Antioxidant activity of DHC-1* – a herbal formulation

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Journal of Ethnopharmacology 2004; 94, 135-141

Abstract
DHC-1, a multiherbal formulation, was tested for its antioxidant activity in rats. DHC-1 was investigated at dose levels of 100 mg/kg, p.o. and 200 mg/kg, p.o., once daily, for 30 days in normal rats. The levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), lipid peroxidation, membrane bound enzymes like Ca2+ATPase, Mg2+ATPase, Na+K+ATPase, lipids like phospholipid, cholesterol, triglyceride and total proteins were estimated in liver, kidneys and heart. Liver Glucose-6-Phosphate-Dehydrogenase was also determined. The serum levels of GOT, GPT, alkaline phosphatase, lactate dehydrogenase and bilirubin were also estimated. The decrease in the serum enzymes may be due to the membrane stabilizing action of DHC-1. The inhibition of lipid peroxidation and enhancement of antioxidant enzymes (SOD and catalase) along with reduced glutathione by DHC-1 may be attributed to the antioxidant potential of various ingredients present in the formulation. Thus, it can be concluded that DHC-1 exhibits an antioxidant activity and could prove beneficial in the treatment of various disorders associated with the involvement of reactive oxygen species.

Key words: Antioxidant; Ca2+ATPase; Mg2+ATPase; Na+K+ATPase; DHC-1

1. Introduction
Free radical reactions have been implicated in the pathology of many human diseases including atherosclerosis, ischaemic heart disease, ageing process, inflammation, diabetes, immunodepression, neurodegenerative condition and other disease conditions (Maxwell, 1995). These free radicals, which are atoms or molecules with an unpaired electron, are capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, proteins and free amino acids, lipids and lipoproteins, carbohydrates and connective tissue macromolecules (Hemmani and Parihar, 1998).

Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease (Barry, 1991). A number of plants have been reported to possess antioxidant effects. The antioxidant properties of Bacopa monnieri (Bhattacharya et al., 2000; Tripathi et al., 1996), Emblica officinalis (Bhattacharya et al., 1999; Mathur et al., 1996), Glycyrrhiza glabra (Hatano et al., 1991), Mangifera indica (Ghosal et al., 1996) and Syzygium aromaticum (Deans, et al., 1995) were earlier investigated and were found to possess free radical scavenging property. Some of the ingredients were also found to produce significant induction in the levels of various endogenous antioxidant enzymes.

The present study was aimed to investigate the effects of DHC-1, an herbal formulation, on the anti-oxidant enzymes and markers of free radical generation in normal rats.

*DHC-1 is marketed as Oxitard
2. Material and methods

2.1 Plant Materials

*Bacopa monnieri, Emblica officinalis, Glycyrrhiza glabra, Mangifera indica and Syzygium aromaticum* were procured from a local supplier and identified by Dr. Kannan, Ph.D., Botanist, The Himalaya Drug Company, Bangalore. Samples were retained for reference purpose at the R & D herbarium.

2.2 Composition

Each gram of DHC-1 contains:

<table>
<thead>
<tr>
<th>Herbs</th>
<th>Voucher code</th>
<th>Part used</th>
<th>Qty.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacopa monnieri</em> Linn. (Scrophulariaceae)</td>
<td>HDHB-157</td>
<td>Whole plant</td>
<td>200 mg</td>
</tr>
<tr>
<td><em>Emblica officinalis</em> Gaertn. (Euphorbiaceae)</td>
<td>HDHB-143</td>
<td>Fruit</td>
<td>200 mg</td>
</tr>
<tr>
<td><em>Glycyrrhiza glabra</em> Linn. (Papilionaceae)</td>
<td>HDHB-174</td>
<td>Roots</td>
<td>200 mg</td>
</tr>
<tr>
<td><em>Mangifera indica</em> Linn. (Anacardiaceae)</td>
<td>HDHB-17</td>
<td>Bark</td>
<td>200 mg</td>
</tr>
<tr>
<td><em>Syzygium aromaticum</em> Linn. (Myrtaceae)</td>
<td>HDHB-208</td>
<td>Flower bud</td>
<td>200 mg</td>
</tr>
</tbody>
</table>

2.3 Sources of Fine Chemicals

1,1,3,3-tetraethoxypropane, crystalline beef liver catalase and superoxide dismutase were obtained from Sigma Chemicals, St. Louis, M.O., U.S.A. Thiobarbituric acid, tris buffer, sucrose, ATP, reduced glutathione, 1-amino-2-naphthol-4-sulphonic acid, 5,5'-dithiobis (2-nitro benzoic acid) and bovine serum albumin were obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. Trichloroacetic acid, ammonium molybdate, citric acid monohydrate, sodium nitrate, sulphanilic acid, hydrogen peroxide, copper sulphate, sodium potassium tartarate, sodium metaperiodate, ethanol and Folin’s phenol reagent were obtained from S.D. Fine Chemicals, Mumbai, India. Sodium hydroxide, sodium carbonate, sodium bicarbonate, magnesium chloride, sodium chloride, potassium chloride, calcium chloride, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, chloroform, ether, hydrochloric acid and conc. sulphuric acid were purchased from Qualigens Chemicals Ltd., Mumbai, India. Ethylenediaminetetraacetic acid disodium salt and epinephrine bitartrate were obtained from BDH Chemicals, Mumbai, India.

2.4 Animals

Forty eight Wistar rats of either sex weighing between 150-225 gms were used for the study. During the course of the experiment the animals were fed with standard pellet diet *ad libitum* and had free access to water. Animal experiments were approved by the Social Justice and Empowerment Committee for the purpose of control and supervision of experiments on animals, Ministry of Government of India, New Delhi.

2.5 Experimental procedure

The animals were divided into two sets of 24 each. In the first set of experiment, the animals were divided into three groups of eight animals each, which are as follows:

- **Group 1:** Represented control that received 5 ml/kg b.wt. of vehicle (1% CMC) orally for 30 days.
- **Group 2:** Rats received the drug DHC-1 at a dose of 100 mg/kg b.wt. as an aqueous suspension, once daily, orally for 30 days.
- **Group 3:** Rats received DHC-1 at a dose of 200 mg/kg b.wt., once daily, orally for 30 days.

*DHC-1 is marketed as Oxitard*
After completion of 30 days of drug administration the rats were anaesthetized with ether and blood was collected from the orbital plexus. Serum was separated and kept at 4°C until use. The animals were then sacrificed and organs such as liver, kidneys and heart were removed, processed and homogenized in Tris buffer (10 mM, pH 7.4) at a concentration of 10% w/v.

The homogenates were centrifuged at 10000 X g at 4°C for 20 minutes using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assays of lipid peroxidation, endogenous antioxidant enzymes and G-6-P-D. The sediment was resuspended in ice cold Tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes and proteins.

2.5.1 Tissue estimations
2.5.1.1 Determination of Lipid Peroxidation (MDA content)
It was estimated using the method described by Slater and Sawyer (1971). 2.0ml of the tissue homogenate (supernatant) was added to 2.0ml of freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, the precipitate was separated by centrifugation and 2.0ml of clear supernatant solution was mixed with 2.0ml of freshly prepared 0.67% thiobarbituric acid (TBA). The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately cooled in an ice bath for 5 minutes. The colour developed was measured at 532nm against reagent blank. Different concentrations (0-23nM) of standard malondialdehyde were taken and processed as above for standard graph. The values are expressed as nM of MDA/mg protein.

2.5.1.2 Determination of Superoxide Dismutase (SOD)
Superoxide dismutase was estimated using the method developed by Misra and Fridovich (1972). 0.5ml of tissue homogenate was diluted with 0.5ml of distilled water, to which 0.25ml of ice-cold ethanol and 0.15ml of ice-cold chloroform were added. The mixture was mixed well using cyclo mixer for 5 minutes and centrifuged at 2500 rpm. To 0.5ml of supernatant, 1.5ml of carbonate buffer (0.05M, pH 10.2) and 0.5ml of EDTA solution (0.49M) were added. The reaction was initiated by the addition of 0.4ml of epinephrine (3mM) and the change in optical density/minute was measured at 480nm against reagent blank. SOD activity was expressed as units/mg protein. Change in optical density per minute at 50% inhibition of epinephrine to adrenochrome transition by the enzyme is taken as the enzyme unit. Calibration curve was prepared by using 10-125 units of SOD.

2.5.1.3 Determination of Catalase (CAT)
Catalase was estimated by the method of Hugo Aebi as given by Colowick et al (1984). To 2ml of diluted sample 1ml of hydrogen peroxide (30 mmol/l) was added to initiate the reaction. The blank was prepared by mixing 2ml of diluted sample (similar dilution) with 1ml of phosphate buffer (50mmol/l; pH 7.0). The dilution should be such that the initial absorbance should be approximately 0.500. The decrease in absorbance was measured at 240nm. Catalase activity was expressed as µmoles of H₂O₂ consumed/min/mg protein.

2.5.1.4 Determination of Reduced glutathione
Reduced glutathione was determined by the method of Moron et al (1979). Equal volumes of tissue homogenate (supernatant) and 20% trichloroacetic acid were mixed. The precipitated fraction was centrifuged and to 0.25ml of supernatant, 2ml of 0.6mM 5,5'-dithiobis (2-nitro benzoic acid) reagent was added. The final volume was made up to 3ml with phosphate buffer (0.2M, pH 8.0). The colour developed was read at 412nm against reagent blank. Different

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concentrations (10-50 µg) of standard glutathione were taken and processed as above for standard graph. The amount of reduced glutathione was expressed as µg of GSH/mg protein.

2.5.1.5 Determination of Membrane Bound Enzymes and Inorganic Phosphorus
Membrane bound enzymes namely Na\(^+\)K\(^+\)ATPase, Ca\(^{2+}\)ATPase and Mg\(^{2+}\)ATPase were assayed according to the methods of Bonting (1970), Hjerken and Pan (1983) and Ohinashi et al. (1982) respectively. The inorganic phosphorus was estimated by the method of Fiske and Subbarow (1925).

Total proteins were determined by the method of Lowry et al (1951). Liver G-6-P-D was estimated by the method of Gowenlock et al (1988a).

2.5.2 Serum estimations
SGPT and SGOT levels were estimated by Reitman and Frankel (1957) method. Serum alkaline phosphatase was determined by the method devised by King (1965). Total bilirubin estimation was done using the method of Jendrassik and Grof as mentioned by Shull et al (1980). Lactate Dehydrogenase activity was estimated by the method of Gowenlock (1988b).

2.5.3 Lipid estimations
In the second set of experiment, the animals were divided into three groups as previously mentioned and were given similar treatment for 30 days. After 30 days of drug administration the animals were sacrificed and organs such as liver, kidneys and heart were removed. Lipids were extracted from these tissues by the method of Folch et al (1955). Triglyceride was estimated by the method of Foster and Dunn (1973). Phospholipid and cholesterol were determined by the method of Stewart (1980) and Zlatkis et al (1953) respectively.

2.6 Statistical analysis
Results of all the above estimations have been indicated in terms of mean ± SEM. The difference between means was analyzed by Student’s ‘t’ test. Minimum level of significance was fixed at \( p \leq 0.05 \).

3. Results
3.1 Effect of DHC-1 on tissue parameters
Administration of the drug DHC-1 (200 mg/kg) rats resulted in a significant (\( p < 0.05 \)) increase in superoxide dismutase and catalase enzymes in liver. It also significantly (\( p < 0.05 \)) elevated the levels of reduced glutathione in liver. The drug produced a decrease in the level of lipid peroxidation in all organs but a significant (\( p < 0.01 \)) decrease was produced only in the heart. Amongst the three membranes bound enzymes, the drug DHC-1 (200 mg/kg) produced a significant (\( p < 0.01 \)) increase in the level of Ca\(^{2+}\)ATPase in liver. The drug (200 mg/kg) also significantly (\( p < 0.05 \)) increased the protein content of liver, heart and kidneys (Tables 1a-1c).

3.2 Effect of DHC-1 on serum parameters
The drug (200 mg/kg) significantly (\( p < 0.05 \)) reduced the levels of SGPT, SGOT and alkaline phosphatase. The drug also significantly (\( p < 0.01 \)) reduced the levels of bilirubin at both the doses. However, there was no significant change produced in the levels of lactate dehydrogenase enzyme (Table 2).

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3.3 Effect of DHC-1 on lipids
The drug (100 mg/kg and 200 mg/kg) significantly \( p<0.05 \) and \( p<0.01 \) reduced the level of triglyceride in heart. However, the levels of cholesterol and phospholipid were not much affected by the drug (Table 3).

<table>
<thead>
<tr>
<th>Tissue parameters</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>SOD (Unit/min/mg protein)</td>
<td>2.66 ± 0.63</td>
</tr>
<tr>
<td>Catalase (µmoles of H\textsubscript{2}O\textsubscript{2} consumed/min/mg protein)</td>
<td>404.25 ± 9.89</td>
</tr>
<tr>
<td>Reduced Glutathione (µg of GSH/mg protein)</td>
<td>2.02 ± 0.09</td>
</tr>
<tr>
<td>Lipid peroxidation (mmoles of MDA/min/mg protein)</td>
<td>52.59 ± 3.46</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}ATPase (µmoles of inorganic phosphorus liberated/mg protein)</td>
<td>3.15 ± 0.12</td>
</tr>
<tr>
<td>Mg\textsuperscript{2+}ATPase (µmoles of inorganic phosphorus liberated/mg protein)</td>
<td>2.34 ± 0.10</td>
</tr>
<tr>
<td>Na\textsuperscript{+}K\textsuperscript{+}ATPase (µmoles of inorganic phosphorus liberated/mg protein)</td>
<td>4.75 ± 0.26</td>
</tr>
<tr>
<td>Liver G-6-P-D (U/mg protein)</td>
<td>5.15 ± 0.64</td>
</tr>
<tr>
<td>Proteins (mg/gm of tissue)</td>
<td>123.20 ± 3.09</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SEM for groups of eight animals each. Experimental groups were compared with the corresponding values of Control group. * \( p<0.05 \); # \( p<0.01 \)

<table>
<thead>
<tr>
<th>Tissue parameters</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>SOD (Unit/min/mg protein)</td>
<td>2.59 ± 0.36</td>
</tr>
<tr>
<td>Catalase (µmoles of H\textsubscript{2}O\textsubscript{2} consumed/min/mg protein)</td>
<td>244.77 ± 6.23</td>
</tr>
<tr>
<td>Reduced Glutathione (µg of GSH/mg protein)</td>
<td>1.97 ± 0.14</td>
</tr>
<tr>
<td>Lipid peroxidation (mmoles of MDA/min/mg protein)</td>
<td>55.91 ± 2.41</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}ATPase (µmoles of inorganic phosphorus liberated/mg protein)</td>
<td>4.55 ± 0.20</td>
</tr>
<tr>
<td>Mg\textsuperscript{2+}ATPase (µmoles of inorganic phosphorus liberated/mg protein)</td>
<td>3.63 ± 0.06</td>
</tr>
<tr>
<td>Na\textsuperscript{+}K\textsuperscript{+}ATPase (µmoles of inorganic phosphorus liberated/mg protein)</td>
<td>10.21 ± 0.31</td>
</tr>
<tr>
<td>Proteins (mg/gm of tissue)</td>
<td>72.82 ± 0.16</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SEM for groups of eight animals each. Experimental groups were compared with the corresponding values of Control group. * \( p<0.05 \); # \( p<0.01 \)

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### Table 1c: Effect of DHC-1 on the levels of tissue parameters in heart

<table>
<thead>
<tr>
<th>Tissue parameters</th>
<th>Group</th>
<th>Control</th>
<th>DHC-1 (100 mg/kg)</th>
<th>DHC-1 (200 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (Unit/min/mg protein)</td>
<td></td>
<td>2.23 ± 0.72</td>
<td>2.36 ± 0.98</td>
<td>2.53 ± 0.22</td>
</tr>
<tr>
<td>Catalase (µmoles of H$_2$O$_2$ consumed/min/mg protein)</td>
<td></td>
<td>46.91 ± 5.79</td>
<td>53.91 ± 5.09</td>
<td>64.37 ± 12.53</td>
</tr>
<tr>
<td>Reduced Glutathione (µg of GSH/mg protein)</td>
<td></td>
<td>1.05 ± 0.07</td>
<td>1.09 ± 0.08</td>
<td>1.14 ± 0.15</td>
</tr>
<tr>
<td>Lipid peroxidation (nmol of MDA/min/mg protein)</td>
<td></td>
<td>33.47 ± 2.56</td>
<td>25.41 ± 2.82</td>
<td>17.49 ± 1.62*</td>
</tr>
<tr>
<td>Ca$^{2+}$ATPase (µmol of inorganic phosphorus liberated/mg protein)</td>
<td></td>
<td>2.57 ± 0.19</td>
<td>2.67 ± 0.19</td>
<td>2.73 ± 0.16</td>
</tr>
<tr>
<td>Mg$^{2+}$ATPase (µmol of inorganic phosphorus liberated/mg protein)</td>
<td></td>
<td>2.38 ± 0.12</td>
<td>2.53 ± 0.20</td>
<td>2.72 ± 0.12</td>
</tr>
<tr>
<td>Na$^{+}$K$^{+}$ATPase (µmol of inorganic phosphorus liberated/mg protein)</td>
<td></td>
<td>4.85 ± 0.20</td>
<td>5.10 ± 0.45</td>
<td>5.10 ± 0.13</td>
</tr>
<tr>
<td>Proteins (mg/gm of tissue)</td>
<td></td>
<td>123.76 ± 0.27</td>
<td>138.45 ± 8.18</td>
<td>241.22 ± 10.95*</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SEM for groups of eight animals each. Experimental groups were compared with the corresponding values of Control group. *p<0.05; #p<0.01

### Table 2: Effect of DHC-1 on the levels of serum parameters of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>Control</th>
<th>DHC-1 (100 mg/kg)</th>
<th>DHC-1 (200 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT (U/ml)</td>
<td></td>
<td>21.33 ± 1.12</td>
<td>17.67 ± 1.15</td>
<td>16.33 ± 0.73*</td>
</tr>
<tr>
<td>SGOT (U/ml)</td>
<td></td>
<td>66.33 ± 2.09</td>
<td>60.50 ± 1.10</td>
<td>56.00 ± 2.93</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/l)</td>
<td></td>
<td>135.03 ± 11.22</td>
<td>108.74 ± 4.81</td>
<td>87.92 ± 5.33</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td></td>
<td>305.75 ± 21.64</td>
<td>255.75 ± 9.54</td>
<td>231.50 ± 5.54</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td></td>
<td>0.38 ± 0.03</td>
<td>0.23 ± 0.01*</td>
<td>0.16 ± 0.02*</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SEM for groups of eight animals each. Experimental groups were compared with the corresponding values of Control group. *p<0.05 and #p<0.01

### Table 3: Effect of DHC-1 on the levels of lipid parameters in various organs of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Organs</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>5.90 ± 0.05</td>
<td>5.86 ± 0.03</td>
<td>5.19 ± 0.04</td>
</tr>
<tr>
<td>DHC-1 (100 mg/kg)</td>
<td></td>
<td>7.18 ± 0.16</td>
<td>6.08 ± 0.21</td>
<td>12.93 ± 0.12</td>
</tr>
<tr>
<td>DHC-1 (200 mg/kg)</td>
<td></td>
<td>4.44 ± 0.44</td>
<td>6.83 ± 0.24</td>
<td>1.10 ± 0.07</td>
</tr>
<tr>
<td>DHC-1 (100 mg/kg)</td>
<td></td>
<td>5.88 ± 0.03</td>
<td>5.76 ± 0.05</td>
<td>5.35 ± 0.15</td>
</tr>
<tr>
<td>DHC-1 (200 mg/kg)</td>
<td></td>
<td>5.15 ± 0.07</td>
<td>8.25 ± 0.13</td>
<td>3.39 ± 0.52*</td>
</tr>
<tr>
<td>DHC-1 (100 mg/kg)</td>
<td></td>
<td>3.02 ± 0.24</td>
<td>5.76 ± 0.33</td>
<td>1.07 ± 0.04</td>
</tr>
<tr>
<td>DHC-1 (200 mg/kg)</td>
<td></td>
<td>2.88 ± 0.41</td>
<td>4.71 ± 0.21</td>
<td>0.90 ± 0.03</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SEM for groups of eight animals each. Experimental groups were compared with the corresponding values of Control group. *p<0.05; #p<0.01

There was no significant difference in body weight gain between the three different groups.

*DHC-1 is marketed as Oxitard*
3.4 Chemical analysis
Chemical analysis of DHC-1 is also proving quality of DHC-1 (Figures 1-6).

Figure 1. High performance thin layer chromatogram fingerprint of *Bacopa monnieri.*

Figure 2. High performance thin layer chromatogram fingerprint of *Emblica officinalis.*

Figure 3. High performance thin layer chromatogram fingerprint of *Glycyrrhiza glabra.*

Figure 4. High performance thin layer chromatogram fingerprint of *Mangifera indica.*

Figure 5. High performance thin layer chromatogram fingerprint of *Syzygium aromaticum.*

Figure 6. High performance thin layer chromatogram fingerprint of DHC-1.

4. Discussion and Conclusions
Superoxide dismutase (SOD) and catalase (CAT) enzymes are the important endogenous antioxidant enzymes. Superoxide dismutases superoxide radical to form hydrogen peroxide and water. At high concentrations, hydrogen peroxide is removed by the enzyme catalase whereas at lower concentrations it is removed by reacting with reduced glutathione (Hemnani and Parihar, 1998). Glutathione is also an important inhibitor of free radical mediated lipid peroxidation (Meistor, 1983). DHC-1 (200 mg/kg) resulted in a significant increase in the liver SOD, catalase and reduced glutathione levels as compared to the control animals, which suggests its antioxidant activity. DHC-1 has also been reported to exhibit antioxidant activity in ulcerated animals using pylorus-ligation and ethanol-induced ulcer models.

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experimental models related to lesion pathogenesis with production of reactive oxygen species (Bafna and Balaraman, 2004).

Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids that eventually results in destruction of membrane often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore, it is not surprising that membrane lipids are susceptible to peroxidative attack (Cheesman, 1993). The study revealed a significant decrease in lipid peroxidation in heart, which suggests its protective effect against myocardial necrotic changes.

Glucose 6-phosphate dehydrogenase (G-6-P-D) is one of the several hepatic carbohydrate metabolizing enzymes, which is important in providing reduced nicotinamide adenine dinucleotide phosphate (NADPH) for lipogenesis (Fitch and Chaikoff, 1960; Rohnstad and Katz, 1966). The activity of this enzyme is correlated with the rate of lipogenesis. Changes in the activity of this enzyme therefore may affect the utilization of ingested carbohydrates or the process of lipogenesis.

Na\(^+\)K\(^+\)ATPase, Ca\(^{2+}\)ATPase and Mg\(^{2+}\)ATPase are membrane bound enzymes. Depending on the alteration in lipid composition; the fluidity and thus the activities of these enzymes vary (Floreani et al., 1981). Increased enzyme activity was reported with decreased phospholipid activity. A change in membrane cholesterol content affects its fluidity (Kumari et al., 1990). The drug increased the activity of Ca\(^{2+}\)ATPase, which may be due to the reduction in lipid content.

Serum transaminases such as GOT and GPT are liberated into the serum after extensive tissue damage. The heart muscle is rich in SGOT whereas liver is rich in both the enzymes. Increased levels are thus indicators of myocardial ischemia and liver damage (Reitman and Frankel, 1957). The reduction in these enzymes by the drug may be due to the prevention of release of these enzymes by virtue of its membrane stabilizing activity.

Serum alkaline phosphatase estimations are of interest in the diagnosis of hepatobiliary diseases and bone diseases associated with increased osteoblastic activity (King, 1965). The drug significantly reduced the levels of this enzyme at the higher dose (200 mg/kg). The drug was also found to significantly \((p<0.01)\) reduce serum bilirubin as compared to normal rats. This shows the protective effect of the drug in liver.

Elevated levels of cholesterol and triglycerides are associated with atherosclerosis, nephrosis, diabetes mellitus, obstructive jaundice and myxedema. Hypertriglyceridemia is associated with metabolic consequences of hypercoagulability, hyperinsulinaemia, insulin resistance and glucose tolerance (Godkar, 1996). Higher the lipid higher is the risk for oxidative damage. The drug DHC-1 (200 mg/kg) showed decrease in triglyceride levels.

With all these findings it can be concluded that DHC-1 exhibits an antioxidant activity by inhibition of lipid peroxidation and enhancement of antioxidant enzymes and reduced glutathione and thus can be used in the treatment of various disorders where reactive oxygen species are involved.

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Acknowledgements
We are thankful to The Himalaya Drug Company, Bangalore, India, for providing financial assistance and the drug, DHC-1 for successfully carrying out this work.

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