

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

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Abstract: Carbon tetrachloride (CCl₄) 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 CCl₄-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 CCl₄ 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 CCl₄ 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 CCl₄ treated rats. However, treatment with ALA attenuated the adverse changes in the CCl₄-induced rats. Our findings suggest that ALA protects the liver and kidney against CCl₄-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 (CCl₄) 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 CCl₄ 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 peroxyxynitrite (Trujillo and Radi, 2002), nitric oxide (Vriesman *et al.*, 1997), hydroxyl radical, superoxide anion (Suzuki *et al.*, 1991), peroxy 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 CCl₄ toxicity, the present study was undertaken to explore the influence of ALA on CCl₄ 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 CCl₄ intoxicated rats.

MATERIALS AND METHODS

Drugs and chemical reagents: ALA, CCl₄, Heparin, Phenobarbital and olive oil were obtained from Sigma

(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 20-25°C 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.

CCl₄-induced acute liver damage model: Twenty four (24) animals were divided into four groups: Group 1 (control), Group 2 (CCl₄ treatment), Group 3 (ALA treatment), Group 4 (CCl₄ + ALA treatment). Each group has six animals. Rats from Groups 2 and 4 were given CCl₄ 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 H₂O₂ 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 CCl₄

	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 CCl₄ group

Table 2: Effects of ALA on creatinine and urea level in the kidney of rats treated CCl₄

	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 CCl₄ group

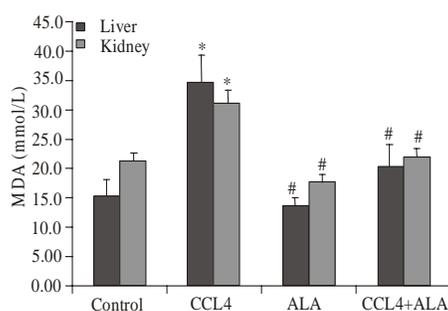


Fig. 1: Effect of ALA on MDA level in the liver and kidney of rats treated with CCl₄. The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; [#]: p<0.05 compared with CCl₄ group

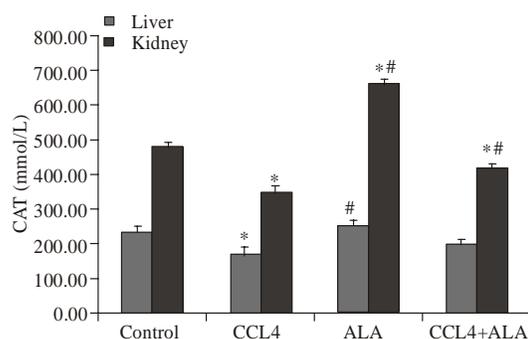


Fig. 3: Effect of ALA on CAT activity in the liver and kidney of rats treated with CCl₄. The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; [#]: p<0.05 compared with CCl₄ group

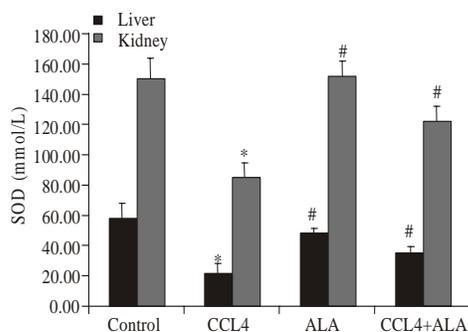


Fig. 2: Effect of ALA on SOD activity in the liver and kidney of rats treated with CCl₄. The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; [#]: p<0.05 compared with CCl₄ group

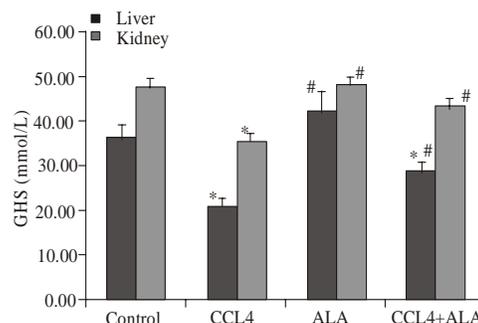


Fig. 4: Effect of ALA on GSH activity in the liver and kidney of rats treated with CCl₄. The values represent mean±SEM (n = 6); *: p<0.05 compared with control group; [#]: p<0.05 compared with CCl₄ group

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

shown in Table 1. The test (CCl₄) group showed a significant increase in serum level of ALT, AST, ALP and bilirubin, respectively. The prophylactic (CCl₄+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.

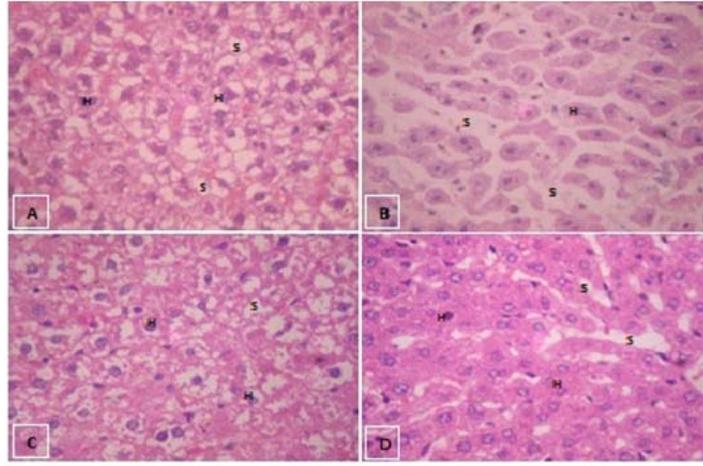


Fig. 5: Histopathologic sections of the liver; (a) control rats showing hepatocytes [H] close to the sinusoid [S] arranged to form a dense spongeliike structure; (b) CCl₄-treated rats showing scanty hepatocytes, vacuolization and disorganised sinusoidal structures associated with necrosis; (c) no disorganization in the basic structural components in ALA-treated rats; (d) hepatocytes of rats treated simultaneously with CCl₄ and ALA showing lesser degree of disorganization [H&E, x40]

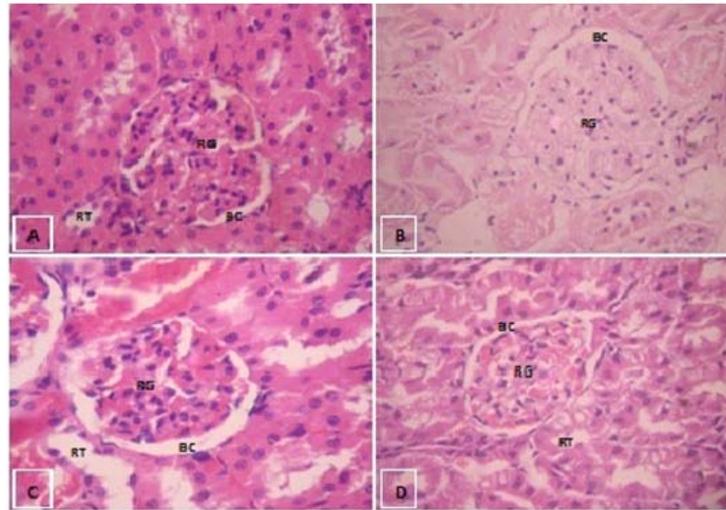


Fig. 6: Histopathologic sections of the kidney; (a) control rats showing the renal glomerulus [RG], Bowman's capsule [BC] and renal tubules [RT]; (b) CCl₄-treated rats showing massive cellular disruptions; (c) ALA-treated rats showing regular and normal cellular structures similar to control rats; (d) rats treated with CCl₄ 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 CCl₄ test group. However, these elevations were attenuated in CCl₄+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 CCl₄ 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 shows 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 CCl₄ 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+CCl₄ co-treatment were significantly higher than the test group. While the CAT activity in both the liver and kidney tissues from ALA+CCl₄ co-treatment were significantly higher than the test rats treated with CCl₄ 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 CCl₄ 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 spongeli-like structure (Fig. 5A). After CCl₄ 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 CCl₄ and ALA showed less histological alterations and a remarkable improvement compared to the CCl₄-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). CCl₄ treatment however produced significant adverse morphological changes with sloughing of renal glomerular structure, vacuolization and necrosis (Fig. 6B). Co-treatment of CCl₄ 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 CCl₄ 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.

CCl₄ 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). CCl₄ toxicity is largely due to free radical mediated damage via lipid peroxidative degradation of the biomembranes ultimately leading to severe damage to many organs in the body (Lavanya *et al.*, 2009). CCl₄ is metabolized to form highly reactive and unstable trichloromethyl radicals (.CCl₃) (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 CCl₄ treated rats. Administration of ALA to CCl₄-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 CCl₄ treated rats. CCl₄ 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 CCl₄ treated rats. This suggests that ALA protects the liver from CCl₄ toxicity by inhibiting the elevation of liver amino transferases. Similarly, the bilirubin level was significantly increased in the CCl₄ treated rats compared with the control rats. Values of bilirubin level obtained in the CCl₄-treated rats administered with ALA were comparatively lower than the CCl₄ only group. This further demonstrates the beneficial effects of ALA on CCl₄-induced oxidative liver toxicity and is in agreement with previous reports (Kamalakaran *et al.*, 2005).

From the result in the present study, CCl₄ 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 CCl₄-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 CCl₄-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 CCl₄-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 CCl₄ 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 CCl₄ 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 CCl₄ in the liver and kidney cells. Furthermore, administration of ALA to CCl₄-treated rats produced a significant increase in the activities of GSH, SOD and CAT, respectively. Therefore, ALA enhances the antioxidant enzyme activities in CCl₄ 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 CCl₄ 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 CCl₄-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 CCl₄ administration. However, co-administration of CCl₄ with

ALA, respectively attenuated the degree of tissue damage in both organs. These results demonstrate that ALA confers a protective effect against CCl₄- induced hepatic and renal damage.

In conclusion, our data strongly suggest that ALA exerts a protective effect against CCl₄-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 CCl₄-induced oxidative damage. Although the detailed mechanisms are not known and remain to be further elucidated, the observed protective activities of ALA against CCl₄ toxicity may involve its antioxidant and/or free radical scavenging potentials.

REFERENCES

- Adelman, R.D., W.L. Spangler, F. Beasom, G. Ishizaki and G.M. Conzelman, 1981. Frusimide enhancement of nelticimin nephrotoxicity in dogs. *J. Antimicrob. Chemother.*, 7: 431-440.
- Adeneye, A.A., 2009. Protective activity of the stem bark aqueous extract of *Musanga Ceropiodes* in carbon tetrachloride-and acetaminophen-induced acute hepatotoxicity in Rats. *Afr. J. Trad. CAM*, 6: 131-138.
- Aebi, H., 1984. Catalase in vitro. *Methods Enzymol.*, 8: 121-126.
- Bast, A. and G.R. Haenen, 2003. Lipoic acid, a multifunctional antioxidant. *Biofactors*, 17: 207-213.
- Basu, S., 2003. Carbon tetrachloride-induced lipid peroxidation: Eicosanoid formation and their regulation by antioxidant nutrients. *Toxicol.*, 189: 113-127.
- Baynes, J.W., 1991. Role of oxidative stress in development of complication in diabetes. *Diab.*, 40: 406-412.
- Bilgin, H.M., M. Atmaca, B.D. Obay, S. Ozekinci, E. Tasdemir and A. Ketani, 2011. Protective effects of coumarin and coumarin derivatives against carbon tetrachloride-induced acute hepatotoxicity in rats. *Exp. Toxicol. Path.*, 63: 325-332.
- Borek, C., 2001. Antioxidant effect of aged garlic extract. *J. Nutr.*, 131: 1010-1015.
- Fadhel, Z.A. and S. Amran, 2002. Effects of black tea extract on tetrachloride-induced lipid peroxidation in liver, kidneys and testes of rats. *Phytother Res.*, 16(1): 28-32.
- Ganie, S.H., E. Haq, A. Hamid, Y. Qurishi, Z. Mahmood and B.A. Zargar, 2011. Carbon tetrachloride induced kidney and lung tissue damages and antioxidant activities of the aqueous rhizome extract of *podophyllum hexandrum*, *BMC Complem. Alt. Med.*, 11: 17-23.

- Guyot, C.S., L.C. Combe, E. Doudnikoff, P. Bioulac-Sage, C. Balabaud and A. Desmouliere, 2006. Hepatic fibrosis and cirrhosis: The (myo) fibroblastic cell subpopulations involved. *Int. J. Biochem. Cell Biol.*, 38: 135-151.
- Henry, R.J., 1974. *Clinical Chemistry, Principles and Techniques* Harper and Row. 2nd Edn., Academic Press, New York, pp: 545.
- Husain, K., B.R. Scott, S.K. Reddy and S.M. Somani, 2001. Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. *Alcohol*, 25: 89-97.
- Jacob, S., E.J. Henriksen, H.J. Tritschler, H.J. Augustin and G.J. Dietze, 1996. Improvement of insulin-stimulated glucosedisposal in type 2 diabetes after repeated parenteral administration of thioctic acid. *Exp. Clin. Endocrinol. Diab.*, 104: 284-288.
- Kagan, V.E., A. Shvedova, E. Serbinova, S. Khan, C. Swanson, R. Powell and L. Packer, 1992. Dihydrolipoic acid-a universal antioxidant both in the membrane and in the aqueous phase. Reduction of peroxy, ascorbyl and chromanoxyl radicals. *Biochem. Pharmacol.*, 44: 1637-1649.
- Kamalakannan, N., R. Rukkumani, K. Aruna, P.S. Varma, P. Viswanathan and V. Padmanabhan, 2005. Protective effect of N-acetyl cysteine in carbon tetrachloride-induced hepatotoxicity in rats. *Iran. J. Pharmacol. Ther.*, 4: 118-122.
- Lavanya, G., R. Sivajyothi, P. Manjunath and R. Parthasarathy, 2009. Fate of biomolecules during carbon tetrachloride induced oxidative stress and protective nature of Ammoniac baccifera Linn: A natural antioxidant. *Int. J. Green Pharm.*, 3: 300-305.
- Loguercio, C. and A. Federico, 2003. Oxidative stress in viral and alcoholic hepatitis. *Free Radical Biol. Med.*, 34: 1-10.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Manjrekar, A.P., V. Jisha, P.P. Bag, B. Adhikary, M.M. Pai, A. Hegde and M. Nandini, 2008. Effect of phyllanthus niruri Linn treatment on liver kidney and testes in CCl₄ induced hepatotoxic rats. *Ind. J. Exp. Biol.*, 46: 514-520.
- Murayama, H., M. Ikemoto, Y. Fukuda, S. Tsunekana and A. Nagata, 2007. Advantage of serum type L arginase and ornithine carbamoyltransferase in the evaluation of acute and chronic liver damage induced by thioacetamide in rats. *Clin. Chim. Acta*, 375: 63-68.
- Okhawa, H., N. Ohishi and K. Yagi, 1997. Assay of lipid peroxides in animal tissues by thiobabaturic acid reaction. *Anal. Biochem.*, 95: 351-358.
- Packer, J., T. Slater and R. Wilson, 1978. Reactions of the carbon-tetrachloride-related peroxy free radical with amino acids: Pulse radiolysis evidence. *Life Sci.*, 23: 2617-2620.
- Packer, L., K. Kraemer and G. Rimbach, 2001. Molecular aspects of lipoic acid in the prevention of diabetes complications. *Nutrition*, 17: 888-895.
- Patton, C.J. and S.R. Crouch, 1977. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal. Chem.*, 44: 464-469.
- Pradeep, H.A., S. Khan, K. Ravikumar, M.F. Ahmed, M.S. Rao, M. Kiranmai, D.S. Reddy, S.R. Ahmed and M. Ibrahim, 2009. Hepato-protective evaluation of Anogeissus latifolia: In vitro and in vivo studies. *World J. Gastroenterol.*, 15: 4816-4822.
- Rahnau, K.J., H.P. Meissnert, J.R. Finn, M. Reljanovic, M. Lobisch, K. Schütte, D. Nehdrich, H.J. Tritschler, H. Mehnert and D. Ziegler, 1999. Effects of 3-week oral treatment with antioxidant thioctic acid (α -lipoic acid) in symptomatic diabetic polyneuropathy. *Diabet Med.*, 16: 1040-1043.
- Recknagel, R.O., E.A. Glende Jr. and R.S. Britton, 1991. *Free Radical Damage and Lipid Peroxidation* In: Meeks, R.G., (Ed.), *Hepatotoxicology*. CRC Press, Florida, pp: 401-436.
- Scott, B.C., O.I. Aruoma, P.J. Evans, C. O'niel, A. Van der Vliet, C.E. Cross, H. Tritschler and B. Halliwell, 1994. Lipoic and dihydrolipoic acids as antioxidants: A critical evaluation. *Free Rad. Res.*, 20: 119-133.
- Shay, P.K., R.F. Moreau, E.J. Smith, A.R. Smith and T.M. Hagen, 2009. Alpha-Lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. *Biochimica Biophysica Acta*, 1790: 1149-1160.
- Sies, H., 1999. Glutathione and its role in cellular functions. *Free Rad. Biol. Med.*, 27: 916-21.
- Stal, P. and J. Olson, 2000. Ubiquinone: Oxidative Stress and Liver Carcinogenesis. In: Kavan, V.E. and D.J. Quinn (Eds.), *Coenzyme Q: Molecular Mechanisms in Health and Disease*. CRC Press, Boca Raton, pp: 317-329.
- Sun, M. and S. Zigmam, 1978. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autooxidation. *Anal. Biochem.*, 90: 81-89.
- Suzuki, Y.J., M. Tsuchiya and L. Packer, 1991. Thioctic acid and dihydrolipoic acid are novel antioxidants which interact with reactive oxygen species. *Free Rad. Res. Comm.*, 15: 255-263.
- Trujillo, M. and R. Radi, 2002. Peroxynitrite reaction with the reduced and the oxidized forms of lipoic acid: New insights into the reaction of peroxynitrite with thiols. *Arch. Biochem. Biophys.*, 397: 91-98.

- Uchiyama, M. and M. Mihara, 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal. Biochem.*, 86: 271-278.
- Van Dooran, R., C.M. Liejdekker and P.T. Handerson, 1978. Synergistic effects of phorone on the hepatotoxicity of bromobenzene and paracetamol in mice. *Toxicol.*, 11: 225-233.
- Vriesman, M.F., G.R. Haenen, G.J. Westerveld, J.B. Paquay, H.P. Voss and A.A. Bast, 1997. A method for measuring nitric oxide radical scavenging activity. Scavenging properties of sulphur containing compounds. *Pharm. World Sci.*, 19: 283-286.
- Yingming, P., L. Ping, W. Hengshan and L. Min, 2004. Antioxidant activities of several chinese medicinal herbs. *Food Chem.*, 88: 347-350.
- Ziegler, D., H. Nowak, P. Kempler, P. Vargha and P.A. Low, 2004. Treatment of symptomatic diabetic polyneuropathy with the antioxidant-lipoic acid: A meta-analysis. *Diabet. Med.*, 21: 114-121.