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Biochemical, Immunological, and Histopathological Assessment of Carbon Tetrachloride (CCl_4)-Induced Hepato-Renal Toxicity in Rats

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Abstract. **General Background** Carbon tetrachloride (CCl_4) is a well-established experimental toxicant known to induce liver and kidney injury through oxidative and inflammatory mechanisms. **Specific Background** CCl_4 metabolism generates reactive free radicals that disrupt biochemical homeostasis, provoke immune imbalance, and produce characteristic histopathological damage in hepatic and renal tissues. **Knowledge Gap** Despite extensive use of CCl_4 models, integrated evaluation combining biochemical, immunological, and histopathological parameters remains limited. **Aims** This study aimed to assess dose-dependent hepato-renal toxicity of CCl_4 in rats using biochemical markers, cytokine profiling, and tissue histology. **Results** CCl_4 exposure increased serum AST, ALT, urea, creatinine, cholesterol, and triglycerides, elevated TNF-α and IL-6, reduced IL-10, and induced marked hepatic and renal histopathological alterations. **Novelty** The study provides a consolidated multi-system assessment highlighting immune dysregulation alongside biochemical and structural injury. **Implications** These findings underscore the importance of combined biochemical, immunological, and histopathological approaches for mechanistic evaluation of chemical-induced hepato-renal toxicity..

Keywords: Carbon Tetrachloride Toxicity, Hepatorenal Injury, Oxidative Stress, Proinflammatory Cytokines, Histopathological Alterations

Highlights:

1. Dose-dependent enzyme, lipid, urea, and creatinine elevations indicate progressive liver and kidney dysfunction.
2. Pro-inflammatory mediators increased while anti-inflammatory response declined, demonstrating marked immune imbalance.
3. Tissue examinations revealed cellular degeneration, inflammatory infiltration, fibrosis, and tubular damage severity increased with dose.

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Introduction

Carbon tetrachloride (CCl_4) is a toxic organochloride chemical compound, long studied for its toxic effects on key organs, especially the liver and kidneys. Synthesized as early as the 19th century, it has been produced by multiple industrial methods, using methane as the primary feedstock today. CCl_4 has been utilized, historically, as a refrigerant, as a cleaning and drying agent, in the production of other chemicals, and as a solvent for oils, fats, paints, rubber and wax [1]. It is also used as a basis in producing insecticides [2].

Humans may be exposed to CCl_4 by ingestion, inhalation and dermal absorption. Acute toxicity can present as CNS (central nervous system) depression and GIT (gastrointestinal tract) signs with nausea and vomiting. At higher doses the high level of hepatotoxicity and nephrotoxicity could develop, which may in extreme cases lead to death induced by multi-organ failure. Liver damage appears within minutes to hours after exposure in the form of altered lipid metabolism leading to fatty degeneration, hepatocellular necrosis, and fibrosis [3]. This is usually accompanied dysglycemia due to increased glucose levels, and impaired activity of microsomal enzymes which in turn inhibits protein synthesis. The kidneys are not spared, but display neutritis, tubular injury, oliguria, and renal failure, often due to the liver injury [4].

Central to the toxicity of CCl_4 is its metabolism. While a fraction of the compound is exhaled as is, the rest is subject to biotransformations mediated by cytochrome P450 (predominantly CYP2E1), resulting in the formation of reactive trichloromethyl free radicals. These radicals trigger lipid peroxidation, lead to damage to DNA and proteins, and loss of cellular integrity [5]. In addition to hepatotoxic and nephrotoxic pathways, growing evidence suggests that CCl_4-induced organ damage is closely linked to immune dysregulation mediated by proinflammatory, and anti-inflammatory cytokines. Upon CCl_4 metabolism and free radical formation, liver Kupffer cells and renal immune cells are activated, leading to excessive secretion of inflammatory factors such TNF-α and IL-6, which promote the inflammatory response, hepatociyte death, and renal dysfunction. Conversely, decreased level of anti-inflammatory cytokines, such as interleukin-10, contribute to the amplification of tissue damage and impaired repair mechanisms. These cytokine-dependent responses highlight the immune aspect of CCl_4 toxicity and underscore the importance of assessing inflammatory mediators when studying its pathogenesis [6]. In particular, their binding to DNA bases such as adenine and guanine is likely to drive genotoxicity. Chronic or work-related exposure to CCl_4 is linked to higher rates of cancers, particularly for the liver, lung, and hematopoietic system. Additionally, epidemiological and experimental evidence also supports its tumorigenic potential in the liver duration and duration of exposure influences risk severity [7].

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Methodology

A. Experimental Procedures

1.1. Animals

In this research, 30 normal male Wistar-origin rats, weighing approximately 180–200 grams were used. The animals were housed in controlled conditions, with room temperature set at 20–21°C and relative humidity maintained at approximately 40%. A 12-hour light/dark cycle was followed for each rat. The experimental rats were maintainedon standard laboratory, diet and drinking water (ad lib(throughout the experiment. Animal handling and experimental procedures were conducted in accordance with the institution's approved ethical guidelines concerning the maintenance, and utilization of laboratory animals.

1.2. Study Design

a) Wistar rats were randomly assigned to three classified groups:-(10 animals per group):
b) Group 1 (control group): Olive oil was administered intraperitoneally (IP) twice weekly.
c) Group 2 (low dose CCl_4): 0.1 ml/100 g of body weight of carbon tetrachloride (approximately 1 ml/kg), diluted 1:1 with olive oil, was administered via intraperitoneal injection twice weekly.
d) Group 3 (high-dose CCl_4): 0.5 ml/100 g body weight CCl_4 (≈5 ml/kg), diluted in olive oil at a 1:1 ratio, was injected IP twice weekly.
e) Treatment lasted 4 weeks.

1.3. Sample Collection

At the end of the study rats were anesthetized with isoflurane and sacrificed, via cardiac puncture. Blood samples were collected to prepare serum, then allowed to clot and centrifuged. Immediately after tissue preservation (ITP), liver and kidney sections were isolated, rinsed with saline, and fixed in10% neutral formalin, for histopathological analysis.

1.4. Biochemical Analysis

The levels of aspartat aminotransferase (AST), alanin aminotransferase (ALT), urea, and creatinine in serum samples were determined using an automated chemistry analyzer, (Mindray BS-120, Shenzhen., China). A separate amount of serum was kept for determination of total cholesterol and triglycerides using commercial kits (Randox; UK; Diamond–Gordon International)

1.5. Immunological Analysis

concentrations of TNF-α, IL-6, IL-10 in serum were measured by ELISA kits, (R and D Systems, USA), according to the manufacturer's procedure, Absorbance was examining at 450 nm, and cytokine concentrations were calculated using a standard curve.

1.6. Histopathological Examination

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Liver and kidney samples, were embedded in paraffin wax, sliced into 5-micrometer-thick sections, and then dyed with hematoxyline-eosin. Histopathological changes, such as ,necrosise, vacuolation, fibrosis, and inflammatory infiltration, were examined using a light microscope.

1.7. Statistical Analysis

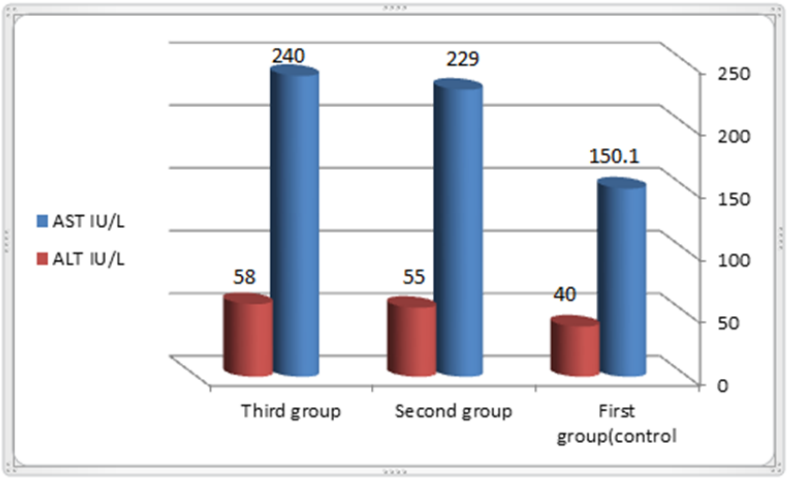
Results were expressed, as mean ± standard error of the mean. Comparisons between groups were performed using one-way ANOVA, followed by Tukeye's post hoc test. The statistical significance level, was considered significant at a confidence level of less than 0.05 ($p < 0.05$).

Results

A. Biochemical Result

1.1 Effect on AST and ALT Enzyme Activity

As shown in graph (1), AST and ALT enzyme levels gradually increased after carbon tetrachloride (CCl_4) injections at doses of 0.1 ml and 0.5 ml in both experimental sets. This increase in liver enzymes demonstrates unequivocal hepatocyte injury. CCl_4 is metabolised to reactive free radicals, which attack cell membranes and change enzyme structure, which results in necrosis. The leak of ALT, and AST in the blood shows the oxidative striss and lipid peroxidation caused by the CCl_4 exposure [8].



Graph 1.displays the effect of carbon tetrachloride (CCl_4) on both AST and ALT enzymes.

1.2 Changes in serumurea and creatinine concentrations

As displayed in graph 2, serum urea and creatinine levels increased significantly in in each of the experimental groups receiving CCl_4 as opposed to the normal control group. This increase indicates reduced kidney function, most likely caused by nephritis and glomerular injury. The damage is linked to oxidative stress and protein breakdown associated with CCl_4 exposure [9].

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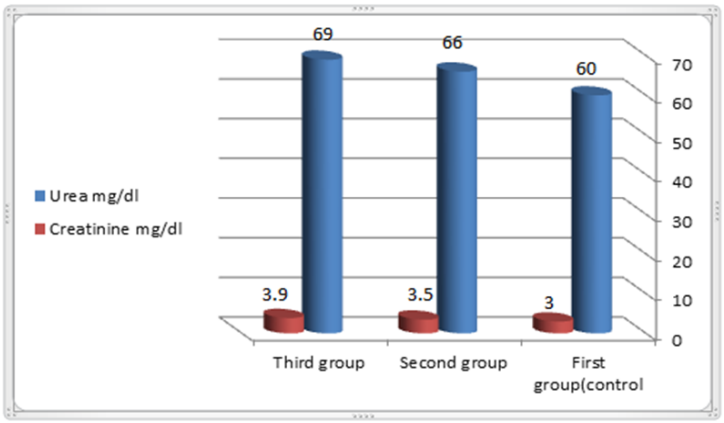
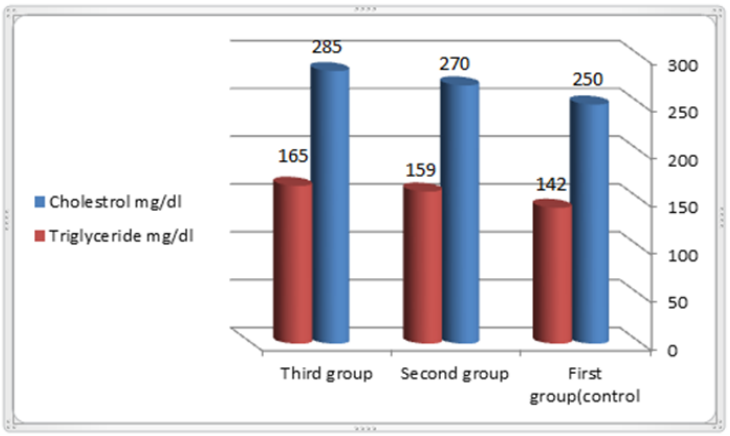


Graph 2.displays the effect of carbon tetrachloride (CCl_4) on creatienine and urea levels.

1.3 Changes in serumcholesterol and triglyceride levels

As shown in graph 3, rats exposed to CCl_4 had significantly higher levels of total cholesterol and triglycerides compared with the control group. This effect is likely due to CCl_4 interfering with normal lipid metabolism, which decreases fat utilization and promotes cholesterol buildup, leading to further disturbance of hepatic lipid balance [10].



Graph 3.displays the effect of carbon tetrachlorid (CCl_4) on both cholesterol and triglycerides.

B. Immunological Findings

In addition to increasing cellular biochemical and histological changes, CCl_4 exposure induces significant immune disturbances as well. In a dose-dependent way serum levels of the pro-inflammatory cytokine TNF-α (from 21.5 to 98.6 pg/ml) and IL-6 (from 14.3 to 74.5 pg/ml) increased (Table 1). In comparison, the anti-inflammatory cytokine IL-10 decreased from 33.7 to 13.4 pg/mL. Such a pro-inflammatory shift indicates an excessive inflammatory response that damages liver and kidney beds. Cytokine imbalance similar to this has been described in experimental models of chemical-induced hepatotoxicity using similar doses and time points [11]

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Table 1. Effect of CCl_4 on serum cytokines (Mean ± SE, n=10)

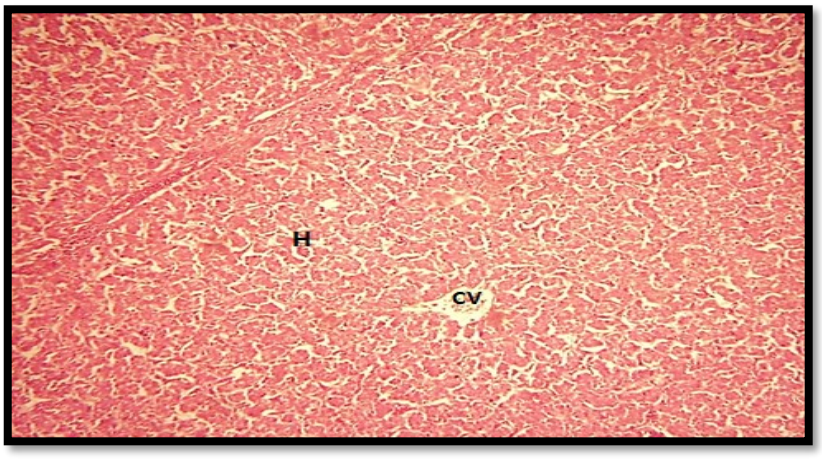
Cytokine	Control Group	Low-dose CCl_4 (0.1 ml/100 g BW)	High-dose CCl_4 (0.5 ml/100 g BW)
TNF-α (pg/mL)	21.5 ± 1.8	55.2 ± 3.9 **	98.6 ± 5.1 ***
IL-6 (pg/mL)	14.3 ± 1.1	42.8 ± 2.9 **	74.5 ± 4.3 ***
IL-10 (pg/mL)	33.7 ± 2.4	22.1 ± 1.9 **	13.4 ± 1.2 ***

C. Histopathological Results

3.1 Histopathological alterations on liver tissues

The liver sections of the control group (Photomicrograph4) showed a normal histological structure with intact central and portal veins, and hepatocytes arranged regularly in cords. By comparison, groups receiving CCl_4 showed distinct pathological alterations. The livers of treated rats (Figure 5) displayed prominent histopathological features such as hepatocellular degeneration, vacuolation and infiltration of inflammatory cells and some foci of fibrosis and hepatocytes enlargement. With more severe liver damage in the high-dose group (Photomicrograph6), the fatty degeneration around the central vein was prominent with extensive fibrotic regions.

The hepatic injury detected might be related to the metabolism of CCl_4 into extremely reactive free radical. Radicals that damage cellular DNA, proteins, and lipids, causing disruption of hepatocyte integrity. And, oxidative stress and excessive lipid peroxidation amplify the injury and play a role in progressive liver injury [12].



Photomicrograph (4) (H&E staining) shows the typical architecture of hepatic tissue, composed of the centrilobular vein (CV) and hepatocyte plates (H).

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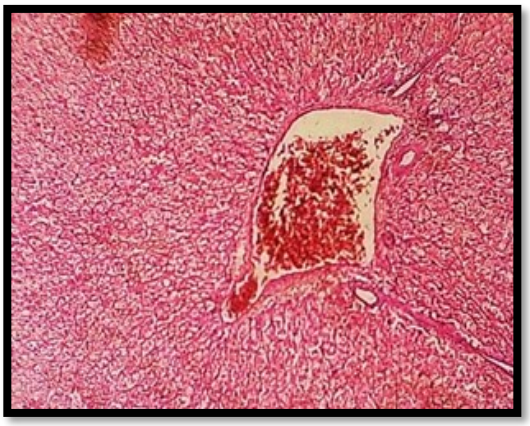

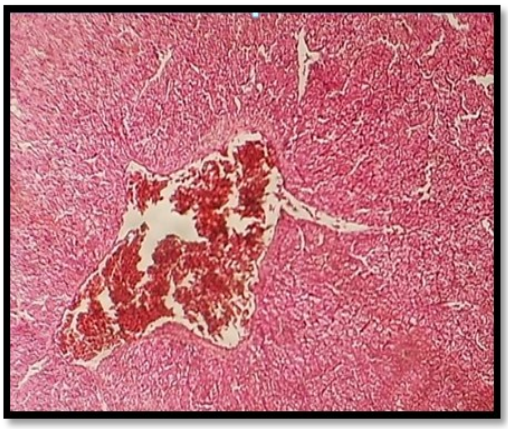

Photomicrograph (5 and 6) (H&E staining) demonstrate an alteration in the structure of hepatic parenchyma, which is represented by the occurrence of necrosis and vacuolation, the appearance of inflammatory cells and fibrosis in some areas, in addition to the appearance of vacuoles accompanied by hepatocyte hypertrophyand fatty degeneration around the central vein.

3.2 Histopathological Findings (Kidney)

Kidney section of control animal under (Photomicrograph7) light microscope reveals normal anatomical features, including renal cortex, nephrons, glomeruli, renal tubules and medullary capillaries. Alternatively, the kidneys obtained from the CCl_4-treated rats had significant pathological alterations. In both Figures 8 and 9, CCl_4 exposure resulted in glomerular degeneration and tubular dilatation, along with the accumulation of inflammatory cells. In the high-dose group, lesions were more obvious with focal necrosis and tumor-like proliferative changes and the injury increased with the increased dose.

The lipophilicity of CCl_4, which favors the accumulation of the compound in the liver and also in the kidney, explains these renal changes. For the free radicals generated by its metabolic activation; free radicals bind to microsomal and cellular macromolecules, lead to lipid peroxidation, membrane damage, and disruption of cell homeostasis. Moreover, toxic metabolites produced by anaerobic reactions continuously induce kidney injury, which is then characterized by inflammation and proliferative lesions [13].

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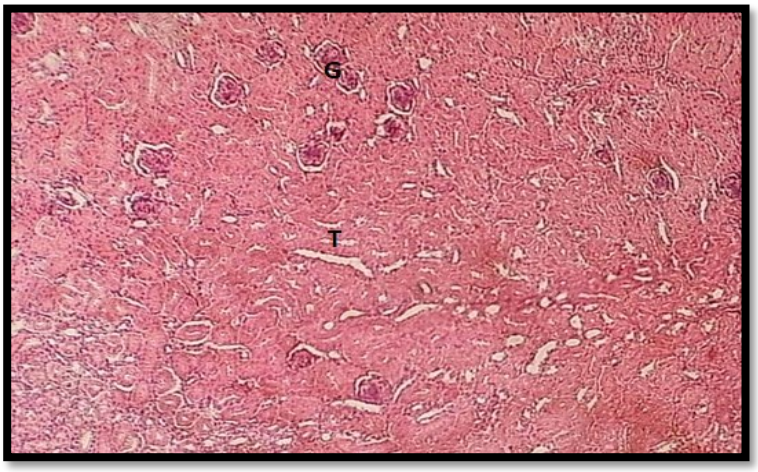
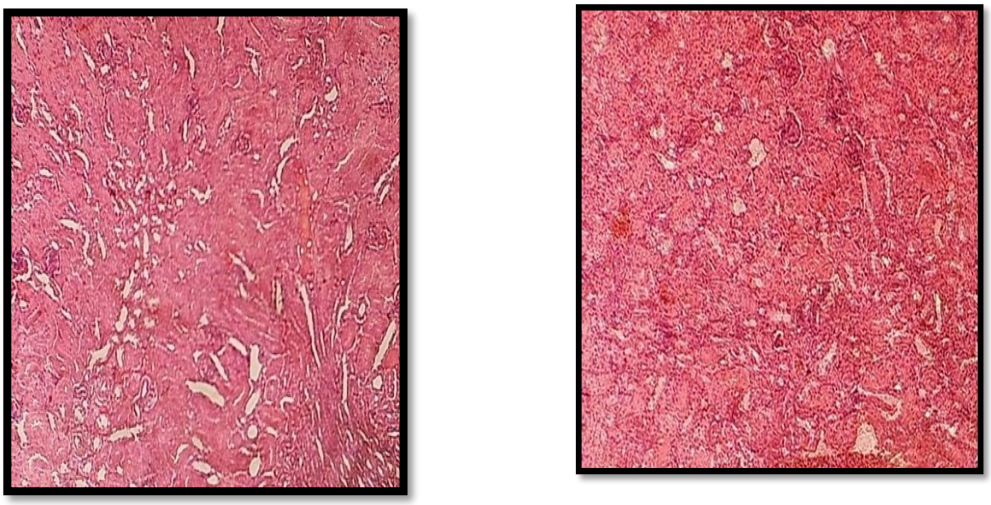


Photomicrograph (7) (H&E staining) demonstrates the typical structure the normal architecture of kidney parenchyma, which characterized by the cortical region that contains which includes the renal tubules, renal glomeruli, and the medullary region.



Photomicrograph (fig 8 and 9) H&E staining: demonstrate alterations in the typical architecture off the renal parenchyma. These alterations involve the occurrence of tubular damage, the attack of inflammatory cells, and bleeding.

Discussion

In regard to its biochemical, histological, and immunological effects, our study demonstrates that carbon tetrachloride (CCl_4) induces extensive hepatonephrotoxicity in the rat, and therefore CCl_4 represents a classic paradigm of experimental liver and kidney toxicity. Serum levels of: AST, ALT, cholesterol, triglycerides, urea, and creatinine increased significantly in all treated groups, suggesting that liver and renal dysfunction were occurring. These outcomes confirm the previous results [14], showing raised levels of aminotransferases and cholesterol, accompanied with vacuolation and fibrosis of liver tissue.

As such, histological analysis demonstrates similar biochemical changes occur in our

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study. CCl_4-stressed rats exhibited hepatocellular necrosis, vacuolar degeneration and hepatic fibrosis in line with earlier reports of widespread hepatic injury [15]. Change in kidneys such as Gomerular shrinkages and vascular congestion induced by CCl_4 were also comparable with previous finding about neohepatoprotective effect of some plant extract against CCl_4-induced nephrotoxicity (23). Together these findings suggest extra-hepatic systemic organ involvement.

One important innovation in this study is the new use of immunological markers. This was associated with elevated levels of TNF-α and IL-6 and decreased IL-10 in a dose-dependent manner, establishing a unique pro-and anti-inflammatory cytokine imbalance. In this regard it elaborates on immune dysregulation as the centre of CCl_4 action and specifies the finding by [16] that reporting that cytokines mediate hepatic injury in CCl_4 toxicity and that silymarin ameliorates both these effects through oxidative and immune pathways.

We additionally noted the very close interconnectedness of oxidative stress and inflammation events. Our finding is in agreement with [17] who reported that CCl_4-induced oxidative stress increased the level of lipid peroxidation as well as production of nitric oxide leading to an enhanced inflammatory response. Here, the reduction of IL-10 reinforces this imbalance and implies a shift toward a strong pro-inflammatory environment. In a similar manner, CCl_4 induces imbalance of hepatic biochemical homeostasis and an immune-mediated injury mediated through up-regulation of pro-inflammatory cytokines, consistent with here bridging oxidative stress with inflammation [18].

Collectively, these results offered a wide perspective of our knowledge on the interaction between oxidative stress and immune dysregulation detected in hepatic and renal injury in CCl_4-exposed rats. Finally, they highlight that dual-targeting of oxidative and inflammatory pathways constitutes a novel therapeutic strategy [19]. Such a dual approach could provide potential strategies for minimizing CCL toxicity$_4$.

Limitations

This study has several limitations that merit mention [20]. First, we did not perform molecular tests such as gene expression of cytokines or antioxidant enzymes, which could better have elucidated the mechanism of CCl_4 toxicity. Second, the small sample size may limit the generalizability of the results [21], [22], [23]. Larger numbers of animals and more sophisticated analytical tools–including transcriptomic and proteomic profiling, in conjunction with treatment trials–will be necessary to more fully elucidate the potential mechanisms of dysregulation of oxidative stress, immune imbalance, and tissue injury in the early stages of injury following CCl_4 exposure [24], [25].

Conclusion

We have shown that carbon tetrachloride (CCl_4) is relatively toxic to the liver but we also confirm that it can also hurt the kidney. This disease is associated with oxidative stress and lipid peroxidation, and immune dysfunction. Elevated parameters of liver and kidney function (AST, ALT, urea, creatinine, cholesterol, and triglycerides) are also present,

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along with associated histological changes and cytokine abnormalities (elevated TNF-α and IL-6, and decreased IL-10), highlights that CCl_4 toxicity involves relevant multimodal effects. The findings indicate that co-treatment with antioxidants and anti-inflammatories may provide protection from this type of injury.

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