Antioxidant DL-alpha lipoic acid as an attenuator of adriamycin induced hepatotoxicity in rat model

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Protective efficacy of DL-alpha lipoic acid on adriamycin induced hepatotoxicity was evaluated in rats. Adriamycin toxicity, induced by a single injection (ip; 15 mg/kg body wt), was expressed by an elevation in alanine transaminase, aspartate transaminase, bilirubin levels in serum and alkaline phosphatase, lactate dehydrogenase, alanine transaminase, aspartate transaminase activity in hepatic tissue. Adriamycin produced significant increase in malondialdehyde levels indicating tissue lipid peroxidation and potentially inhibiting the activity of antioxidant, reduced glutathione and antioxidant enzymes, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, glucose-6-phosphate dehydrogenase. The present results showed that pretreatment with lipoic acid [75 mg/kg body wt/day (ip), 24 h prior to administration of adriamycin] significantly restored various cellular activity suggesting the antioxidant potential of lipoic acid in ameliorating the hepatotoxicity induced by adriamycin.

Keywords: Adriamycin, Hepatotoxicity, Lipoic acid

Adriamycin is considered to be one of the most compelling drugs against a wide range of tumors. However, its clinical potential is contraindicated due to severe cytotoxic side effects. Based on in vitro model of toxicity using isolated hepatocytes and liver microsomes, adriamycin has been shown to undergo redox cycling between semiquinone and quinone radicals during its oxidative metabolism. Inherent high risk of developing cardiomyopathy and liver function abnormalities has severely restricted the application of this useful antitumor drug.

Since the free radical produced during the metabolism of the drug is considered to be responsible for alteration induced in various cellular enzyme activities, lipid peroxidation, antioxidant, antioxidant enzymes, the effect of antioxidant lipoic acid, which intercepts the toxic free radicals was taken for investigation.

Lipoic acid is a naturally occurring compound, which is an essential component of oxidative metabolism, participating as protein bound lipoamide in alpha keto acid dehydrogenase complexes in mitochondria. Role of lipoic acid (LA) a mitochondrial “metavitamin” in intermediary metabolism may not be confined to its cofactor role, but may well extend to certain reactions in lipid biosynthesis where it replaces coenzyme A for activating fatty acids prior to acylation and has been established as a universal antioxidant due to its high singlet oxygen quenching constant.

Supplementation of lipoic acid leads to its reduction to produce the more active antioxidant molecule dihydrolipoic acid. No evidence suggests teratogenic or carcinogenic effect of lipoic acid. Therefore, the present study was undertaken to evaluate the protective efficacy of lipoic acid in mitigating adriamycin induced hepatotoxicity considering lipid peroxidation, antioxidants and marker enzymes as major parameters of the study.

Materials and Methods

Materials — DL-alpha lipoic acid (LA) was purchased from Sigma Chemicals, St. Louis, USA. All other chemicals were of analytical grade procured from local commercial sources.
Animal model — Adult male rats of Wistar strain (12-14 weeks old) weighing 150 ± 20 g were obtained from Veterinary College, Chennai. Animals were maintained as per the guidelines of Institutional Animal Ethics Committee. They were kept at 26° ± 2° C under 12 h day/night cycle in the animal house. They were fed standard rat pellet diet (M/s. Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and were given access to water ad libitum.

Experimental design — Test animals were divided into four groups of six rats in each group. Group I (control) received only normal saline throughout the course of the experiment. Group II (adriamycin) received single injection (ip) of adriamycin (15 mg/kg body wt/day). Group III received single injection (ip) of lipoic acid (75 mg/kg body wt/day). Group IV received a single injection (ip) of lipoic acid as in group III, but 24 hr prior to administration of adriamycin through tail vein. After 4 days, the animals were sacrificed by cervical decapitation under ether anesthesia and liver was excised immediately and washed with ice-cold saline. A homogenate (10%) of washed tissue (liver) was prepared in 0.01 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 12,000 g for 30 min using high-speed cooling centrifuge (Remi) at 4° C. The blood samples collected in plain centrifuge tubes were kept in inclined position to allow complete clotting of blood and then centrifuged at 2,500 rpm for 30 min. The resultant clear supernatant was pipetted out and preserved in small vials in the freezer and the biochemical investigations were carried out.

Enzyme assays — The method of King was followed to assay alkaline phosphatase, lactate dehydrogenase, aspartate transaminase and alanine transaminase. Lipid peroxidation was assayed by the method of Devasagayam in which MDA released served as the index of lipid peroxidation. In ascorbate-induced lipid peroxidation, the peroxidation system contained 0.2 ml of a solution containing ferric chloride (3.4 mg), adenosine diphosphate (9.8 mg) and ascorbate (8.8 mg) in 2 ml, whereas in ferrous sulphate induced lipid peroxidation, the peroxidation system contained 0.2 ml of 10 mM ferrous sulphate. 1, 1, 3, 3-tetra ethoxy propane MDA bis (diethyl acetal) was used as the standard. Reduced glutathione, antioxidant enzymes, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and glucose-6-phosphate dehydrogenase were assayed by using the method as described earlier. Serum bilirubin was also assessed by the method of Lathe and Ruthven.

Data analysis — The results were computed statistically (SPSS Software Package) using one-way ANOVA. Post-hoc testing was performed for inter-comparison using LSD. Values were considered significant at P<0.05.

Results
Liver and serum enzymes — Adriamycin administered rats (Group II animals) showed significant decrease in the activities of various liver enzymes and significant increase in serum enzymes and bilirubin.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver enzymes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>0.32 ± 0.02</td>
<td>0.19 ± 0.02*</td>
<td>0.29 ± 0.03</td>
<td>0.30 ± 0.03*</td>
</tr>
<tr>
<td>LDH</td>
<td>1.85 ± 0.19</td>
<td>1.28 ± 0.13*</td>
<td>1.90 ± 0.19</td>
<td>1.82 ± 0.19*</td>
</tr>
<tr>
<td>AST</td>
<td>1.06 ± 0.10</td>
<td>0.55 ± 0.06*</td>
<td>1.08 ± 0.10</td>
<td>1.04 ± 0.12*</td>
</tr>
<tr>
<td>ALT</td>
<td>1.42 ± 0.12</td>
<td>0.89 ± 0.089*</td>
<td>1.40 ± 0.15</td>
<td>1.41 ± 0.16*</td>
</tr>
<tr>
<td>Serum enzymes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>64.42 ± 7.73</td>
<td>83.92 ± 8.39*</td>
<td>65.19 ± 7.17</td>
<td>65.32 ± 7.18*</td>
</tr>
<tr>
<td>AST</td>
<td>72.16 ± 7.71</td>
<td>93.26 ± 11.19*</td>
<td>71.32 ± 8.55</td>
<td>71.29 ± 7.18*</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.49 ± 0.05</td>
<td>0.58 ± 0.04*</td>
<td>0.49 ± 0.05</td>
<td>0.48 ± 0.05*</td>
</tr>
</tbody>
</table>

*Significant at P<0.05

One unit of enzyme activity is expressed as ALP – μ mole × 10⁻¹ of phenol; LDH – μ mole of pyruvate; ALT, AST – μ mole × 10⁻¹ of pyruvate; BR – mg per dL.

Comparisons were made between *groups I and II; groups I and III; groups I and IV and *groups II and IV.

Table 1 — Effect of adriamycin (ADR) and lipoic acid (LA) on liver enzymes alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) and serum enzymes ALT, AST and bilirubin (BR)

[Values are mean ± SD of 6 rats]
(Table 1) that were restored to near normal upon lipoic acid pretreatment in Group IV animals.

**Lipid peroxidation, antioxidant and antioxidant enzymes** — Effect of adriamycin administration on tissue lipid peroxidation, antioxidant and antioxidant enzyme activities is shown in Tables 2, 3. Lipid peroxidation (basal, ascorbate and ferrous sulphate induced) was significantly increased in adriamycin administered animals with a concomitant fall in antioxidants, reduced glutathione, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-S-transferases and glucose-6-phosphate dehydrogenase (Group II; Table 3). Pretreatment with lipoic acid was efficient in counteracting the adriamycin induced membrane damage brought about by lipid peroxidation induced by free radicals, which was conspicuous with a significant decline in lipid peroxidation and increase in the status of antioxidant and antioxidant enzymes (Group IV).

**Discussion**

Adriamycin by virtue of its quinone groups under aerobic conditions can undergo one-electron reduction to generate semiquinone radical. This semiquinone radical can rapidly react with oxygen to form superoxide radicals like hydroxyl radical that participates in the peroxidation of membrane lipids leading to increased malondialdehyde formation\(^2^1\). Generation within membrane and lipoproteins of peroxy and alkoxy radicals, aldehydes and other products of lipid peroxidation affects liver to a greater extent, causing formation of high molecular mass protein aggregates within the membrane. Hence increased level of malondialdehyde is a factual indicator of LPO\(^2^2\). This was in agreement with our present study where we observed hike in lipid peroxidation in liver in adriamycin administered rats.

Thiols are thought to play a pivotal role in protecting cells against lipid peroxidation\(^2^3\). Lipoic acid, a universal antioxidant, has been reported to be effective in scavenging hydroxyl radical generated by Fenton-type reactions and also a scavenger of peroxy and superoxide radical\(^2^4\) thus, rendering protection against adriamycin induced lipid peroxidation. The dose of lipoic acid that suppressed lipid peroxidation expressed as – nmole of malondialdehyde released per mg protein.

Comparisons were made between *groups I and II; groups I and III; groups I and IV and *groups II and IV.

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Basal</td>
<td>1.53 ± 0.18</td>
<td>2.11 ± 0.23*</td>
<td>1.55 ± 0.15</td>
<td>1.58 ± 0.14*</td>
</tr>
<tr>
<td>Ascorbate-induced</td>
<td>4.39 ± 0.42</td>
<td>6.65 ± 0.63*</td>
<td>4.40 ± 0.48</td>
<td>4.44 ± 0.41*</td>
</tr>
<tr>
<td>Ferrous sulphate-induced</td>
<td>7.60 ± 0.73</td>
<td>10.95 ± 0.98*</td>
<td>7.62 ± 0.66</td>
<td>7.67 ± 0.74*</td>
</tr>
</tbody>
</table>

*Significant at \(P<0.05\)

Lipid peroxidation expressed as – nmole of malondialdehyde released per mg protein.

Comparisons were made between *groups I and II; groups I and III; groups I and IV and *groups II and IV.

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<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>3.97 ± 0.47</td>
<td>2.08 ± 0.18*</td>
<td>3.99 ± 0.39</td>
<td>3.94 ± 0.43*</td>
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<tr>
<td>CAT</td>
<td>318.23 ± 35.00</td>
<td>169.38 ± 16.93*</td>
<td>321.64 ± 38.59</td>
<td>320.16 ± 28.81*</td>
</tr>
<tr>
<td>SOD</td>
<td>7.88 ± 0.94</td>
<td>4.24 ± 0.46*</td>
<td>7.93 ± 0.79</td>
<td>7.91 ± 0.87*</td>
</tr>
<tr>
<td>GPx</td>
<td>5.61 ± 0.56</td>
<td>2.21 ± 0.22*</td>
<td>5.58 ± 0.61</td>
<td>5.55 ± 0.66*</td>
</tr>
<tr>
<td>GR</td>
<td>0.31 ± 0.03</td>
<td>0.18 ± 0.01*</td>
<td>0.32 ± 0.03</td>
<td>0.29 ± 0.03*</td>
</tr>
<tr>
<td>GST</td>
<td>1.23 ± 0.12</td>
<td>0.58 ± 0.06*</td>
<td>1.22 ± 0.14</td>
<td>1.19 ± 0.10*</td>
</tr>
<tr>
<td>G6PD</td>
<td>2.46 ± 0.27</td>
<td>1.94 ± 0.21*</td>
<td>2.41 ± 0.24</td>
<td>2.42 ± 0.29*</td>
</tr>
</tbody>
</table>

*Significant at \(P<0.05\)

Enzyme activities are expressed as GSH – \(\mu\)g per mg protein; CAT - nmol of \(\text{H}_2\text{O}_2\) consumed per min per mg protein; SOD - units per mg protein. (One unit is equal to the amount of enzyme required to inhibit autoxidation of pyrogallol by 50%); GPx - \(\mu\)g of GSH utilized per min per mg protein; GR - nmole of NADPH oxidised per min per mg protein; GST - nmole of CDNB - GSH conjugate formed per min per mg protein; G6PD - nmole of NADPH formed per min per mg protein.

Comparisons were made between *groups I and II; groups I and III; groups I and IV and *groups II and IV.
adriamycin induced toxicity was fixed based on the earlier studies by Malarkodi et al.25.

Skibska et al.26 have speculated that during oxidative stress, catalase activity decreases, hydrogen peroxide accumulates and thereby more peroxidation of lipids is favoured. A decline in the activity of superoxide dismutase on adriamycin administration in the liver of rat has been reported by el Missiry et al.27 that upholds with the present findings.

Reduced glutathione (GSH) exhibits its antioxidant effect by reacting with superoxide radical and hydroxyl radical following the formation of oxidized glutathione (GSSG) and reduces peroxides in the non-enzymatic reaction. Reduced glutathione is a major non-enzymic antioxidant for regulating intracellular free radical concentration28. Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of pentose phosphate pathway, catalyzes the synthesis of riboses for nucleic acid production and is the principal intracellular source of NADPH. NADPH in turn is used as reducing equivalent to maintain reduced glutathione stores, which are used to scavenge reactive oxygen species. Reduced G6PD activity is associated with increased cellular reactive oxygen species accumulation resulting in depletion of glutathione stores and enhanced oxidative stress29. Glutathione-S-transferase (GST) is a group of multifunctional isoenzymes, which play an important role in detoxification of toxic electrophiles by catalyzing the conjugation of these electrophiles with glutathione. GST is considered as first line of defense against oxidative injury along with other antioxidant enzymes, decomposing O₂ and H₂O₂ before interacting to form the more reactive OH⁻. In the present study the activities of reduced glutathione and reduced glutathione dependent enzymes were reduced significantly in rats administered adriamycin. Glushkov et al.30 has reported the decline in activities of reduced glutathione, glutathione reductase, glutathione-S-transferase, glucose-6-phosphate dehydrogenase and glutathione peroxidase in adriamycin administered rats which is in consonance with the present observation. Lipoic acid pretreatment resulted in the increased availability of reducing equivalents possibly due to improved glucose-6-phosphate dehydrogenase activity thereby significantly increasing the activity of glutathione reductase with subsequent regeneration of reduced glutathione pool.

Alanine transaminase and aspartate transaminase are regarded as markers of liver injury, since liver is the major site of metabolism. Several researchers have reported decreased activities of alanine transaminase and aspartate transaminase in liver due to adriamycin toxicity31, which is consistent with the present findings. Decline in the activities of liver alkaline phosphatase and lactate dehydrogenase in adriamycin injected animals noticeably demonstrated cellular damage31 which correlates with the present findings.

Elevation of serum levels of alanine transaminase and aspartate transaminase in adriamycin injected animals is attributed by lipid peroxidation in the liver. Influence of adriamycin toxicity, therefore, reveals leakage of these enzymes from damaged liver cells. In the present study, we have observed increase in serum alanine transaminase and aspartate transaminase. Ito et al.32 have observed similar increments in the levels of aspartate transaminase and alanine transaminase in the serum of adriamycin administered rats. The serum level of total bilirubin in the present study has been found to be increased in adriamycin injected animals, which is in agreement with the results of Liss et al.33 who suggested that increase in bilirubin is a clear marker of hepatic dysfunction.

Preventive administration of lipoic acid to adriamycin injected animals counteracted the hepatic dysfunction attributed by adriamycin. Treatment with lipoic acid appreciably reduced the abnormal changes induced by adriamycin and restored the biomarker’s value of oxidative stress and hepatotoxicity towards near normal. This was substantiated by increase in the activities of liver and serum enzymes, antioxidant enzymes and level of antioxidants along with decreasing levels of malondialdehyde.

In view of the present study, it can be concluded that lipoic acid played a role as an antioxidant and also improved the detrimental state of liver cells which unraveled its use as a possible mitigator/attenuating agent of adriamycin induced hepatotoxic side effects.

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References