



## Original Article

## Hepatotoxicity Induced by Carbon Tetrachloride in Experimental Model

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## ABSTRACT

The present study is the first attempt to evaluate the hepatotoxicity induced by carbon tetrachloride (CCl<sub>4</sub>) in experimental model. It poses a significant hazard to one's health. It is also one of the leading sources of toxicity in critical organs such as the lungs, kidneys, liver, and brain.

**Objective:** To assess the hepatotoxicity of carbon tetra chloride in albino rats. **Methods:** The research was conducted at the Department of Zoology, University of Okara. The experiment was conducted at the animal home of the Department of Zoology, University of Okara. There were two groups created: a control group and an experimental group. The experimental group was treated with CCl<sub>4</sub>. The rats were fed 30% diluted carbon tetrachloride with normal saline as a control group to test the harmful effect on the liver profile. This was accomplished through a 12-day trial. Sampling or dissection was done after 12 days. Rats were dissected, and their liver was punctured to obtain a blood sample and organ collection. After sampling was taken by puncturing the Rats' liver, the samples were examined by a machine called Micro-Lab 300.

**Results:** Histopathological studies also proved that the liver of rats was damaged. The hepatotoxic dose of CCl<sub>4</sub> also raised the serum AST, ALP, ALT, and bilirubin levels. Total levels of AST, ALP, ALT, and Bilirubin were higher than usual, indicating that CCl<sub>4</sub> has a toxic effect on the liver profile of rats. **Conclusions:** This study suggested that CCl<sub>4</sub> induced toxicity in rat liver.

## INTRODUCTION

Carbon tetrachloride (CCl<sub>4</sub>) is a chlorinated hydrocarbon that is clear, colourless, volatile, and highly stable. Its inhalation can cause kidney and liver degeneration and central nervous system depression [1]. CCl<sub>4</sub> was first synthesised in 1839 by the French chemist Henri Victor Regnault by reacting chlorine and chloroform. Currently, it is mainly produced from methane. Recently, it has been widely used in fire extinguishers, as a precursor to refrigerants, and as a cleaning agent [2]. Although it is a common environmental pollutant, workers are at high risk of exposure to high levels of CCl<sub>4</sub> through inhalation and skin contact. On the other hand, the general population may be exposed to low levels of CCl<sub>4</sub> through inhalation in the atmospheric environment [3]. It is a well-known toxin frequently used in pre-clinical experiments for xenobiotic-

induced hepatotoxicity. It induced hepatic damage in mice [4]. CCl<sub>4</sub>-induced liver toxicity leads to necrosis and fatty liver conditions, ultimately causing tissue injury. The process is mediated by several underlying mechanisms, including metabolic activation, generation of reactive free radical metabolites, lipid peroxidation, covalent binding, and disruption of calcium homeostasis [5]. Carbon tetrachloride (CCl<sub>4</sub>) is known for its hepatotoxic effects, causing severe liver damage such as necrosis and steatosis. This is due to the release of free radicals, including trichloromethyl (CCl<sub>3</sub>) and per oxy trichloromethyl (OCCl<sub>3</sub>) radicals, which produce lipid peroxides that damage cell membranes. It also alters enzyme activity and plays a significant role in liver damage. CCl<sub>4</sub> is an essential substance involved in tissue injury, and

its mechanism of hepatotoxicity, especially in necrosis and fatty liver, has been a challenging topic for researchers in various fields for the past 50 years [6, 7]. According to the World Health Organization (WHO), carbon tetrachloride causes hepatomas and hepatocellular carcinomas in mice and rats. These harmful effects are caused by toxic metabolites of  $\text{CCl}_4$ , which are produced by reactions catalysed by specific cytochrome enzymes such as CYP2E1 and CYP3A4. It should be noted that the doses required to induce liver tumours are higher than those required to induce cell toxicity [8]. Animal studies have indicated a positive correlation between CYP2E1 activity and the extent of liver injury due to  $\text{CCl}_4$  exposure. However, no conclusive data exist on the relationship between CYP2E1 or CYP3A4 activity and human toxicity. Administration of  $\text{CCl}_4$  changes liver tissue and causes an increased serum hepatic marker enzyme activity, which is associated with higher lipid peroxidation levels [9]. Carbon tetrachloride ( $\text{CCl}_4$ ) is a potent toxic agent that affects multiple organs, including the kidneys, testicles, brain, heart, lungs, and especially the liver. It is a hepatotoxic solid with nephrotoxic and prooxidant properties, making it a commonly used agent to induce liver injury, hepatocellular carcinoma, hepatic fibrosis/cirrhosis, chemical hepatitis, renal failure, and nephrotoxicity in experimental animals [10]. In rats, administration of  $\text{CCl}_4$ -induced liver injury is characterised by significant changes in serum hepatic enzymes such as AST, ALT, and ALP, as well as changes in liver function biomarkers, oxidant parameters, and inflammation [11]. The study examined the effects of  $\text{CCl}_4$  on male albino rats, looking specifically at hepatotoxicity, lipid peroxidation, and haematological changes. Results revealed increased alpha-fetoprotein levels and changes in hepatic function biomarkers, such as increased levels of transaminases (AST, ALT) [12].  $\text{CCl}_4$  is essential for investigating the underlying mechanisms of hepatotoxic effects, including fatty degeneration, fibrosis, hepatocellular death, and carcinogenesis. Its ability to damage hepatocytes is attributed to its similarity to the oxygen gradient in the liver lobule in  $\text{CCl}_4$ -induced injury. Thus, making it a valuable tool for understanding liver function and pathogenesis [13].  $\text{CCl}_4$ -induced hepatotoxicity resulted in elevated plasma transaminase levels and liver damage. Women showed greater susceptibility to this form of liver injury than men [14]. After four weeks of  $\text{CCl}_4$  administration, significant changes were observed in the liver. In the centrilobular area, hepatocytes underwent necrotic changes with infiltration of ceroid pigment-laden macrophages, causing inflammation. This region also shows increased hepatic stellate cells (HSCs) surrounding the central vein and displaying enlarged nuclei. Fibrotic changes in the

centrilobular area were detected by reticulin and Sirius red staining [15].  $\text{CCl}_4$  is a highly effective compound for inducing hepatotoxicity in experimental rats and is commonly used for this purpose because of its hepatotoxic properties [16]. Because of its effectiveness, chemical toxin-induced liver damage has been extensively studied using animal models [17, 18]. When animals are injected with  $\text{CCl}_4$  in their peritoneal cavity,  $\text{CCl}_4$  interacts with key cellular molecules such as proteins, lipids, and nucleic acids that are structurally and biologically important. This interaction results in liver damage and dysfunctioning [19]. Twenty-four hours after  $\text{CCl}_4$  injection, typical oxidative stress-induced centrilobular necrotic acute liver injuries were observed, including loss of body weight and development of small nodules with a corresponding increase in liver weight on gross examination with an increase in serum AST and ALT levels and notable deficiency of endogenous antioxidants and antioxidative enzymes, with centrilobular necrosis, and oxidative stress markers [20].

## METHODS

$\text{CCl}_4$  was purchased freshly from a market. A 30% concentrated solution of  $\text{CCl}_4$  was prepared using distilled water. The experimental model used in this study was the male albino Wistar adult mice weighing about 180-200g. They were purchased from a local market and were kept in the animal house of the Department of Zoology, University of Okara, approximately one week before the start of the experiment. The mice were housed in four to five cages under the standard conditions of 25-27°C, humidity minimum of 44% under 12 hours of light and dark. Rats were nourished with rat feed containing 20% of protein along with water available at the libitum. Rats were given  $\text{CCl}_4$  doses in the daytime. Doses can only be given once the rats have attained a size of 30g for two weeks (Table 1). During the experiment, the mice were placed randomly, five per cage, divided into two groups, one of the control group and the other of the experimental group. The animals were randomly placed into 5 groups, with 3 rats in each group. The control group in which non affected animals not affected with  $\text{CCl}_4$ -intoxication. The experimental group in which the animals are operated with  $\text{CCl}_4$  during solutions. The solutions were prepared just at the beginning of the experiment. The animals in the experimental group made exposed to the model substance carbon tetrachloride by oral consumption. Continuous for two weeks, given in day time.

**Table1:** List of Groups, Doses, Days, and Amount of Dose

Groups	Doses	Days	Amount
Group 1	Normal Saline	12 Days	1 ml
Group 2	30 % $\text{CCl}_4$	12 Days	200mg/kg

The rats were euthanised with chloroform and weighed before dissection. Blood samples were collected in vacutainer tubes using 23 G1 syringes after a cardiac puncture. Dissected out heart, liver, and brain were washed with ice-cold saline. As for the requirements of tissue homogenisation, weighed organs were stored at  $-20^{\circ}\text{C}$  after the dissection of 1x1 cm tissues was obtained. After this, they were placed in a petri dish containing 0.9% saline solution. For further processing, sections were placed in a glass containing 10% formalin solution. Blood samples were centrifuged for 15 minutes at  $4^{\circ}\text{C}$  at 10,000 rpm to isolate the serum. The diagnostic kits were used to estimate AST and ALT levels to analyse serum samples of rats. Took 800 ul of reagent (R1) and 100ul of the sample. Mixed and added 200 $\mu\text{l}$  reagent (R2) after 1 minute. Mixed and read absorbance at 340nm after 1 min. Read absorbance again after 1, 2, and 3 min. From the absorbance reading, calculated AA/min and multiplied by the corresponding factors (1745).  $\Delta\text{A}/\text{min} \times \text{factor} = \text{ALAT (GPT)activity}[U/1]$ .

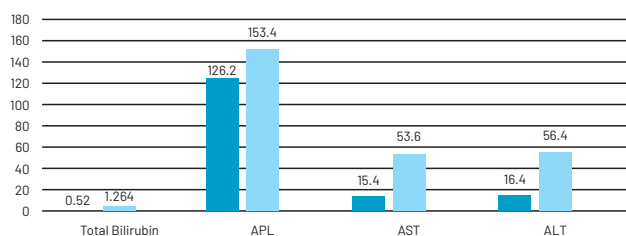
## RESULTS

$\text{CCl}_4$  is a toxic compound that exerts toxic effects on the liver. The analysis showed that  $\text{CCl}_4$  exposure disrupts regular physiological features by disturbing the standard values of ALP, AST, ALT, and bilirubin.

**Table 2:** Mean comparison between the healthy and experimental group

	Healthy N=15	Diseased N= 15
Total bilirubin	.52 00 $\pm$ 0.6188	1.2640 $\pm$ .04735
ALP	126.2000 $\pm$ 8.78212	153.40 $\pm$ 3.17850
AST	15.4000 $\pm$ 1.21420	53.6 $\pm$ 1.3876
ALT	16.4000 $\pm$ 1.36905	56.400 $\pm$ 1.526

The estimation level of bilirubin of the  $\text{CCl}_4$  group (.5200  $\pm$  0.6188) is usually increased than the standard control (1.2640  $\pm$  0.04735). The estimation level of ALP in serum (126.200  $\pm$  8.78212) is typically increased to the control level of (153.40  $\pm$  3.17850). The estimation level of AST in serum (15.4000  $\pm$  1.21420) is typically increased to the control level of (53.6  $\pm$  1.3876). The estimation level of ALT in serum (16.4000  $\pm$  1.36905) is generally increased to the control level of (56  $\pm$  1.526)(Table 2).



**Figure 1:** Mean Comparison of the Normal Group with the Diseased Group

The treatment group's descriptive statistics at the significant variation of ( $P > 0.05$ ) were done to determine the

mean and standard deviation of Bilirubin, ALP, AST, and ALT. One sample t-test was applied to determine the confidence interval of the mean difference of bilirubin, ALP, AST, and ALT (Figure 1, Table 3-5).

**Table 3:** Descriptive statistics values of Bilirubin, ALP, AST, ALT

	N	Minimum	Maximum	Mean	Std. Deviation
Bilirubin	15	.98	1.53	1.2640	.18337
ALP	15	133.00	168.00	153.4000	12.31027
AST	15	47.00	60.00	53.6000	5.38251
ALT	15	49.00	64.00	56.4000	5.91366

**Table 4:** One-Sample Test Show Mean, No of Bilirubin, ALP, AST, ALP

	N	Mean	Std. Deviation	Std. Error Mean
Bilirubin	15	1.2640	.18337	.04735
ALP	15	153.4000	12.31027	3.17850
AST	15	53.6000	5.38251	1.38976
ALT	15	56.4000	5.91366	1.52690

**Table 5:** Mean difference of Bilirubin, ALP, ALT, AST

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Bilirubin	26.697	14	.000	1.26400	1.1625	1.3655
ALP	48.262	14	.000	153.40000	146.5828	160.2172
AST	38.568	14	.000	53.60000	50.6193	56.5807
ALT	36.938	14	.000	56.40000	53.1251	59.6749

At 0.05 significant level (.536\*), the value shows the association between the ALP and ALP. At 0.05 significant level (.613\*), the value shows the correlation between the AST and ALT. At 0.01 significant level (-.715\*\*) value shows the association between the AST and ALP (Table 6).

**Table 6:** Correlation between Bilirubin, ALP, AST, and ALT

		Bilirubin	ALP	AST	ALT
Bilirubin	Pearson Correlation	1	.536*	.304	.439
	Sig. (2-tailed)		.040	.271	.102
	N	15	15	15	15
ALP	Pearson Correlation	.536*	1	-.250	.613*
	Sig. (2-tailed)	.040		.370	.015
	N	15	15	15	15
AST	Pearson Correlation	.304	-.250	1	-.715**
	Sig. (2-tailed)	.271	.370		.003
	N	15	15	15	15
ALT	Pearson Correlation	.439	.613*	-.715**	1
	Sig. (2-tailed)	.102	.015	.003	
	N	15	15	15	15

## DISCUSSION

$\text{CCl}_4$  is a toxic compound which exerts toxic effects on the liver. The analysis shows that  $\text{CCl}_4$  exposure disrupts regular physiological features by disturbing the standard values of ALP, AST, ALT, and Bilirubin. The chemical industry commonly uses  $\text{CCl}_4$  as an organic solvent, and it is also well-documented as an experimental inducer of

hepatotoxicity. In this study, mice treated with  $\text{CCl}_4$  suffered damage to the hepatocyte membrane, releasing hepatocyte cytosolic enzymes. This was evidenced by significant increases in serum marker enzymes (AST, ALT, and ALP) associated with acute liver damage. Elevated serum levels of AST and ALT are specific markers and showed acute liver damage, while elevated ALP levels indicated hepatobiliary damage. ALT is the most specific marker of liver damage, while AST is abundant in cardiac muscle, kidney, testes, and skeletal muscle. ALP is abundant in growing bone. Therefore, elevated serum levels of these enzymes indicated disease affecting any extrahepatic tissues [21]. Studies have indicated that  $\text{CCl}_4$  significantly affects ALT and AST serum levels. Elevation of these liver enzymes can be attributed to acute hepatocyte injuries caused by  $\text{CCl}_4$  [22]. In assessing  $\text{CCl}_4$ -induced hepatotoxicity, serum AST, ALT, and ALP activity levels were used as indices.  $\text{CCl}_4$ -treated animals showed a significant increase in serum AST, ALT, and ALP activity levels compared to the standard group. This indicated that hepatotoxicity induced by  $\text{CCl}_4$  was evident in the animals [23]. Elevated serum ALT and AST levels indicated severe liver damage and were significantly higher in the  $\text{CCl}_4$ -treated group than in the control group. This increase in ALT and AST activities resulted from  $\text{CCl}_4$  exposure [24]. Control animals treated with  $\text{CCl}_4$  exhibited a significant elevation in ALP levels. This finding was consistent with a study by Prakash and colleagues [25]. Serum levels of ALT and AST were measured and found to be significantly elevated after  $\text{CCl}_4$  injection. Furthermore, the concentration of both ALT and AST peaked after injecting  $\text{CCl}_4$  [26]. Elevated levels of ALT and AST serum activities and elevated bilirubin levels indicated early acute hepatic damage. In cases of  $\text{CCl}_4$ -induced hepatic cell injury, ALT and AST serum activities are markedly elevated. In contrast, bilirubin levels are elevated within 24 hours of treatment—these biochemical markers serve as essential indicators for early detection of hepatic damage [23]. Acute hepatotoxicity was observed due to increased serum AST and ALT activities by  $\text{CCl}_4$ . Our study found a significant reversal of AST and ALT level changes, consistent with previous findings [27]. In response to administering a toxic dose of  $\text{CCl}_4$ , the control group of rats showed elevated levels of serum transaminases, especially AST and ALT. These are essential indicators of liver damage and function [28].

## CONCLUSIONS

The serum AST and ALT activity results from  $\text{CCl}_4$ -induced toxicity revealed significant increases in rats' livers. Serum enzyme assay (AST, ALT, and ALP) results of albino Wistar rats exposed to  $\text{CCl}_4$ -induced hepatotoxicity showed

significant differences, and the serum AST, ALT, and ALP activity levels were significantly higher.

## Authors Contribution

Conceptualization: FM, MKAK

Methodology: FM, MKAK

Formal analysis: FM, MKAK

Writing-review and editing: FM, MKAK

All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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