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Carbontetrachloride induced toxicity in liver of albino mice

KEYWORDS: Carbontetrachloride, Hepatatoxicity, AKP, Aminotransferases, Albino mice

Prerna Vohra | **Navneet Kumar Gupta**

Department of Biology and Environmental Sciences, CSK HP KV Palampur, Himachal Pradesh

ABSTRACT *Carbon tetrachloride (CCl4) intoxication in rodents is a commonly used model of both acute and chronic liver injury. Carbon tetrachloride induced hepatic injury as measured by alterations in serum enzymes and histopathological changes were investigated in Swiss Albino mice (Musmusculus). Mice were divided into two groups: control group and carbon tetrachloride administered group. A single dose of CCl4 (0.8 ml/kg body weight, sc) was given and induced hepatotoxicity was investigated, when sacrificed at 1, 3, 7 days of treatment and 1, 3 and 6 months post treatment. The hepatotoxicity was manifested biochemically as significant elevation of the enzyme activities of serum alanine aminotransferase (ALT, EC: 2.6.1.2), aspartate aminotransferase (AST, EC: 2.6.1.1), alkaline phosphatase (ALP, EC: 3.1.3.1) and bilirubin after 1, 3 and 7 days post treatment. While after 1, 3 and 6 months, levels of serum enzymes and bilirubin were reached to their normal or near normal values. The toxicity was further evidenced by histopathological changes as hydropic degeneration, congestion of sinusoids, vacuolation of cytoplasm and centrilobular necrosis in the liver of mice. It is concluded that CCl4 is a potent hepatotoxin with a dose of 0.8ml/kg body weight.*

Introduction

Liver, the largest organ in vertebrate body is the major site of intense metabolic activities. It is the main organ responsible for multitude of essential functions and plays an essential role in the metabolism of foreign compounds entering the body (Athar *et al*. 1997). Carbontetrachloride (CCl_4) is a potent hepatotoxin in a variety of experimental animals and man. Prolonged administration of CCl_4 leads to fibrosis, cirrhosis and hepatic carcinoma.$CCl_4$is widely used in scientific research to evaluate hepatoprotective agents. It is metabolized in the liver by cytochrome P-450 to trichloro methyl radical that reacts with Glutathione to form a Glutathione containing radical and causes various pathological and toxicological manifestations (Connor *et al*. 1990). The enzymes L-alanine amino transferase (L-ALT), L-aspartate amino transferase (L-AST), alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) are often used in assessing the integrity of liver (Medhat*et al*. 2002). Histological observation of liver tissue of CCl_4 treated mice show fatty degeneration, damage of parenchyma cells, cirrhosis, steatosis and hydropic regeneration of liver tissue. CCl_4 intoxicated rats show a significant increase in the relative weight of liver when compared to normal rats. No information is available on carbontetrachloride induced hepatic injury in mice. Therefore, the present investigation is undertaken to estimate the level of serum enzymes and histopathological changes in the liver of mice.

Materials and Methods:

Chemicals: Carbon tetrachloride was purchased from Qualikems Fine Chemicals Private Limited New Delhi

Procurement and maintenance of animals

Swiss albino mice (*Musmusculus*) procured from IVRI, Palampur were bred under controlled conditions of temperature (25±2^{0}C) and light (14hrs.light and 10 hrs. darkness).The animals were given pelleted standard mice feed obtained from Hindustan Lever's Ltd., New Delhi and water ad libitum.

Treatment:

The animals were administered carbon tetrachloride (0.8ml/kg body weight, sc).

Animals were sacrificed at 1, 3 and 7 days, 1, 3 and 6 months post treatment intervals. Normal healthy looking mice (without carbon tetrachloride treatment) showing no sign of morbidity were used as control.

Biochemical studies:

Serum separated from the blood when animals were sacrificed at intervals of 1,3 and 7 days and 1,3 and 6 months was used for the estimation of levels of enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Bilirubin by method of **Bergmeyer and Brent, 1974.**

Histopathological examination:

Histological studies of the liver were done according to the method of Etim *et al*. (2008)

Liver was excised immediately after sacrificing animals by cervical dislocation, and a portion of the liver tissue was fixed in 10% buffered neutral formalin solution. Following 16-24 hours of fixation, tissues were washed in running water, dehydrated through ascending grades of alcohol (30%,50%,70%,90% and absolute alcohol), cleared in xylene and embedded in paraffin wax (m.pt.58-60^{o}C), followed by their microtome sectioning at 5-7µ.

Tissue sections stretched on albumenized slides were dewaxed, hydrated through descending grades of alcohol (absolute alcohol, 90%, 70%, 50% and 30%), water and stained in Delafield's Haematoxylin. Differentiation was done in acid water and bluing in 0.7 per cent ammonia water. Dehydration was done by passing sections through ascending grades of alcohol (30%, 50%, 70%). Eosin staining was done in 90% alcohol for 5 minutes. The sections were dehydrated in two volumes of absolute alcohol followed by their clearing in xylene. Finally the sections were mounted in Canada balsam. After staining, the sections were observed under light microscope for histopathological changes and photographs were taken.

Statistical Analysis:

Experimental results were expressed as mean±S.D. Statistical analysis was performed by one way analysis of variance (ANOVA).

Results:

Biochemical studies:

Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) activity showed an increasing trend during early intervals (from Day1 to Day

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7) after CCl_4 administration. And in the later intervals (from month1 to month 6) the levels of these three enzymes returned to normal or near normal values. (Table 1) In case of Bilirubin, an increase in level was observed from day 1 to day 7. The level decreased at late intervals of 1,3 and 6 months and has values near normal or control values.

Histopathological Examinations:

Liver histological observations of control rats showed radially arranged hepatic cords around the central vein. (Fig2A). However the microscopic examination of carbontetrachloride treated rat liver revealed many dose dependent degenerative changes of variable degrees in many areas of liver. Histopathological effects of 1, 3, 7 and 1,3 and 6 months post treatment of carbontetrachloride are presented in fig 1B,C. Carbontetrachloride treated rats showed extensive fatty change, congested sinusoids distended hepatocytes, haemorrhage vacuolated cytoplasm and fatty degeneration. The recovery towards normalisation of serum enzymes and histological architecture is due to regenerative capacity of the liver. (Nakata *et al.*1985).The results obtained for elevated levels of serum enzymes and Bilirubin were in accordance with Ogeturk*et al.* (2004); Sultana *et al.* (2005); Krishnakumar*et al.* (2008) and Das *et al.*(2008). (Fig 2 B and C)

Discussion

The levels of serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) showed an increasing trend during early intervals (from Day1 to Day 7) after CCl_4 administration. And in the later intervals (from month1 to month 6) the levels of these three enzymes returned to normal or near normal values. In case of Bilirubin, an increase in level was observed from day 1 to day 7. The level decreased at late intervals of 1,3 and 6 months and has values near normal or control values.

Serum levels of AST, ALT and ALP are the quite sensitive indicators to evaluate the degree of hepatic damage. Marked increase in release of hepatic enzymes into the blood stream is often associated with massive necrosis of the liver. CCl_4 is known to cause marked elevation of serum enzymes. In the present study a significant increase in level of ALT, AST, ALP and serum bilirubin was observed after 1, 3, 7 days of exposure of CCl_4 indicating considerable hepatocellular injury. The maximum values of all the four parameters ALT(227.730U/L), AST(285.370U/L), ALP(977.108U/L) and Bilirubin(1.516mg/dl) was recorded at 7th Day post-treatment when compared to control and minimum values were obtained after 6 months for the above parameters, 57.660U/L, 43.660U/L, 36.899U/L and 0.287mg/dl respectively.

Histopathological studies of liver showed marked lesions in the form of oedema and degranulation and vacuolation of hepatocytoplasm. In few cells the nuclei were dislocated and some cells were enucleated (without nucleus).Sinusoids became congested and fatty degeneration was also seen. Slight hydropic degeneration of some hepatocytes was also seen. There were many pyknoticnuclei with condensed chromatin. Sinusoids showed further congestion. The Kuppfer cells showed hypertrophy and their number increased The Kuppfer cells showed hypertrophy. The sinusoid walls show numerous Kupffer cells, focal hepatic necrosis, hemorrhage, fibroplasia in portal triad. Histopathological examination demonstrated that CCl_4 treated group induced hydropic degeneration, congestion of sinusoids, distended hepatocytes and vacuolation of cytoplasm (Arhoghro*et al.*2009).

Table 1
Effect of carbontetrachloride on Alanine aminotransferase (ALT), Aspartate aminotransferase(AST), Alkaline phosphatase(ALP) and Bilirubin in plasma of control and treated rats.

	Control	1 day	3 day	7 day	1 month	3 month	6 month
ALT(U/L)	57.537 ±1.195	72.279* ±0.805	144.361* ±0.845	227.730* ±1.028	59.108* ±0.649	58.437 ±0.210	57.660 ±0.350
AST(U/L)	43.192 ±0.512	65.800* ±1.704	268.120* ±0.743	285.370* ±1.127	44.644* ±0.481	43.686 ±0.518	43.660 ±0.527
ALP (U/L)	35.794 ±0.622	125.532* ±0.984	576.105* ±0.554	977.108* ±0.876	38.033* ±0.570	37.282* ±0.758	36.899* ±0.564
BIL (mg/dl)	0.170 ±0.068	0.232* ±0.017	0.822* ±0.063	1.516* ±0.061	0.322* ±0.045	0.319* ±0.040	0.287* ±0.050

Values are expressed in units of ALT, AST and ALP as Unit/litre and of bilirubin as mg/dl.

All values are mean± SD of six individual observations.

* The values are significantly different from control at P<0.05.

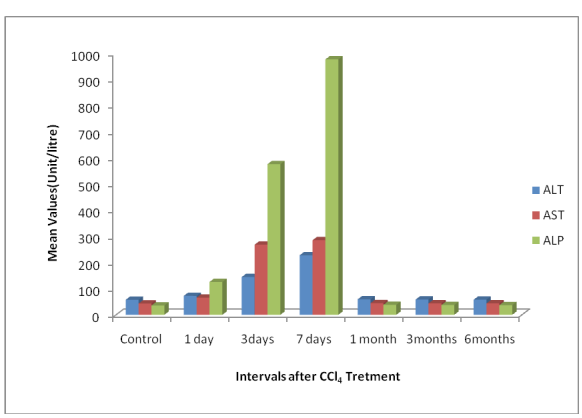
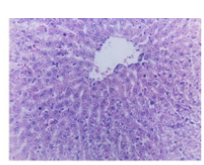
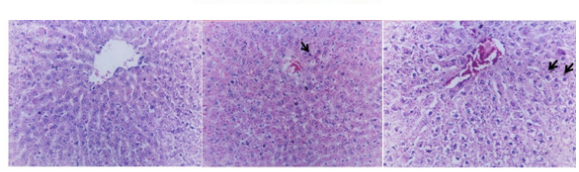


Fig 1 Comparison of Alanine aminotransferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP)



A. T.S of the liver of control



B.Histopathology of liver 1,3 and 7days after the administration of CCl_4

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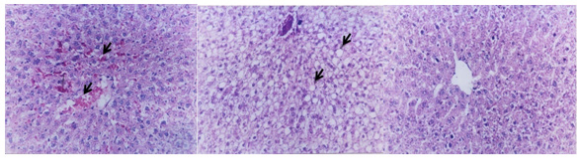

C. Histopathology of liver1, 3 and 6 months after the administration of CCl_4

REFERENCE

Arhoghro, E. M, Ekpo, K.E., Anosike, E. O. and Ibeh G. O (2009). Effect of aqueous extract of Bitter leaf (VernoniaAmygdalina Del) on carbon tetrachloride (CCl4) induced liver damage in albino wistar rats. European Journal of Scientific Research.26(1).122-130 | Athar, M., Hussain, Z.S. and Hassan, N., (1997). Drug metabolizing enzymes in the liver.In:Rana SVS, Taketa K, editors. Liver and Environmental xenobiotics, New Delhi: Narosa Publishing House | Bergmeyer, H.U., and Brent, E, (1974) Methods of Enzymatic Analysis,Bergmeyer,H.U (Ed.) VerlagChemieWeunheun, Academic Press, New York.2: 735-760 | Connor, H.D.,Lacagnin, L., Knecht, K.T., Thugman, R.G. and Mason, R.P.(1990). Reaction of glutathione with a free radical metabolite of carbon tetrachloride. Molecular Pharmacology37:443. | Das S., Sarma G. and Barman S. (2008).Hepatoprotective activity of aqueous Extract Of fruit Pulp OfCassia Fistula against carbon tetrachloride (CCl4) induced liver damage in albino rats. Journal of Clinical and Diagnostic Research 2:1133 -1138 | Etim, O.E.,Akpan, E.J. and Usoh, I.F. (2008). Heptotoxicity of Carbon tetrachloride: Protective effect of Gongronemalatifolium.Pakistani Journal of Pharmaceutical Science21 (3): 268-274 | Krishnakumar, N.M.,Latha, P.G.,Suja, S.R.,Shine, V.J.,Shyamal, S.,Anuja, G.I.,Sini, S.,Pradeep, S.,Shikha, P.Unni, P.K.S. and Rajasekharan, S. (2008) Hepatoprotective effect of Hibiscus Hispidissimus Griffith, ethanolic extract in paracetamol and CCl4 induced hepatotoxicity in Wistar rats. Indian Journal of Experimental Biology46: 653-659 | Medhat, A.,Shehata, M. and Magder, S. (2002) .Hepatitis C in a community in Upper Egypt.Risk factors for infection.American Journal of Tropical Medical Hygiene.66:633-638 | Nakata, R., Tsukamoto, I., Miyoshi, M. and Kojo, S. (1985). Liver regeneration after carbon tetrachloride intoxication in the rat.Biochemical Pharmacology 34(4): 586-588 | Ogeturk, M.,Kus, I.,Kavakli, A.,Zararsiz, I.,Ilhan, N. and Sarsilmaz, M., (2004). Effects of melatonin on carbon tetrachloride-induced changes in rat serum.Journal of Physiology and Biochemistry 60: 205-10 | Sultana, S., Ahmad, S., Khan, K. and Jahangir, T. (2005). Effect of Emblica officinalison CCl4 induced hepatic toxicity and DNA synthesis in Wistar rats .Indian Journal of Experimental Biology 43:430-436