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HEPATOTOXIC EFFECTS OF CARBON TETRATCLORIDE ON ADULT WISTAR RATS

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ABSTRACT

This work aimed at demonstrating the histopathological effects of carbon tetrachloride (CCL_4) on the liver cells of adult wistar rats following oral administration. Twenty adult wistar rats of weights 190-220kg were used. They were designated into four groups (A, B, C, D) of five animas each. Group A served as the control and received 0.5ml of distilled water. The experimental groups B,C and D were orally administered with 0.1ml, 0.2ml and 0.3ml of carbon tetracholoride respectively for a period of 21 days. Twenty four hours after the last administration, the animals were weighed and trimmed down to a size of 3mm x 3mm and fixed in zenkers fluid for histological studies. The final body weight of the experimental groups were significantly lower ($P<0.001$) than the control. Histological results showed distortions of liver architecture in the experimental groups compared with the control. The present study indicated dose-dependent thus our findings therefore suggest that chronic oral consumption of carbon tetrachloride may put the liver at risks of adverse histopathological conditions.

INTRODUCTION

Carbon tetrachloride is a manufactured chemical that does not occur naturally. It is a clear liquid with a sweet smell that can be detected at low levels [1]. Carbon tetrachloride is ozone-depleting [2] and a greenhouse gas [3]. Exposure to high concentrations of carbon tetrachloride can affect the central nervous system, degenerate the liver [4] and kidneys [5] and may result (after prolonged exposure) in coma and even death [6] Chronic exposure to carbon tetrachloride can cause liver [7,8] and kidney damage and could result in cancer [9]. The California environmental protection Agency (CalEPD) has established a chronic reference exposure level of 0.04 milligram per cubic meter (mg/m^3) for carbon tetrachloride based on the effects in guinea pigs. The CalEPA reference exposure level is a concentration at or below which adverse health effects are not likely to occur. It is not a direct estimator of risk, but rather a reference point to gauge the potential effects. At lifetime exposures increasingly greater than the reference exposure level, the potential for adverse health effects increases[10]. Acute animal tests in rats, mice, rabbits and guinea pigs have demonstrated carbon tetrachloride to have low toxicity from inhalation exposure, low to moderate toxicity from ingestion and moderate toxicity from dermal exposure [11]. Like many other volatile substances, carbon tetrachloride is prone to misuse by inhalation or oral consumption due to its possible depressant effect upon the central nervous system. These facts call for more studies to demonstrate the hepatotoxic effects of carbon tetrachloride. Hence, this study is aimed at investigating the histopathologic changes in the liver tissues of rats following exposure to different doses of carbon tetrachloride.

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MATERIALS AND METHOD

Twenty adult wistar rats weighing 190-22kg were used. They were allowed for a period of seven days for acclimatization under normal temperature (27^{oC} – 30^{oC}) before their weights were taken. They were fed ad libitum with water and guinea feed pallets from Agro feed mill Nigeria ltd.

The animals were allocated into four groups of five animals each. The groups were designated as group A, B, C and D. Group A served as the control and received 0.5ml of distilled water. The experimental groups B, C and D received 0.1ml, 0.2ml and 0.3ml of carbon tetrachloride respectively. The drug was administered once in a day between the hours of 2-3pm for a period of twenty one days.

The drugs were administered orally using intubations method. After the twenty first day, the animal was weighed and their weight recorded. Twenty four hours after the last administration the animals were anaesthetized under chloroform vapour and were dissected. Liver tissues were removed from the animals and weighed. They were trimmed down to a size of 3mm x 3mm thick and fixed in zenkers fluid for four 4 hours for histological studies.

TISSUE PROCESSING

For easy study of sections under microscope, the tissues passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in zenkers fluid. The tissues remained in the fluid for four (4) hours. After fixation the tissues were washed overnight under a stream tap water. Dehydration of the fixed tissues was carried out in different percentages of alcohol 50%, 70%, and 90% absolute. After dehydration, tissues were cleared in zylenefor two (2) hours after which infiltration was due in molten paraffin wax at a temperature of 60^{oC} for two (2) hours each in two changes and then sectioned. I made use of hematoxylin and eosin method for histological studies.

RESULT

MORPHOMETRIC ANALYSIS OF BODY WEIGHT

MORPHOMETRIC ANALYSIS OF LIVER WEIGHTS

The relative liver weight for the experimental groups treated with carbon tetrachloride were significantly higher (P<0.001) than the control

HISTOPATHOLOGICAL FINDINGS

Photomicrograph of liver showing portal tract and surrounding hepatocytes arranged in plates, converging towards it. Hepatic plates are separated by spaces called sinusoids. Normal histology.

Photomicrograph of the liver showing fatty degeneration of the hepatocytes and subsequent fibrosis. Hepatocellular macro steatosis and fibrosis

Periportal hepatocytes are lost and replaced by fibrous tissues, adjacent cells have undergone fatty degeneration, intermediate zone shows near normal hepatocytes.

Photomicrograph of the liver showing complete distortion of the cyto architecture of the liver. No Hepatic cells nuclei are visible. Hepatocellular necrosis.

Table1. Comparison of mean initial and final bodyweight change in all the groups (A, B, C and D) (Mean ± SEM given for each measurement)

	GPA	GP.B	GP.C	GRD	F.Ration	Prob of Sig
Initial Body Wt.	190.50±4.60	195.20±5.20	199.40 ±4.70	220.70 ±5.60	72.210	<0.001
Final Body Wt.	200.10 ± 6.20	180.70±4.60	168.40 ± 5.10	175.30±6.70	44.420	<0.001
Wt. Change	10.00±5.70	15.00 ±6.10	31.20 ±7.10	50.30 ± 4.80	11.15	<0.001

The final body weight for the experimental groups showed a statistically significant decrease (P<0.001) compared with the control.

Table2: Comparison of mean relative liver weight in all the groups (A,B,C& D) (mean ±SEM given measurement)

	GP. A	GP.B	GP.C	GP.D	F-Ration	Prob. of Sig
Liver Wt.	4.90±0.250	10.40±0.120	15.70±0.040	19.50 ±0.080	73.80	<0.001

The relative liver weight for the experimental groups treated with carbon tetrachloride were significantly higher (P<0.001) than the control.

Fig. 1. Micrograph 1 (Group A- Control)

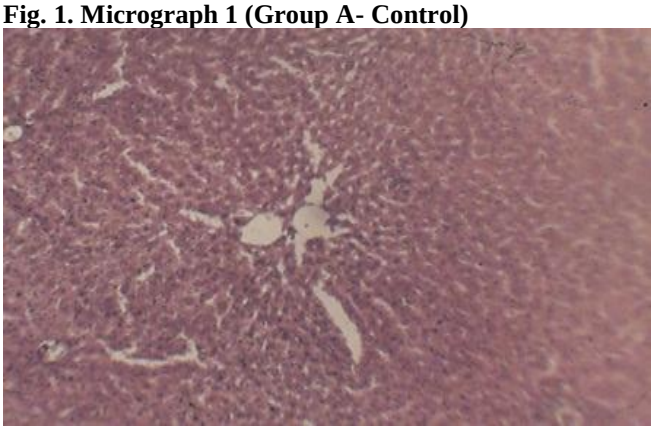


Fig. 2. Micrograph 2 (Treated with 0.1ml of CCL_4)

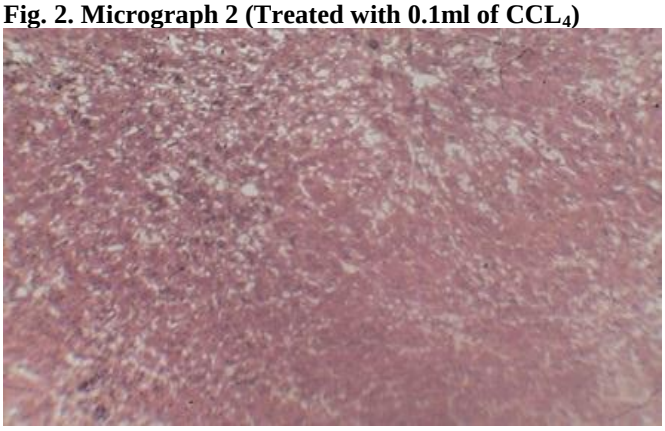


Fig. 3. Micrograph 2 (Treated with 0.2ml of CCL_4)

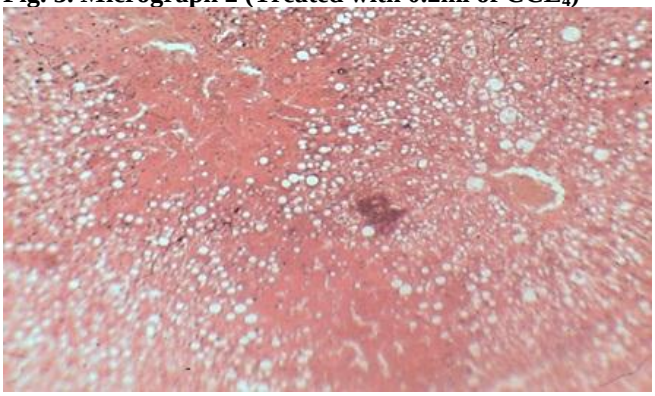
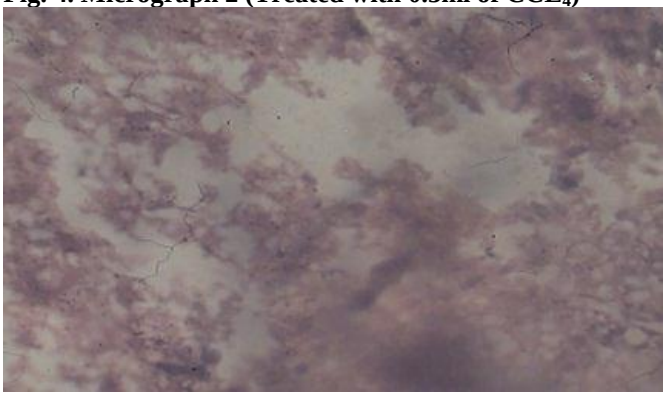


Fig. 4. Micrograph 2 (Treated with 0.3ml of CCL_4)



DISCUSSION

Carbon tetrachloride has toxicological effect on the liver, kidney and other visceral organs. Studies on the toxic effects of this chemical on the liver have been reported [1]. These reports have all presented carbon tetrachloride as a hepatotoxin.

The result of this study showed that there was a weight difference in the control group (A) compared with the experimental groups (B,C & D). This could have been physiological as the only group exposed to only water and food. Comparing the result of weight difference reveals sever loss of weight by the experimental groups (B,C& D). This is probably as a result of loss appetite by the animals in the groups. There was an also significant difference in weight of the experimental groups. The group B body weight were significantly lower ($P<0.001$) than groups C and D. The body weight of animals in group C were also significantly lower ($P<0.001$) than the group D.

The relative organ weights also showed significant differences in groups. There was relative increase in liver in groups. There was relative increase in liver weight of animals in the experimental groups treated with CCL_4, compared with the control(A). This organ weight increase was irrespective of the fact that there were total body weight loss in the experimental groups compared with the control (A). There was a significant difference in organ weights of the experimental groups (B, C & D). The organ weight of group D animals were significantly higher ($P<0.001$) than groups B and C while C was significantly higher ($P<0.001$) than group B.

The results of this study agree with previous researchers that carbon tetrachloride has toxicological effect on the liver of wistar rats (*Rattus norvegicus*). These results tend to agree with walker et al 2000 [12, 4, 13, 14] that higher concentration of carbon tetrachloride can lead to degeneration of the liver cells.

CONCLUSION

In conclusion, the present study indicated that a long exposure to small and high dosages of carbon tetrachloride caused alterations in the histological of the liver of wistar rats. Some of the histopathological effects on the liver cells were either mild on the rats administered with 0.1ml of carbon tetrachloride but marked in those exposed to high-dose of carbon tetrachloride, thus indicating dose-dependent effect.

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