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IDEAL HEPATOTOXICITY MODEL IN RATS USING CARBON TETRACHLORIDE (CCL_4)

[1]A. J. Alhassan, [1]M .S. Sule, [1]S. A. Aliyu and [2]M. D. Aliyu
[1]Department of Biochemistry, Bayero University, P M.B. 3011, Kano, Nigeria.
[2]Jigawa Research Institute, Kazaure, P. M. B. 5015, Kazaure, jigawa, Nigeria.
Correspondence author: ajalhassan@yahoo.com

ABSTRACT
A study to produce ideal Hepatotoxicity rats' models using varying concentrations of carbon tetrachloride (CCl_4) was carried out. A total of seventy five rats were divided into five (5) groups of twenty five (25) rats each; rats in group I are negative control, were not induced with lipid peroxidation. Rats in groups II, III, IV and V were induced with lipid peroxidation and liver damage using, 90, 105 and 120mg/Kg body weight CCl_4 respectively. Five (5) rats were removed from each group after 48 hours, 72 hours (3 days) and 144 hours (6 days) of inducement with CCl_4 and sacrificed for blood sample collection. Sera obtained were analyzed for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) activities, and for Malondialdehyde (MDA), total protein (TP) and albumin (ALB) concentrations. After 48hours, rats treated with various concentrations of CCl_4 had mean serum activities/ levels of AST, ALT, ALP, MDA, TP and ALB significantly higher ($p< 0.05$) than normal rats. The control group had 39.5±0.71, 11.5±2.12, 36±7.07U/L, 0.0769 ± 0.015 µM, 43.5±3.54, 19 .00 ± 2.83 g/L respectively. Rats treated with 75mg of CCl_4 showed activities/level non significant ($p>0.05$) increase of serum AST, ALT, ALP, and MDA after 96 hours of CCl_4 treatment compared with control group. However, rats treated with 90, 105 and 120mg/kg of CCl_4 showed persistently high serum AST, ALT, ALP in U/L, MDA in µM/L, TP and ALB in g/L. even at 144 hours after the treatment. Ideal hepatotoxicity rat model using CCl_4 requires a dosage that can cause massive liver damage.

Key Words: carbon tetrachloride, hepatotoxicity rats modeling, natural healing.

INTRODUCTION

A model organism is a species that is extensively studied to understand a particular biological phenomena, with the expectation that discoveries made in the organism model will provide insight into the workings of other organisms (Stanley and Mark, 2005). In particular, model organisms are widely used to explore potential causes and treatments for human disease when human experimentation would be unfeasible or unethical. This strategy is made possible by the common descent of all living organisms, and the conservation of metabolic and developmental pathways and genetic material over the course of evolution (Fox, 1986). Model organisms are often chosen on the basis that they are amenable to experimental manipulation. This usually will include characteristics such as short life-cycle, techniques for genetic manipulation (inbred strains, stem cell lines, and methods of transformation) and non-specialist living requirements. For example the rat is particularly useful as a toxicology model. Studying model organisms can be informative, but care must be taken when generalizing from one organism to another.

Biological materials, particularly membranes, contain high concentrations of unsaturated lipids. In the presence of a free radical initiator and oxygen they may be oxidized. This process, known as lipid peroxidation, has been implicated as a general biological degenerative reaction, and may be an important *in vivo* process (Pryor, 1973; Meyers, 1973; Milvy, 1973; Pla and Witschi, 1976; Tappel, 1972). CCl_4 induces lipid peroxidation and liver damage (Robbins and Cotran, 2006). Various concentrations of CCl_4 could be used to cause various degree of liver damage, thereby generating an ideal hepatotoxicity model organism. This study intends to define concentration(s) of CCl_4 which can cause massive liver damage and delay natural healing. An earlier Study had shown that using certain concentration of CCl_4 to induce liver damage but natural healing started at three days of the liver damage inducement (Shehu, 2008).

MATERIALS AND METHOD

EXPERIMENTAL DESIGN

Experimental Animals

Albino rats weighing 105 – 120 were obtained from Pharmacology Department, ABU Zaria and kept in the animal house of Physiology Department, Bayero University Kano, Nigeria. The rats were kept under the same atmospheric conditions and fed with green leaves, carrots and water for about two weeks to acclimatize. An initial study was conducted to determine the dose range of the concentration of CCl_4 to be used for the study. Seventy five (75) rats were obtained and divided into five (5) groups of twenty five (25) rats each. The rats in group I are negative control, they were not induced with lipid peroxidation and liver damage.

Rats in groups II, III, IV and V were induced with lipid peroxidation and liver damage using 75, 90, 105 and 120mg/Kg body weight of CCl_4 respectively administered intramuscularly. Five (5) rats were removed from each group after 48, 72 and 144 hours of inducement respectively and sacrificed by decapitation.

Biochemical Analysis

Blood sample was collected inside centrifugation tubes and allowed to clot for five to ten minutes at room temperature. The sample was later centrifuged to obtain sera. Sera samples were tested for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities by the method of Reitman and Frankel (1957), serum alkaline phosphatase activity by the method of Rec (1972), serum Malondialdehyde (MDA) concentration by the method of Hunter *et al.,* (1963) modified by Gutteridge and Wilkins (1982) and serum total protein and albumin assessed by Biuret and BCG methods respectively.

Statistical Analysis

Results were statistically analyzed using t – test SPSS software developed by Microsoft Inc. p value of 0.05 was considered as significant level.

RESULTS AND DISCUSSION

Tables 1, 2 and 3 present the serum activities/levels of ALT, AST, ALP, MDA, TP and ALB for groups of rats administered with 75, 90, 105 and 120 mg/Kg body weight of CCl_4 after 48, 72 and 144hours respectively.

ALT and AST are non plasma specific enzymes requiring pyridoxal phosphate as coenzyme for deamination of aspartic acid and alanine respectively, mostly in the liver (Price and Stevens, 2003; Vasudevan and Sreekumari, 2005). Very high values of ALT are seen in acute hepatitis, either toxic or viral in origin. Both ALT and AST levels are increased in liver diseases with ALT>> AST (Vasudevan and Sreekumari, 2005), this observation confirms the result of this study (Table I). ALP is a non specific enzyme which hydrolyses aliphatic, aromatic or hetrocyclic phosphate compounds. ALP is produced by osteoblast of bone, intestinal mucosa. Mild increase in ALP was reported in children because of the increased osteoblastic activity and in pregnant women due to placental isoenzyme. Moderate increase in ALP level is seen in hepatic diseases such as infective hepatitis, alcoholic hepatitis or hepato cellular carcinoma. Very high activity of serum ALP than normal may be noticed in exterahepatic obstruction or cholestasis. ALP is also produced by epithelial cells of billiary canaliculi and obstruction of bile with consequent irritation of epithelial cells leads to secretion of ALP into serum. This observation also agrees with the increase in ALP in this study (Table 1).

Serum albumin is quantitatively the most important protein synthesized by the liver, and reflects the extent of functioning liver cell mass. Since albumin has a fairly long half life of 20 days, it is not a good indicator of acute liver diseases (Vasudevan and Sreekumari, 2005), result of this study confirms this observation (Table 1). However, values of albumin (ALB) and the total protein (TP) provide information to rule out any case(s) of chronic and infectious hepatitis among the group of experimental rats used.

Malondialdehyde (MDA) is a degradative product of peroxidation of polyunsaturated fatty acids (PUFA) in cell membrane. Presence of MDA in serum is often an indicator of oxidative stress. Detection of higher levels of MDA in the serum of CCl_4 treated rats than in control rats confirms the report that CCl_4 induces lipid peroxidation and liver damage (Robbins and Cotran, 2006), and the level of the MDA generated increases with increase in CCl_4 dosage as indicated in Tables I, II, and III. This signifies that the degree of lipid peroxidation and liver damage increases with increase in CCl_4. The rats intramuscularly injected with 75, 90, 105 and 120 mg/Kg body weight of CCl_4 in 48hours had mean serum AST, ALT ALP, MDA, TP and ALB levels significantly higher ($p<0.05$) than those in control rats and the increase in these parameters was shown to be proportional to concentration of CCl_4 administered (Table I). This therefore, confirms the statement that various concentrations of CCl_4 can cause various degrees of liver damage.

The rats treated with 75mg/Kg CCl4 had mean serum activity of AST, ALT, and ALP and concentration of MDA not significantly higher ($p>0.05$) than those in control rats after 72hours (Table 2). This could be due to natural healing that possibly occurred in 72hours after 75mg/Kg CCl_4 treatment and it confirms the fact that liver cells are capable of regenerating naturally (), especially when the toxic agent is eliminated. The trend was maintained in 144hours in which rats treated with 90, 105 and 120 mg/Kg had mean serum activity of AST ALT, ALP MDA, TP and ALB significantly higher ($p<0.05$) than those in control rats (tables 2 and 3). This indicates that using high dosage of CCl_4 give rise to an ideal hepatotoxicity model rats and which may persist for longer time.

Table 1: Serum levels of biochemical parameters in rats after 48 hrs. of intramuscular administration of CCl_4.

Group/treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	MDA (µM)	TP (g/L)	ALB (g/L)
Group I Negative control	$39.5\pm0.71^{a,b,c,d}$	$11.5\pm2.12^{e,f,g,h}$	$36\pm7.07^{i,j}$	$0.0769\pm0.015^{k,l,m,n}$	$43.5\pm3.54^{o,p,q,r}$	$19\pm2.83^{s,t,u,v}$
Group II (75mg/kg CCl_4)	51 ± 0.00^{a}	71.5 ± 6.36^{e}	45 ± 1.41	0.1413 ± 0.046^{k}	66 ± 0.00^{o}	29 ± 0.00^{s}
Group III (90mg/kg CCl_4)	62.5 ± 0.71^{b}	76 ± 0.00^{f}	52 ± 0.00	0.2735 ± 0.916^{l}	75.5 ± 2.12^{p}	36.5 ± 0.71^{t}
Group IV (105mg/kg CCl_4)	73 ± 0.00^{c}	102 ± 4.24^{g}	62.5 ± 0.71^{i}	0.3587 ± 0.046^{m}	78.5 ± 0.71^{q}	34 ± 1.41^{u}
Group V (120mg/kg CCl_4)	145 ± 7.07^{d}	116 ± 0.00^{h}	95 ± 2.83^{j}	0.4239 ± 0.015^{n}	87 ± 1.41^{r}	37 ± 0.00^{v}

Values are mean ± standard deviation, Similar superscripts are significant at $P< 0.05$. n = 5 for each group.

Table 2: Serum levels of biochemical parameters in rats after 72 hours of intramuscular administration of CCl_4.

Group/treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	MDA (µM)	TP (g/L)	ALB (g/L)
Group I negative control	36±1.41^{a,b,c}	8.5±2.12^{d,e,f}	34±1.41^{g,h,i}	0.0869±0.00j^{j,k}	43.5±3.54^{l,m,n,o}	19.5±2.12^{p,q,r,s}
Group II (75mg/kg CCl_4)	36±2.83	13±0.00	34.5±0.71	0.0870±0.031	55.5±0.71^{l}	26±4.24^{p}
Group III (90mg/kg CCl_4)	48±0.00^{a}	63±5.66^{d}	40±0.00^{g}	0.0899±0.088	75.5±2.12^{m}	34±4.24^{q}
Group IV (105mg/kg CCl_4)	54.5±2.12^{b}	76±0.00^{e}	46.5±0.71^{h}	0.1739±0.00^{j}	77.5±0.71^{n}	37±1.41^{r}
Group V (120mg/kg CCl_4)	99±1.41^{c}	97±2.83^{f}	98±0.00^{i}	0.2500±0.015^{k}	80±0.00^{o}	34.5±3.54^{s}

Values are mean ± standard deviation, Similar superscripts are significant at P< 0.05. n = 5 for each group.

Table 3: **Serum levels of biochemical parameters in rats after 144 hrs. of intramuscular administration of CCl_4.**

Group/treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	MDA (µM)	TP (g/L)	ALB (g/L)
Group I negative control	38.5±2.12^{a,b,c}	13±0.00^{d,e,f}	34±1.41^{g,h}	0.1304±0.062	47±1.41^{i,j,k,l}	24.5±3.54^{m}
Group II (75mg/kg CCl_4)	38.5±0.71	11.5±2.12	33.5±0.71	0.1631±0.015	63±4.24^{i}	26.5±4.95
Group III (90mg/kg CCl_4)	46±0.00^{a}	29±0.00^{d}	38±2.83	0.1848±0.015	68±2.83^{j}	31±2.83
Group IV (105mg/kg CCl_4)	52±2.83^{b}	27±0.71^{e}	43±0.00^{g}	0.1739±0.00	72±2.83^{k}	34±4.24
Group V (120mg/kg CCl_4)	77±1.41^{c}	52±2.83^{f}	68.5±2.12^{h}	0.1848±0.015	77.5±0.85^{l}	36±0.00^{m}

Values are mean ± standard deviation, Similar superscripts are significant at P< 0.05. n = 5 for each group.

Conclusion

Based on the result of this study it can be concluded that using low doses of CCl_4, natural healing of the liver occurs 72hours after administration. However, high doses of the CCl_4 (90 – 120 mg/Kg) can induce massive liver damage and may persist for longer period compared to lower dosage, hence giving rise to an ideal hepatotoxicity rats model.

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