Cardioprotective effect of *Urtica parviflora* leaf extract against doxorubicin-induced cardiotoxicity in rats

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[ABSTRACT] **AIM:** The present study evaluated the cardioprotective property of the hydroethanol extract of *Urtica parviflora* leaf material (EEUP) against doxorubicin-induced cardiotoxicity in rats. **METHODS:** Cardiotoxicity was produced by doxorubicin administration (15 mg·kg⁻¹ i.p. for 21 days). The rats received EEUP at 200 and 400 mg·kg⁻¹ b.w. (i.p.) daily for 21 days. After 24 h, serum cardiac biomarkers, i.e. creatine phosphokinase (CPK) and lactate dehydrogenase (LDH); serum lipid profiles, like high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG); serum biochemical parameters, viz. aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP); myocardial antioxidant parameters, viz. malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were measured. **RESULTS:** EEUP treatment significantly (*P* < 0.01) and dose dependently protected the myocardium by decreasing the elevated level of MDA; elevating the diminished levels of GSH, SOD, CAT and HDL, with a concomitant decrease in the elevated levels of LDL, and TG. EEUP also significantly (*P* < 0.01) reduced the increased activities of AST, ALT, ALP, CPK and LDH. The results revealed that EEUP demonstrated dose dependent cardioprotective efficacy by restoration of the serum biomarkers profile and antioxidant property. **CONCLUSION:** From the present study, *U. parviflora* leaf extract showed promising cardioprotective effect against doxorubicin-induced cardiotoxicity in Wistar rats. **[KEY WORDS]** Doxorubicin; Cardioprotective; *Urtica parviflora*; Antioxidant; Leaf material; Serum biomarkers


1 Introduction

Doxorubicin (adriamycin) is an anthracycline compound and highly efficacious anticancer drug that is widely used for treatment of various neoplastic diseases[¹]. However, its clinical use very often becomes a limiting factor in anticancer therapy due to its high irreversible cardiotoxicity. Congestive heart failure, cardiomyopathy and electrocardiographic changes were demonstrated after cumulative doxorubicin administration[²]. Doxorubicin-induced cardiotoxicity has been shown to be mediated through several different mechanisms, including membrane lipid peroxidation[³], free radical formation[⁴], mitochondrial damage[⁵], decreased activity of Na⁺–K⁺ adenosine triphosphate[⁶], and increases in serum total cholesterol, triglyceride and low density lipoproteins[⁷].

*Urtica parviflora* Roxb. (Urticaceae), commonly known as Sishnu in Nepalese, Nettle in English, and Paharah-bichuti in Bengali is a monoeious, perennial herb consisting of long stoloniferous rhizomes found in forests and amongst taller herbaceous vegetation, at 1 700–2 800 meters, partly shady, moist places of evergreen forests, along the streams and roadsides of Nepal, Bhutan, Western China and in Northern India. In India, it is mainly found in the Uttarakhand, Kashmir, Tamil Nadu, Assam and Sikkim states[⁸-⁹]. The leaves of the plant have stinging hairs, causing irritation and rashes to the skin. Its leaves are used as a culinary vegetable (after treatment with boiling water) in Darjeeling and the Sikkim Himalayan region of India, and the plant is commercially cultivated and sold in these regions to be used as vegetable. Young cooked leaves are very palatable and nutritious food, rich in vitamins and minerals, especially of α-tocopherol and vitamin C[⁹]. The plant has been traditionally used for several medicinal purposes. Its seed oil is edible, as well as medicinal...
for sciatica, rheumatism, and several skin ailments; a hair wash from the leaf extract is believed to avoid baldness. The leaves are used in dysentery, joint pain and liver disorders[9]. The fresh roots are employed for the treatment of fractures of the bone and dislocations of the joints[9]. Leaves and inflorescences are prescribed as a general tonic, and as a cleaning agent after parturition[10]. Its leaves and stems produce an inflammatory rash, accompanied by a considerable burning and itching sensation that is attributed to the presence of histamine and 5-hydroxytryptamine[11]. Previous research studies have reported the hepatoprotective[12], hypoglycemic[13], and in vitro antioxidant potential of U. parviflora[14]. The present study attempted the evaluation of the cardioprotective effect of U. parviflora leaf extract in a doxorubicin-induced cardiotoxicity model in Wistar rats.

2 Materials and Methods

Plant material The mature leaves of Urtica parviflora Roxb. (Urticaceae) were collected during the month of August 2009 from Majhitar, East Sikkim, India. The plant species was identified and authenticated from the Botanical Survey of India, Sikkim Circle, Gangtok, Sikkim, India, and a voucher specimen (No. HPI/121) was retained in the Pharmacology Department of Himalayan Pharmacy Institute, Majhitar, Sikkim, India for future reference.

Drugs and chemicals Doxorubicin was obtained from Get Well Pharmaceuticals, New Delhi, India. Superoxide dismutase, catalase references were obtained from Sigma-Aldrich, Germany and reduced glutathione was from Loba Chemie, Mumbai, India. Enzyme analyzing reagent kits were obtained from Span Diagnostics Ltd., Surat, India and Transasia Bio-medicals Ltd., Solan, India. All of the other chemicals used for the biochemical estimation were of analytical grade obtained commercially.

Preparation of extract Air-dried fresh leaves (218 g) were powdered in a mechanical grinder, and the powdered plant material was extracted with 50% ethanol by maceration at room temperature 24–26 °C for 96 h. Then the solvent was completely removed under reduced pressure to yield the dry extract (EEUP yield: 6.50%) which was stored in a vacuum desicator for future use. Preliminary phytochemical studies on EEUP indicated the presence of flavonoids, steroids, glycosides and tannins[15].

Animals Adult male Wistar albino rats (100–150 g) were used for the present study. The rats were grouped and housed in polyacrylic cages (38 cm × 23 cm × 10 cm), with not more than four animals per cage and maintained under standard laboratory conditions (temperature (25 ± 2) °C and dark/light cycle 14/10 h) for seven days prior to commencement of the experiment. They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee, Himalayan Pharmacy Institute (IAEC Reg. No. HPI/09/60/0071).

Acute toxicity EEUP was administered orally to male Swiss albino mice to evaluate the acute toxicity as per the reported method[16].

Experimental design The rats were divided into 4 groups (n = 6). Group I served as normal control and received normal saline 5 mL·kg−1 body weight i.p. Except for group I, doxorubicin was administered (5 mg·kg−1 body weight i.p.) to all other groups of animals in three equal injections on the 7th, 14th and 21st days for a total cumulative dose of 15 mg·kg−1 body weight. Group II served as the doxorubicin control. Groups III and IV received EEUP at doses of 200 and 400 mg·kg−1 body weight i.p. daily for 21 consecutive days.

Enzyme assays Twenty-four hours after the last treatment, blood samples were collected by cardiac puncture from all of the groups of rats. Serum samples were separated for the estimation of cardiac biomarkers, viz. creatine phosphokinase (CPK)[17] and lactate dehydrogenase (LDH)[18]; lipid profile like cholesterol[19], triglycerides[20], high density lipoprotein (HDL), low density lipoprotein (LDL)[21]; serum enzymes like aspartate amino transferase (AST)[22], alanine amino transferase (ALT)[23], alamine amino transferase (ALT)[23], and alkaline phosphatase (ALP)[24]. After collection of blood, all animals were sacrificed by cervical dislocation, and heart vessel was isolated and homogenized in buffered isotonic saline for estimation of myocardial antioxidant parameters, including malondialdehyde (MDA)[25], superoxide dismutase (SOD)[26], catalase (CAT)[27], and reduced glutathione (GSH)[28].

Histopathological studies The hearts form the experimental animals were fixed in 10% buffered formalin and were processed for microtome sectioning. Sections of about 5 µm thickness were stained with haematoxylin and eosin for histopathological studies.

Statistical analysis All results are expressed as the mean ± standard error of the mean (SEM). The results were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test using GraphPad InStat version 3.05 (GraphPad Software, USA).

3 Results

Acute toxicity: The EEUP did not show any toxic effects or deaths up to the dose of 4 000 mg·kg−1, b.w., p.o.

Serum lipid levels: Animals treated with doxorubicin produced a significant (P < 0.01) increase in cholesterol, triglycerides and LDL levels, and a slight increase in the HDL levels as compared to the normal control group. However, treatment with EEUP significantly (P < 0.01) decreased the cholesterol, triglycerides, HDL and LDL levels as compared to the doxorubicin control group (Table 1).

Serum enzyme biomarkers: Doxorubicin-treated animals showed a significant (P < 0.01) increase in CPK, LDH, AST, ALT, and ALP levels as compared to the normal control group. After treatment with EEUP, the levels of
CPK, LDH, AST, ALT and ALP were significantly (P < 0.01) restored towards normal in a dose dependent manner (Tables 2 and 3).

### Table 1  Effect of EEUP on cholesterol, triglycerides, HDL and LDL level in doxorubicin-treated rats (mean ± SEM, n = 6)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cholesterol / (mg dL⁻¹)</th>
<th>Triglycerides / (mg dL⁻¹)</th>
<th>HDL / (mg dL⁻¹)</th>
<th>LDL / (mg dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>112.60 ± 1.13</td>
<td>176.46 ± 0.96</td>
<td>23.54 ± 0.29</td>
<td>50.06 ± 0.73</td>
</tr>
<tr>
<td>Dox (5 mg kg⁻¹)</td>
<td>199.02 ± 1.09</td>
<td>195.91 ± 0.84</td>
<td>35.21 ± 0.61</td>
<td>85.69 ± 1.29</td>
</tr>
<tr>
<td>EEUP (200 g kg⁻¹) + Dox</td>
<td>136.46 ± 0.83</td>
<td>175.13 ± 0.67</td>
<td>28.36 ± 0.44</td>
<td>61.75 ± 0.88</td>
</tr>
<tr>
<td>EEUP (400 g kg⁻¹) + Dox</td>
<td>137.625 ± 1.081</td>
<td>177.99 ± 1.11</td>
<td>25.11 ± 1.31</td>
<td>71.63 ± 0.72</td>
</tr>
</tbody>
</table>

P < 0.01 vs normal control and doxorubicin control.

Dox: Doxorubicin.

### Table 2  Effect of EEUP on CPK and LDH in doxorubicin-treated rats (mean ± SEM, n = 6)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CPK / (IU L⁻¹)</th>
<th>LDH / (IU L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>137.13 ± 1.99</td>
<td>134.33 ± 1.64</td>
</tr>
<tr>
<td>Dox (5 mg kg⁻¹)</td>
<td>631.70 ± 9.39a</td>
<td>221.78 ± 1.29a</td>
</tr>
<tr>
<td>EEUP (200 mg kg⁻¹) + Dox</td>
<td>361.72 ± 2.80b</td>
<td>178.41 ± 1.21b</td>
</tr>
<tr>
<td>EEUP (400 mg kg⁻¹) + Dox</td>
<td>206.87 ± 2.28b</td>
<td>166.73 ± 1.04b</td>
</tr>
</tbody>
</table>

P < 0.01 vs normal control and doxorubicin control.

Dox: Doxorubicin.

### Table 3  Effect of EEUP on AST, ALT and ALP in doxorubicin-treated rats (mean ± SEM, n = 6)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AST / (IU L⁻¹)</th>
<th>ALT / (IU L⁻¹)</th>
<th>ALP / (IU L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5.55 ± 0.36</td>
<td>14.63 ± 0.46</td>
<td>48.11 ± 0.65</td>
</tr>
<tr>
<td>Dox (5 mg kg⁻¹)</td>
<td>23.62 ± 0.87a</td>
<td>55.27 ± 0.71a</td>
<td>64.43 ± 1.57a</td>
</tr>
<tr>
<td>EEUP (200 mg kg⁻¹) + Dox</td>
<td>16.36 ± 0.69b</td>
<td>29.63 ± 0.63b</td>
<td>34.68 ± 1.18b</td>
</tr>
<tr>
<td>EEUP (400 mg kg⁻¹) + Dox</td>
<td>11.60 ± 0.39b</td>
<td>22.29 ± 0.57b</td>
<td>18.04 ± 0.78b</td>
</tr>
</tbody>
</table>

P < 0.01 vs normal control and doxorubicin control.

Dox: Doxorubicin.

### Table 4  Effect of EEUP on MDA, GSH, CAT and SOD in doxorubicin-treated rats (mean ± SEM, n = 6)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MDA / (nmol/g of wet tissue)</th>
<th>GSH / (µg/g of wet tissue)</th>
<th>CAT / (Units/mg of protein)</th>
<th>SOD / (Units/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>13.73 ± 0.53</td>
<td>36.95 ± 1.66</td>
<td>25.95 ± 0.81</td>
<td></td>
</tr>
<tr>
<td>Dox (5 mg kg⁻¹)</td>
<td>44.96 ± 1.32</td>
<td>63.27 ± 0.75</td>
<td>9.93 ± 0.69</td>
<td></td>
</tr>
<tr>
<td>EEUP (200 mg kg⁻¹) + Dox</td>
<td>34.55 ± 0.69a</td>
<td>21.28 ± 1.01</td>
<td>15.96 ± 0.62</td>
<td></td>
</tr>
<tr>
<td>EEUP (400 mg kg⁻¹) + Dox</td>
<td>24.74 ± 1.30</td>
<td>20.72 ± 0.39</td>
<td>18.04 ± 0.78</td>
<td></td>
</tr>
</tbody>
</table>

P < 0.01 vs normal control and doxorubicin control.

Dox: Doxorubicin.

### Antioxidant status:
The MDA level was increased, whereas the GSH, SOD and CAT activities were significantly (P < 0.01) decreased in the doxorubicin-treated group as compared with normal control animals. In the EEUP-treated groups, there was significantly lower MDA and higher GSH, CAT and SOD levels as compared to the doxorubicin control group (Table 4).

### Cardiac histopathology:
Figs. 1-4 illustrate the histopathological changes in the heart tissue of normal control and doxorubicin-treated rats. The heart sections from normal control rats (Fig. 1) show normal cardiac structure with intact myocardium and coronary vessels. In contrast, the heart sections from doxorubicin-treated rats (Fig. 2) exhibit marked cardiac damage characterized by myocardial necrosis, fibrosis, and vascular congestion. The heart sections from EEUP-treated rats (Figs. 3 and 4) show partial restoration of normal cardiac structure with reduced myocardial necrosis and improved vascular patency compared to the doxorubicin control group.
pathological assessments of the cardiac segments of all experimental rats. Doxorubicin administration caused disorganization of normal cellular architecture of the heart. EEUP treatment reduced such changes in a dose related manner almost similar to that of the normal control.

4 Discussion

The present work was aimed to study the cardioprotective activity of the hydroethanol extract of *U. parviflora* leaf (EEUP) in doxorubicin-induced cardiotoxicity in rats. The results of this study revealed that EEUP at the doses of 200 and 400 mg kg\(^{-1}\) body weight dependently and significantly ameliorated the cardiotoxicity by restoring serum and myocardial biochemical parameters towards the normal values.

The existing experimental evidence suggests that doxorubicin-induced myocardial oxidative stress is due to the generation of free radicals in the heart tissues\(^{[29]}\). In biological systems, doxorubicin is enzymatically reduced to the doxorubicin semiquinone radical. This semiquinone radical directly transfers its electron to molecular oxygen, generating free radicals, namely, superoxide and hydrogen peroxide\(^{[30]}\). This free radical generation plays an important role in the cardiotoxicity of doxorubicin. Cardiac tissue is especially susceptible to free radical injury because of the lower activities of the free radical detoxifying mechanisms, such as SOD, CAT and GSH. Further, doxorubicin also has a high affinity for the phospholipid component of the mitochondrial membrane in cardiac myocytes, leading to selective accumulation of doxorubicin in the heart tissue\(^{[31]}\). The doxorubicin-induced mitochondrial injury is critical to the heart because it would presumably have extreme adverse effects on the contractile functioning of the cardiac myocytes by producing alterations in the energy metabolism\(^{[32]}\).

EEUP treatment was able to reduce the doxorubicin-induced cardiotoxic manifestations in multiple ways. Increase in the level of plasma triglycerides, total cholesterol and low density lipoproteins in the doxorubicin-treated group indicate doxorubicin may be interfering with metabolism or the biosynthesis of lipids. Treatment with EEUP showed a reduction in serum lipid profile levels in a dose related fashion. The lipid lowering effect of EEUP may be due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor mediated catabolism of LDL cholesterol, and an increase in the uptake of LDL from blood by the liver\(^{[33]}\).

A deficiency of oxygen supply or glucose may damage the myocardial cells, and the cell membrane becomes permeable or ruptures, resulting in leakage of enzymes from cardiac tissues to the blood. It has been reported that doxorubicin-induced free radical generation triggers membrane degradation and disruption of cardiac myocytes, which can lead to elevations of LDH and CPK in the serum\(^{[34-35]}\). In the present study, an increase in the activities of LDH, CPK, AST, ALP and ALT was observed in doxorubicin-treated rats. Treatment with EEUP decreased the enzyme activities in serum and restored the same in the heart. This could be due to a protective or membrane-stabilizing effect of EEUP on the myocardium, reducing the cardiac damage, and thereby restricting the leakage of these enzymes. The present results are in good agreement with those reported by previous workers\(^{[36-37]}\).

Cardioprotective activity of EEUP was further supported by EEUP-induced activation of myocardial non-enzymatic and enzymatic endogenous antioxidant defense mechanisms. Cellular GSH depletion is closely related to the lipid peroxidation and disturbance of Ca\(^{2+}\) influx induced by toxic agents. Overproduction of oxidative free radicals enhances lipid peroxidation. Here it was evident by an increase in MDA, which is the end product of lipid peroxidation. Intraportal administration of EEUP in doxorubicin-treated rats reduced the MDA level and elevated the GSH content to near-normal levels, which prevented degradation of cellular macromolecules and thus cell disruption, probably by decreasing the Ca\(^{2+}\) influx\(^{[38]}\). The present study has shown that doxorubicin induced lipid peroxidation and decreased the levels of protective antioxidant enzymes in the heart tissues. Treatment with EEUP significantly reduced the lipid peroxidation and increased the activities of SOD and CAT which detoxify superoxide and hydrogen peroxide radicals, respectively\(^{[39]}\). These results indicated the protective effect of EEUP on doxorubicin-induced cardiotoxicity by boosting the endogenous non-enzymatic and enzymatic antioxidant systems, which entailed scavenging of oxidative free radicals.

Histopathological examination of different cardiac sections revealed that doxorubicin caused anomalous histological changes in the cardiac tissue. However, EEUP treatment prevented the changes and maintained the histological structure almost similar to that of normal control.

Preliminary phytochemical studies showed the presence of flavonoids, tannins, glycosides and steroids in EEUP. Flavonoids and tannins are well known polyphenolic natural antioxidants the presence of which may be responsible for the antioxidant role of EEUP and protection of myocardial tissues from doxorubicin-induced oxidative injury.

In the present investigation, administration of EEUP to doxorubicin-intoxicated rats demonstrated prominent reduction in serum biomarker enzymes, normalization of serum lipid profiles compared to doxorubicin control rats in a dose-dependent manner. Also, EEUP treatment resulted in significant modulation of lipid peroxidation, endogenous non-enzymatic (GSH) and enzymatic (SOD and CAT) antioxidant and detoxification systems. Therefore, it can be concluded that the hydroethanol extract of the leaves of the edible plant *Urtica parviflora* is remarkably effective against doxorubicin-induced cardiotoxicity in Wistar rats, plausibly by virtue of its lipid lowering property and augmenting endogenous antioxidant mechanisms.
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References