Original Article

Anatomy

Hepatoprotective Effects of Curcuma Longa against Carbon Tetrachloride Induced Liver Injury in Rats

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ABSTRACT

Objective: To investigate the protective effect of Curcuma longa (CL) against carbon tetrachloride (CCl_4) induced liver injury in adult male Wistar rat model.

Study Design: Experimental/Analytical study

Place and Duration of Study: Animal House, Isra University Hyderabad from March to December 2013.

Subjects and Methods: Forty five adult male Wistar rats were divided into three groups; Group 1. controls received 0.9% isotonic saline, Group 2. received CCl_4 orally (1.9mg/kg) mixed in olive oil, and Group 3. received the CCl_4+CL (250mg/kg) 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, Fischer`s LSD 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 CCl_4+CL groups (p=0.0001). Liver enzymes and histology were deranged significantly in CCl_4 group compared to controls and CCl_4 +CL group (p=0.0001). The CCl_4+CL group showed less elevation of liver enzymes and derangement in liver histology 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 Curcuma longa protects against oxidative damages caused by carbon tetrachloride induced liver injury in rat model.

Key Words: Curcuma longa, Carbon Tetrachloride, Liver injury.

INTRODUCTION

Curcuma longa (CL) is a rhizomatous perennial herb that belongs to the family Zingiberaceae, native to South Asia and is commonly known as turmeric.[1] In Sindhi, it is commonly known as “Hade”. The turmeric plant is a popular ingredient for preparing culinary dishes. In addition, it is used as herbal remedy due to the prevalent belief that the plant has medical properties. In folk medicine, the rhizome juice from C. longa is used in the treatment of many diseases such as anthelmintic, asthma, gonorrhea and urinary, and its essential oil is used in the treatment of carminative, stomachic and tonic.[2] In traditional medicine, several plants and herbs have been used experimentally to treat liver disorders, including liver cirrhosis. [3,4] C. longa possesses antioxidant[5], anti-tumor[6], antimicrobial[7], anti-inflammatory[8], wound healing[9], and gastroprotective activities.[10]

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.[11]

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[11, 12] causing anatomical disruption of liver architecture and physiological disturbances.[13] The hepatocyte injury causes leakage of cytoplasmic and mitochondrial enzymes in the blood streams.[14]

The cytoplasm and mitochondrial enzymes of hepatocytes are clinically used as markers of liver injury, and for monitoring and treating the liver diseases. 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.[11,15]

The previous studies have shown that the aqueous extract of C. longa has hepatoprotective activity against carbon tetrachloride toxicity[19]. The present study aims to investigate the possible hepatoprotective effects of the ethanolic extract of Curcuma longa rhizomes against Carbon tetrachloride (CCl_4)-induced hepatotoxicity in adult male Wistar rat model.

MATERIALS AND METHODS

The present original study was conducted at the animal house of Isra University from March to December 2013. Adult male Wistar rats of 250-300 grams were included while female rats and rats weighing <250 grams or >300 grams were excluded from the study protocol. The animal's house is well equipped with essential facilities like an optimal room temperature with 55-60% humidity and exposure to 12 hour light-dark cycles. The fresh alfalfa and clean water are provided freely. The rats were divided into three groups;

Group 1. Control Group (n=15) Rats received 0.9% isotonic saline orally on alternate day for three successive weeks and served as control group,

Group 2. Carbon tetrachloride Group (n=15) Rats were given CCl_4 orally mixed in olive oil on alternate day for three successive weeks and

Group 3. Experimental Group (n=15) Rats received Curcuma longa (250 mg/kg) and CCl_4 on alternate days for three successive weeks

Experimental Details: The CL was purchased from Medical store of Isra University Hospital. The Curcuma longa was administered in a dose of 250 mg/kg orally.[16] 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.[15] The animals were sacrificed using standard method as described by Nayak et al. (2006)[17] In order to examine the liver tissue, the liver of the sacrificed animals was removed promptly and preserved in formaldehyde.

Blood sampling: The blood samples were collected from tail at twenty four hours of experimental period. Sera were separated by centrifugation at 300xs for ten minutes. Serum samples were used to estimate liver enzymes.

Biochemical 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).[18]

The data was analyzed on SPSS version 21.0 (IBM corporation). The continous variables were presented as mean±SD using one-way ANOVA and Fischer`s LSD test. 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 among groups as indicated by blood enzyme levels in animal groups. The serum LDH, ALP, and ALT, AST of Rats treated with carbon tetrachloride were found elevated compared with control group after three weeks, with a highly significant p-value (p=0.001).

The CCl_4+ Curcuma longa (CL) 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+CL group shows significant reduction in the liver enzyme elevation compared to CCl_4 group alone (p=0.001). The finding shows significant hepatoprotection by the CL in CCl_4 induced injury. The liver enzyme assays among different groups are shown in table.1.

Table. 1. Biochemical liver parameters in controls, *CCl_4 and CCl_4+ Curcuma longa (CL) groups (n=45)

Groups	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	ALP (IU/L)
Group. 1 (Controls)	48.9± 3.19	91.3± 15.1	712.4± 41.7	83.6± 8.71
Group. 2 (*CCl_4)	189.6± 11.91	479.7 ± 19.9	2278.8± 117.6	165.1± 8.02
Group. 3 (*CCl_4 + CL)	87.7± 17.92	181.3± 18.3	1938.6± 141.3	135.7± 18.1

*Carbon tetrachloride

Table. 2. Histology of liver injury of controls, *CCl_4 and Curcuma longa (CL) groups (n=45)

Groups	Inflammatory cell	Congestion	Vacuolar degeneration	Necrosis
Group. 1 (Controls)	0	0	0	0
Group. 2 (*CCl_4)	++++	++++	+++	++++
Group. 3 (*CCl_4 + CL)	+++	++	++	++

*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 were seen in control group. 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. The centrilobular hepatocytes show hydropic changes and necrosis, while midzonal and peripheral hepatocytes show vacuolar degeneration and fatty changes in CCl_4 group. In CCl_4+CL animals, liver tissue sections reveal less significant derangement of hepatocytes cords, hepatocytes damage and necrosis was limited compared with CCl_4 group.

DISCUSSION

The present study is an original research work, which investigates the effect of Curcuma longa (CL) 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 CL as evidenced by biochemical and histological marker of liver injury.

Curcumin, the most common antioxidant constituent of Curcuma longa rhizome extract, was reported to enhance apoptosis of damaged hepatocytes which might be the protective mechanism whereby curcumin down-regulated inflammatory effects and fibrogenesis of the liver. [19]

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.[20] The Hurkkeri et al.[20] 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.[21]

The ethanolic extract of C. longa rhizomes showed a significant hepatoprotective effect when orally administrated in doses of 250 mg/kg. The main constituents of CL extract are the flavonoid curcumin and various volatile oils, including tumerone, atlantone, and zingiberene.[1] The hepatoprotective effects of turmeric and curcumin might be due to direct antioxidant and free radical scavenging mechanisms, as well as the ability to indirectly augment glutathione levels, thereby aiding in hepatic detoxification.[22] The volatile oils and curcumin of C. longa exhibit potent anti-inflammatory effects.[23]

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. [24,25]

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.[26,27] The carbon tetrachloride is metabolized to free radical during its metabolism and detoxification in smooth endoplasmic reticulum by the cytochrome P450.[28] The findings of present study are highly comparable to a recent study of Salma et al.[1] The Salma et al[1] reported hepatoprotective effects of CL in thiacetamide induced liver cirrhosis in rat models. The present study concludes that the Curcuma longa decreases the carbon tetrachloride induced oxidative stress and liver damage.

CONCLUSION

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

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