DOI: https://doi.org/10.54393/mjz.v4i01.62

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
Volume 4, Issue 1 (Jan-June 2023)

Original Article

Evaluation of Cardiac Profile in CCl4 Induced Toxicity in Albino Rats

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ARTICLE INFO

Key Words:
Cardiac Profile, CCl4, Toxicity, Albino Rats

How to cite:
Murtaza, S. ., & Ahmad Khan, M. K. . (2023). Evaluation of Cardiac Profile in CCl4 Induced Toxicity in Albino Rats: Cardiac Profiling in CCl4 Induced Toxicity in Albino Rats. MARKHOR (The Journal of Zoology), 4(01). https://doi.org/10.54393/mjz.v4i01.62

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Received Date: 8th April, 2023
Acceptance Date: 26th June, 2023
Published Date: 30th June, 2023

ABSTRACT

The use of consumer and fumigant products like CCl_4 has been phased down, leaving only industrial usage. It is extremely harmful to one's health. It's also one of the most toxic substances in vital organs like the lungs, kidneys, liver, heart, and brain. **Objective:** To evaluate the cardiac profile in CCl_4-induced toxicity in albino rats. **Methods:** The research was conducted at animal home of Department of Zoology, University of Okara. The targeted victims were albino rats. There were two types of groups created: control group and experimental group. The rats were fed 30 percent diluted carbon tetrachloride with normal saline as a control group to see if it had an adverse effect on their cardiac profile. A 12-day trek was used to accomplish this. After 12 days, samples were taken or dissected to assess serum Creatine Kinase (CPK), (CK-MB), and (LDH). The samples were analyzed by a machine called Micro-Lab 300 after they were taken by puncturing the Rats' hearts. **Results:** Abnormal increased level for Creatine Kinase (CPK)(199.20 ± 1.93) was observed after the administration of CCl4 compared to normal control (71.60 ± 4.04). When CCl_4 was injected, the level of CK-MB was abnormally high (34.00 ± 1.21) compared to the normal control (16.00± .84). The level of LDH increased abnormally (291.60 ± 5.01) when CCl_4 was given, compared to the normal control (250.20± 2.16). **Conclusions:** The levels of Creatine Kinase (CPK), CK-MB, and LDH were found to be greater than normal, showing that CCl_4 is hazardous to rats cardiac profile.

INTRODUCTION

The study of how harmful chemicals or physical factors affect living things is known as toxicology. Scientists who study the poisonous effects of substances and the cellular, biochemical, and molecular mechanisms behind those effects are known as toxicologists [1]. Chronic toxicity, or the production of adverse effects after long-term exposure to a contaminant or other stressor, is a crucial feature of aquatic toxicology [2]. Changes in development, reproduction, or actions are sources of adverse effects associated with chronic toxicity. Carbon tetrachloride (CCl_4) is a non-polar organic solvent that has a pleasant ether-like odour that can be perceived at low concentrations [3]. Carbon tetrachloride (CCl_4) is a well-known model compound for creating chemical tissue toxicity by generating free radicals in a variety of tissues. It is a transparent, colorless, volatile, thick, and nonflammable liquid [4]. Inhalation of its vapours, dermal absorption following direct skin touch, or ingestion are the most common causes of human toxicity; it may also be ingested intentionally as a suicidal agent. CCl_4 damages cells in a variety of tissues, mostly the liver, kidneys, and lungs [5]. In the liver, the enzyme cytochrome P_{450} catalyses that the conversion of CCl_4 to other toxic metabolites. The hepatic microsomal ethanol-oxidizing system (MEOS) contains this enzyme [6]. The liver, kidneys, and lungs are all affected in most cases of CCl_4 poisoning. Cardiovascular biomarkers have become crucial tools in cardiology during the past 50 years, functioning as primary and secondary prevention, acute myocardial infarction (AMI) diagnosis and treatment, and heart failure (HF) diagnosis and risk assessment. We are about to enter a time where using biomarkers to direct care will help us better manage our patients. After it was established that AST was released from ischemic cardiac myocytes, lactate dehydrogenase

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(LDH) became another potential biomarker for identifying myocardial ischemia [7]. The calcium-mediated interaction between actin and myosin is regulated by the cardiac regulatory proteins troponin T (cTnT) and troponin I (cTnI). Specific genes code for the cardiac versions of these regulatory proteins, which may be unique to the myocardium. Outside of the myocardium, no cTnI has been discovered [8]. There are three types of Creatine kinase, including CK-MB. The heart, muscles, and other organs all contain CK. The small intestine, brain, and uterus are among them. Injured heart muscle cells release CK-MB into the bloodstream during a heart attack [9]. CK-MB levels can also be high in the absence of acute myocardial infarction (AMI), and this is owing to increased amounts of B subunit synthesis in injured skeletal muscle, much as it occurs during fetal development [10, 11]. This study was carried out to evaluate these cardiac markers including LDH, CPK, CK-MB and the effect of CCL_4 on toxicity of these cardiac markers.

METHODS

In this study, male Albino Wistar adult rats weighing 180-200 g were used. The animals were bought from a local market and housed in the Department of Zoology at the University of Okara animal house. They were housed in normal conditions with 12-hour light/dark cycles at a plastic cage under controlled temperature of 25 ± 3 °C and a minimum relative humidity of 44-55%. Before and during the experiment, all of the rats were given free access to food, water and libitum. All of the animals' weights were checked twice a week. The doses were all administered in the morning (Table 1).

Table 1: Dose Schedule for Rats

Groups	Doses	Days	Amount
Group 1	Normal Saline	12 Days	1 ml
Group 2	30 % CCl4	12 Days	200mg/kg

Before the treatment, the rats were weighed. Before the experiment, the animals were housed in these facilities for at least one week. For this experiment, carbon tetrachloride CCl_4 was used. The measured chemical carbon tetrachloride CCl_4 was purchased locally and stored in the Department of Zoology's laboratory at the University of Okara. The chemical has been 30 % diluted. In distilled water, the stock solutions were produced. All other chemicals and solutions were of pro-analysis quality and collected from standard commercial sources. The rats were divided into two groups at random. A control group Co and an experimental group exposed to 30 % CCl_4 for two weeks via oral gavage. The control group consisted of four animals, while the experimental group consisted of six. The rats were placed in a desiccator with chloroform and a small incision was made in the abdominal wall with sharp scissors to anaesthetize them. The internal organs were then exposed by cutting the muscular coating on the sides. To keep the exposed organs of the animal from drying out, a 0.9% pyrogen-free sodium saline solution was poured on them. The dissections were carried out in sterile conditions, with tissues such as the heart, kidneys, liver, spleen, and intestine being removed. Normal feed was given to rats for 24 before dissection. Rats were euthanized with chloroform. Rats were weighted before dissection. Dissect the rats and by cardiac puncture blood samples were collected in vacutainers by using 23 G1 syringes. Liver and brain were dissected out, washed with ice cold saline to remove debris. Organs were weighted and store at -20 ◦C for tissue homogenate tests. The blood samples were centrifuged for 15 minutes at 4 C at 10,000rpm. The serum was isolated and stored at -20℃.

RESULTS

Abnormal increased level for Creatine Kinase (CPK) (199.20 ± 1.93) was observed after the administration of CCl4 compared to normal control (71.60 ± 4.04) indicating disruption in the normal Creatine Kinase (CPK) level. When CCl4 was injected, the level of CK-MB was abnormally high (34.00 ± 1.21) compared to the normal control (16.00± .84), showing that the normal CK-MB level had been disrupted. The level of LDH increased abnormally (291.60 ± 5.01) when CCl4 was given, compared to the normal control (250.20± 2.16) indicating a disturbance in the normal LDH level (Table 2).

Table 2: Statistical Analysis of Creatine Kinase (CPK), CKMB, and LDH Diseased Value with Normal Values

	Healthy (n=10)	Diseased (n=15)
Creatine Kinase (CPK)	71.6000 ± 4.04475	199.2000 ± 1.93760
CK-MB	16.0000 ± .84515	34.0000 ± 1.21890
LDH	250.2000 ± 2.16729	291.6000 ± 5.01882

The minimum, maximum, mean and standard deviation (n=15) of Creatine kinase, CKMB, and LDH were calculated statistically (Table 3).

Table 3: Descriptive Statistics of Cardiac Markers

	N	Minimum	Maximum	Mean	Std. Deviation
Creatine kinase	15	189.00	211.00	199.2000	7.50428
CK-MB	15	28.00	41.00	34.0000	4.72077
LDH	15	268.00	315.00	291.6000	19.43781

A one-sample t-test was used to calculate the confidence interval for mean differences in Creatine kinase, CK-MB, and LDH (Table 4).

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Table 4: Results of the One-Sample Test Show the Interval Difference of Creatine-kinase, CK-MB, And LDH

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Creatine kinase	102.808	14	.000	199.20000	195.0443	203.3557
CK-MB	27.894	14	.000	34.00000	31.3857	36.6143
LDH	58.101	14	.000	291.60000	280.8357	302.3643

Creatine kinase shows a correlation between LDH; however, CK-MB only indicates a correlation between Creatine kinase. LDH shows no correlation between Creatine kinase, CK-MB, and LDH (Table 5).

Table 5: Correlations analysis among the Creatine kinase, CK-MB, and LDH

Correlations				
		Creatine kinase	CK-MB	LDH
Creatine kinase	Pearson Correlation	1	.327	.604*
	Sig. (2-tailed)	-	.235	.017
	N	15	15	15
CKMB	Pearson Correlation	.327	1	.325
	Sig. (2-tailed)	.235	-	.238
	N	15	15	15
LDH	Pearson Correlation	.604*	.325	1
	Sig. (2-tailed)	.017	.238	-
	N	15	15	15

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

DISCUSSION

The goal of this study was to see if CCl_4 has any negative impact on the Cardiac profile of Albino Rats. Rats were given 30 percent diluted CCl_4 to eat. Throughout the study, total Creatine Kinase (CPK), CK-MB, and LDH were all monitored. According to this finding, the parameters were considerably higher in the CCl_4 treated rats than in the control rats. CCl_4 ingestion causes toxicity in Albino Rats by disrupting the normal Cardiac levels. Similar result and observation were obtained by Ohta *et al.*, 1997; Jayakumara 2008 and Botsoglou *et al.*, 2009 [12-14]. According to them that injection of CCl_4 to rats caused an increase in lactate dehydrogenase (LDH) and creatine kinase (CK), resulting in oxidative cardiac tissue damage. Similar result and observation were obtained by Kurian *et al.*, and Prabhu *et al.*, [15, 16]. According to them the cardiac profile, as well as serum levels of Creatine Kinase (CPK), CK-MB, and LDH, exhibited significant increases in CCl_4-treated rats. Excessive release of CK and CK-MB in the serum of rats was caused by CCl_4 poisoning, and this result aligns with the widely published study that Dox-induced free radical generation triggers cardiac myocyte rupture and membrane peroxidation, which increased serum CK-MB levels. Similar observation and results were obtained by Shahzad *et al.*, 2019 [17]. according to his results the cardiac profile as well as serum levels of CK-MB, LDH, were abnormal due to CCl_4 toxicity. As a result, an abnormal cardiac profile was to be observed. Similar observation and results were obtained by Chrostek and Szmitkowski, 1989, according to the findings; CCl_4 toxicity causes an increase in cardiac profile as well as serum levels of creatine kinase (CPK) and lactate dehydrogenase (LDH) [18]. Similar observation and results were obtained by Chen *et al.*, 2011; according to these results that injury to the heart causes an increase in cardiac profile as well as serum levels of lactate dehydrogenase LDH and CK-MB to be discharged into the bloodstream [19]. Similar observation and results were obtained by Elberry *et al.*, 2010, according to the findings, demonstrated an increase in serum levels of CK, CK-MB, and LDH, which are important cardiac enzymes for the assessment of cardiotoxicity and congestive heart failure [20]. Similar observation and results were obtained by Rajadurai and Prince, 2010, according to these results the cardiac marker enzymes like creatine kinase-MB (CK-MB), creatine kinase (CK), and lactate dehydrogenase (LDH) increased in serum levels and are released into the blood stream due to damage to the heart [21]. Similar observation and results were obtained by PacÃ *et al.*, 2015, According to these results the increase in the concentration of enzymes CK-MB and LDH are released into the blood stream due to damage to the heart [22]. Similar results were obtained by Mansour and Hasan, 1966, the quantity of Cardiac profile increase in serum levels of creatine kinase (CK), lactate dehydrogenase (LDH) enzymes are released into the blood stream due to heart injury [23]. Similar results were obtained by Al-Shabanah *et al.*, 1966 and Chopra *et al.*, 1995. The enzyme increases CPK and LDH leak from necrotic heart cells to serum levels as a result of the damage, which are essential indicators of cardiac injury [24, 25]. Similar observation and results were obtained by Nemmar *et al.*, 2015, according to these findings; blood levels of CK-MB and LDH were elevated, indicating early and late cardiac damage [26]. Similar observation and results were obtained by Cavas and Tarhan and Karras and Kane, according to these findings LDH and CK-MB tissue levels were observed with increase in the current investigation [27, 28]. Similar observation and results were obtained by

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Potluri *et al.*, 2004; this hypothesis was supported by the current study's findings of increased serum CK-MB and LDH levels [29]. CK-MB is a marker for heart failure in theory. The release of this marker appears to be triggered by ventricular remodeling, continuing myocyte degeneration, coronary artery disease, and a reduction in coronary reserve. Similar observation and results were obtained by Zarei and Shivanandappa 2013; according to their findings increased serum levels of CK-MB and LDH were seen [31]. Similar observation and results were obtained Budas *et al.*, 2019, according these results the cardiac profile as well as serum levels of CK-MB, LDH, are aberrant due to CCl4 toxicity. As a result, there will be an aberrant cardiac profile [31]. A one-sample t-test was used to calculate the confidence interval for mean differences in Creatine kinase, CK-MB, and LDH (Table 4).

CONCLUSIONS

CCl4 is a harmful substance that has a negative effect on the lipid profile. CCl4 exposure disturbed normal physiology in this study, resulting in aberrant Creatine Kinase (CPK), CK-MB, and LDH levels in the treated group's blood. Between the control and treatment groups, significant differences in hematological parameters were discovered. The levels of Creatine Kinase (CPK), CK-MB, and LDH were found to be greater than normal, showing that CCl4 is hazardous to rats cardiac profile.

Authors Contribution

Conceptualization: SM
Methodology: MKAK
Formal analysis: SM
Writing-review and editing: SM, MKAK

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

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

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