hepatoprotective effect of Cichorium intybusextract on these biochemical markers might be associated with its bioactive constituents including chlorogenic acid and chicoric acid that can inhibit CYP2E1-mediated bioactivation of CCl_4 to the highly reactive trichloromethyl radical (CCl_3 •). They also promote stabilization of hepatocyte membranes through a membrane phospholipid increase [12] . These results are similar with who demonstrated that ALT, AST in Iraqi rats were significantly decreased by 63% for ALtl or 58% for AS without Cichorium intybusaqueous extract. Hepatocyte cells are responsible for the

synthesis of ~90% plasma proteins, comprising include albumin, fibrinogen and clotting factors. The significant reductions in total protein and albumin levels seen in the CCl_4 group reflects a marked dysregulation of hepatic synthetic function due to disruption of Edoplasmic reticular & ribosomal functions responsible for proteins synthesis [1]. Clinically, hypoalbuminemia causes decreased colloid osmotic pressure and may present with peripheral edema and ascites in more advanced liver disease. Cichorium intybusextract treatment improved the level of serum levels total protein and albumin ($P < 0.050$). This amelioration might be linked to diminishing loss of hepatocyte and premier regeneration restoring damaged hepatic structure through activation of PI3K/Akt signaling pathway along with upregulated expression levels of Insulin-like growth factor-1 (IGF-1) as previously reported by [13]. Oxidative stress is a core mechanism of CCl_4 −induced hepatotoxicity. The trichloromethyl radical (CCl_3 •) initiates a series of lipid peroxidation reactions that lead to the formation of malondialdehyde (MDA), 4-hydroxynonenal (HNE), as well as other toxic secondary products which are typically measured with thiobarbituric acid reactive substances assay [5]. The notably increased levels of MDA found in the CCl_4 group (5.68 nmoL/mL vs 1.24nmoL/mLl controls) strongly demonstrates a marked degree of lipid peroxidation and loss of membrane integrity at cellular level.Operation depletion of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidise GPx is associated with the exhaustion of endogenous antioxidant defence mechanism pathophysiological response to excessive generation reactive oxygen species. SOD converts high concentration superoxide radicals to hydrogen peroxide, which is then decomposed by CAT into water and oxygen; GPx detoxifies lipid hydroperoxides with reduced glutathione as a cofactor [14] [11] .

Mainly, chlorogenic acid, chicoric acid and caffeic acids are known as a potent hydrogen atom transfer or free-radical scavengers agents that is responsible for strong inhibition on lipid peroxidation chain reactions of Cichorium intybusextract. In addition, these phytochemicals are confirmed to activate the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway by suppressing oxidative stress-mediated inflammatory reactions and promote antioxidant genes of heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase-1(NQO1), glutamate cysteine ligase catalytic subunit(GCLC) The results are in line with those of [15], reported a substantial improvement of antioxidant status after consumption of commercial Cichorium intybusextracts in humans. Free radicals stimulation, in the context of chemical hepatitis overview Inflammation plays a crucial role in promoting chemical-induced hepatic injury; free radical can trigger intrinsic NF-κB signalling on Kupffer cells and infiltrate inflammatory cell which produce wave of proinflammatory cytokines including TNF-α ; IL-6, and IL -1β mostly [9] . TNF-α activates apoptosis pathways through its death receptor TNFR1 and via caspase-8/caspase 3, while IL6-plasma levels enhance systemic inflammatory response with the JAK/STAT3 pathway. It is in agreement with the study of Mahran et al., where sharp increase seen TNF-α (92.6 vs 18.4 picograms/ml) and in IL-6 as well (68.4 vs 12.2picogram/sml). (2023) observing similar values with the significant positive correlation of these two cytokines and increased liver enzymes (r=0.87, 0.83 respectively). One reason this is highly effective at preventing these cytokines, is the compounds intybin and lactucine both inhibit NF-κB activation by inhibiting

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IKKβ kinase therefore stopping the production of inflammatory cytokines right where they start [16] . The loss of body mass in the CCl_4 group mirrors both the systemic effects caused by advanced chemical hepatitis: metabolic disorders and a decrease in appetite induced by increased levels of inflammatory cytokines (inflammatory cachexia), diminished intestinal absorption due to deficiency bile secretion, along with disturbances glycogen storage as well metabolism. However, increased liver weight reflects a combination of three factors: inflammatory edema; accumulation of fat (Hepatic steatosis) in the cytoplasm; and Kupffer cell hypertrophy and inflammatory infiltration [5] . The Cichorium intybusextract inversely relates with the disorders proportional with the dose, reflecting both a system-wide improvement in general health and hepatic metabolic functions regularity. The study results exhibit a clear and significant dose-response trend in the physiological parameters across all of those examined; namely, every single parameter showed better performance at 400 mg/kg than 200 mg/kg ($p<0.05$ for each respective third vs fourth group comparison). This complete broader coverage is achieved whether concerning oxidation + inflammation+direct cellular damage with the higher content of phenolic compounds and sesquiterpenes in comparison to a lower dose that achieves only partial inhibition [17] [18]

Interestingly, treatment with the high dose (400 mg/kg) completely or almost normalized oxidative stress markers (MDA, SOD, CAT and GPx), as well as pro-inflammatory cytokines without statistically significant differences when comparing with control group values $p > 0.05$. The latter suggests that the high dose was optimal for maximal therapeutic reduction of both oxidative and inflammatory damage. Which pattern of response is consistent with the work by [11] and [14] . revealed the full spectrum of hepatoprotective activity of aqueous Cichorium intybusextracts in experimental models for non-alcoholic fatty liver disease and hepatic fibrosis. The results from this study strongly concur with those of [19] Cichorium intybusshowed similar effects to milk thistle in decreasing liver enzyme activities, with another study by Moon et al concluded that Cichorium intybushas little more beneficial effect on oxidative stress parameters as compared to milk thistle (Silybum marianum), the gold –standard herbal hepatoprotector [20]. Likewise, our results are consistent with those of [21] subsequently confirmed the liver-sparing effects of Cichorium intybusin a similar CCl_4 induced hepatic damage model, complemented by findings from those of [20] utilized a nano formulated Cichorium intybusextract and found improved therapeutics with lower doses. However, the present investigation has some limitations: first it is based solely on an experimental rat model. Clearly, reasonable extrapolation of these results to humans is limited by the need for further well-designed and controlled clinical studies in order to prove therapeutic efficacy alongside determining appropriate dosage regimens and assessing any potential herb-drug interactions

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Table (1): Comprehensive Summary of Physiological and Biochemical Findings in Experimental Groups

Parameter	Control	CCl_4	CCl_4 + 200 mg/kg	CCl_4 + 400 mg/kg
ALT (U/L)	28.4 ± 2.1	189.6 ± 12.4***	112.3 ± 8.6†	67.8 ± 5.3†††
AST (U/L)	32.1 ± 2.8	210.4 ± 14.2***	128.7 ± 9.1†	79.2 ± 6.1†††
ALP (U/L)	78.2 ± 5.4	245.8 ± 18.3***	156.4 ± 11.2†	102.7 ± 7.8†††
GGT (U/L)	18.6 ± 1.8	89.4 ± 7.2***	52.1 ± 4.1†	31.8 ± 2.9†††
Total Bilirubin (mg/dL)	0.48 ± 0.04	2.84 ± 0.22***	1.62 ± 0.14†	0.91 ± 0.08†††
Total Protein (g/dL)	7.2 ± 0.3	4.8 ± 0.4***	6.1 ± 0.3†	6.9 ± 0.3†††
Albumin (g/dL)	4.1 ± 0.2	2.4 ± 0.2***	3.2 ± 0.2†	3.8 ± 0.2†††
MDA (nmol/mL)	1.24 ± 0.09	5.68 ± 0.44***	3.21 ± 0.28†	1.98 ± 0.16†††
SOD (U/mL)	42.8 ± 3.2	18.4 ± 1.8***	28.6 ± 2.4†	37.9 ± 3.1†††
CAT (U/mL)	28.4 ± 2.1	11.2 ± 1.0***	18.9 ± 1.5†	25.6 ± 1.9†††
GPx (U/mL)	38.6 ± 2.8	14.8 ± 1.2***	24.2 ± 1.9†	34.1 ± 2.6†††
TNF-α (pg/mL)	18.4 ± 1.4	92.6 ± 7.8***	54.2 ± 4.6†	28.8 ± 2.4†††
IL-6 (pg/mL)	12.2 ± 1.0	68.4 ± 5.4***	38.6 ± 3.2†	19.2 ± 1.6†††
Liver Weight (g)	6.8 ± 0.4	11.2 ± 0.7***	9.1 ± 0.5†	7.4 ± 0.4†††

Values are expressed as Mean ± SD (n = 8 animals per group).

***P < 0.001 compared with the Control group.

†P < 0.05 and †††P < 0.001 compared with the CCl_4 -treated group

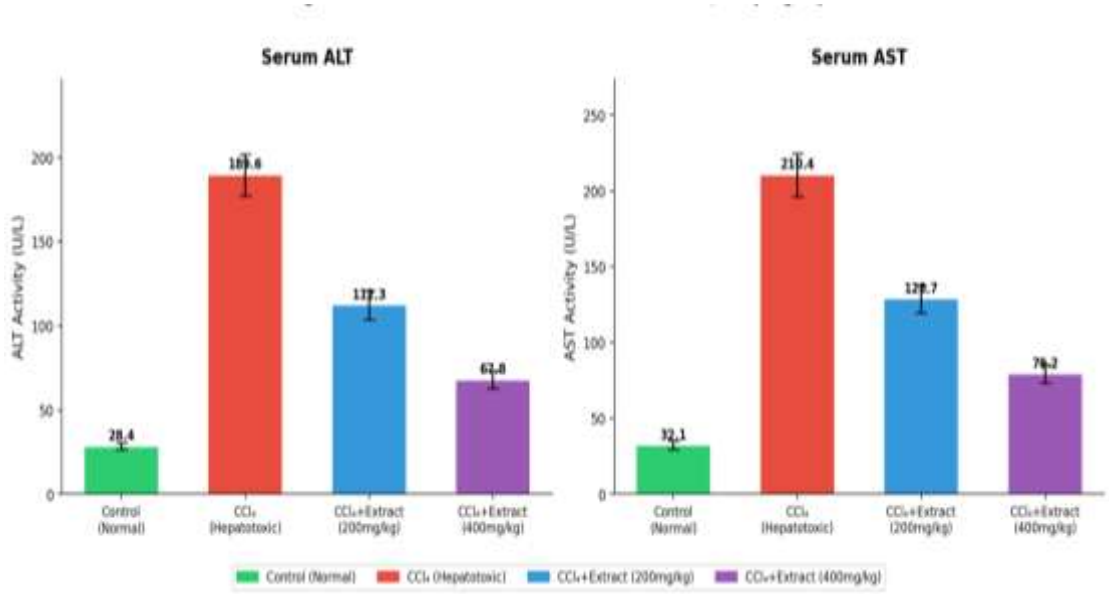

Figure 1: Serum ALT and AST levels (U/L). Mean ± SD

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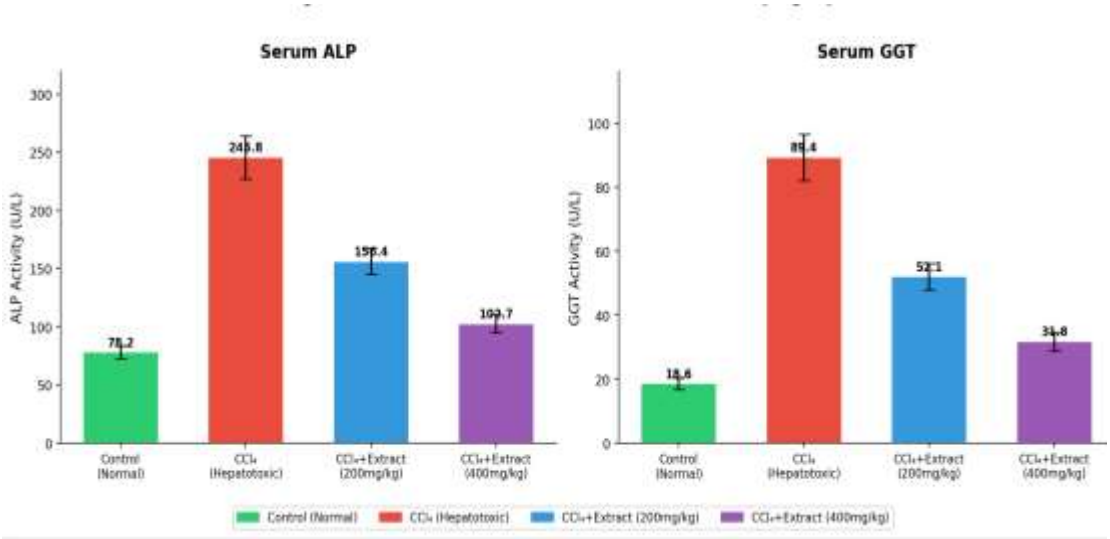

Figure 2: Serum ALP and GGT levels (U/L). Mean ± SD

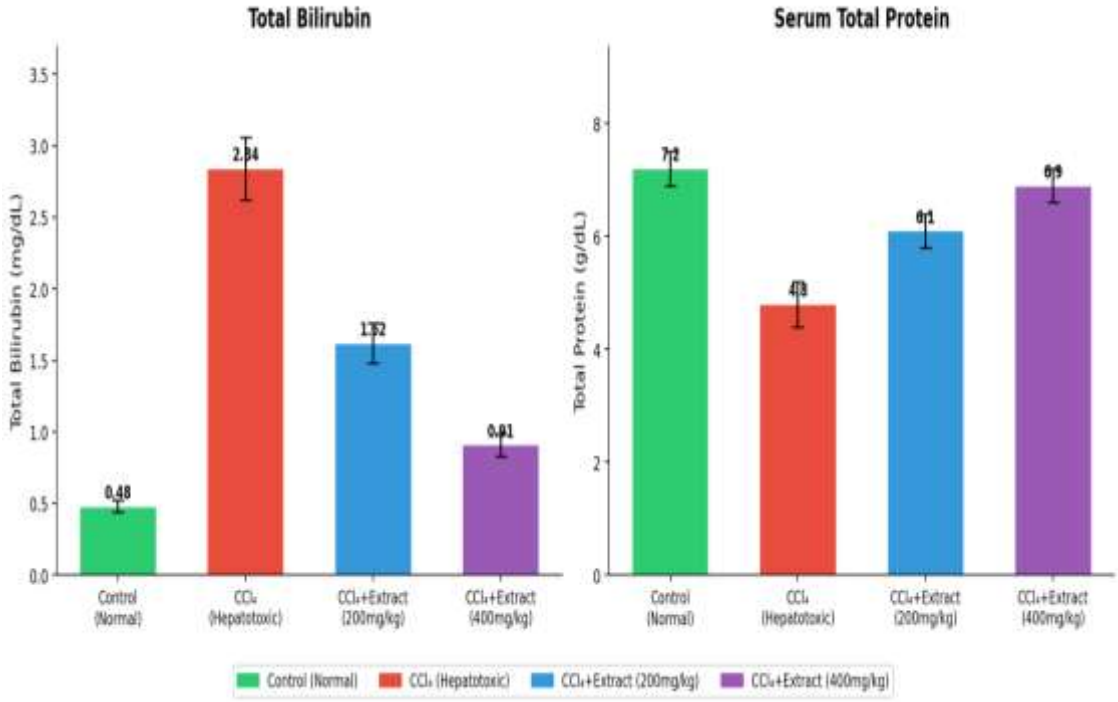

Figure 3: Total bilirubin (mg/dL) and serum total protein (g/dL). Mean ± SD

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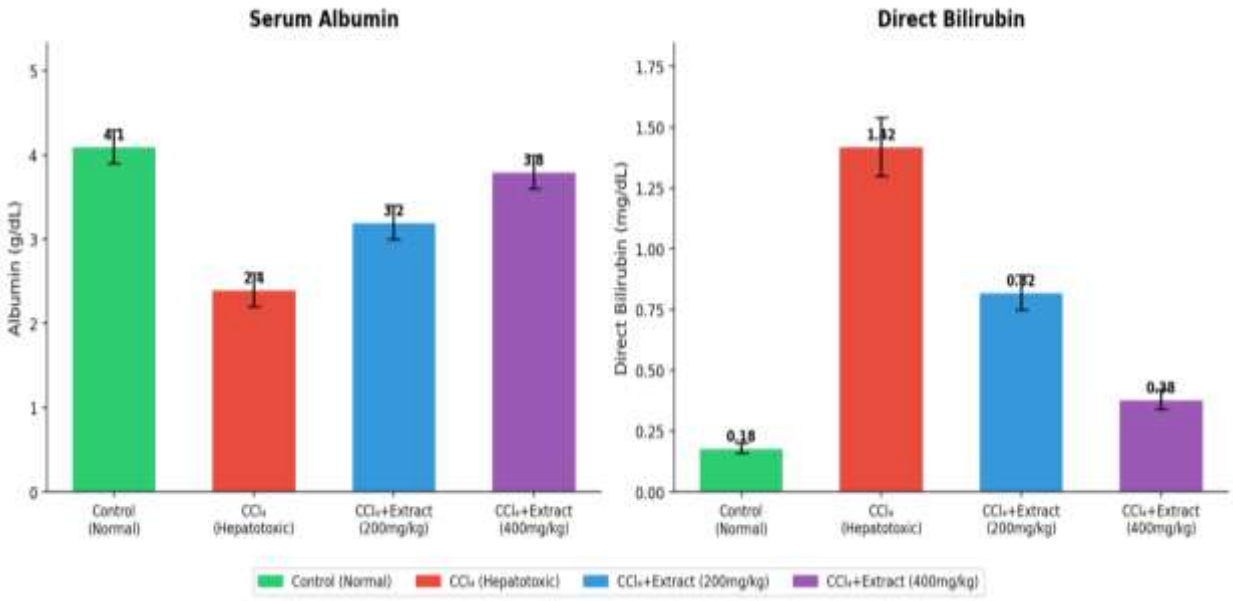

Figure 4: Serum albumin (g/dL) and direct bilirubin (mg/dL). Mean ± SD

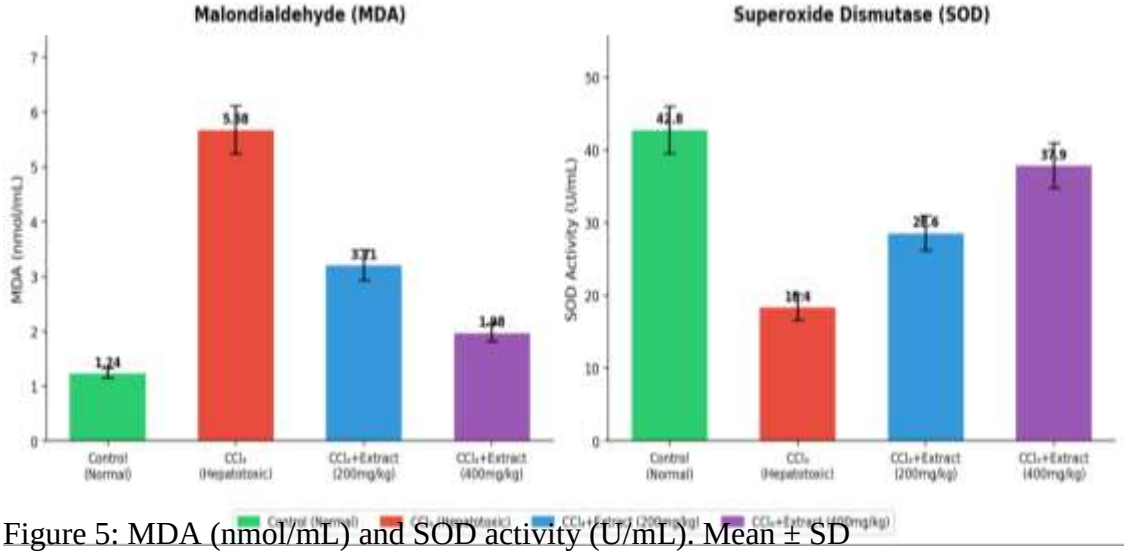

Figure 5: MDA (nmol/mL) and SOD activity (U/mL). Mean ± SD

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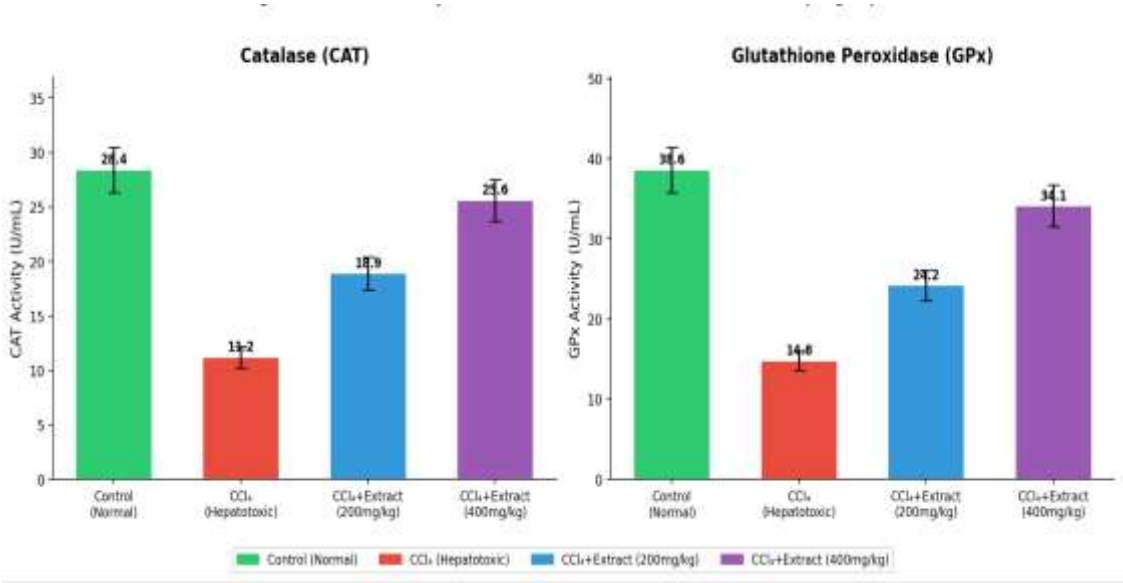

Figure 6: Catalase (CAT) and GPx activity (U/mL). Mean ± SD

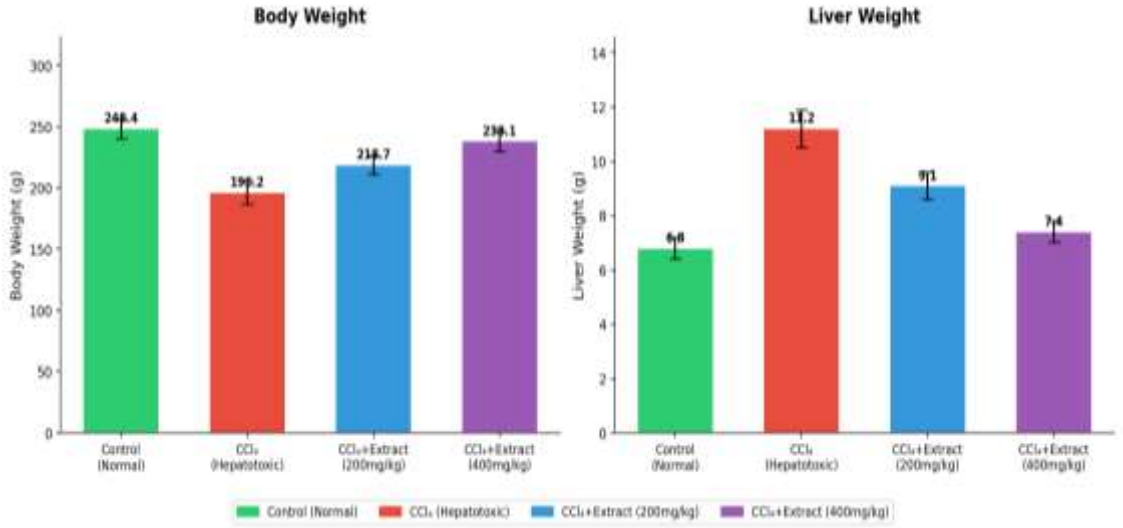

Figure 7: Body weight (g) and liver weight (g). Mean ± SD.

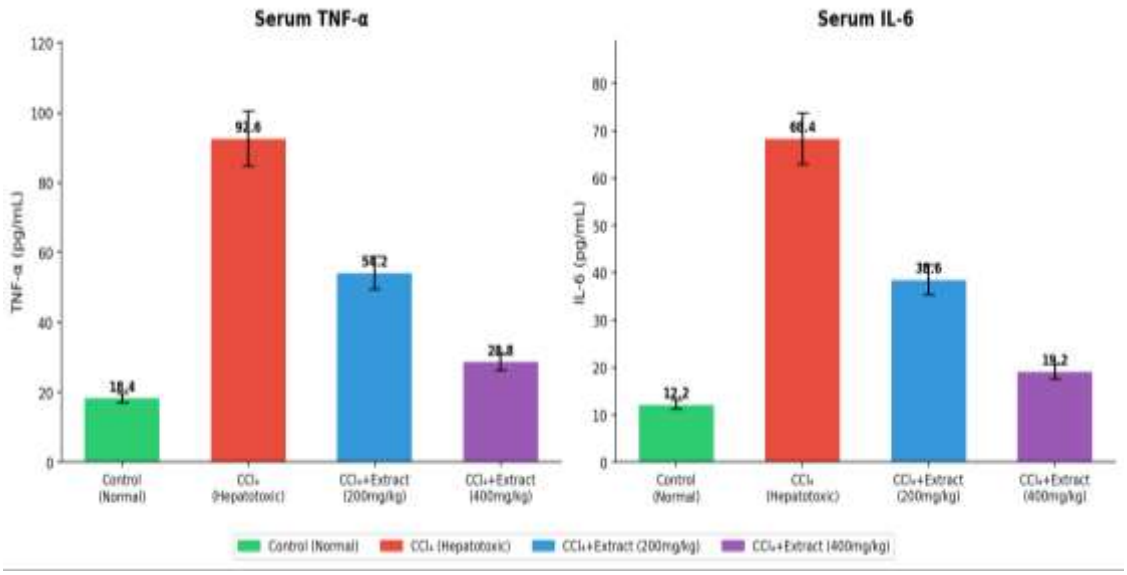

Figure 8: Inflammatory cytokines TNF-α and IL-6 (pg/mL). Mean ± SD

Conclusion

The aqueous extract of chicory roots (*Cichorium intybus*) has a clear protective and therapeutic effect on the liver in a chemically induced hepatitis model using CCl_4 in rats. The extract significantly reduces liver enzymes ALT, AST, ALP, and GGT, as well as bilirubin ($p<0.001$), and proportionally increases total protein and albumin with the dose. The extract enhances the internal antioxidant system by increasing the activity of SOD, CAT, and GPx and reducing MDA levels, indicating the inhibition of oxidative stress. The extract inhibits the excessive inflammatory response by significantly reducing the cytokines TNF-α and IL-6, indicating its role in inhibiting the NF-κB pathway. The high dose (400 mg/kg/day) outperforms the low dose in all physiological parameters, achieving a near-complete restoration of normal levels in oxidative stress indicators and cytokines.

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