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 hepatoxicty 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 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 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...
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 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) 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 the concentration of urea (mg/dL) was determined.

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 the concentration of urea (mg/dL) was determined.

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 (DTNB) 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
### 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 from a dense spongelike structure; (b) CCl₄-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 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], Bowmans 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 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 CCl₄ in the prophylactic group produced in
Co-treatment of CCl₄ with ALA however resulted in the glomerular structure, vacuolization and necrosis (Fig. 6B). Morphological changes with sloughing of renal remarkable improvement compared to the CCl₄-treated ALA showed less histological alterations and a disorganization of sinusoidal structures associated with vacuolization, very scanty hepatocytes and damage was observed with classic histology of massive 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.

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 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 (Kamalakannan 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 \(\text{CCl}_4\)-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 \(\text{CCl}_4\) 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 \(\text{CCl}_4\) 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 \(\text{CCl}_4\) in the liver and kidney cells. Furthermore, administration of ALA to \(\text{CCl}_4\)-treated rats produced a significant increase in the activities of GSH, SOD and CAT, respectively. Therefore, ALA enhances the antioxidant enzyme activities in \(\text{CCl}_4\) 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 \(\text{CCl}_4\) 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 microscopic image of the histological sections of the liver and kidney from the \(\text{CCl}_4\)-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 \(\text{CCl}_4\) administration. However, co-administration of \(\text{CCl}_4\) with ALA, respectively attenuated the degree of tissue damage in both organs. These results demonstrate that ALA confers a protective effect against \(\text{CCl}_4\)-induced hepatic and renal damage.

In conclusion, our data strongly suggest that ALA exerts a protective effect against \(\text{CCl}_4\)-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 \(\text{CCl}_4\)-induced oxidative damage. Although the detailed mechanisms are not known and remain to be further elucidated, the observed protective activities of ALA against \(\text{CCl}_4\) toxicity may involve its antioxidant and/or free radical scavenging potentials.

REFERENCES


