Original Article

Investigating Hepatoprotective Effects of Pentoxifylline in Carbon Tetrachloride Induced Liver Injury in Rat Model

Kashif Rasheed Shaikh[1], Sadia Tabassum[2], Shumaila Shaikh[3], Shagufta Memon[4], Shumail Saeed Siddiqui[5] and Umair Ali Soomro[6]

ABSTRACT

Objective: Investigating the hepatoprotective effects of Pentoxifylline (PTX) in carbon tetrachloride (CCl_4) induced liver injury in rat model.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Animal house of Sindh Agriculture University Tando Jam and Isra University from January 2017 to August 2017.

Materials and Methods: 45 male rats were selected by purposive sampling through criteria of inclusion. Animals were housed in stainless steel cages under standard conditions. Rats were divided into Group A (control), Group B (CCl_4) and Group C (CCl_4 + PTX 200 mg/kg). Blood samples were centrifuged to get sera for the estimation of serum bilirubin, creatinine and liver enzymes. Liver tissue sections (3-5µ thickness) were stained with H & E and Microscopic tissue findings were noted. Statistical software SPSS version 22.0 (IBM corporation) was used for statistical analysis ($P \leq 0.5$).

Results: Serum bilirubin and creatinine in control group in experimental groups B and C were raised ($P<0.05$). Pentoxifylline treated group C rats revealed reduction of 65% in ALT, 58% in AST, 48% LDH and 71% ALP ($P<0.05$). Liver histology was also improved in PTX treated group C ($P<0.05$).

Conclusion: Pentoxifylline shows hepatoprotective potential against chemical induced liver injury, however mechanism of action remains to be elucidated.

Key Words: Carbon tetrachloride, Rat model, Liver injury, Pentoxifylline

Citation of article: Shaikh KR, Tabassum S, Shaikh S, Memon S, Siddiqui SS, Soomro UA. Investigating Hepatoprotective Effects of Pentoxifylline in Carbon Tetrachloride Induced Liver Injury in Rat Model. Med Forum 2020;31(7):55-59.

INTRODUCTION

The Pentoxifylline (PTX) is one of the methylxanthine drugs that have been used in clinical practice for the medicinal purpose. Sole indication of PTX is for the peripheral arterial diseases (PAD). PTX is used for treating the PAD for dilating the occluded arteries since decades back.[1-3] PTX is prescribed as vasodilator agent for the dilating the arteries in PAD, particularly in those suffering from severe intermittent claudicating (IC).

[1.] Department of Pharmacology / Biochemistry[2], Suleman Roshan Medical College, Tando Adam, Sindh, Pakistan.
[3.] Department of Biochemistry / Physiology[4], Khairpur Medical College, Khairpur Mirs, Sindh, Pakistan.
[5.] Department of Pathology / Hematology[6], Indus Medical College, Tando Muhammad Khan, Sindh, Pakistan.

Correspondence: Dr. Kashif Rasheed Shaikh, Associate Professor of Pharmacology, Suleman Roshan Medical College, Tando Adam, Sindh, Pakistan.
Contact No: 0333 710 33 24
Email: mailboxKxm@gmail.com

Received: January, 2020
Accepted: April, 2020
Printed: July, 2020

It`s pharmacological effects are exerted through blockade of the phosphodiesterase-4 type (PDE-4) that leads to improvement in blood flow through microcirculation and clogged capillaries, perfusing the tissues better. Increased blood supply to tissues improves the cell functioning. Anti- inflammatory effect has been reported by previous studies.[1-3] Anti inflammatory effect has been exploited for treating the liver affections. Hepato protective effects have been proved in previous studies.[1-3] Liver is a major glandular organ destined for metabolic reactions. Anatomically, it is described as the largest glandular organ of human body. The functional parenchyma cells are called the '*hepatocyte*'. Hepatocytes are engaged in the handling, distribution and re-distribution of digested food. Thus liver is metabolically active gland involved in biochemical reactions. Metabolic reactions of carbohydrate, protein and lipids are the major biochemical reactions.[4] Beside this, liver is involved in the detoxification of toxins, poisons and drugs. It also scavenges and neutralizes oxygen derived free radicals, called the reactive oxygen species (ROS). Many of acute and chronic liver diseases have been caused by these ROS. The ROS are involved in causing inflammatory and non- inflammatory liver diseases. Inflammatory diseases include viral and alcoholic

hepatitis; while non- inflammatory diseases include; ischemia/reperfusion induced liver injury, non-alcoholic steatohepatitis, non- alcoholic fatty liver disease, drug induced liver necrosis, and toxin induced hepatotoxicity and cholestasis. Anti tubercular drug toxicity has been observed commonly in the clinical practice.[5] Many of xenobiotics are detoxified by the liver. Thus liver is the seat of detoxification mainly because of two reasons; first – it neutralizes the intestinal toxins reaching to it through the porto-systemic circulations mixed in the digested and absorbed nutrients; and second – it is the major site of detoxification of toxins and drugs and their excretion through the entero- hepatic pathway. In cases of liver diseases, the toxins and drugs skip detoxification, enter porto-systemic circulation and interfere with the metabolic reaction in the body.[6,7] Liver disease in experimental animal models of chemical induced injury have been studied for the hepatoprotective effect of drugs and herbs. One such liver disease model is induced by carbon tetrachloride (CCl_4) to in animal models to analyze the hepatoprotective effects of drugs. Animal models are used for the deliberate induction of liver injury in laboratory animals for research purpose. Many drugs are tested in laboratory animals of their therapeutic significance. CCl_4 induces hepatocellular damage through the ROS generation. The ROS damages cell membrane, and nucleus, etc through lipid peroxidation.[7] ROS generation is one of the postulated mechanism of liver injury beside others. ROS medicated cell injury releases liver enzymes that are measured for research purpose. The ROS annihilates the hepatocyte cell membrane by oxidation and lipid peroxidation.[7-9] Both, liver micro anatomy and physiological functions are altered CCl_4 treated animal models. Microscopy shows the basic liver architecture in animal models.[7-9] Cytoplasmic and mitochondrial enzymes leak into the systemic circulation that are measured for determining the extent of liver injury. Liver enzymes are biomarkers of liver injury in drug and toxin induced animal models. They are also of clinical value in monitoring and treating acute and chronic liver diseases.[10] In the present experimental study, carbon tetrachloride induced liver injury rat model was prepared for researching the pharmaco-therapeutic effects of Pentoxifylline and its possible hepatoprotective effects were evaluated through the liver enzymes estimation.

MATERIALS AND METHODS

The present experimental study was conducted at the animal house of Sindh Agriculture University Tando Jam and Isra University on rat model. The study covered time period from January 2017 to August 2017. The research proposal for the present thesis based study was already taken from the ethical committee. Adult rats were selected by purposive sampling through criteria of inclusion of; body weight 150 – 200 grams, male rats, Wistar bred, active mobile and feeding well. While female rats and rats of different body weight, not feeding well, and feeling immobile and lazy were excluded from the study. Animals were kept under standard conditions. They were housed in stainless steel cages with nozzles of water bottles. Humidity and room temperature were held at 55-60% and 25 ^{0}C respectively. 12/12 hour dark light cycle was maintained. Water was available freely 24 hours. Similarly the diet was fresh chaw. Pentoxifylline was purchased from Pharmacy of the institute. It was given in doses of 200 mg/kg orally daily.[1] Carbon tetrachloride was dissolved in olive oil in equal (1:1) ratio. It was given in dose of 1.9 ml/kg orally daily for 21 days.[1] Rats were divided into control and experimental groups. Control group were tagged as Group A (n=15) - received 0.9% isotonic saline orally for 21 days, Experimental control Group B (n=15) – treated with CCl_4 orally. Drug was mixed in olive oil for 21 days, and Experimental Group C (n=15) - rats were given CCl_4 on alternate day + Pentoxifylline (200 mg/kg) orally daily for 21 days. The blood samples were collected by lancet from the retro orbital space at twenty four hours of experimental period. Blood was centrifuged to separate sera that were used for liver enzymes estimation. Liver enzyme assays kits were purchased and sera were run on Hitachi Chemistry Analyzer for the enzyme measurement. Serum Bilirubin, Serum Creatinine, ALT (Alanine transaminase), AST (Aspartate transaminase), ALP (Alkaline phosphatase) and LDH (lactate dehyderogenase) were estimated. Rats were sacrificed by cervical dislocation.[11] Abdomen was dissected in midline, peritoneum was sectioned and liver was approached. Liver pieces were collected and stored in 4% formaldehyde filled plastic jars. Tissue pieces were embedded in paraffin blocks. Tissue sections of approximately 3-5µ thickness were prepared with microtome. Tissue sections were stained H & E (Haematoxylin and Eosin staining). Microscopic slides were prepared and mounted on light microscopy. Microscopic tissue findings were noted according to the grading of; normal = 0, mild tissue architecture injury = 1+, moderate tissue architecture injury = 2+, severe tissue architecture injury = 3+ and very severe tissue architecture injury = 4+. Mild, moderate, severe and very sever tissue injury was defined as swollen and pale cytoplasm, vacuolated cytoplasm, myelin sheaths, and pyknotic nuclei with eosinophilic cytoplasm respectively.[12] Statistical software SPSS version 22.0 (IBM corporation) was used for statistical analysis, using one- way analysis of variance (1-ANOVA) for Gaussian distributed numerical variables. Post- Hoc Fischer LSD was used for difference between individual groups for continuous variables. Chi-square

test tested the categorical variables. Variable were analyzed at 95% confidence interval (P≤0.5).

RESULTS

Serum bilirubin, serum creatinine, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) are shown in the table 1. Results of experimental groups B and C were compared with control group A. Serum bilirubin in control group A was 0.73± 0.31 mg/dl compared to 6.12±1.91 mg/dl in group B and 4.01± 1.13 mg/dl (P<0.05). Serum creatinine in control group A was 0.65 ± 0.21 mg/dl compared to 3.15±0.81 mg/dl in group B and 2.01±0.39 mg/dl (P<0.05). Alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) showed manifold rise in group B compared to Pentoxifylline treated group C and control group A (P<0.001). Pentoxifylline treated group C rats revealed a decrease of 65% in ALT, 58% in AST, 48% LDH and 71% ALP (P<0.05). Experimental group B revealed +3 to +4 grade very severe liver injury compared to +1 to +3 grade of severe liver injury in Pentoxifylline treated group C (P<0.05). Histological microphotographs 1-3 show the findings in groups A, B and C.

Table No. 1: Bilirubin, Creatinine and Liver Enzymes in Animal Groups

Parameter	Groups			P-value
	Group A	Group B	Group C	
S. Bilirubin (mg/dl)	0.73± 0.31	6.12±1.91	4.01± 1.13	0.0001
S. Creatinine (mg/dl)	0.65 ± 0.21	3.15±0.81	2.01±0.39	0.0003
Alanine transaminase (IU/L)	35.87±3.10	199.7 ±11.9	131.3±11.3	0.0001
Aspartate transaminase (IU/L)	41.07±3.11	187.3±13.7	110.3±10.3	0.0001
Lactate dehydrogenase (IU/L)	145.2±9.30	435.3±21.5	210.3±31.73	0.0001
Alkaline phosphatase (IU/L)	99.87±5.71	189.6±11.01	135.1±15.3	0.0001

Table No. 2: Histological findings in Animal Groups

Parameter	Groups			P-value
	Group A	Group B	Group C	
Inflammation	0	+4	+3	0.0001
Congestion	0	+3	+2	0.0003
Vacuolar degeneration	0	+4	+3	0.0011
Pyknotic nuclei	0	+4	+2	0.001
Eosinophilic cytoplasm	0	+4	+2	0.001
Necrosis	0	+3	+1	0.0001

*****0=normal, +1= mild injury, +2= moderate injury, +3 severe injury, +4 very severe injury

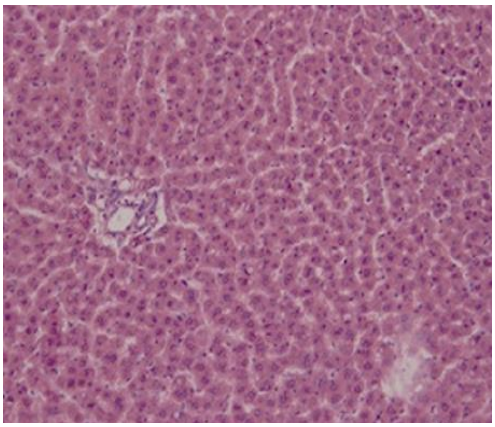

Figure No.1: Microscopy of control group A showing normal anatomical architecture of liver

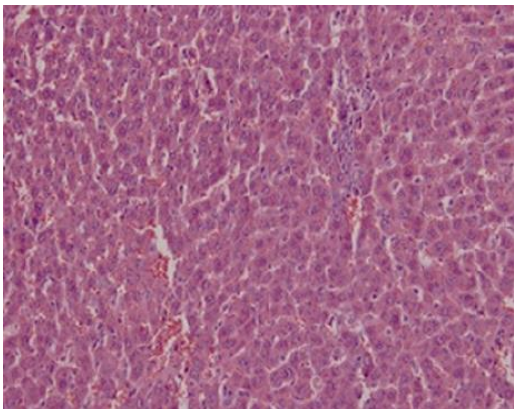

Figure No.2: CCl_4 group showing distorted tissue architecture, inflammatory cell infiltrate and necrosis

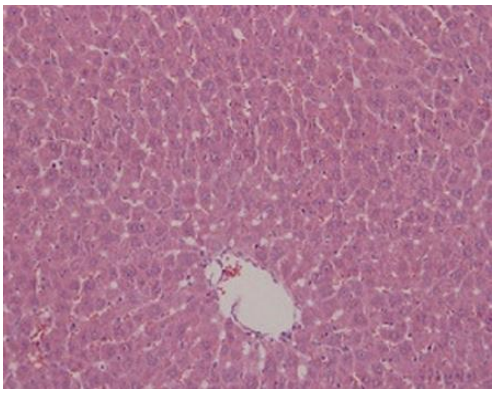

Figure No.3: Pentoxifyllline treated group C showing hepatocyte cords with congestion in sinusoids & few lymphocytic infiltrations.

DISCUSSION

The present experimental animal study investigated the hepatoprotective effects of Pentoxifylline (PTX) against chemical (CCl_4) induced liver injury in rat model. The present study reproved the hepatoprotective effects of Pentoxifylline (PTX) against chemical (CCl_4) induced liver injury. The findings are in agreement with previous studies.[1,4,5] The present study rejects null hypothesis (H_0) because the statistically significant differences were observed in both biochemical and histological markers of liver injury among control and experimental groups ($P<0.05$). The serum bilirubin and creatinine shows major improvement in the PTX treated rats group C compared to group B ($P<0.05$). The findings are in agreement with previous studies.[1,4,5] A previous study[1] analyzed the hepatoprotective effects of Pentoxifylline in ecstasy treated liver damage and reported positive findings. The hepatoprotective effective of above study supports the finding of present study. Pharmacological efficacy of PTX has been reported in alcoholic hepatitis,[13] hepatic encephalopathy[14,15] and hepatorenal syndrome.[16] A previous study[17] reported hepatoprotective effect similar to the present study. That previous study further added that the PTX prevents liver fibrosis through inhibition of cytokine and pro-collagen- I gene expression, as both facilitate fibrosis. Another previous study[18] reported pharmacological efficacy of PTX in non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NALFD). They reported[18] that the PTX ameliorates liver aminotransferase and PTX inhibits tumor necrosis factor-α (TNF-α) that is profibrinogenic cytokine. The findings amelioration of liver aminotransferase of above study is consistent with the present study as hepatoprotective effect has been observed as indicated by biochemical and histological parameters (table 1 and 2). 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. This is in agreement with previous studies[19.20] that reported the carbon tetrachloride is proven to cause liver damage with concomitant rise in liver enzymes that are the reliable indices of liver parenchyma damage and leakage of enzymes into general circulation.[19.20] In present study, the Pentoxifylline treated group C rats revealed a decrease of 65% in ALT, 58% in AST, 48% LDH and 71% ALP ($P<0.05$). Experimental group B revealed +3 to +4 grade very severe liver injury compared to +1 to +3 grade of severe liver injury in Pentoxifylline treated group C ($P<0.05$). These findings are in keeping with previous studies.[17.20] In carbon tetrachloride induced liver injury in group B shows severe rise in liver cytoplasmic and mitochondrial enzymes and liver tissue injury. This is in agreement with a previous study.[21] Above study[21] reported the carbon tetrachloride induces sever liver tissue injury with multifold rise in liver aminotransferase in experimental rats. The finding of severe rise in ALT, AST, LDH and ALP indicates severe cellular injury of liver with release of cytoplasmic and mitochondrial enzymes into the general circulation. This finding is in keeping with previous studies.[17-21] The histological findings of inflammation, congestion, vacuolar degeneration, pyknotic nuclei, eosinophilic cytoplasm and necrosis was prominently observed in the carbon tetrachloride treated group B and these findings shows improvement in the PTX treated group C. Pyknotic nuclei and eosinophilic cytoplasm are indices of severe hepato-cellular injury caused by carbon tetrachloride. These findings are in agreement with previous studies.[23,24] The evidence based findings of present study correlates well with previous studies, hence PTX may be used for clinical purpose for those suffering from chemical induced liver injury.

CONCLUSION

It is concluded that the Pentoxifylline has hepatoprotective potential against chemical induced liver injury. However, mechanism of hepatoprotective effect of Pentoxifylline remains to be elucidated. Pentoxifylline may be used for those suffering from chemical induced liver injury.

Author's Contribution:

Concept & Design of Study:	Kashif Rasheed Shaikh
Drafting:	Sadia Tabassum, Shumaila Shaikh
Data Analysis:	Shagufta Memon, Shumail Saeed Siddiqui, Umair Ali Soomro
Revisiting Critically:	Kashif Rasheed Shaikh, Sadia Tabassum
Final Approval of version:	Kashif Rasheed Shaikh

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. 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 methamphetamine (MDMA or Ecstasy). Ir J Basic Med Sci 2013; 16: 922-27.
2. Türkyılmaz Z, Hatipoğlu A, Yüksel M, Aydoğdu N, Hüseyinova G. Comparison of effects of melatonin, pentoxifylline and dimethyl sulfoxide in experimental liver ischemia-reperfusion injury by three different methods. Eur Res J 2019;5(1): 148-158
3. Yucel B , Coruh A, Deniz K. Salvaging the Zone of Stasis in Burns by Pentoxifylline: An Experimental Study in Rats. J Burn Care Res 2019; 40 (2): 211–9.
4. Essawy AE, Abdel-Moneim AM, Khayyat LI and 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.
5. Wafay H, El-Saeed G, El-Toukhy S, Youness E, Ellaithy N, Agaibi M and 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.
6. Patil S, Maheshwari S. Efficacy of pentoxifylline in the management of oral submucous fibrosis. J Orofac Sci 2014; 6: 94-8.
7. Gupta K, Mahakalkar C, Maple M, Deshpande S, Ladhha P, Jain N. A comparative study of cilostazol and Pentoxifylline in intermittent claudication in peripheral arterial disease. J Datta Meghe Inst Med Sci Univ 2017;12: 11-6.
8. Luo M, Dong L, Li J, Wang Y, Shang B. Protective effects of pentoxifylline on acute liver injury induced by thioacetamide in rats. Intl J Clin Exp Pathol 2015; 8(8): 8990–6.
9. Bektas S, Karakaya K, Can M, Bahadir B, Guven B, Erdogan N, et al. The effects of tadalafil and pentoxifylline on apoptosis and nitric oxide synthase in liver ischemia/reperfusion injury. Kaohsiung J Med Sci 2016; 32 (7): 339-47.
10. Kor A, Shahroozian E, Ahmadi- Hamedani M, Naeimi S. The Modulatory Effects of Pentoxifylline in Biochemical Changes Induced By 17α-Ethinyl Estradiol in the Rat Model. Iranian J Toxicol 2018; 12 (4): 5-9.
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 and 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. Al-Zahra JIA, Ismael DK, Al-Shawi NN. Preventive effects of different doses of pentoxyfilline against ccl4-induced liver toxicity in rats. Iraqi J Pharm Sci 2017;18:39-45.
14. 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.
15. Amini M, Runyon BA. Alcoholic hepatitis 2010: a clinician's guide to diagnosis and therapy. World J Gastroenterol 2010; 21 (16):4905-4912.
16. Mc Hutchison 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.
17. 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.
18. 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.
19. Rajesh M and Latha M. Preliminary evaluation of anti-hepatotoxic activity of Kamilari, a polyherbal formulation. J. Ethnopharmacol 2004; 91: 99-104.
20. Bashandy S, Al-Wasel S. Carbon tetrachloride-induced hepatotoxicity and nephrotoxicity in rats: Protective role of vitamin C. J Pharm Toxico 2001; 16(30): 283-92.
21. Cruz Jr RJ, Yada-Langui MM, de Figueiredo LF, Sinosaki S. Rocha e Silva M. The synergistic effects of pentoxifylline on systemic and regional perfusion after hemorrhage and hypertonic resuscitation. Anesth Analg 2006; 102:1518e24.
22. Luo M, Dong L, Li J, Wang Y, Shang B. Protective effects of pentoxifylline on acute liver injury induced by thioacetamide in rats. Int J Clin Exp Pathol 2015; 1(8):8990e6.
23. Ji Q, Zhang L, Jia H, Xu J. Pentoxifylline inhibits endotoxin induced NF-kappa B activation and associated production of proinflammatory cytokines. Ann Clin Lab Sci 2004; 34: 427e36.
24. Aslan A, Karagüzel G, Çelik M, Uysal N, Yücel G, Melikoglu M. Pentoxifylline Contributes to the Hepatic Cytoprotective Process in Rats Undergoing Hepatic Ischemia and Reperfusion Injury. Eur Surg Res 2001; 33:285–90.