Original Article

Pentoxifylline on Liver

Pentoxifylline Protects against Carbon Tetrachloride Induced Liver Injury in Adult Male Wistar Rat Model

1. Maria Kazi 2. Muhammad Bachal Pandhiani 3. Salman Ahmad Farsi Kazi 4. Saif-ur-Rehman 5. Haji Kjan Khoharo

1. Asstt. Prof. of Biochemistry, Al-Tibri Medical College, Karachi 2. Asstt. Prof. of Anatomy, Al-Tibri Medical College, Karachi 3. Asstt. Prof. of Forensic Medicine, Faculty of Medicine and Allied Medical Sciences, Isra University, Hyderabad 4. Asstt. Prof. of Physiology, Faculty of Medicine and Allied Medical Sciences, Isra University, Hyderabad 5. Asstt. Prof./Consultant Physician, Dept. of Physiology/Medicine, Faculty of Medicine and Allied Medical Sciences, Isra University, Hyderabad

ABSTRACT

Objective: To investigate the protective effect of Pentoxifylline (PTX) in carbon tetrachloride (CCl_4) induced liver injury in adult male Wistar rat model.

Study design: Experimental/Analytical study

Place and Duration: Animal House, Isra University Hyderabad from May to December 2012.

Subjects and Methods: Forty five adult male Wistar rats were divided into three groups; Group A. controls received 0.9% isotonic saline, Group B. received CCl_4 orally (1.9mg/kg) mixed in olive oil, and Group C. received the PTX+ CCl_4. Blood samples were collected for liver biochemical assays. The animals were sacrificed, liver tissue, after fixation in 4% formaldehyde, was embedded in paraffin. Tissue sections of 5µ thickness were subjected to haematoxylin and eosin staining and were assessed by light microscopy. The data was analyzed on SPSS 21.0 using one-way ANOVA, Tukey-Cramer and Chi-square tests. A p-value of ≤ 0.05 was taken statistically significant.

Results: The liver biochemical and histological findings reveal statistically significant differences among the controls, CCl_4 and PTX+ CCl_4 groups (p=0.0001). Liver enzymes and histology was deranged significantly in CCl_4 group compared to controls and PTX+ CCl_4 group (p=0.0001). The CCl_4+PTX group shows less elevation of liver enzymes and derangement in liver histology when compared to CCl_4 group (p=0.001). The histological findings of congestion, inflammatory cell infiltrate, vacuolar degeneration and necrosis are found prominent in CCl_4 group.

Conclusion: The Pentoxifylline protects against oxidative damages caused by carbon tetrachloride induced liver injury in rat model.

Key words: Carbon tetrachloride, Liver injury, Pentoxifylline

INTRODUCTION

Liver is the largest gland of human body. The parenchyma cells of liver are known as the hepatocyte, which performs biochemical and metabolic functions.[1]

The oxygen is a precursor of a number of free radicals which are collectively known as the reactive oxygen species (ROS). The ROS are implicated in the pathogenesis of most liver diseases both inflammatory and non-inflammatory like ischemia/reperfusion injury, chronic viral hepatitis, alcoholic hepatitis, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and cholestasis.[2] The liver is a metabolically active organ, and protects against drug toxicity for two reasons 1). As it is interposed between porto-systemic circulation i.e. between absorption of intestinal contents containing toxins. 2). Hence preferred site of toxin metabolism and elimination through intestine. Drug induced liver injury poses body systems to possible injury if toxins access to systemic circulation.[3,4]

Carbon tetrachloride (CCl_4) is a hepatotoxic compound. The CCl_4 has been has been used extensively in laboratory animals for induction of liver injury, elucidate the underlying mechanism of liver injury and hepatoprotective effects of various therapeutic agents.[5]

One of the postulated mechanism of CCl_4 induced liver injury is the formation of ROS. The ROS disrupts the hepatocyte at cell membrane level through the lipid peroxidation[5,6] causing anatomical disruption of liver architecture and physiological disturbances.[7] The hepatocyte injury causes leakage of cytoplasmic and mitochondrial enzymes in the blood streams.[8]

The cytoplasmic and mitochondrial enzymes are clinically used as markers of liver injury, and for monitoring and treating the liver diseases also. The liver enzymes which appear in the blood as a result of liver injury include; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) are important enzymes that are often employed in assessing liver injury.[1,5]

The Pentoxifylline (PTX) is a well known methyl xanthine. The PTX is a non-specific inhibitor of phosphodiesterase-4 type. The PTX is used clinically in peripheral arterial disease with intermittent

claudication. The mechanism underlying its therapeutic effects seems to be related to improvement of microcirculation, tissue perfusion and cell functions. The PTX is reported to possess anti-inflammatory and hepatoprotective effects against chemical-induced liver injury like alcohol.[4,9,10]

The present study aims to investigate the possible protective effects of Pentoxifylline (PTX) against Carbon tetrachloride (CCl_4)-induced hepatotoxicity in experimental animals.

MATERIALS AND METHODS

An experimental study was conducted at the animal house of Isra University on rabbit model over a period of one year, from January to December 2012. Adult male Wistar rats of 250-300 grams were included in the study. Female rats, weight <250 grams or >300 grams were excluded from the study. Animals were housed in animal house at an optimal room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. The chaw like fresh alfalfa and clean water are provided freely. The rats were divided into three groups;

Group A. Control Group (n=15) Rats received 0.9% isotonic saline orally on alternate day for three successive weeks and served as control group,

Group B. Carbon tetrachloride Group (n=15) Rats were given CCl_4 orally mixed in olive oil on alternate day for three successive weeks and

Group C. Experimental Group (n=15) Rats received PTX (200 mg/kg intraperitoneally) and CCl_4 on alternate days for three successive weeks

Experimental Details: The PTX was purchased from Medical store of Isra University Hospital. The PTX was administered in a dose of 200 mg/kg intraperitoneally.[4] Carbon tetrachloride was purchased from scientific drug store at Hyderabad City. The CCl4 dissolved in olive oil as vehicle (1:1 Ratio) at a dose level of 1.9 ml/kg orally on alternate day for three successive weeks and sacrificed at the end of their respective period of time.[1]

Sacrifice of animals: The animals were sacrificed using standard method as described by Nayak et al. (2006)[11] In order to examine the liver tissue, the liver of the sacrificed animals was removed promptly and preserved in formaldehyde.

Blood sample Processing: The blood samples were collected from peripheral veins at twenty four hours of experimental period. Sera were separated by centrifugation at 300xs for ten minutes. Serum samples were used to determine liver enzymes.

Liver enzyme assay: Liver enzyme assays were determined for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) using commercially available diagnostic kits.

Histological studies: After fixation in 4% formaldehyde, samples were embedded in paraffin. Sections of 5µ thickness were subjected to haematoxylin and eosin Hepatic morphology was assessed by light microscopy. A total of five sections for each liver tissue sample were observed under light microscope.

In H & E staining, damaged hepatocytes graded as 0= normal, += mild damage (swollen and pale cytoplasm), ++= moderate damage (vacuolated cytoplasm), +++= severe damage and ++++= very severe damage (pyknotic nucleus and eosinophil cytoplasm).[12]

The data was analyzed on SPSS version 21.0 (IBM corporation). The continous variables were presented as mean±SD using one-way ANOVA and Tukey-Cramer test for multiple comparisons. Chi-square test was used for categorical variables. A p-value of ≤ 0.5 was taken statistically significant.

RESULTS

The present study observes major differences in liver injury between and among groups as indicated by blood enzyme levels in different animal groups. The LDH, ALP, and ALT, AST in serum of Rats treated with carbon tetrachloride were found elevated compared with control group after three weeks, with a highly significant p-value for multiple comparisons (p=0.001). The CCl_4+PTX group shows a significant reduction in the liver enzymes compared with the CCl_4 group (p=0.001) and control group (p=0.001). The animals CCl_4+PTX group shows significant reduction in the liver enzyme elevation compared to CCl_4 group alone (p=0.001). The finding shows significant hepato protection by the PTX in CCl_4 induced injury. The liver enzyme assays among different groups are shown in table.1.

Table No. 1: Liver enzyme levels in controls, *CCl_4 and CCl_4+ Pentoxifylline groups

Groups	LDH (IU/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Group. A (Controls)	711.5± 51.7	93.6± 8.91	48.9± 3.19	91.2± 16.81
Group. B (CCl_4)	2778.8± 139.6	167.1± 8.02	189.6± 11.91	499.7 ± 21.9
Group. C (CCl_4 + PTX)	2138.6± 153.3	136.7± 18.14	87.7± 17.92	171.3± 19.3

*Carbon tetrachloride

Different parameters of histological score of liver injury are shown in Table. 2. The Liver sections of the control group animals show intact central venules and hepatocytes arranged in compact cords. Normal looking hepatocytes with prominent nucleus, nucleolus and well preserved cytoplasm are seen in control group (Figure.1). On the contrary, the CCl_4 group shows derangement of hepatocytes cords, hydropic changes

with congestion of central venules and sinusoids, and abundant inflammatory cell infiltration (Figure.2).

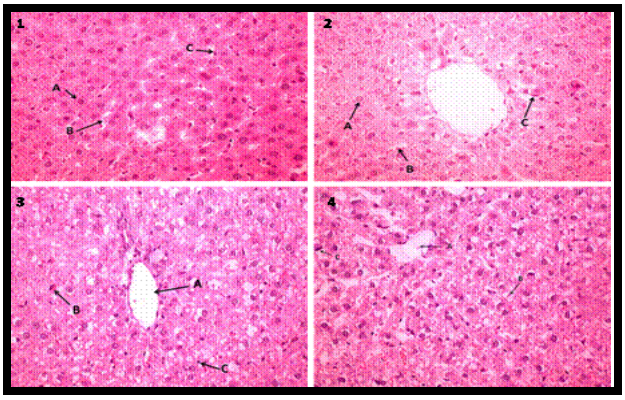

Figure No.1: Liver slide of control group shows normal looking hepatocytes arranged in cords. Central vein (CV) is shown (A) separated by sinusoids

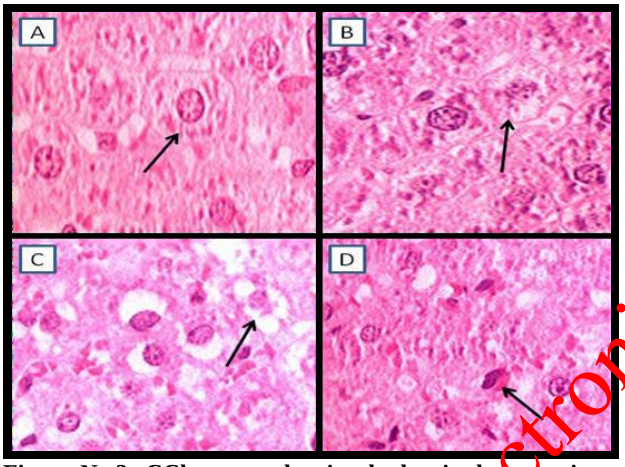

Figure No.2: CCl_4 group showing hydropic degeneration (arrow), inflammatory cell infiltrate and necrosis

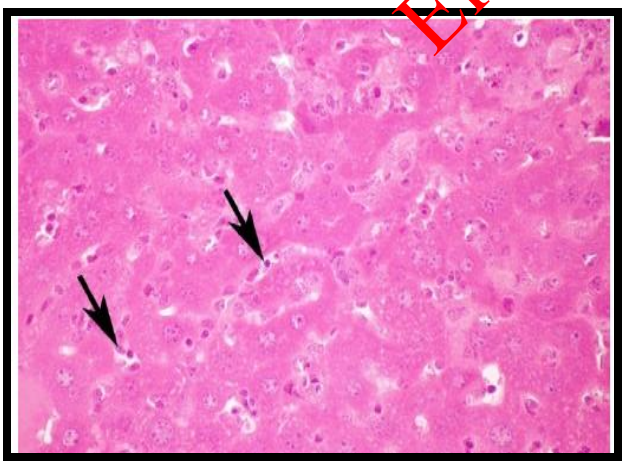

Figure No.3: CCl_4 + Pentoxifyllline group showing normal hepatocyte arranged in cords with congested sinusoids, and few lymphocytic infiltrations.

The centrilobular hepatocytes show hydropic changes and necrosis, while midzonal and peripheral hepatocytes show vacuolar degeneration and fatty changes in CCl_4 group (Figure 2). In CCl_4+PTX animals, liver tissue sections reveal less significant derangement of hepatocytes cords, hepatocytes damage and necrosis was limited compared with CCl_4 group (Figure.3).

Table No. 2: Histology of liver injury of controls, *CCl_4 and CCl_4+ Pentoxifyllline groups

Groups	Inflammatory cell infiltrate	Congestion	Vacuolar degeneration	Necrosis
Group. A (Controls)	0	0	0	0
Group. B (CCl_4)	++++	++++	+++	++++
Group. C (CCl_4 + PTX)	+++	++	++	++

*Carbon tetrachloride

DISCUSSION

The present study is an original research work, which investigates the effect of Pentoxifyllline (PTX) on carbon tetrachloride (CCl_4) induced liver injury in adult male Wistar rats. The Null hypothesis is rejected because the study observes hepatoprotective effects of PTX as evidenced by biochemical and histological marker of liver injury. The present study shows liver damage caused by the carbon tetrachloride as indicated by serum levels of liver enzymes compared to control group in rat model. The carbon tetrachloride induced liver injury with release of liver enzymes is comparable finding to reported previously by Hurkkeri et al.[13] The Hurkkeri[13] reported elevated hepatocyte enzyme of liver as a consequence of CCl_4 induced liver injury in animal model. The release of large quantities of cytoplasmic and mitochondrial enzymes of liver is a clinical indicator of hepatocyte cell membrane damage and rupture sufficient to produces change in enzyme levels in blood.[14] The effect of PTX has been studied on the hepatic encephalopathy,[15,16] hepatorenal syndrome,[17] and alcoholic hepatitis by some researchers.[18] PTX inhibits profibrogenic cytokine and procollagen I expression, [19] decreases the AST and ALT and its anti-TNF-α improves symptoms of liver tissue in patients with Non-alcoholic steatohepatitis (NASH) Non-alcoholic fatty liver disease (NALFD). [20]

The present study shows that the damage of liver caused by CCl_4 is evident by the rise in serum enzymes levels beside the histological changes in liver tissue. Administration of CCl_4 significantly increases the serum levels of liver enzymes; LDH, ALP, ALT and AST, which are indices of hepatocyte damage and leakage of enzymes from cells. [21,22]

The histological examination of present research study correlates in parallel to disturbance in biochemical markers of liver injury. The histology of liver tissue shows disruption of liver architecture, hepatocytes, hepatic lobules and arrangement of hepatocytes in

cords. The hepatocytes show findings of cellular injury with marked cytoplasmic vacuolization. The injured hepatocytes show pyknotic nuclei with lymphocyte infiltrations. The pyknotic nuclei are a sign of severe cellular injury caused by a toxin like carbon tetrachloride. The histological and biochemical findings of present study are comparable to those mentioned previously.[23-,25] The carbon tetrachloride is metabolized to free radical during its metabolism and detoxification in smooth endoplasmic reticulum by the cytochrome P450.[26] The Movassaghi, et al [4] (2013) studied hepatoprotective effects of PTX on ecstasy induced liver damage and reported effects of PTX. The PTX reduced liver injury in this previous study by reducing apoptosis and fibrosis caused by ecstasy in rat liver model. The present study concludes that the PTX decreases the carbon tetrachloride induced liver damage.

CONCLUSION

The Pentoxifylline protects against oxidative damages caused by carbon tetrachloride induced liver injury in rat model. The Pentoxifyllline may be used as an effective protector against chemical induced liver damages; however, further studies are warranted.

REFERENCES

1. Essawy AE, Abdel-Moneim AM, Khayyat LI, Elzergy AA. Nigella sativa seeds protect against hepatotoxicity and dyslipidemia induced by carbon tetrachloride in mice. J Appl Pharm Sci 2012;2 (10):021-5.
2. Wafay H, El-Saeed G, El-Toukhy S, Youness E, Ellaithy N, Agaibi M, Eldaly S. Potential effect of garlic oil and silymarin on carbon tetrachloride induced liver injury. Austr J Basic Appl Sci 2012; 6 (3):409-14.
3. Russmann S, Kullak-Ublick GA, Grattagliano I. Current concepts of mechanisms in drug-induced hepatotoxicity. Curr Med Chem 2009; 16:3041-53.
4. Movssaghi S, Sharifi ZN, Mohammadzadeh F, Soleimani M. Pentoxifylline protects the rat liver against fibrosis and apoptosis induced by acute administration of 3,4-Methyleneoxy methamphe-tamine (MDMA or Ecstasy). Ir J Basic Med Sci 2013; 16: 922-27.
5. Obi FO, Omogbal LA, Orlafo OS, Ovat OD. Effect of a Short Time Post Carbon Tetrachloride Treatment Interval on Rat Plasma Enzyme Levels and Percentage Mortality. J Applied Sci Environ Mgt 2001; 5: 5-8.
6. Muriel P, Albo N, Perez-Alvarez VM. Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp Biochem Physiol C Toxicol Pharmacol 2001;130: 219-26.
7. Rasha SA, Ashraf AA, Aly R. Carbon tetrachloride-induced liver disease in rats: the potential effect of supplement oils with vitamins E and C on the nutritional status. German Med Sci 2009; 7: 1612-3174.
8. Rost DA, Welker DA, Welker J, Millonig G, Berger I, Autschbach F, et al. Liver-homing of purified glucose oxidase: A novel in vivo model of physiological hepatic oxidative stress (H_2O_2). J Hepatol 2007; 46: 482-91.
9. Seiffge D. Pentoxifylline: its influence on interaction of blood cells with the vessel wall. Atherosclerosis 1997;131:27–28.
10. Windmeier C, Gressner AM. Pharmacological aspects of pentoxifylline with emphasis on its inhibitory actions on hepatic fibrogenesis. Gen Pharmacol 1997; 29:181–196.
11. Nayak S, Nalabothu P, Sandiford S, Bhogadi V, Adogwa A. Evaluation of wound healing activity of Allamanda cathartica L. and Laurus nobilis. L. Extracts on rats. BMC Compl Alt Med 2006; 6: 12.
12. Murat-Bilgin H, Atmaca M, Deniz-Obay B, Ozekinci S, Taşdemir E, Ketani A. Protective effects of coumarin and coumarin derivatives against carbon tetrachloride-induced acute hepatotoxicity in rats. Exp Toxicol Pathol 2011; 63(4): 325-30.
13. Hurkkeri VI, Jaiparkash B, Lavhale RV, Karadi RV, Kuppast IJ. Hepatoprotective activity of Anthus Excelsa Roxb leaf extract on experimental liver damage in rats. J Pharmacogn 2002;11: 120-28.
14. Shaarawy SM, Tohamy AA, Elgendy SM Elmageed ZY, Bahnasy A, Mohamed MS, et al. Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity. Int J Biol Sci 2009; 5(6): 549-57.
15. Assimakopoulos SF, Thomopoulos KC, Labropoulou-Karatza C. Pentoxifylline: a first line treatment option for severe alcoholic hepatitis and hepatorenal syndrome? World J Gastroenterol 2009;15: 3194-3195.
16. Amini M, Runyon BA. Alcoholic hepatitis 2010: a clinician's guide to diagnosis and therapy. World J Gastroenterol 2010 21; 16:4905-4912.
17. McHutchison JG, Runyon BA, Draguesku JO, Cominelli F. Person JL, Castracane J. Pentoxifylline may prevent renal impairment (hepatorenal syndrome) in severe acute alcoholic hepatitis. Hepatol 1991; 14:96.
18. Akriviadis E, Botla R, Briggs W, Han S, Reynolds T, Shakil O. Pentoxifylline improves short-term survival in severe acute alcoholic hepatitis: a double-blind, placebo-controlled trial. Gastroenterol 2000; 119:1637-48
19. Raetsch C, Jia JD, Boigk G, Bauer M, Hahn EG, Riecken EO, et al. Pentoxifylline downregulates

profibrogenic cytokines and procollagen I expression in rat secondary biliary fibrosis. Gut 2002; 50:241-7.
20. Li W, Zheng L, Sheng C, Cheng X, Qing L, Qu S. Systematic review on the treatment of pentoxifylline in patients with non-alcoholic fatty liver disease. Lipids Health Dis 2011; 10:49.
21. Rajesh M and Latha M. Preliminary evaluation of anti-hepatotoxic activity of Kamilari, a polyherbal formulation. J Ethnopharmacol 2004; 91: 99-104.
22. Bashandy S, and Al-Wasel S. Carbon tetrachloride-induced hepatotoxicity and nephrotoxicity in rats: Protective role of vitamin C. J Pharm Toxico 2001; 16(30): 283-92.
23. Trivedi P, Mowat A. Carbon tetrachloride-induced hepatic fibrosis and cirrhosis in the developing rat: an experimental model of cirrhosis in childhood. Br J Exp Pathol 1983; 64(1): 25–33.
24. Berman E, House D, Allis J, Simmons J. Hepatotoxic interactions of ethanol with allyl alcohol or carbon tetrachloride in rats. J Toxicol Environ Health 1992;37(1): 161–76.
25. Brandao C, Ferreira H, Piovesana H, Polimeno N, Ferraz J, de-Nucci G. Developmental model of liver cirrhosis in rabbits. Clin Exp Pharma Physiol 2000;27:(12): 987-90.
26. Wang P, Kaneko T, Tsukada H, Nakano M, Nakajima T, Sato A. Time courses of hepatic injuries by chloroform and by carbon tetrachloride comparison of biochemical and histopathological changes. Arch Toxicol 1997; 71: 638-45.

Address for Corresponding Author:
Dr. Haji Khan Khoharo,
Assistant Professor/Consultant Physician,
Department of Physiology/Medicine
Faculty of Medicine and Allied Medical Sciences, Isra University, Hyderabad.
Tel: 0331-2662500