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EFFECT OF VARYING DOSES OF CCL_4 ON SERUM ASPARTATE AMINOTRASNFERASE (AST), ALANINE AMINOTRASNFERASE (ALT), ALKALINE PHOSPHATASE (ALP) ACTIVITIES, SERUM TOTAL PROTEIN AND ALBUMIN LEVELS IN RATS

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ABSTRACT

The effect of varying doses of carbon tetrachloride (CCL_4) on serum AST, ALT, and ALP was investigated. Serum total protein and albumin levels were also measured. Different doses (0.03 mg/kg, 0.06 mg/kg, 0.09mg/kg and 0.12 mg/kg were injected into some rats intraperitoneally for 21 days. The blood of the rats was collected and through standard methods the level of activity of AST, ALT and ALP, serum total protein and albumin were measured.

No deaths occurred at the dose of 0.03 mg/kg CCL_4 Fifty percent mortality rate was recorded at the dose of 0.06 mg/kg, 75% at the dose of 0.09 mg/kg and 100% at dose of 0.12 mg/kg. The 21-day cumulative study showed a significant increase in activities of AST, ALT and ALP ($p \leq 0.001$) in the serum of rats injected with the different doses of CCl_4. The mean values in serum were: for AST, 59.52±0.03, without CCl_4 (control) and 107.67 ± 0.87, 154.10 ± 0.08, 186. 12 ± 0.83, 255.02 ± 0.62 with CCl_4 at 0.03, 0.06, 0.09 and 0.12 mg/kg respectively; for ALT, 54.00 ± 0.15 without CCl_4 (control) and 98.37 ± 0.21, 109. 87 ±0.19, 129.40 ± 0.37, 185.51 ± 3.24 with CCl_4 at 0.03,0.06, 0.09 and 0.12 mg/kg respectively; for ALP, 54.90 ± 0.20 without CCl_4 (control) and 65.20 ± 0.09, 85.34 ± 0.53, 95.90 ± 2.05, 177.15 ± 1.24 with CCl_4 at 0.03, 0.06, 0.09 and 1.2 mg/kg respectively. The increase in activities of AST, ALT and ALP was dose-dependent.

The activities of AST, ALT and ALP were used as markers of hepatotoxicity. The difference in serum total protein albumin levels were not significant ($p \leq 0.001$). It appears from the results of this study that CCl_4 affects the liver by inducing damage to liver cells and so can act as a liver poison.

INTRODUCTION

Carbon tetrachloride (CCl_4) is a clear oily liquid at room temperature. It has an aromatic sweet odour. Humans absorb CCl4 after both oral and inhalation exposure as demonstrated by accounts of poisoning (U. S. EPA, 1989; Seawright and Mclean, 1967). A distribution following oral exposure in rats showed a higher concentration in the liver than in the brain ATSDR, 1989).

No information is available on the metabolism of CCl_4 in humans. However, animals studies have revealed that the molecule is metabolized in the liver by cytochrome p-450 (Sipes et al, 1977). The liver is rich in enzymes such as the amino transferases. Destruction or damage of the liver cells releases the enzymes with consequent rise in their measured values in blood. The aminotransferases are useful in the screening test for detecting liver injury.

Although there is a lot of information on the sensitivity of humans and some experimental animals to CCl_4 by oral inhalation and dermal routes, there is limited available literature documented on the sensitivity of the experimental animals by intraperitoneal route (Sax and Lewis, 1989). This is therefore a study of the in vivo effect of varying doses of CCl_4 on rats through intraperitoneal administration on serum AST, ALT and ALP activities as indices of hepatotoxicity. Serum total protein and albumin levels were also measured. The study is aimed at determining the effect of CCl_4 administration intraperitoneally in rats.

MATERIALS AND METHODS

Sources of animals

Albino rats of about four (4) months of age and weighing about 185± 25g., were obtained from the laboratory animal house of Animal and Environmental Biology and Biochemistry Departments of the university of Port Harcourt. The rats were kept under laboratory conditions in cages before and during the experiments. They were fed with pellet feed (Livestock Feeds, Nig. Ltd., Ikeja, Lagos) and water ad libitum.

Blood collection

Blood was quickly collected from the rats, following decapitation, by cardiac puncture into sample tubes. The blood was allowed to clot and then centrifuged at 3,000g for 10 minutes to obtain the serum. The serum was centrifuged at 3,000g for 10 minutes and the supernatant obtained was stored for analysis.

Toxicity test

Twenty (20) male albino rats were divided into five (5) groups of four (4) rats each. Four groups (2-5) were administered different doses. (0.03 mg/kg, 0.06 mg/kg, 0.09 mg/kg and 0.12 mg/kg) of CCl_4 intraperitoneally. The first group (Group 1) received distilled water and served as the control. The animals were observed continuously for six hours for fatality.

For the twenty-one (21) day toxicity study of CCl_4, forty-eight (48) rats, divided into four (4) groups of twelve rats (12) each, were treated intraperitoneally with CCl_4 mixed with mineral oil (3:1) at doses of 0.03 mg/kg, 0.06mg/kg, 0.09mg/kg and 0.12 mg/kg, respectively. This was done daily for a period of seven (7) days. Another group consisting of seven (7) rats served as control. The animals were observed for fatality after each dose for seven (7) days. They were then kept for another fourteen (14) days for daily observation of cumulative toxicity. During this time, they were fed with water and their usual food. At the end of the 21 days, three (3) rats from each group were sacrificed by decapitation and the test samples collected and prepared for enzyme assays.

Enzyme assays

In vivo effect of different doses of CCl_4 on serum AST, ALT and ALP activities were determined on three rats from each group at varied doses of CCL_4 (0.03mg/kg, 0.06mg/kg, 0.09mg/kg, 0.12mg/kg in the serum.

A pack of thirty (30) strips was used. The foil protecting the

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test area was removed while care was taken not to over-bend the strip. Using the Reflotron pipette, the sample was drawn up avoiding the formation of bubbles. The sample was applied as a drop to the center of the red application zone (indicated) without allowing the pipette tip to touch the zone. The flap was opened after 15 second and the strip placed on the guide and inserted horizontally into the instrument until a click was heard. The flap was then closed.

The display "AST", "ALT" or "ALP" confirmed that the test-specific magnetic code has been correctly read into the instrument. Enzyme activity was measured at 567 nm and the result displayed in IU/litre at 37^{0}C.

Serum total protein determination

Serum protein was determined by the method of Weichselbaum (1946). 0.1ml serum was pipetted into a test tube and 0.5 ml Biuret reagent added. The solution was mixed and incubated for (30) minutes at 25^{0}C and absorbance taken at 546 nm against a reagent blank.

Concentration of total protein in the sample was calculated using the formula:

Concentration (C) g/l=190x Absorbance of sample.

Serum albumin determination

Serum albumin concentration was determined by the method of Doumas et al (1971). Serum albumin binds with Bromocresol green at pH 4.2 to form a coloured compound. The blue colour formed is directly proportional to the amount of albumin present. Into different test tubes was pipetted 5µl of sample, 5µl standard and water. 2.5 ml reagent was then added to the different test tubes. The contents of the tubes were then mixed and stood for 3 minutes. Absorbance was measured against the reagent blank within 60 minutes at 630 nm at room temperature.

RESULTS

The result of the acute toxicity test is shown in Table 1. No deaths occurred at the dose of 0.03mg/kg CCl_4. Fifty percent (50%) mortality rate was recorded at the dose of 0.06mg/kg, 75% at dose of 0.09 mg/kg 100% at dose of 0.12 mg/kg of CCl_4.The deaths occurred within 6 hours of administration.

Table 1: Acute Toxicity Test

Carbon tetrachloride	Number of rats (avg. wt. 185± 25g)	Mortality rate after 6 hrs	% Mortality
0.03	4	0	0.00
0.06	4	2	50.00
0.09	4	3	75.00
0.12	4	4	100.00

The results for the 21-day toxicity study for rats given different doses of CCl_4 intraperitoneally are shown in Table 2. The exposure of the male albino rats to 0.03 mg/kg of CCl_4 by daily intraperitoneal administration caused no death. The only observed effect was a marked general weakness within the first seven days. Body weight was not significantly affected (data not shown). Marked increases were observed in the mean (±SD) activities of the enzymes, AST, ALT, ALP, both in the serum and the liver homogenate of rats treated with different doses of CCl_4 when compared with the control. AST, ALT and ALP activities were significantly higher ($p \leq 0.001$) in the liver than in the serum. A statistical comparison of the mean AST, ALT and ALP activities for all the groups with the control showed a significant increase in the enzyme activities ($p \geq 0.001$). The enzyme activities increased as the CCl_4 dose increased in the order 0.12 mg/kg >0.09 mg/kg >0.06 mg/kg >0.03mg/kg.

There was no significant difference in mean serum total protein and albumin concentration ($p \geq 0.001$).

Table 2: Comparative 21-day toxicity study (ALT, AST, ALP, Protein and Albumin Levels) for rats given different doses of CCl_4 intraperitoneally

Sample	N	TP (g/dl)	ALB (g/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group 1						
(distilled water) control						
Serum	3	6.65±0.10	2.9± 0.04	59.52± 0.30	54.00± 0.15	54.90± 0.20
Liver	3			89.63± o.58	78.40± 0.30	130.00±0.15
Group2						
(0.03mgCCl_4/kg)						
Serum	3	6.80±0.06	2.71±0.05	107.67±0.87	98.37±0.21	65.20±0.09
Liver	3			201.20±0.17	127.20±0.57	142.06±0.34
Group 3						
(0.06mgCCl_4/kg)						
Serum	3	5.87±0.08	2.98±0.001	154.10±0.08	109.87±0.19	85.34±0.53
Liver	3			286.60ss±0.17	177.82±0.82	185.23±0.23
Group 4						
(0.09mg CCl_4/kg)						
Serum	3	7.28±0.05	3.40±0.14	186.12±0.03	129.40±0.37	95.90±2.05
Liver	3			318.82±2.93	198.41±1.45	259.20±0.75
Group 5						
(0.12mgCCl_4/kg)						
Serum	3	7.39±0.32	4.64±0.43	255.02±0.62	185.51±3.24	177.15±124
Liver	3			413.28±2.06	236.01±0.79	305.03±3.41

TP (Total protein); ALB (Albumin g/dl); AST (Aspartate amino transferase, IU/l); ALT (Alanine transferase, IU/l); ALP (Alkaline phoshatase, IU/l); IU/l (international unit per litre); n (size of variables, in this case number of rats)

DISCUSSION

Carbon tetrachloride (CCl administered intraperitoneally affected the rats adversely as shown by death and also the level of activities of the liver enzymes (AST, ALT and ALP). There is a lot information on the sensitivity of humans and some experimental animals to CCl_4 intoxication by oral, inhalation and dermal routes. We are reporting also toxicity or intoxication by intraperitoneally route,. This suggests that the intoxication of CCl_4 is independent of the route of administration. At the doses of 0.06 mg/kg CCl_4, 50% mortality was recorded while at the dose of 0.03 mg/kg there was no death. At higher doses of 0.09 and 0.12 mg/kg, 75% and 100% deaths, respectively, were recorded. This indicates that the effect of CCl_4 on the rats was concentration-dependent. An oral LD_{50} of 2.80 mg/kg and dermal LD_{50}.5.070 mg/kg have been reported by Sax and Lewis (1989).

Marked increases in the activities of the enzyme AST, ALT and ALP in both the serum and liver homogenates were observed. These increases are attributable to CCl_4. Similar elevated levels of serum liver enzymes through dermal exposure were recorded by Perez et al (1987), Barnes and Joues (1967) and Smyth et al (1936).

ATSDR (1989) also reported increased hepatic enzymes activities indicative of liver damage by oral inhalation of CCl_4. All the animals given CCl_4 exhibited signs of hepatotoxicity as measured by increased serum/liver homogenate enzymes activities. The increase in the activities was dose-dependent and was noted to be higher In the liver (Table 2). This agrees with the findings of Bruckner et al (1986) that liver cirrhosis was observed when a high dose (10-33) mg CCl_4 administered to Sprague-Dawley rats in corn oil.

The differences in mean AST, ALT and ALP activities at CCl_4 doses (0.03, 0.06, 0.09 and 0.12 mg/kg) when compared with the control were statistically significant ($p \leq 0.001$). Since CCl_4 is insoluble in water and volatile, the use of mineral oil in this work provided an effective and innocuous vehicle for CCl_4. Previous work by Bruckner et al (1986) showed that corn oil used as solvent/vehicle increased the toxicity of CCl_4.

The differences in the mean total protein and albumin levels for rats treated with the different doses of CCl_4 were not significantly different ($p \leq 0.001$) when compared with the control. This may suggest that changes in the serum protein and albumin concentrations lack sensitivity and specificity for the detection of liver disease. The observed variations on serum protein and albumin levels agree with the findings of Rothschild et al (1988).

In conclusion, it can be reported that treatment of rats with CCl_4 resulted in increased hepatic enzyme activities indicative of liver damage. Changes in serum protein and albumin measurements lack sensitivity and specificity for the detection of liver disease. CCl_4 is a hepatotoxin.

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