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Effect of a Short Time Post Carbon Tetrachloride Treatment Interval on Rat Plasma Enzyme Levels and Percentage Mortality

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ABSTRACT : The effect of a short time (3 hours) post carbon tetrachloride treatment interval on rat plasma enzyme levels and percentage mortality have been examined. Relative to their corresponding activities in the plasma of carbon tetrachloride-free rats the activities of plasma L-aspartate aminotransferase, L-alanine aminotransferase and alkaline phosphatase of carbon tetrachloride treated rats were statistically significantly ($P<0.05$) increased when the rats were sacrificed 3 hours post exposure. During this period no mortality occurred. These results indicate that plasma enzyme levels can still be used as indices of carbon tetrachloride-induced tissue damage within a short exposure time rather than a longer post-exposure interval, which carries the risk of an unacceptably high rate of mortality. @ *JASEM*

Naturally carbon tetrachloride (CCl_4) was believed to be found in the troposphere by solar-induced photochemical reactions of chlorinated alkanes (Singh *et al.,* 1975) but this does not appear to be the major source of environmental CCl_4. It has been detected in volcanic emission gases (Isidorov *et al.,* 1990). Several studies have shown that global atmospheric levels of CCl_4 can be attributed to anthropogenic sources alone (Singh, *et al*., 1976). CCl_4 can be produced directly by chlorination of methane, methanol, carbon disulphide, propane, 1,2-dichloroethane and higher hydrocarbons and indirectly as a by-product during the manufacture of other products and compounds and during wood pulp bleaching (US EPA, 1984a).

Carbon tetrachloride like a number of other chemicals can cause cell or tissue necrosis. Tissue damage or death leads to the leakage of the enzyme produced in the affected tissue(s) into the bloodstream (Jaeger et al., 1975; Magos et al., 1982; Siegers et al., 1985). Hence serum or plasma enzyme levels have be used as indices for monitoring chemically induced tissue damages (Ngaha et al., 1989; Lin and Wang, 1986). Following the administration of such chemicals to experimental animals a time interval of 12 – 48 hours is often allowed to elapse before the surviving animals are sacrificed and the plasma/serum enzyme activities analysed (De Zwart et al., 1997).

We adopted this long time interval in a number of studies on the effect of anthocyanin on CCl_4 – induced liver damage (Obi and Ozoemena, 1998; Obi et al., 1998; Obi and Okokoro, unpublished results) and in our experience this was usually accompanied by high mortality rate. Therefore, the purpose of this investigation was to find out whether a short post – CCl_4 treatment interval could lead to higher survival rate and reasonable increase in plasma/serum enzyme levels that could still allow the effect of this chemical agent to be satisfactorily monitored.

MATERIALS AND METHODS

Animals: Ten white albino rats (Wistar strain) used for this experiment were obtained from NIMR, Lagos, Nigeria. They were maintained after purchase for 7 days on rat chow and water *ad libitum* before the commencement of the experiment.

Chemicals: Absolute ethanol and carbon tetrachloride were the products of BDH Chemical Company Ltd (Poole, England) and Hopkins and Williams respectively. Other materials include corn oil (Mazola produced for CPC, UK) and rat chow (Pfizer, Nigeria, Plc).

Treatment of animals: The rats were divided into two experimental groups of 5 rats each. Rats in-group I (normal control) were given 50% aqueous ethanol by gavage (2.5 ml/kg body weight) followed by subcutaneous injection of corn oil (1.5 ml/kg body weight). Rats in group II were given 50% aqueous ethanol followed by 1.5 ml/kg weight of a 1:1 (v/v mixture of carbon tetrachloride and corn oil via the same routes described for group I rats.

Preparation of plasma: Three hours after the last treatment given to each group of rats, each rat was anaesthetised in chloroform saturated chamber. While under anaesthesia the thoracic region was opened to expose the heart. Blood was obtained by cardiac puncture by means of a 5 ml hypodermic syringe and needle and placed in ice-cold heparinized bottles. The blood was centrifuged at 5000 rpm (Spinette – Damon / IEC bench top centrifuge) for 5 min. The plasma samples were collected and left at –20°C until required.

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Plasma protein and enzyme assay: Total plasma protein was quantified by using biuret reagent. L-aspartate aminotransferase (L-AST) and L-alanine aminotransferase (L-ALT) activities in the plasma were determined at 37^oC by colorimetric method of Reitman and Frankel (1957). The activities of alkaline phosphates were determined by the colorimetric method of Plummer (1978) using phenolphthalein monophosphate as substrate. The enzymes were assayed using reagents obtained from enzyme assay kits (QCA Laboratories, Spain).

Statistical analysis: The mean values of the control and test rats plasma activities of a given enzyme were compared using Student's t-test (Elzey, 1971). The significance level was set at P<0.05.

RESULTS AND DISCUSSION

The percentage mortality and survival of rats in our earlier studies involving long post CCl_4 interval and that of the present study of 3 hours post CCl_4 are presented in Table 1. Plasma L-AST, L-ALT and alkaline phosphatase activities in the presence and absence of CCl_4 are presented in Table 2. A significant increase (P<0.05) in the plasma activities of the three enzymes was observed in the CCl_4 – treated rats when compared with that of the CCl_4 – free (control) rats.

Table 1: Percentage mortality and survival of rats exposed to CCl_4

Expt. #	Initial No. of rat	Final No of rats	Post CCl_4 interval (h)	% Mortality	% Survival
I[a]*	10	5	18	50.0	50.0
II[a]*	20	14	18	30.0	70.0
III[a]**	16	10	24	37.5	62.5
IV[b]**	5	5	3	0.0	100.0

[a]I, II & III are previous experiments. {Obi and Ozoemena, 1998 (I); Obi et. al, 1998 (II); Obi and Okokoro-unpublised results (III)}
[b]IV, present study
*CCl_4 administered by intraperitoneal injection (i.p)
**CCl_4 administered by subcutaneous injection (s.i)

Table 2: Effect of CCl_4 on plasma enzyme levels 3 hours post – CCl_4

Group #	Treatment	Plasma Enzyme Activities U/L/mean mg protein Mean $\pm$ SD (n)[a]		
		L – AST	L – ALT	AP
I(Control)	-CCl_4	2.13 $\pm$ 0.10(5)	7.22 $\pm$ 0.22(5)	1.02 $\pm$ 0.47(5)
II(Test)	+CCl_4	2.51 $\pm$ 0.14(4)[b, c]	9.50 $\pm$ 0.34(4)[b]	1.62 $\pm$ 0.58(4)[b]

[a]AP – Alkaline phosphatase; n - number of samples analysed.
[b]Values statistically significantly higher than the corresponding control value (P<0.05).
[c]One of the samples in this group was lost post-sacrifice. Hence n = 4.

When CCl_4 is administered to rats orally, intraperitoneally or subcutaneously it is normal practice to allow 12 – 48 hours to elapse before tissue and blood analyses (De Zwart et al., 1997; Reinke et al., 1988). Within this period maximum levels of serum/plasma L-alanine aminotransferase and L-aspartate aminotransferase (Teschke et al., 1983; Nakata et al., 1985) and glutamate dehydrogenase (Teschke et al., 1983) activities have been demonstrated. In our earlier studies in which a good number of rats died before the expiration of 24 hours the doses of CCl_4 used were in the range of 0.5 – 1.5ml CCl_4/kg body weight. This dose range is not outrageous. Other investigators have used 1.5 ml (Teschke et al., 1983) 2.5 ml (Dingell and Heimberg 1968; Moore et al., 1976) 1.25 ml (Larson and Plaa 1965) and 2.0 ml (Marchand et al., (1970) CCl_4/kg body weight orally. Dose of 3 ml/kg body weight has been used (Hase et al., 1996) subcutaneously which is considerably higher than our dose. Most of these reports are silent on the issue of mortality of the experimental rat, which in a number of cases, are the

same stain of rat we used. However since our doses are well within the dose range used by others we are inclined to attribute the death to the long post CCl_4 interval instead of overdose.

Following intraperitoneal or subcutaneous injection of CCl_4 the rats that did not survive started dying barely 4 hours post exposure. Based on this fact we decided to allow only 3 hours post exposure interval. This time period as reported by others is sufficient for an oral dose to reach peak levels in blood, liver, kidney, brain and muscle (Watanabe *et al.*, 1986; Teschke *et al*., 1983). As the data presented in Table 1 reveals this time interval ensured that the rats remained alive until they were sacrificed. The three enzymes whose activities were analysed are frequently used for assessing liver injury (Ngaha *et al*., 1989; Lin and Wang, 1986; Teschke *et al*., 1983; Nakata et al., 1985; Magos *et al*., 1982; Jaeger et al., 1975; Siegers *et al*., 1985). Theoretically when an agent damages an organ the enzymes it elaborates leak into the bloodstream leading to increased serum or plasma activities of the biomarker enzymes. In order to demonstrate this change unequivocally sufficient post-exposure time, 12 – 48 hours, is allowed (De Zwart *et al*., 1997; Reinke *et al*., 1988). In this study a clear and significant margin was demonstrated between the activities of the enzyme in the plasma of CCl_4 – free rats and CCl_4 – treated ones. The increased enzyme activities in the plasma of CCl_4 – treated rats suggests that the toxicant was able to reach the liver and induce detectable damage within 3 hours. It may, therefore, be worthwhile to consider and adopt a short post – CCl_4 exposure time which not only allows a high survival rate but a reasonable increase in serum/plasma enzyme levels for assessing the toxic potency of this chemical.

A number of substances increase the biotransformation of CCl_4 and potentiates its intoxication. Among the substances are high fat diet and ethanol (Strubelt, 1984; McCay *et al*., 1984; Reinke *et al*., 1988). Ethanol in particular is thought to be responsible for the induction of the cytochrome P_{450} isoenzyme involved in CCl_4 bioconversion to metabolites that initiate lipid peroxidation and attendant tissue damage. Therefore in this study we treated both group of rats with 50% aqueous ethanol simply to achieve reasonable damage within 3 hours of CCl_4 exposure.

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