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RESEARCH ARTICLE

Nephroprotective Roles of L-Cysteine, Silymarin and Ursodeoxycholic acid, against Carbon Tetrachloride induced Nephrotoxicity in Male Albino Rats

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ABSTRACT

Background and objectives: The comparison of histopathological and biochemical effects of L-cysteine, silymarin, and ursodeoxycholic acid, which may have a protective effect on kidney function against carbon tetra chloride (CCl4) induced nephrotoxicity, were investigated in this study.
Methods: Twenty adult male rats were divided into five groups and treated as follows; Group I: control group was administrated 1.5 ml/kg. B.W normal saline (0.9%) orally by gavage, group II: carbon tetrachloride group (CCl4 1.5 ml/kg. B.W), group III: L-cysteine (100mg/kg B.W.) +CCl4 group, group IV: ursodeoxycholic acid (50 ml/kg B.W.) +CCl4 group, and group V: Silymarin 100 mg/kg. B.W with CCl4, the experiment lasted 30 days.
Results: Oxidative stress caused by CCl4 leads to an increase in serum creatinine and urea levels while decreasing the level of uric acid and albumin compared with the control group. L-cysteine, silymarin, and the drug presented ameliorating effects by decreasing the creatinine and urea levels while increasing uric acid and albumin levels. Histopathological results showed that CCl4 caused oxidative damage in kidney tubules in comparison with the control group, while Silymarin and L-cysteine showed nephroprotective effects.
Conclusions: The findings show that CCl4 caused hepatic and renal toxicity, and this toxicity can be attenuated by the administration of L-cysteine, Ursodeoxycholic acid, and silymarin, probably by antioxidant action, although further tests are required to fully assess the antioxidant property of L-cysteine, Ursodeoxycholic acid, and silymarin against CCl4 toxicity.

Keywords: L-cysteine, Silymarin, CCl4, Nephrotoxicity, Ursodeoxycholic acid.

INTRODUCTION

The kidney is an important organ with vital biological functions in the excretion of metabolic waste. It is very sensitive to xenobiotics and metabolites produced by different toxic substances. Reactive oxygen and nitrogen species during xenobiotic metabolism are barely toxic, but they become toxic when generated in an amount that exceeds the ability of the organ to defend its systems (Jacobs and Marnett, 2010, Ahmed et al., 2019).

Carbon tetrachloride (CCl4) is an environmental pollutant with acute toxicity and is widely used on experimental animals for the induction of toxicity in different organs such as kidneys and liver, including alteration in the oxidative stress status (Salman and Randa, 2016).

L- Cysteine (L-Cys) is a thiol-containing amino acid (figure 1) which is a potent antioxidant amino acid found in the tri-peptide glutathione, the scavenger of a wide variety of free radicals. L- Cysteine is a nonessential amino acid, is one of the building blocks of many proteins, and has antioxidant properties as a counterbalance to oxidative stress (O.S.). In the body, L-Cys contributes 2% of total proteins in cell membranes, connective tissue, and the myelin sheaths around neurons, which protect neurons from O.S. and harsh extracellular environmental conditions. Because L-Cys contains a thiol group (-S.H.), this thiol group is responsible for many important

biological functions in the human body. L- Cysteine contains Disulfide Bridge, Disulfide Bridge is a type of covalent bond that plays a role in the folding and stabilization of proteins, thereby supporting the biological activities of proteins.

L-Cysteine may be capable of preventing and minimizing acute kidney injury (Briguori et al., 2011, Piste, 2013, Ahmed, 2016, Clemente Plaza et al., 2018, Hsu et al., 2022). L- Cysteine is one of the important exogenous antioxidants it was reviewed by some workers to evaluate its antioxidant activity on kidney damage by different chemical agents in rats. It also showed to reduce lipid peroxidation and enhance the antioxidant level of the biomarker in rats treated with CCl4. Liver and kidney injury have shown to be improved in rats with toxicity, the improvement mediated through attenuated through the increase in antioxidant capacity when administered some antioxidant agents (Hamza et al., 2015, Hamza and El-Shenawy, 2017, Al-Salmi et al., 2019). Also, L-Cysteine is a source of energy production and biomass (Bonifácio et al., 2021).

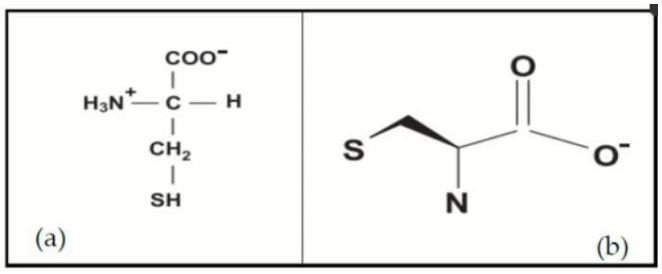

Fig.1: (a) Chemical structure of L-cysteine (b) structural model of L-cysteine (Clemente Plaza et al., 2018)

Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid that comes from animal bile. UDCA has been proven to enhance a variety of metabolic parameters and delay histological progression and prolong transplant-free survival (Harms et al., 2019).

Silymarin is obtained from the Silybum marianum plant, its active component is Silybum which biochemically exhibits many activities such as antioxidant and scavenger against free radicals, as well as anti-inflammatory effects (MacDonald-Ramos et al., 2020). The goal of this research was to determine the biological function of L-Cysteine, silymarin, and Ursodeoxycholic acid, in protecting the kidney against nephrotoxicity caused by CCl4 and its metabolites in Wister albino rats.

SUBJECTS AND METHODS

Experimental animals and design

Twenty male albino rats were obtained from the laboratory animal of the Education college-biology department, at Salahaddin University. The animals were housed in cages in a room with controlled light (12hr dark/12h light), a temperature of 22±3°C, and a standard rat diet and tap water were available ad libitum. The rats were put into five groups at random, each with four rats.

Group I: Control group rats were intraperitoneally injected with normal saline and received 0.5 ml of normal saline orally by intragastric gavage twice a week.

Group II: Rats were intraperitoneally injected with CCl4 (1.5 ml/kg B.W.) twice a week.

Group III: Rats received L-cysteine (100 mg/kg B.W. orally six days/week) and CCl4 intraperitoneally (1.5 ml/kg B.W.) twice weekly.

Group IV: Rats received Ursodeoxycholic acid (50 mg/kg B.W.) six days/week along with CCl4 intraperitoneally (1.5 ml/Kg B.W.) twice weekly.

Group V: Rats received silymarin orally (100 mg/kg B.W. six days/week) with CCl4 injection intraperitoneally (1.5 ml/kg B.W.) twice weekly.

At the end of the experiment which lasted 30 days, the animals were fasted overnight following the final treatment. Later on, all the rats were euthanized with general anesthesia using a combination of xylazine (0.4 ml) and ketamine (0.1ml), samples of blood were drawn by heart puncture into the non-heparinized gel tube for renal function analysis and then the serum was separated by centrifugation and kept frozen until analyses.

Biochemical Tests

Estimation of Uric acid

The serum uric acid was estimated by the enzymatic colorimetric method, using the (liner) diagnostic kit, (LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN), and (U.V./Visible spectrophotometer) (Fossati et al., 1980).

Estimation of creatinine

Creatinine in serum was analyzed by using a creatinine Kinetic colorimetric method fixed time (liner) kit (LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN (Larsen, 1972).

Estimation of Urea

Urea in serum was determined by enzymatic colorimetric method using the urea Berthelot diagnostic liner kit (LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN (Chaney and Marbach, 1962).

Estimation of albumin

The serum albumin was measured by using the (Bromocresol green) Biosystems diagnostic kit (BioSystems S.A. Costa Brava 30, Barcelona (Spain)) (Doumas et al., 1997).

Histological Study

The kidney swere removed and fixed in 10% formal saline, paraffin blocks were created, and 5mm sections were prepared. Hematoxylin and Eosin were used for histological staining; then, the sections were examined and photographed using a light microscope with a built-in camera. All the slides were examined to find the eextent of kidney tissue injury.

Statistical analysis

Overall data were statistically assessed using the SPSS program (Version 17) to compare means between groups using one-way ANOVA followed by Tukey's test. In the statistical analysis, the probability value below 0.05 was considered significant.

RESULTS

Biochemical analysis

As shown in table (1), a significant decline in uric acid for groups (II), (III), (IV), and (V) was found in comparison to the control group, it was noticed that groups (III) and (V) showed statistically higher values compared to group (II), and non-significant values were observed between groups (IV) and (II)

A significant elevation in creatinine level for group (II) was depicted in comparison to the control group. The creatinine concentration for the treated groups (III), (IV), and (V) was found to be near the normal control value with no significant difference with the control group (I). While a significant reduction in creatinine levels of groups (III), (IV), and (V) in comparison to group (II) was found.

Table 1: Serum levels of biochemical parameters (uric acid, creatinine urea, and albumin) in all

Parameters	*Uric acid (mg/dl)*	*P*	*P**	*Creatinine (mg/dl)*	*P*	*P**	*Urea (mg/dl)*	*P*	*P**	*Albumin (g/dl)*	*P*	*P**
Group (I)	1.3±0.011			0.5±0.0035			44±4.89			21±3.33		
Group (II)	0.3±0.0026	S		1.1±0.0029	S		48±5.35	S		15±1.98	S	
Group (III)	0.6±0.0037	S	S	0.5±0.0011	N.S.	S	43±5.22	N.S.	S	19±1.84	N.S.	S
Group (IV)	0.4±0.0013	S	N.S.	0.5±0.0026	N.S.	S	43±2.23	N.S.	S	23±3.00	N.S.	S
Group (V)	1.0±0.0019	S	S	0.65±0.0034	N.S.	S	36±3.19	S	S	20±1.99	N.S.	S

S: Significant

N.S.: Non- Significant

P: Probability between group (I) with groups (II), (III), (IV), and (V).

P*: Probability between group (II) with groups (III), (IV), and (V).

No significant alteration in urea levels for groups (II), (III), and (IV) in comparison with the control group was recorded, while group (V) had significantly lower urea levels when compared to the control group. Also, a significant decrease amount is shown in groups (III), (IV), and (V) when compared to group (II).

The rats of group (II) had significantly lower albumin concentrations than the control group, while no significant difference for groups (III), (IV), and (V) from the control group was observed. On the other hand, Groups (III), (IV), and (V) showed significantly elevated values in comparison to group (II).

Histological Examination

Histopathological findings in all the groups are presented in Figures (2 & 3). CCl4 showed degenerative effects for renal tissue, including shrinkage of the glomerulus and absence of renal space around the glomerulus, which is well defined in (Fig. 2 B and C), cortical congestion between the proximal and distal tubules (Fig. 2 C); also, CCl4 caused accumulation of cast in the lumen of the renal

tubules (Fig. 2 D). Furthermore, the control group's renal structures were intact, and no abnormal symptoms were found in light microscope examinations (Fig. 2 A).

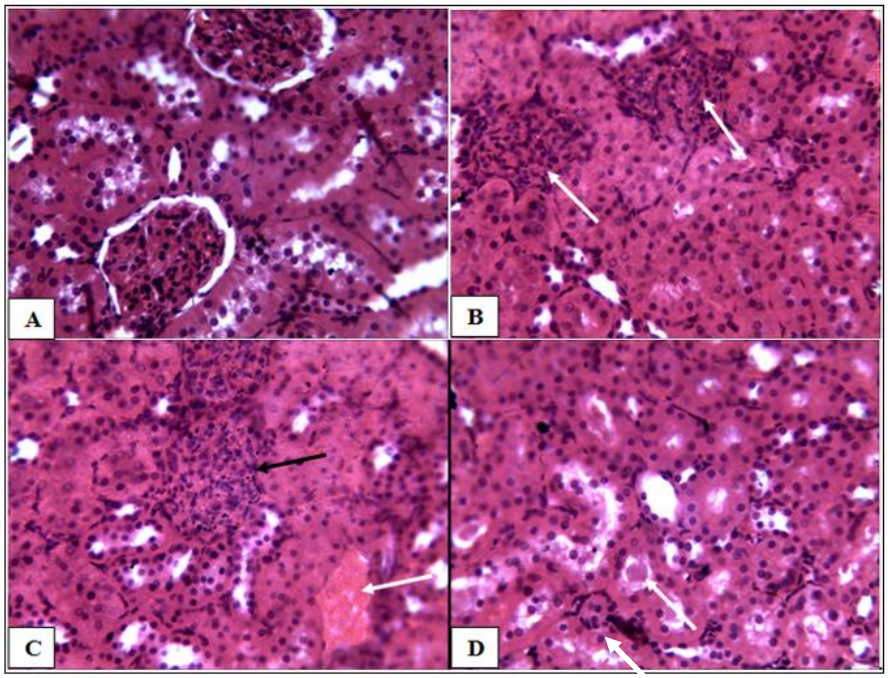

Fig.2 : Section through the kidney of A) Control group, B, C, D) CCl_4 treated group (H&E, 400x)

Administration of L-cysteine and silymarin showed nephroprotective effects against tubular damage as shown in Fig (3 A&B). while the Ursodeoxycholic acid has less protective effects than other treated materials in which degeneration feature in the sections with Ursodeoxycholic acid still found including intraluminal casts, dilation in the lumen of some tubules and absence of the brush border in most of the tubules as shown in Figure (3 C&D) when compared with control group section.

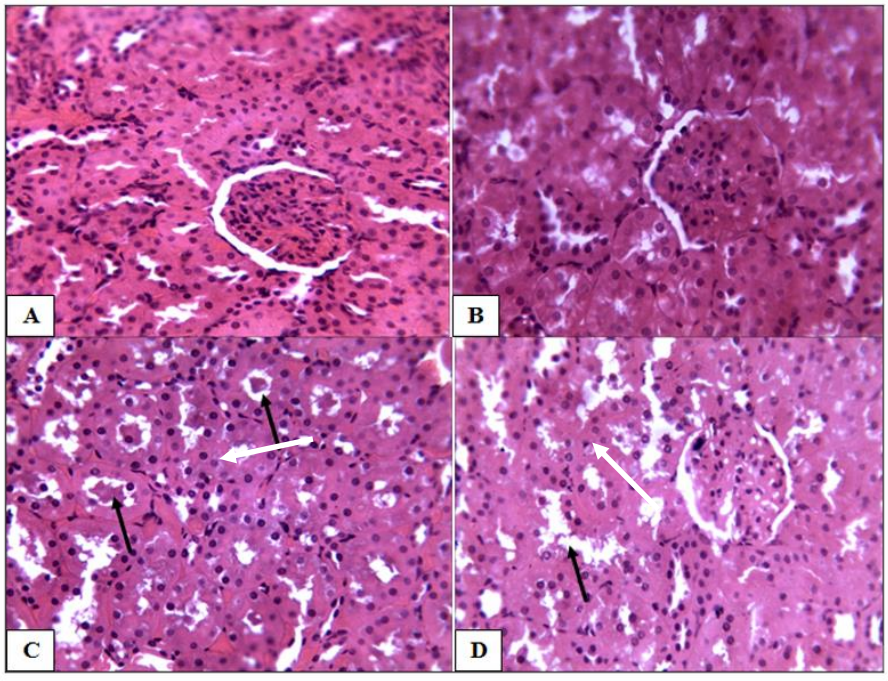

Fig.3 : Section through the kidney of A) L-cysteine supplementation with CCl_4, B) silymarin with CCl_4 and C, D) CCl_4+ Ursodeoxycholic acid (H&E, 400x).

DISCUSSION

The mechanism of CCl4-induced liver injury is well-documented in rat models by many researchers (Ogeturk et al., 2005). It has also been observed that CCl4 administered systemically to rats was scattered in greater quantities within the kidney than in the liver. (Mukai et al., 2002). Numerous research findings have already shown that CCl4 can cause kidney dysfunction and histopathological changes. (Yoshioka et al., 2016).

Comprehensive research has been done on the molecular basis of CCl4-induced kidney damage. CCl4 is particularly hydrolyzed by the cytochrome P450 CYP2E1 enzyme to generate the extremely toxic trichloromethyl free radical and/or trichloromethyl peroxyl free radical. These free radicals cause damage to the kidney because they bind to cell membrane lipids, intracellular proteins, or DNA, causing lipid peroxidation in the cell membrane, Denaturation of proteins, and oxidative DNA damage, which results in necrosis of kidney cells (Hismiogullari et al., 2015).

Generally, kidney function markers are used to determine a renal injury as uric acid and creatinine levels. The level of uric acid reduced in groups (III) and (IV) in comparison to the control group, the effect of L-cysteine and ursodeoxycholic acid is less than silymarin which could increase uric acid in the serum of treated rats. Since uric acid is considered an antioxidant and has a protective effect returning to high values is beneficial (Mohan et al., 2018).

Examination of kidney function revealed that CCl4 had a detrimental impact on the kidney, which was attributable to the elimination of CCl4 metabolites by the kidney and the subsequent damage to kidney tissue. Studies reported that CCl4 initiated lipid peroxidation which reflects the high plasma levels of creatinine, uric acid, and urea (Adewole et al., 2007, Venkatanarayana et al., 2012).

Creatinine levels for all studied groups (III), (IV), and (V) showed no significant differences from the control group, which indicates a defense mechanism against necrosis occurred in the kidney of the treatment groups, while a non-significant increase in serum creatinine in the group(II) was observed, this outcome is in line with results of Mukai et al. (2002). Because of its high CYP content, the liver is commonly assumed to be the primary target organ when CCl4 is administered systemically. Conversely, CYP2E1 metabolizes less than 5% of the most highly prescribed drugs but plays an important role in determining drug toxicity(Urquhart and Nolin, 2020) Furthermore, exposure to CCl4 led to oxidative stress and lipid peroxidation in hepatic and renal tissues(Mazani et al., 2022). Based on these findings, we can assume that kidney injury is due to CCl4 administration.

Regarding serum albumin our data shows that CCl4-intoxicated rats had a significant decrease in serum albumin, this outcome is consistent with the results of Fararh et al. (2016), Considering that the liver is the main organ for protein synthesis, specifically albumin, the decrease is due to toxicity and liver damage due to CCl4 administration. Hypoalbuminemia occurs commonly in association with chronic hepatic disease and liver cirrhosis (Gounden et al., 2018). Also, maybe due to low feed intake, decrease absorption of proteins. In addition, CCl4 affects the kidney which led to albuminuria. While rats treated with the L-cysteine, ursodeoxycholic acid, and silymarin after CCl4 administration maintained close to normal values of serum albumin in comparison with the control group and prevented the CCl4 administration-induced decrease, this means that these substances have been able to stimulate partial liver function recovery, decrease liver inflammation, and provide the best treatment for hepatic fibrosis.

Consistent with our results, Vargas-Mendoza et al. (2014) found that silymarin administration significantly reversed the effect of CCl4 by increasing serum albumin, This could be linked to silymarin's ability to improve ribosome synthesis and stimulate DNA and protein production (Khazaei et al., 2021). Compounds used for treatment; L-cysteine, ursodeoxycholic acid, and silymarin could be useful in the treatment of kidney necrosis, yet silymarin has a better effect on uric acid. L-cysteine and ursodeoxycholic acid showed an improved effect on uric acid which is limited and could be due to the short period for administration. On the other hand, the effect of the three substances used for treatment showed a protective effect due to decrease creatinine levels.

CONCLUSION

In conclusion, the findings show that CCl4 caused hepatic and renal toxicity, and this toxicity can be attenuated by the administration of L-cysteine, Ursodeoxycholic acid, and silymarin, probably by antioxidant action, although further tests are required to fully assess the antioxidant

property of L-cysteine, Ursodeoxycholic acid, and silymarin against CCl4 toxicity.

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