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Original Article

Renal Toxicity Induced by Carbon Tetrachloride in Experimental Model

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ABSTRACT

Carbon tetrachloride (CCl_4) is largely used as a solvent in chemical industries. It is also well known for hepatic and renal toxic actions. It imposes serious health threats. It is also one of the major causes that is toxic for the vital organs like lungs, kidney, liver, brain, etc. **Objective:** To check nephrotoxicity of Carbon Tetrachloride (CCl_4) on Rat Kidneys. **Methods:** The experiment was conducted at the animal house of the Department of Zoology, University of Okara. The targeted animal was Albino Rat. Two groups were designed control and experimental groups. The rats were fed with 30% diluted CCl_4 to check the toxic effect on the kidneys and normal saline to the control group for comparison. A trial for 12 days was conducted for this purpose. Sampling or dissection was done after 12 days to determine serum Urea, Creatinine, and Electrolytes Sodium (Na), and Potassium (K). Rats were dissected and the heart was punctured to take a blood sample and to collect organs. **Results:** We observed the increased values of Urea, Creatinine and Electrolytes, Sodium (Na), and Potassium (K) as compared to normal values, which have proved the renal toxicity was induced by CCl_4 in Albino Rats. All the experimental data were analyzed by using SPSS-19. The level of significance among the various treatments was determined by LSD at a 0.05% level of probability. **Conclusions:** These findings underline the substantial health risks that CCl_4 poses and emphasize the necessity of putting preventative measures and safety regulations in place.

INTRODUCTION

The molecular weight of carbon tetrachloride (CCl_4), which has four Cl atoms surrounding the carbon atom at its center, is 153.8 g/mol [1]. It is a colorless, transparent, volatile liquid. Free radicals generate lipid peroxidation and are thought to be one of the main factors in cell membrane deterioration, which can result in acute and long-term renal injury and a number of clinical conditions [2–5]. Furthermore, research on numerous proven case studies demonstrated that CCl_4 causes renal disorders in people [4]. According to earlier observations, CCl_4 produced oxidative stress in the kidney and lung as well as alterations in the mixed-function oxidases and microsomal cytochromes in the lungs of rats [6]. Human toxicity is typically brought on accidently by ingestion, dermal absorption via direct skin contact, or inhalation of its vapors; it can also be intentionally consumed as a suicidal agent. The liver, kidneys, and lungs are the primary organs in which CCl_4 damages cells [7]. Tetrachloride has been found to mostly harm the liver (swollen, painful liver, alterations in enzyme levels, and jaundice) and kidneys (nephritis, nephrosis, proteinuria) of humans during acute inhalation and oral exposures to high levels of carbon. There have also been reports of central nervous system depression [8]. Even at relatively large dosages, renal damage is rarely seen in animals exposed to carbon tetrachloride. Although the cause of animals' lower sensitivity to renal damage than humans is unknown, it may be related to how differently CCl_4 is metabolized by their kidneys. After 5 weeks of intermittent exposure to an anesthetizing dosage of carbon tetrachloride, the content of vitamin A in the kidneys doubled, and a 10% rise in wet organ weight was noted [9]. However, the concurrently

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caused hepatotoxicity may have overshadowed this vitamin A effect. Rats subjected to 50 ppm for 5–10.5 months and monkeys exposed to 200 ppm for 10.5 months both showed a little renal edema. At an exposure level of 200 ppm, renal tubular degeneration became visible [10]. Through the production of free radicals, it has been noted that carbon tetrachloride (CCl_4) induces renal damage in rats [11, 12] in addition to hepatic toxicity [13]. Another study found that the protein content of renal tissues significantly increased after treatment with CCl_4. When CCl_4 was administered, proteins suffered oxidative damage and accumulated as a result of inadequate proteasomal and lysosomal breakdown, leading to metabolic inefficiency in the kidneys [14]. Similar to earlier investigations, the treatment of rats with the same substance reversed the shifts toward the control rats [12]. According to a histological analysis, CCl_4 treatment caused lipid peroxidation of the lipid structures in the renal tissues, which led to subcellular damages. The CCl_4-induced vasoconstriction results in an ischemia local environment, and SA has the effect of reducing the morphological changes that CCl_4 causes. Similar histological alterations were seen in renal CCl_4-treated rats, and these alterations resulted in a number of cellular impairments, including a decline in membrane integrity. The fact that the severe alterations were not seen in the groups given the same treatment suggests that rats given CCl_4 plus *Launaea procumbens* extracts had lost their protective effects. In other research [11, 12], similar histological observations were described. Free radicals generate lipid peroxidation and are thought to be one of the main factors in cell membrane deterioration, which can result in acute and long-term renal injury and a number of clinical conditions [3-5, 15]. In addition, findings on several case studies with documentation demonstrated that CCl_4 causes renal problems in people [4]. Both the kidney's inner medullary area and outer cortex have noticeable histological alterations. *Digera muricata* extracts appeared to have protective benefits in reducing the morphological alterations caused by CCl_4 in the groups that were not subjected to the severe modifications. Ogeturk *et al.*, (2005) found similar histological abnormalities in the kidneys of CCl_4-treated rats; however, these changes vanished in rats treated with CCl_4 + caffeic acid phenyl ester (CAPE) [3, 11]. In the kidneys of rats given CCl_4, tubular epithelial cell changes, such as vacuolization, atrophy, and ultimately epithelial cell detachment, suggested tubular necrosis. After prolonged exposure to CCl_4, similar histological changes were also discovered in other studies [16]. The capacity of tubular absorption may have been altered as a result of these histological changes, leading to the functional overload of nephrons and eventual renal dysfunction [5].

METHODS

In this investigation, adult male Albino Wistar rats weighing 180-200g were employed. The animals were bought from a nearby market and housed at the zoo's animal house at the University of Okara (Pakistan). Throughout the trial, they were kept in cages with four or five rats each, at a temperature of 25 3 °C, with 12-hour light/dark cycles and a minimum relative humidity of 44%. Rat Chow (20% crude proteins) and water were available at all times. Every dose was administered in the morning (Table 1). The animals were kept in these facilities for at least one week before the experiment.

Table 1: List of Groups, Doses, Days and Amount of Doze

Groups	Doses	Days	Amount
Group 1	Normal Saline	12 Days	1ml/kg
Group 2	30 % CCl4	12 Days	1ml/kg

Carbon Tetrachloride (CCl_4) was used for this study. The tested substance, carbon tetrachloride (CCl4), was bought from a nearby market and stored in the zoology department's lab at the University of Okara in Pakistan. The chemical is diluted by 30%. distilled water was used to produce the stock solutions. Freshly manufactured stock solutions were used for all of the working solutions. The other substances that were used were all of pro-analysis quality and came from conventional commercial sources. The number of rats were randomly assigned into two groups, a control group (Co) having fifteen (15) rats and an experimental group having the same number of rats as in the control group with exposure of 30% CCl_4 mixed with normal saline through oral gavage for 12 days. The experimental rats were anesthetized by putting in a desiccator exposed to chloroform and a small incision was made to cut the abdominal wall with sharp scissors. Then the muscular layer was cut on the sides to expose the internal organs. 0.9% pyrogen-free sodium saline solution was poured on the exposed organs of the animal to avoid drying. The dissections were done in completely aseptic conditions and tissues (heart, kidneys, liver, spleen & intestine) were excised. Normal feed was given to rats for 24 before dissection. Rats were euthanized with chloroform. Dissect the rats and by cardiac puncture, blood samples were collected in vacutainers by using 23 G1 syringes. Kidneys were dissected out, washed with ice-cold saline to remove debris. Organs were weighed and store at -20 ºC for tests. Centrifuge the blood samples at 10,000rpm for 15 minutes at 4 ºC. The serum was separated and store at -20 ºC. Urea, creatinine, and electrolytes (Na and K) in serum only write your parameters were estimated by using standard AMP diagnostic kits (Stattogger Strasse 31b 8045 Graz, Austria). For the analysis of serum samples

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of rats, the diagnostic kits were used to estimate urea, creatinine, and electrolytes levels in serum samples. Creatinine was determined using the Bartels & Böhmer (1971) technique [17]. The sample's creatinine combines with picrates in an alkaline solution to create a colorful complex at a wavelength of 500 nm. The amount of urea in the sample was determined using the Tabacco et al., (1979) method, which produced a colored complex that could be quantified by spectrophotometry (LKB, Sweden) at 600 nm [18]. Biochemical parameters measured were sodium (Na), potassium (K) using standard kits (Eve's Inn Diagnostics, Vadodara, India). Take blood in clot vile as a sample. Let it clot put it on the incubator for 5 to 6 minutes. Let it clot then centrifuge it 2,3 mint and then red blood cells came to the bottom and serum float over the cells. Then take the serum to test RTF (Urea, Creatinine, and BUN) and Serum Electrolyte. Before performing any test, we have to give washings to the machine to clean it. Apparatus that was used during the serum sample tests are Micro – Lab 300, Reagent Hemans or Diesis Reagent 1 and Reagent 2, Yellow and Blue Tip, Pipette 100ml and 1000ml Take Glass Tube, before using the tip and glass tube clean it well with tissue paper. Reagent 1and Reagent 2 were used 400ml and 100ml respectively by using the yellow tip. The total was 500ml. 5 ml serum was taken and after shaking it well, was read in Micro-Lab 300 by setting it at Chapter Urea. After two or three minutes, trial results appeared on the screen of Micro-Lab. The sample was taken in Clot Vile. Incubated it by incubator for 5 to 6 minutes to clot the blood then centrifuge it for 2 to 3 minutes. After centrifugation, plasma and blood cells were separated. Micro – Lab 300, Reagent Hemans or Diesis Reagent 1 and Reagent 2, yellow and blue tips were used, Pipette 100 ml and 1000ml were used. Take Glass Tube. Before using tips and glass tubes cleaned it well with tissue paper. After taking the sample in a clot vile, incubated it to clot the sample and then centrifuged it. Sodium reagent was taken in the amount of 1000ml and a 1000ml pipette was used. Incubate it at 35°C for 7 minutes. The sodium chapter was set at Micro-Lab 300. Samples were read through the machine and trial results were appeared on the screen. Samples were taken in clot vile. 500ml reagent was taken in the glass tube and 10-µl plasma was used. Incubated for three minutes and the potassium chapter was set at Micro-Lab 300. Results appeared on the screen. The computer program SPSS - 19 conducted a one-way analysis of variance to evaluate the treatment effects. LSD was used to calculate the significance level for each of the treatments at a level of probability of 0.05%.

RESULTS

As we know Carbon Tetrachloride (CCl_4) is a toxic compound and this toxic compound also exerts toxicity on the kidney. The analytical analysis showed that CCl_4 exposure alternates the normal physiology disturbing the normal values of urea, creatinine, Na, and K in the blood of the treated groups. Significant differences were observed in the hematology parameters between the control and treatment groups. We have found that those individuals who were exposed to CCl4 Urea levels are increased in them. Serum urea level was increased in treated groups (44.60 ± 1.68) in comparison to the control group by the value of (22.60 ± 0.95). Abnormally increased level of Creatinine was observed in CCl4 administered rats by the value of (1.386 ± 0.094) in comparison to normal control by the value of (0.88 ± 0.03) indicating the increased toxicity in rat's kidneys. An abnormally increased level of Sodium (Na) was observed in CCl4 administered rats in treated groups (139.20 ±.685) in comparison to normal control by the value of (136 ± 0.392) indicating the increased toxicity in rat's kidneys. An abnormally increased level of Potassium (K) was observed in CCl4 administered rats (4.722 ± 0.578) in comparison to normal control by the value of (3.68 ± 0.392) indicating the increased toxicity in rat's kidneys (Table 2 and Figure 1).

Table 2: Mean comparison between healthy and diseased group

	Healthy (n=15)	Diseased (n=15)	p-value
Total Urea	22.60 ± 0.95	44.60 ± 1.68	0.00
Creatinine	0.88 ± 0.03	1.386 ± 0.094	0.00
Na	136 ± 0.392	139.20 ±.685	0.00
K	3.68 ± 0.392	4.722 ± 0.578	0.00

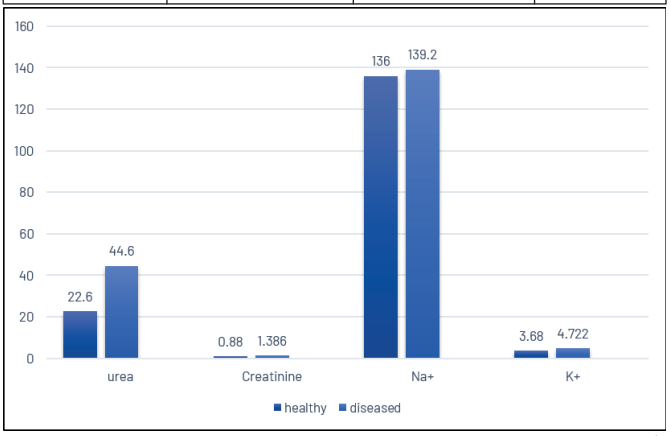

Figure 1: Mean comparison of the diseased urea, creatinine, Na^+, and K^+ with healthy group

One sample t-test was applied to determine the confidence interval of mean difference of Urea, Creatinine, Na, and K as shown in Table 3.

Table 3: T-Test Analysis

	N	Mean ± SD	Std. Error Mean
Urea	15	44.60±6.50	1.67843
Creatinine	15	1.38±0.36	.09411
Na	15	139.20±2.65	.68452
K	15	4.72±0.22	.05778

Table 4 shows confidence interval of the mean difference

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of urea, creatinine, Na, and K. Upper and lower confidence interval for urea was 41.00 and 48.19 respectively. Upper and lower confidence interval for creatinine was 1.18 and 1.58 respectively. Upper and lower confidence interval for Na was 137.73 and 140.66 respectively. Upper and lower confidence interval for K was 4.59 and 4.84 respectively.

Table 4: Mean difference of urea, creatinine, Na, and K

Test Value = 0						
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Total Urea	26.572	14	.000	44.60000	41.0001	48.1999
Creatinine	14.728	14	.000	1.38600	1.1842	1.5878
Na	203.353	14	.000	139.20000	137.7318	140.6682
K	81.727	14	.000	4.72200	4.5981	4.8459

Descriptive statistics were done of the treatment group at the significant variation of ($p > 0.05$) to determine the mean and standard deviation of urea, creatinine, Na, and K (Table 5).

Table 5: Descriptive statistics of the urea, creatinine, Na, and K

	N	Minimum	Maximum	Mean	Std. Deviation
Urea	15	38.00	55.00	44.6000	6.50055
Creatinine	15	.93	2.00	1.3860	.364472
Na	15	136.00	143.00	139.2000	.65115
K	15	4.40	5.01	4.7220	.22377

The (-.578*) value shows the association between urea and K, similarly the (.613*) indicates the correlation among the Na and K ions (Table 6).

Table 6: The correlation between the variables was determined by Pearson statistical analysis, at the significance level of 0.05

		Urea	Creatinine	Na	K
Urea	Pearson Correlation	1	-.211	-.492	-.578*
	Sig. (2-tailed)	-	.449	.062	.024
	N	15	15	15	15
Creatinine	Pearson Correlation	-.211	1	-.338	-.379
	Sig. (2-tailed)	.449	-	.217	.164
	N	15	15	15	15
Na	Pearson Correlation	-.492	-.338	1	.613*
	Sig. (2-tailed)	.062	.217	-	.015
	N	15	15	15	15
K	Pearson Correlation	-.578*	-.379	.613*	1
	Sig. (2-tailed)	.024	.164	.015	-
	N	15	15	15	15

*. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

This study was conducted to assess the toxicity of carbon tetrachloride (CCl_4) on albino rat kidneys. 30% diluted CCl_4 mixed with regular saline was fed to rats as food. The values of urea, creatinine, and the electrolytes sodium (Na) and potassium (K) were examined during this study. The findings demonstrated that the parameters in CCl_4-treated rats were considerably greater than those in control rats. When CCl_4 is consumed, the body's normal balance is disrupted, which results in kidney damage in albino rats. Similar findings were noted by Ogeturk *et al.*, Ozturk *et al.*, Simerville and Bhattacharya and Lun, (2005) [3, 16, 19, 20]. They looked at high levels of urea and creatinine in the urine as the key signs of renal damage brought on by CCl_4 treatment. In their experiment, they discovered that the serum creatinine level doesn't increase until at least half of the kidney nephrons had been compromised or lost. Both Adewole *et al.*, and Bhadauria *et al.*, noted comparable outcomes. They claim that the CCl_4-treated rats had significantly higher serum urea, creatinine, and BUN values, which is associated with decreased creatinine clearance [5, 21]. Similar findings and observations were made by Khan *et al.*, and Xu *et al.*, (2010) [11, 22]. They claimed that clinical chemistry data demonstrated that CCl_4 caused substantial increases in serum BUN and creatinine, which were consistent with published results and suggested potential renal damage. Eden *et al.*, noted comparable outcomes. They claimed that the elevated levels of renal creatinine caused by CCl_4 indicated that the chemical was responsible for several impairments of renal functions, including increased energy expenditure and reduced renal utilization of freshly synthesized creatinine [23]. Huxtable recorded comparable outcomes. He claimed that CCl_4 exposure significantly raised the levels of urine, electrolytes (Na & K), and creatinine in the rat kidney [24]. We saw higher amounts of each of these factors in this investigation. Adewole *et al.*, noted comparable outcomes. They claimed that chronic injection of CCl_4 resulted in considerable kidney oxidative stress and marked impairment of renal functioning [5]. With reduced creatinine and BUN clearance, serum creatinine and blood urea nitrogen (BUN) concentrations were considerably increased in CCl_4-treated rats. Melatonin (MEL) lowered the high levels of serum creatinine and BUN and greatly increased the clearance of creatinine and BUN. The capacity for tubular absorption may have been altered, leading to a functional overload of nephrons and associated renal dysfunctions, according to theory. Similar results were noted by Vengal Rao *et al.*, They claim that giving CCl_4 to normal rats caused kidney toxicity because it increased serum levels of creatinine and uric acid, which are indicators of the risk of impaired renal function and gout, respectively [25].

CONCLUSIONS

These findings underline the substantial health risks that CCl_4 poses and emphasize the necessity of putting preventative measures and safety regulations in place.

Authors Contribution

Conceptualization: MFB
Methodology: MKAK

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Formal analysis: MFB
Writing-review and editing: MFB, 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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