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Protective Effects of Alpha Lipoic Acid on Carbon Tetrachloride-Induced Liver and Kidney Damage in Rats

A.O. Morakinyo, G.O. Oludare, A.A. Anifowose and O.A. Adegoke
Department of Physiology, College of Medicine of the University of Lagos, Lagos, Nigeria

Abstract: Carbon tetrachloride (CCl_4) is a well known toxicant and exposure to this chemical is known to induce oxidative stress by the formation of free radicals. The present study investigates the *in vivo* effects of alpha lipoic acid (ALA) on CCl_4-induced hepatic and renal toxicities. Twenty-four Sprague-Dawley rats were divided into four groups of 6 animals each and treated for 10 consecutive days. Group 1 was given olive oil only. Group 2 received CCl_4 intra-peritoneally (i.p.) at a dose of 0.8 mg/kg as a 30% olive oil solution. Group 3 was given ALA only at a dose 25 mg/kg. Group 4 was given both CCl_4 and ALA, respectively. At the end of experiment, the antioxidant status in both the liver and kidney tissues were estimated by determining the activities of antioxidant enzymes; reduced glutathione, superoxide dismutase, catalase as well as the level of lipid peroxidation via thiobarbituric reactive substance. The liver and kidney functions tests were also performed in addition to their histopathological evaluation. Results obtained showed significant adverse changes in the levels of all measured parameters in CCl_4 treated rats. However, treatment with ALA attenuated the adverse changes in the CCl_4-induced rats. Our findings suggest that ALA protects the liver and kidney against CCl_4-induced damage through its significant effects on the antioxidant activities.

Key words: Alpha lipoic acid, antioxidants, carbon tetrachloride, hepatotoxicity, nephrotoxicity, oxidative stress

INTRODUCTION

Carbon tetrachloride (CCl_4) intoxication in animals is an experimental model of oxidative stress induced hepatotoxicity and nephrotoxicity (Recknagel *et al*., 1991; Loguercio and Federico, 2003). There is excessive generation of free radicals such as trichloromethyl and trichloromethyl peroxide radicals from the metabolic conversion of CCl_4 by cytochrome P-450 (Stal and Olson, 2000), which consequently induces oxidative changes to many cellular bio-molecules including lipid peroxidation of cell membrane in many tissues (Basu, 2003). Alpha Lipoic Acid (ALA) is a naturally occurring antioxidant and plays a fundamental role in metabolism. ALA has been shown to affect cellular processes, alter redox status of cells, and interact with thiols and other antioxidants (Packer *et al*., 2001). ALA is a unique antioxidant because it has beneficial effects on energy production, and is also an essential cofactor of mitochondrial complexes. Infact, there is evidence that ALA and its metabolites are capable of scavenging a variety of reactive oxygen species such as peroxynitrite (Trujillo and Radi, 2002), nitric oxide (Vriesman *et al*., 1997), hydroxyl radical, superoxide anion (Suzuki *et al*., 1991), peroxyl radical (Kagan *et al*., 1992), and hydrogen peroxide (Scott *et al*., 1994). Furthermore, ALA appears to regenerate other endogeneous antioxidants (Bast and Haenen, 2003) and has a unique property of neutralizing free radicals without itself being consumed in the process (Shay *et al*., 2009).

Considering its role in various biochemical processes, many researchers have shown keen interest in the pharmacological effects of ALA in the therapy of many diseases that has oxidative pathophysiology (Jacob *et al*., 1996; Rahnau *et al*., 1999; Ziegler *et al*., 2004). Given that oxidative stress plays a fundamental role in CCl_4 toxicity, the present study was undertaken to explore the influence of ALA on CCl_4 induced hepatic and renal toxicity. To this end, the radical scavenging activity of ALA was evaluated by estimating the activities of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), as well as the extent of lipid peroxidation in both the liver and kidney tissue homogenates. In addition, the study also examined the protective effects of ALA on liver and kidney functions in CCl_4 intoxicated rats.

MATERIALS AND METHODS

Drugs and chemical reagents: ALA, CCl_4, Heparin, Phenobarbital and olive oil were obtained from Sigma

Corresponding Author: A.O. Morakinyo, Department of Physiology, College of Medicine of the University of Lagos, Surulere 23401, Lagos, Nigeria, Tel.: +2348055947623

(USA). Others reagents such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were from Boehringer, Germany. All the other chemicals and test kits used were of analytical grade.

Animals: Male Sprague-Dawley rats aged 12 weeks weighing 190-220 g were obtained from the Laboratory Animal House of the College of Medicine of the University of Lagos. Animals were allowed to acclimate for seven days; they were fed with standard pellet diet and water *ad libitum* at 20-25ºC under a 12 h light/dark cycle. Food was withdrawn one day before the experiment but water continued to be provided. All animal handling and experiment protocols complied with the international guidelines for laboratory animals.

CCl_4-induced acute liver damage model: Twenty four (24) animals were divided into four groups: Group 1 (control), Group 2 (CCl_4 treatment), Group 3 (ALA treatment), Group 4 (CCl_4 + ALA treatment). Each group has six animals. Rats from Groups 2 and 4 were given CCl_4 intra-peritoneally (i.p.) at a dose of 0.8 mg/kg (0.5 ml/kg) as a 30% olive oil solution while Group 1 received 0.5 mL/kg of olive oil. ALA was dissolved in distilled water to form a solution and administered at a dose of 25 mg/kg orally via an oral cannula to rats in Groups 3 and 4. All treatment lasted for 10 consecutive days. Twenty-four (24) hours after the last administration, blood samples were collected by cardiac puncture from the animals, placed in heparinized tubes, allowed to clot and centrifuged at 3000×g for 10 min to obtain sera which were used to determine ALT, AST, ALP, bilirubin, urea and creatinine levels. Immediately after blood collection, the animals were sacrificed by cervical dislocation. The liver and kidney of each rat was promptly removed and processed for histological and oxidative studies.

Serum ALT, AST, ALP and bilirubin analyses: Estimation of ALT, AST, ALP and Bilirubin levels, respectively in serum samples were measured with Boehringer Mannheim kits and a UV-rate auto-analyzer (Hitachi 736-60, Japan).

Determination of serum urea and creatinine level: Blood urea and creatinine levels were measured in all samples of serum using standard kits (Randox Laboratories, UK). Urea level was estimated using the method of Patton and Crouch (1977). In alkaline medium, the ammonium ions released by urease react with salicylate and hypochloride to form green indophenols. The absorbance of samples and standards were measured by spectrophotometer at 580 nm against a reagent blank and the concentration of urea (mg/dL) was determined. Creatinine level was measured according to the procedure of Henry (1974). The rate of complex formation was measured photometrically at 492 nm, and the concentration of serum creatinine was measured as mg/dL.

Preparation of liver and kidney homogenate: Prior to oxidative analyses, liver and kidney samples were homogenized (10% w/v) in 0.1M phosphate buffer (pH 7.0). The homogenate was then centrifuged at 10,000 rpm for 15 min and the supernatant used for the determination of the lipid peroxidation level and antioxidant enzyme activities. The protein content was determined by Lowry's method (Lowry *et al*., 1951).

Measurement of MDA level: As a marker of lipid peroxidation, the level of malondialdehyde (MDA) in the tissue (liver and kidney) homogenate was measured by the method of Uchiyama and Mihara (1978), as Thiobarbituric Acid Reactive Substances (TBARS). The development of a pink complex with absorption maximum at 535 nm is taken as an index of lipid peroxidation.

Measurement of SOD, CAT and GSH activities: The activity of the superoxide dismutase (SOD) enzyme in the homogenate was determined according to the method described by Sun and Zigmam (1978). The reaction was carried out in 0.05M sodium carbonate buffer pH 10.3 and was initiated by the addition of epinephrine in 0.005N HCl. Catalase (CAT) activity was determined by measuring the exponential disappearance of H_2O_2 at 240 nm and expressed in units/mg of protein as described by Aebi (1984). The reduced glutathione (GSH) content of the sperm homogenate was determined using the method described by Van Dooran *et al*. (1978). The GSH determination method is based on the reaction of Ellman's reagent 5, 5' dithiobis-2-nitrobenzoic acid (DNTB) with the thiol group of GSH at pH 8.0 to produce 5-thiol-2-nitrobenzoate which is yellow at 412 nm. Absorbance was recorded using Agilent UV-Visible Spectrophotometer in all measurement.

Histopathological analysis: Liver and kidney samples were immediately collected and fixed in 10% buffered formaldehyde solution for a period of at least 24 h before histopathological study. Samples were then embedded in paraffin wax and five-micron sections were prepared with a rotary microtome. These thin sections were stained with hematoxylin and eosin (H&E), mounted on glass slides with Canada balsam (Sigma, USA) and observed for pathological changes under a binocular microscope.

Statistical analysis: Data were presented as mean and standard error of mean (SEM). When one-way ANOVA showed significant differences among groups, Tukey's

Table 1: Effects of ALA on serum AST, ALT, ALP and bilirubin levels, respectively in the liver of rats treated with CCl_4

	Control	CCl_4	ALA	CCl_4+ALA
AST (u/L)	197.95±5.00	486.62±18.30*	171.63±4.05$^{\#}$	289.63±15.33*$^{\#}$
ALT (u/L)	60.13±10.08	119.64±8.56*	54.82±4.96$^{\#}$	83.88±7.20$^{\#}$
ALP (u/L)	193.01±20.86	299.79±22.84*	207.16±13.37$^{\#}$	233.82±11.05
Bilirubin (mg/dL)	1.58±0.14	2.52±0.10*	1.508±0.10$^{\#}$	2.09±0.09*$^{\#}$

The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; $^{\#}$: p<0.05 compared with CCl_4 group

Table 2: Effects of ALA on creatinine and urea level in the kidney of rats treated CCl_4

	Control	CCl_4	ALA	CCl_4+ALA
Creatinine (mg/dL)	197.95±1.20	486.62±1.51*	171.63±7.75*$^{\#}$	289.63±4.95*$^{\#}$
Urea (mg/dL)	60.13±0.73	119.64±0.30*	54.82±0.72*$^{\#}$	83.88±0.85*$^{\#}$

The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; $^{\#}$: p<0.05 compared with CCl_4 group

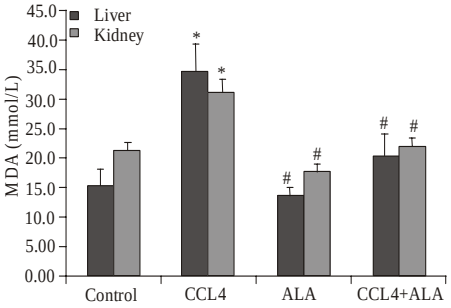

Fig. 1: Effect of ALA on MDA level in the liver and kidney of rats treated with CCl_4. The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; $^{\#}$: p<0.05 compared with CCl_4 group

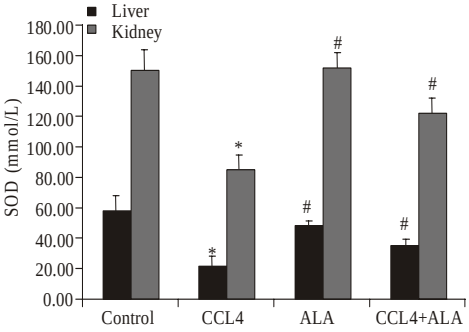

Fig. 2: Effect of ALA on SOD activity in the liver and kidney of rats treated with CCl_4. The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; $^{\#}$: p<0.05 compared with CCl_4 group

post hoc test was used to determine the specific pairs of groups that were statistically different. A level of p<0.05 was considered statistically significant. Analysis was performed with the GraphPad Instat Version 3.05 (GraphPad Software, San Diego California, USA).

RESULTS

Serum ALT, AST, ALP and bilirubin level: Effect of ALA on serum ALT, AST, ALP and bilirubin activities, respectively in rats from various treatment groups are

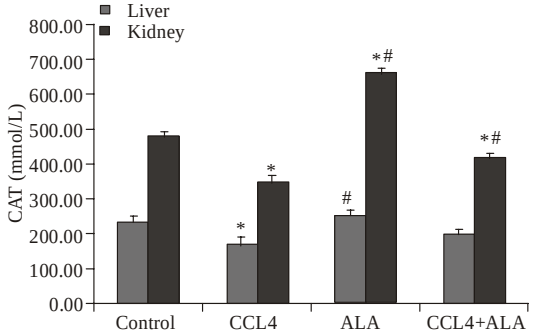

Fig. 3: Effect of ALA on CAT activity in the liver and kidney of rats treated with CCl_4. The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; $^{\#}$: p<0.05 compared with CCl_4 group

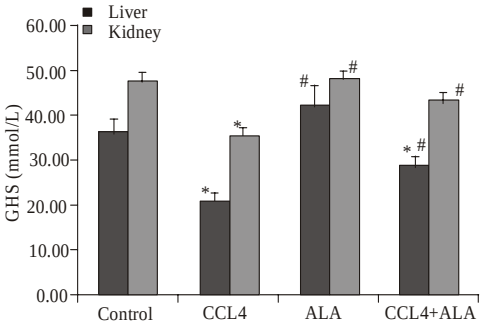

Fig. 4: Effect of ALA on GSH activity in the liver and kidney of rats treated with CCl_4. The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; $^{\#}$: p<0.05 compared with CCl_4 group

shown in Table 1. The test (CCl_4) group showed a significant increase in serum level of ALT, AST, ALP and bilirubin, respectively. The prophylactic (CCl_4+ALA) group showed an improvement in liver functioning as shown by a significant decrease in the level of all measured liver enzyme activities. A marginal increase was observed in the level of liver enzymes in the ALA treated group which was however not significantly different from the control group.

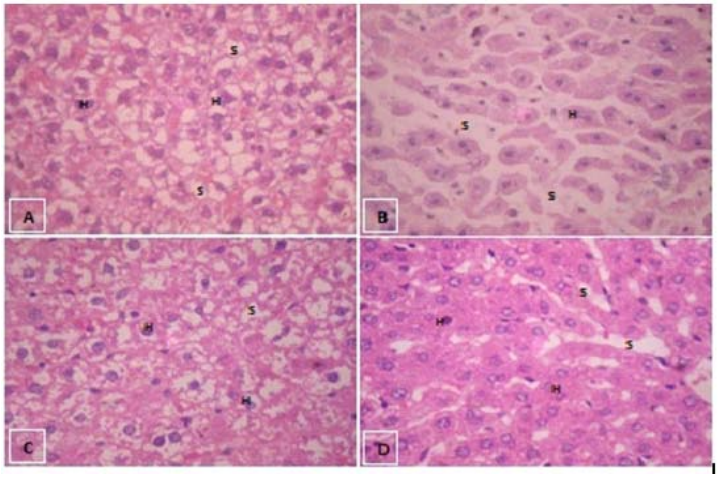

Fig. 5: Histopathologic sections of the liver; (a) control rats showing hepatocytes [H] close to the sinusoid [S] arranged to from a dense spongelike structure; (b) CCl_4-treated rats showing scanty hepatocytes, vacoulization and disorganised sinusoidal structures associated with necrosis; (c) no disorganization in the basic structural components in ALA-treated rats; (d) hepatocytes of rats treated simultaneously with CCl_4 and ALA showing lesser degree of disorganization [H&E, x40]

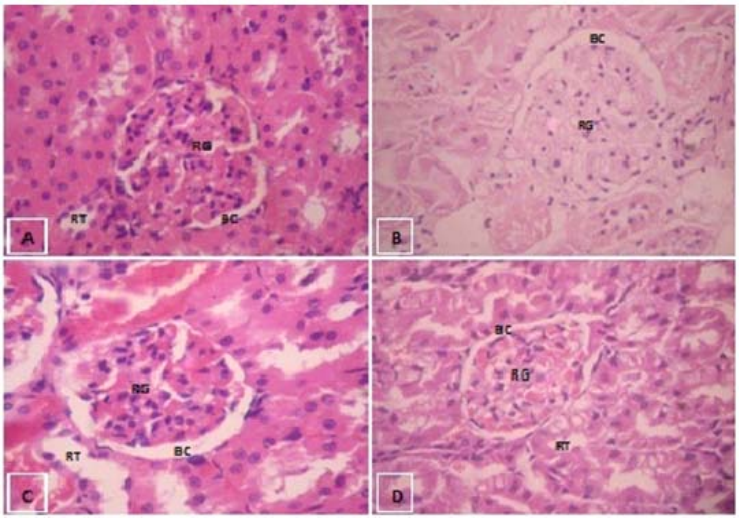

Fig. 6: Histopathologic sections of the kidney; (a) control rats showing the renal glomerulus [RG], Bowmans capsule [BC] and renal tubules [RT]; (b) CCl_4-treated rats showing massive cellular disruptions; (c) ALA-treated rats showing regular and normal cellular structures similar to control rats; (d) rats treated with CCl_4 and ALA simultaneously showed lesser cellular disorganization and disruptions [H&E, x40]

Serum creatinine and urea level: In Table 2, it can be seen that the level of creatinine and urea were significantly elevated in the CCl_4 test group. However, these elevations were attenuated in CCl_4+ALA rats, although the values were still statistically higher than the control values.

MDA level: Figure 1 gives the MDA levels in the liver and kidney homogenates. The levels of MDA were significantly increased in the test groups. Co-treatment of ALA with CCl_4 in the prophylactic group however indicated a significant attenuation of these elevations. Although the MDA levels were significantly higher than the control level, these values were also significantly lower than the test group, this show that ALA confers a level of protection on the animals against lipid peroxidation in both organs.

SOD activities: A significant decrease in the activity of SOD was found in the test groups compared with the control in both the liver and kidney tissues. Co-treatment of ALA with CCl_4 in the prophylactic group produced in

a significant increase in the activities of SOD. However, the activity of SOD in the prophylactic group was still significantly lower than the control values (Fig. 2).

CAT activities: The activities of CAT are shown in Fig. 3. There was a significant decrease in the CAT activity of the test groups when compared with the control. However, CAT activities in both the liver and kidney tissues from ALA+CCl_4 co-treatment were significantly higher than the test group. While the CAT activity in both the liver and kidney tissues from ALA+CCl_4 co-treatment were significantly higher than the test rats treated with CCl_4 only, it was much lower and significantly different from the control rats.

GSH activities: Figure 4 depicts the GSH activities in the experimental rats. There was a significant decrease in the GSH activity of the test group in both liver and kidney tissues. Co-treatment of ALA with CCl_4 in the prophylactic group however indicated a significant attenuation of these reductions. Meanwhile, the activity of GSH in the prophylactic group was significantly lower than the control values (Fig. 2).

Histological observation: Histologically, liver samples from the control rats stained with H&E showed normal architecture with the basic structural arrangement of the hepatocytes in close proximity with the sinusoids and the presence of Kupffer cells to form a dense spongelike structure (Fig. 5A). After CCl_4 treatment, significant liver damage was observed with classic histology of massive vacuolization, very scanty hepatocytes and disorganization of sinusoidal structures associated with necrosis (Fig. 5B). The group co-treated with CCl_4 and ALA showed less histological alterations and a remarkable improvement compared to the CCl_4-treated group (Fig. 5D). The kidney section of the control rat stained with H&E showed apparent normal histological features with normo-cellular glomerular structures displayed on a background containing tubules (Fig. 6A). CCl_4 treatment however produced significant adverse morphological changes with sloughing of renal glomerular structure, vacuolization and necrosis (Fig. 6B). Co-treatment of CCl_4 with ALA however resulted in the attenuation of the adverse changes showing less vacuolization, detachment of renal tubular cells and degeneration (Fig. 6D). The ALA group showed normal liver (Fig. 5C) and kidney (Fig. 6C) architecture similar to their respective control group.

DISCUSSION

Using rats treated with CCl_4 as a model of hepatic and renal toxicities, we showed ALA as an effective protective agent against oxidatively mediated damage to the kidney and liver.

CCl_4 has been shown to have hepatotoxic and nephrotoxic potentials (Ganie *et al*., 2011). The extent of hepatic and renal damage is usually assessed by the increased serum level of liver enzymes and renal function markers respectively (Recknagel *et al*., 1991; Adeneye, 2009). CCl_4 toxicity is largely due to free radical mediated damage via lipid peroxidative degradation of the bio-membranes ultimately leading to severe damage to many organs in the body (Lavanya *et al*., 2009). CCl_4 is metabolized to form highly reactive and unstable trichloromethyl radicals ($.CCl_3$) (Fadhel and Amran, 2002), which bind to the unsaturated fatty acids of membrane lipids covalently, resulting in the formation of chloroform and lipid radicals (Packer *et al*., 1978).

Serum urea and creatinine have been documented to be effective and reliable markers of renal functions (Adeneye, 2009). An increased serum level of these markers is indicative of renal damage (Adelman *et al*., 1981). The present study showed an increase in the serum levels of urea and creatinine in CCl_4 treated rats. Administration of ALA to CCl_4-treated rats however caused a significant reduction in the serum level of urea and creatinine thereby reversing the biochemical alterations towards normal values. As a measure of liver function test; AST, ALT, ALP and bilirubin (which are known biomarkers of the liver) were evaluated in CCl_4 treated rats. CCl_4 administration caused a significant increase in the serum level of the liver enzymes (namely AST, ALT and ALP, respectively) compared with control rats. An elevated level of these enzymes is indicative of liver damage (Murayama *et al*., 2007), and the administration of ALA in this study caused a significant decrease in the level of these liver markers in CCl_4 treated rats. This suggests that ALA protects the liver from CCl_4 toxicity by inhibiting the elevation of liver amino transferases. Similarly, the bilirubin level was significantly increased in the CCl_4 treated rats compared with the control rats. Values of bilirubin level obtained in the CCl_4-treated rats administered with ALA were comparatively lower than the CCl_4 only group. This further demonstrates the beneficial effects of ALA on CCl_4-induced oxidative liver toxicity and is in agreement with previous reports (Kamalakannan *et al*., 2005).

From the result in the present study, CCl_4 administration caused a significant increase in lipid peroxidation indexed by the MDA level; and decrease in antioxidant status shown by reduced activities of SOD, CAT and GSH in CCl_4-treated rats. These findings are indicative of oxidative stress and are in agreement with previous reports (Husain *et al*., 2001). The disruption of antioxidant balance in the liver of the CCl_4-treated rats correlated with the severe damage as shown by the rise in serum levels of AST, ALT and ALP, respectively.

Similarly, the increased serum level of urea and creatinine which have been documented to be effective and reliable markers of renal functions correlated well with the oxidative perturbations.

The susceptibility of the liver and kidney cells to CCl_4-induced oxidative insult is within plausible explanation in view of the fact that failure of the antioxidant mechanism to prevent excessive free radical damage leads to lipid peroxidation and ultimately tissue damage. Therefore, the rise in the serum level of the liver enzymes for instance, may be attributed to the damaged structural integrity of the liver, because these enzymes are located in the cytoplasm and are released into circulation after cellular damage (Bilgin *et al*., 2011). Decrease in the activities of SOD, CAT and GSH, respectively increase the susceptibility of cells to various oxidative attacks and also several biochemical alterations. It is known that deficiency of these endogenous enzymes within a living cell can down-regulate or inhibit the dismutation of harmful superoxide anion, decomposition of hydrogen peroxide and compromise several defence processes against free radicals and peroxides (Okhawa *et al*., 1997; Sies, 1999; Baynes, 1991). It therefore appears that CCl_4 not only generates excessive free radicals and toxic peroxides but also inhibits the activities of endogenous antioxidants.

Treatment with ALA was found to protect the liver and kidney against CCl_4 toxicity. This protection was evidenced by the reduction in the concentration of the lipid peroxidation marker, MDA. Antioxidants have been shown in literature to inhibit the peroxidation of lipid structures in cells (Borek, 2001; Yingming *et al*., 2004), in a similar pattern, ALA inhibits lipid peroxidation capacity of CCl_4 in the liver and kidney cells. Furthermore, administration of ALA to CCl_4-treated rats produced a significant increase in the activities of GSH, SOD and CAT, respectively. Therefore, ALA enhances the antioxidant enzyme activities in CCl_4 treated rats and thereby confers protection against reactive species and/or free radicals. Evidence suggests that these enzymatic antioxidant systems confer protection on the cell against oxidative insults such as highly reactive superoxides and oxygen reactive species (Baynes, 1991).

Furthermore, treatment with CCl_4 has been shown to induce the necrosis of hepatocytes and accumulation of inflammatory cells (Guyot *et al*., 2006; Pradeep *et al*., 2009). In the present study, the microscopical image of the histological sections of the liver and kidney from the CCl_4-treated rats showed extensive disruptions in the histo-architecture of these organs. It is worth noting that the present biochemical findings correlated with the histological observations in the liver and kidney which clearly revealed severe alterations in the hepatic and renal cells normal histological features after CCl_4 administration. However, co-administration of CCl_4 with ALA, respectively attenuated the degree of tissue damage in both organs. These results demonstrate that ALA confers a protective effect against CCl_4- induced hepatic and renal damage.

In conclusion, our data strongly suggest that ALA exerts a protective effect against CCl_4-induced toxicity in the liver and kidney by scavenging free radicals and regenerating endogenous antioxidants. The oxidative and histological evaluations along with biochemical investigations were suggestive of the hepato- and nephro-protective activities of ALA on CCl_4-induced oxidative damage. Although the detailed mechanisms are not known and remain to be further elucidated, the observed protective activities of ALA against CCl_4, toxicity may involve its antioxidant and/or free radical scavenging potentials.

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