ABSTRACT
Myocardial infarction is the common presentation of the ischemic heart disease. Herbal medicines is getting more importance in the treatment of heart diseases because the modern synthetic medicines have limitation in their use due to side effects. Present study was designed to investigate the protective effect of Hygrophila auriculata L. (family: Acanthaceae) against cardiotoxicity induced by doxorubicin. Administration of doxorubicin (25 mg/kg i.p.) induced cardiomyopathy by significant elevation in serum creatine kinase (CK), lactate dehydrogenase (LDH), triglycerides, cholesterol & lipid peroxidation activities with a corresponding decrease in SOD, CAT, GSH level in tissue homogenate. Oral administration of methanolic extract of Hygrophila auriculata leaves (100, 200 & 400mg/kg) & it’s different fraction like petroleum ether fraction, n-butanol fraction, chloroform fraction & ethyl acetate fraction (100 & 200 mg/kg) prior to doxorubicin produced a significant cardioprotective activity. Methanolic extracts of Hygrophila auriculata leaves & its n-butanol fraction at dose 200 mg/kg shows reduction in mortality & restoration of altered cardiac marker enzymes. The histopathological studies also supported the protective properties of Hygrophila auriculata leaves, animals pre-treated with Hygrophila auriculata leaves extract showed a marked protective effect with decreased necrotic zones and revealed normal cardiac muscle bundles. Present study showed that Hygrophila auriculata leaves methanolic extract & its n-butanol fraction significantly
restores most of the biochemical and histopathological parameters. These result indicates that Hygrophila auriculata leaves methanolic extract have significant cardioprotective activity.

KEYWORDS: Cardioprotective, Doxorubicin, Hygrophila auriculata, Myocardial infarction.

INTRODUCTION
Myocardial infarction is the common presentation of the ischemic heart disease. It occurs when myocardial ischemia surpasses the critical threshold level for an extended time resulting in irreversible myocardial cell damage. Although clinical care is improved, public awareness is raised and health innovations are widely used, myocardial infarction still remains the leading cause of death worldwide.\[1\] According to the World Health Organization, it will be the major cause of death in the world by the year 2020.\[2\] In India, the number of patients being hospitalized for myocardial infarction, commonly known as heart attack, is increasing in the past 35 years and male patients have shown a more striking increase.\[3\]

Herbal medicines are represented as the most important field of alternative medicines all over the world. Hygrophila auriculata (K. Schum) Heine (family: Acanthaceae) is described in the Ayurvedic literature as Ikshura, Ikshagandha and Kokilasha having eyes like the kokila or the Indian cuckoo. Almost all parts of the plant are recorded to be therapeutically valuable in the Ayurvedic, Unani and Allopathic systems of medicine. Almost all parts of the plant are recorded to be therapeutically valuable in the Ayurvedic, Unani and Allopathic systems of medicine. Extracts from different part of this species has shown several pharmacological properties such as anti-inflammatory activity,\[4\] analgesic,\[5\] antibacterial activity,\[6\] neuroprotective potential,\[7\] anti-cancer activity,\[8\] diuretic activity,\[9\] antidiabetic activity, hepatoprotective and antioxidant activities.\[10\] Aerial parts of the plant have been reported to contain lupeol, stigmesterol and butelin while the seeds of the plant are reported to contain mainly fatty acids, reducing and non-reducing sugars, polyphenolics (flavonoids and tannins), steroids, saponins, triterpenes.

Doxorubicin-induced cardiac toxicity is characterized by ventricular wall thinning and dilation of the left ventricular chamber. The variety of pathogenic mechanisms such as mitochondrial dysfunction, apoptosis of the cardiac myocytes and alteration in calcium handling have been shown to be involved in doxorubicin-induced cardio-myopathy. The present study was conducted to evaluate the Cardioprotective potential of the Hygrophila
auriculata leaves against doxorubicin induced cardiac toxicity in rats. The existing drugs can cure most of the diseases. Still there is a never ending search for finding new drugs in the hope that it would yield drugs with lesser side effects and better therapeutic benefits than the existing drugs. Heart plays a vital role in regulation of physiological processes. There are numerous plants and polyherbal formulations claimed to have cardioprotective activities. The growing concerns in the recent past over the toxic effects of various synthetic drugs have forced pharmaceutical researchers and physicians to use herbal drugs. Present study deals with pharmacological evaluation of leaves of Hygrophila auriculata L. with special reference to Cardioprotective potential in animal models.

MATERIALS AND METHODS

Plant collection

The Hygrophila auriculata leaves were collected from Ratnagiri district of Maharashtra in the month of Oct-Nov 2011. The Hygrophila auriculata leaves were authenticated by Dr. S. G. Bhave, HOD from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, College of forestry, Dapoli, Ratnagiri. The leaves were dried under shade and then powdered with a mechanical grinder. The powder of plant was passed through sieve No.30 and stored in airtight containers for further use.

Extraction and Fractionation of methanolic extract of Hygrophila auriculata leaves:

The dried powder of leaves was defatted with petroleum ether (60-80°C) in a Soxhlet apparatus & further defatted material extracted with methanol. The solvent was recovered by distillation under low pressure and the resulting semisolid mass was dried using rotary flash evaporator. The methanolic extract of H. auriculata (MEHA) was taken in a round bottom flask of simple condenser and further fractionated using solvents of increasing polarity viz. petroleum ether (400 ml×3wash), n-butanol (400 ml×3wash), chloroform (400 ml×3wash) & ethyl acetate (400 ml×3wash). From all the four fractions the solvent was removed using a rotary vacuum evaporator under reduced pressure. The methanol extract was thus fractionated into petroleum ether soluble fraction, n-butanol soluble fraction, chloroform soluble fraction & ethyl acetate soluble fraction.

Chemical used

Doxorubicin (Oncodria, Sun Pharma Lab. Ltd.)
Experimental setup
Albino rats of Wistar strain weighing 150-200gm were procured from listed suppliers National Institute of Bioscience, Pune. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ab libitum*. All the animals were acclimatized for a week before use. The experimental protocol was approved by Institutional Animal Ethics Committee (Reg.No.1092/ac/07/CPCSEA/02/2012). Acute oral toxicity test was carried out according to OECD guidelines 423.[11] From acute toxicity study of MEHA at 100, 200 & 400 mg/kg doses of whole extract & entire fractions of MEHA at doses 100, 200 mg/kg body weight were selected for actual study. In cardioprotective studies the test extracts were pre-treated before inducing Cardiac damage with DOX. [12] The methanolic extract & entire fractions were prepared in the form of emulsion with gum acacia. The rats were divided into following groups with 6 animals in each.

Group-I Received vehicle control gum acacia (2%, w/v acacia 5 ml/kg p.o.)
Group-II Received vehicle control gum acacia (2%, w/v acacia 5 ml/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-III Received MEHA (100mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-IV Received MEHA (200mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-V Received MEHA (400mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-VI Received Pet.ether fraction of MEHA (100mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-VII Received Pet.ether fraction of MEHA (200mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-VIII Received *n*-butanol fraction of MEHA (100mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-IX Received *n*-butanol fraction of MEHA (200mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-X Received chloroform fraction of MEHA (100mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-XI Received chloroform fraction of MEHA (200mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-XII Received ethyl acetate fraction of MEHA (100mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.
Group-XIII Received ethyl acetate fraction of MEHA (200mg/kg p.o) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.

Estimation of Biochemical Cardiac Marker Enzymes
At the end of the experiment period (28 days), 48 hrs after DOX injection, all the rats were anesthetized and then sacrificed by cervical decapitation. Blood was collected and serum and plasma were separated by centrifugation. Heart tissue was excised immediately and rinsed in ice-chilled normal saline. A known weight of the heart tissue was homogenized in 5.0 ml of 0.1 M Tris–HCl buffer (pH 7.4) solution. The homogenate was centrifuged and the supernatant tissue homogenate & blood serum were used for the estimation of various biochemical parameters like creatine kinase (CK), superoxide dismutase (SOD), catalase (CAT), levels of glutathione (GSH), triglycerides, Cholesterol, lactate dehydrogenase (LDH) & lipid peroxidation.

Determination of creatine kinase (CK) \[^{13}\]
0.75 mL of double distilled water, 0.05 mL of serum, 0.1 mL of ATP (0.0185 M in Tris-HCl buffer), 0.1 mL of magnesium-cystine reagent and 0.1 mL of creatine (240 mM) containing tubes were incubated at 37°C for 20 min. The tubes were centrifuged and the supernatant was used for the estimation of phosphorus by Fiske and Subbarow method (1925). 1.0 mL of the supernatant was made up to 4.0 mL with distilled water and 1.0 mL of 2.5% ammonium molybdate was added. This was incubated at room temperature for 10 min and 0.4 mL of ANSA was added. The colour developed was read spectrophotometrically at 640 nm after 20 min. The enzyme activity was expressed as U/L for serum.

Determination of Superoxide dismutase (SOD) \[^{14}\]
0.5 ml of liver homogenate was diluted with 0.5 ml of distilled water. To this, 0.25 ml ethanol and 0.15 ml of chloroform, all reagents chilled, were added. The mixture was shaken for 1 minute and centrifuged at 2000 rpm. The enzyme in the supernatant was determined. To 0.5 ml of the supernatant, 1.5 ml of buffer was added. The reaction was initiated by the addition of 0.4 ml epinephrine and change in optical density per minute was measured at 470 nm in a Double beam UV-VIS spectrometer (Perkin Elmer), Germany. SOD activity was expressed as U/l.
Determination of Catalase (CAT)\textsuperscript{[15]}

0.1 ml of liver homogenate was taken, to which 1.0 ml of each phosphate buffer and hydrogen peroxide were added and a timer started. The reaction was arrested by the addition of 0.2 ml dichromate acetic acid reagent. Standard hydrogen peroxide in the range of 4 to 20 µm were taken and treated similarly. The tubes were heated in a boiling water bath for 10 minutes. The green color developed was read at 570 nm in a Double beam UV-VIS spectrometer (Perkin Elmer), Germany. Catalase activity was expressed as U/l.

Determination of Glutathione peroxidase (GSH-P\textsubscript{x})\textsuperscript{[14]}

0.2 ml each of EDTA, sodium azide, reduced glutathione, H\textsubscript{2}O\textsubscript{2}; 0.4 ml of buffer and 0.1 ml of enzyme (liver homogenate) were mixed and incubated at 37°C for 10 minutes. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged. To 0.5 ml of supernatant, 3.0ml of sodium hydrogen phosphate and 1.0 ml of DTNB were added and the color developed was read at 412 nm immediately in a Double beam UV-VIS spectrometer (Perkin Elmer), Germany. Graded concentrations of the standard were also treated similarly. Glutathione peroxidase activity, in serum is expressed as µg/mg.

Determination of triglycerides (TG)\textsuperscript{[16]}

Take 0.1 ml of the serum made up the volume to 4.0 ml with isopropanol. Mixed well and added 400 mg of silicic acid. Placed them in a mechanical shaker and centrifuged. To 2.0ml of the supernatant added 0.6 ml of saponification reagent and incubated at 60-70°C for 15 min. After cooling added 1.0 ml of sodium metaperiodate and mixed well. Then added 0.5 ml of acetyl acetone reagent and mixed again. Incubated the tubes at 50°C for 30 mints & after cooling read the colour at 405 nm. Standard tripalmitin (20-100 jig) were taken in tubes and treated similarly. Triglycerides were expressed as mg/100 ml in serum and mg/g in tissues.

Determination of total cholesterol (TC)\textsuperscript{[17]}

0.5 mL of the serum was evaporated to dryness. To the extract/0.2 mL of serum, 5.0 mL of ferric chloride-acetic acid reagent was added. After mixing well, 3.0 mL of concentrated sulphuric acid was added and the absorbance was read at 560 nm after 20 min. A series of standards containing cholesterol in the range of 3-15g were made to 5.0 mL with the reagent and a blank containing 5.0 mL of the reagent was prepared. The values were expressed as mg/dL for serum.
**Determination of lactate dehydrogenase (LDH)**\(^{[18]}\)

Placed 1.0 ml buffered substrate and 0.1ml sample into each of two tubes. Added 0.2 ml water to the blank & to test added 0.2ml of NAD. Mixed and incubated at 37°C for 15 mints. Exactly after 15 min, 1.0 ml of dinitrophenyl hydrazine was added to each (test and control) & left for further 15 min. Then added 10 ml of 0.4N Sodium hydroxide and the color developed was read immediately at 440 nm. A standard curve with sodium pyruvate solution with the concentration range 0.1- 1 µmole was taken. LDH activity in serum was expressed as µmoles of pyruvate liberated/L and in liver homogenate as nmoles of pyruvate liberated/minute/mg protein.

**Determination of Lipid peroxidation (LPO)**\(^{[19]}\)

1 ml of the tissue homogenate was mixed with 2.0 mL of TCA-TBA-HCl reagent. The mixture was kept in a boiling water bath for 15 min, cooled, the tubes were centrifuged at 1000 rpm for 10 min and the colour developed in the supernatant was measured in a spectrophotometer at 535 nm against a reagent blank. A series of standard solutions in the concentration range of 2.5-10 mmoles were treated in a similar manner. The absorbance of chromophore was read at 535 nm against a reagent blank. Values are expressed as mmoles/mg tissue and nmoles/mg protein for mitochondrial heart.

**Histopathological examination of heart section**

Formalin fixed heart embedded in paraffin wax were sectioned at 5µm thickness and stained with haematoxylin & eosin for routine assessment of histopathological changes. One coded slides from each group were examined by a pathologist blinded to the nature of treatments. Microscopic evaluation was done. Cardiac histopathological changes were recorded.

**STATISTICAL ANALYSIS**

All the values are presented as mean ± SEM (standard error of mean) for six rats. Statistical significance of differences between the control and treatments groups were assessed by One-way ANOVA followed by Dunnett’s multiple comparison test using the “Stat” statistics computer program. A difference in the mean values of P<0.05 was considered to be statistically significant.

**RESULT AND DISCUSSION**

Medicinal plants have recently become a focus of interest because they may play key roles in treating a majority of heart disease with minimal or no side effects. Therefore, present study
was designed to examine the cardioprotective actions of whole methanolic extract of *Hygrophila auriculata* L. leaves & sub-fraction of methanolic extracts against DOX (Doxorubicin) induced cardiotoxicity. The anthracycline antibiotic DOX is one of the most effective chemotherapeutic agents against a wide variety of cancers. Present study have shown that intra-peritoneal administration of DOX produced signs of cardiomyopathy as it was manifested by excessive fluid accumulation that found in pleural, pericardial and peritoneal cavities together with ventral edema and enlargement of liver and kidneys. Cardiac dysfunction associated with DOX is due to cardiac cell apoptosis resulted from reactive oxygen species (ROS) produced by DOX.

It was found that creatine kinase iso-enzyme and lactate dehydrogenase are most specific highly sensitive markers for myocardial cell injury. Animal treated with DOX shows extremely elevated level of these enzymes. The mechanism for the release of these markers seems to be from oxidative damage of DOX to cardiac tissue and the subsequent release of its contents into circulation. Normalization of CK-MB and LDH elevated levels and increasing percentage of survivors by *Hygrophila auriculata* leaves methanolic extracts confirms the cardio-protective effects. The results observed in pre-treatment of *Hygrophila auriculata* leaves methanolic extract with respect to induction of cardiotoxicity using doxorubicin were given in Table No. 1. Rats treated with DOX developed significant heart damage and it was well indicated by change in levels of cardiac marker enzymes in serum & tissue homogenate.

A marked elevation in triglycerides & total cholesterol level were observed in the group treated with DOX and they were significantly high when compared with the normal values. The CAT, SOD, GSH levels in the heart homogenate were also significantly altered in the group received DOX alone.

The groups received the pre-treatment of *Hygrophila auriculata* leaves methanolic extract at dose levels of 100, 200 & 400 mg/kg body weight significantly controlled the altered level of biochemical cardiac markers. The extract at a dose level of 200 mg/kg exhibited a sharp decrease in the serum enzyme levels. As well as pretreatment with *Hygrophila auriculata* leaves methanolic extract shows significant increase in SOD, CAT & GSH level in tissue homogenate as shown in table no.01. LPO is the oxidative degradation of polyunsaturated fatty acids and is a basic membrane damage process. It involves the formation of lipid peroxide radicals, oxidation of unsaturated lipids and the eventual destruction of membrane lipids producing a variety of breakdown products and deleterious effects. These deleterious
processes can be prevented by reducing or destroying the formation of free radicals which are continuously formed in the biological.[24] Malondialdehyde is a major lipid peroxidation end product, increased malondialdehyde content may contribute to increased generation of free radicals and/or decreased activities of antioxidant system. The results presented in this study indicate that methanolic extracts of *H. auriculata* (100, 200 & 400 mg/kg) pretreatment could counteract.
Table no.01 Effect of methanolic extract of *Hygrophila auriculata* L. leaves on cardiac markers of control and doxorubicin induced cardiotoxicity in rat.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK  (IU/L)</th>
<th>SOD (IU/L)</th>
<th>CAT  (IU/L)</th>
<th>LDH (IU/L)</th>
<th>TG  (mg/dl)</th>
<th>TC  (mg/dl)</th>
<th>GSH  (ug/mg protein)</th>
<th>LPO  (nM of MDA/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>258.4±1.96</td>
<td>252±2.47</td>
<td>14.4±0.50</td>
<td>104.6±2.27</td>
<td>65.2±1.74</td>
<td>77.6±1.20</td>
<td>11.2±0.37</td>
<td>1.62±0.14</td>
</tr>
<tr>
<td>DOX (25 mg/kg)</td>
<td>360±13.0</td>
<td>190.4±2.11</td>
<td>4.6±0.40</td>
<td>197.6±2.04</td>
<td>182.6±3.12</td>
<td>205±4.53</td>
<td>4.4±0.24</td>
<td>5.82±0.10</td>
</tr>
<tr>
<td>MEHA (100mg/kg)</td>
<td>329.0±22.29</td>
<td>208.1±10.31</td>
<td>5.4±0.24</td>
<td>175.6±5.66</td>
<td>150.3±63.54</td>
<td>148.4±8.37</td>
<td>5.2±0.37</td>
<td>4.14±1.22</td>
</tr>
<tr>
<td>MEHA (200mg/kg)</td>
<td>277.1±12.67</td>
<td>220.3±19.62</td>
<td>9.32 ± 0.5</td>
<td>124.7±28.46</td>
<td>109.2 ± 5.28</td>
<td>109.2±5.18</td>
<td>9.0±0.31</td>
<td>3.66±0.77</td>
</tr>
<tr>
<td>MEHA (400mg/kg)</td>
<td>296 ± 49.98</td>
<td>208.5±19.10</td>
<td>7.2±0.37</td>
<td>156.6±4.25</td>
<td>114.0±40.15</td>
<td>175.6±5.66</td>
<td>6.0±0.21</td>
<td>4.23 ± 0.05</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± S.D. (n=6) and analyzed by one way ANOVA followed by Dunnett’s comparison test. P values: $a$<0.05, $b$<0.01, $c$<0.001, when compared to toxic DOX treatment.

Doxorubicin-induced elevation in malondialdehyde content. The decreased level of malondialdehyde in heart tissues and serum might be due to the enhanced activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase). It was quite possible that the free radicals induced by Doxorubicin were effectively neutralized and/or scavenged, resulting in the cardioprotective effect of methanolic extracts of *H.auriculata*.
Table no.02 shows that administration of DOX lead to alteration of cardiac marker enzyme levels in serum of control and experimental group. As discussed above Creatine kinase & lactate dehydrogenase plays important role in myocardial infarction. From the result it was observed rat treated with DOX shows significant increase in CK & LDH level as compare to control. However pre-treatment with different fractions of H.auriculata methanolic extracts at doses 100, 200 mg/kg shows decreased in the activity of serum CK & LDH. It was observed that as compare to all the fractions n-butanol fraction shows significant activity by increase (P<0.01) in CK & LDH level as compare to control. Result also revealed that Methanolic extract n-butanol fraction shows significant increase (P<0.05) in serum SOD & CAT level as compared to DOX treated.

Table no.02 elucidates the levels of triglycerides & cholesterol level in serum of normal and experimental rats respectively. Triglycerides & cholesterol level in serum were found to be significantly increased in DOX induced MI rats when compared to control rats. As discussed above serum triglycerides & cholesterol levels plays important role in cardiovascular functions. Result indicates that pre-treatment with different fractions of H.auriculata methanolic extracts at doses 100, 200 mg/kg showed decrease in serum triglycerides & cholesterol level. It was observed that as compare to other fraction n-butanol fraction of both the plant methanolic extract at dose level 100 mg/kg shows significant decrease (P<0.05) in serum triglycerides & cholesterol level as compare to DOX treated group which indicate protective potential of n-butanol fraction.

It has been observed that there was a significant decrease in the level of the tissue GSH in doxorubicin treated group as compared to normal control animals. Pretreatment with different fraction of H.auriculata methanolic extracts raised the myocardial GSH level as compared toxic group. However, n-butanol fraction at dose 100 mg/kg shows significant activity. As discussed above increase in lipid peroxidation indicate the severity of Doxorubicin induced necrotic damage of the heart. From the result lipid peroxidation was found to be significantly increased in animals subjected to DOX exposure. The level of lipid peroxides was decrease in groups of rat pretreated with different fraction of H.auriculata methanolic extract when compared to toxic group. It was observed that n-butanol fraction of plant at dose 100 mg/kg shows significant (P<0.05) decreased in lipid peroxidation activity when compare with DOX treated group indicating the cardioprotective potential n-butanol.
Table No.02 Effect of MEHA sub-fractions on cardiac markers of control and doxorubicin induced cardiotoxicity in rat.

<table>
<thead>
<tr>
<th>Treatment/ Dose</th>
<th>CK (IU/L)</th>
<th>SOD (IU/L)</th>
<th>CAT (IU/L)</th>
<th>LDH (IU/L)</th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>GSH (ug/mg protein)</th>
<th>LPO (nM of MDA/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>97.67±8.7</td>
<td>111.2±9.5</td>
<td>25.50±1.4</td>
<td>96.32±8.61</td>
<td>58.9±9.3</td>
<td>82.1±12.0</td>
<td>8.62±0.32</td>
<td>0.49±0.03</td>
</tr>
<tr>
<td>DOX (25 mg/kg)</td>
<td>176.45±1.7</td>
<td>45.23±3.12</td>
<td>8.0±0.77</td>
<td>185.56±4.54</td>
<td>104.4±16.6</td>
<td>220.3±19.62</td>
<td>5.09±0.47</td>
<td>4.14±1.22</td>
</tr>
<tr>
<td>Pet.Ether fraction of MEHA (100mg/kg)</td>
<td>180.35±2.1</td>
<td>25.20±3.70</td>
<td>8.93±0.6</td>
<td>165.1±0.19</td>
<td>92.9±8.2</td>
<td>195.2±7.91</td>
<td>4.88±0.32</td>
<td>4.23±0.05</td>
</tr>
<tr>
<td>Pet.Ether fraction of MEHA (200mg/kg)</td>
<td>169.40±0.2</td>
<td>35.55±1.55</td>
<td>9.32±0.5</td>
<td>151.2±0.13</td>
<td>85.2±9.3</td>
<td>199.0±8.37</td>
<td>4.4±0.24</td>
<td>4.15±0.03</td>
</tr>
<tr>
<td>n-BuOH fraction of MEHA (100mg/kg)</td>
<td>121.32±2.3b</td>
<td>90.11±1.55a</td>
<td>16.32±0.9a</td>
<td>120.1±25.9a</td>
<td>68.3±6.1b</td>
<td>114.0±40.15b</td>
<td>7.45±0.12b</td>
<td>1.02±0.88b</td>
</tr>
<tr>
<td>n-BuOH fraction of MEHA (200mg/kg)</td>
<td>130.12±5.3a</td>
<td>85.11±2.33a</td>
<td>20.15±1.2b</td>
<td>125.5±11.4a</td>
<td>79.7±36.3a</td>
<td>124.7±28.46b</td>
<td>8.12±0.25b</td>
<td>2.02±1.44a</td>
</tr>
<tr>
<td>CHCl3 fraction of MEHA (100mg/kg)</td>
<td>156.02±2.3</td>
<td>30.11±1.33</td>
<td>8.21±2.3</td>
<td>135.1±13.2</td>
<td>89.4±13.9</td>
<td>200.3±11.02</td>
<td>6.67±0.01</td>
<td>3.00±0.04</td>
</tr>
<tr>
<td>CHCl3 fraction of MEHA (200mg/kg)</td>
<td>155.3±1.0</td>
<td>75.13±2.00a</td>
<td>15.21±1.6a</td>
<td>133.3±0.5a</td>
<td>99.6±8.5</td>
<td>130.2±3.21a</td>
<td>5.37±0.13</td>
<td>3.5±0.02</td>
</tr>
<tr>
<td>mg/kg)</td>
<td>EtOAc fraction of MEHA(100 mg/kg)</td>
<td>135.2±1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.11± 2.11</td>
<td>12.25±0.2</td>
<td>166.11±1.33</td>
<td>88.6 ± 20.3</td>
<td>180.3±16.03</td>
<td>6.29 ± 0.04</td>
</tr>
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</tr>
<tr>
<td></td>
<td>EtOAc fraction of MEHA(200 mg/kg)</td>
<td>155.3±0.1</td>
<td>33.13 ± 1.35</td>
<td>9.21 ±1.3</td>
<td>188.93±0.60</td>
<td>97.2 ± 5.8</td>
<td>215.5±25.30</td>
<td>6.36±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± S.D. (n=6) and analyzed by one way ANOVA followed by Dunnett’s comparison test. P values: <sup>a</sup><0.05, <sup>b</sup><0.01, <sup>c</sup><0.001, when compared to DOX treatment.
The histopathological studies also supported the protective properties of *Hygrophila auriculata* leaves (Fig. No.01). The structural and functional organization of the liver has been described by hepatic lobule and hepatic acinus models respectively. \[25\] The areas of necrosis, inflammatory cell infiltration and degeneration of myocytes were observed in the toxic group. The group of animals pre-treated with *Hygrophila auriculata* methanolic & it’s *n*-butanol fraction showed a marked protective effect with decreased necrotic zones and revealed normal cardiac muscle bundles. There was mild edema but no infarction and inflammatory cells and the cardiac fibers were within the normal limits.

![Fig.01 Photomicrograph of heart section A-](image)

Normal architecture of the cardiac cells was observed with no evidence of microscopic changes in the control group. B-Doxorubicin treated group (25mg/kg i.p.) showing focal confluent necrosis of muscle fiber with inflammatory cell Infiltration, edema with fibroblastic proliferation and phagocytosis was seen. C-Group pre-pretreated with *H. auriculata*...
Neharkar et al. World Journal of Pharmacy and Pharmaceutical Sciences

(100mg/kg) + DOX shows decrease in inflammatory cells. D-Group pre-treated with *H. auriculata* (200mg/kg) + DOX shows small foci of mononuclear collections without muscle damage, mild oedema and necrosis without inflammatory cells. E-Group pre-treated with *H. auriculata* (400mg/kg) + DOX showing mild degree of necrosis and less infiltration of inflammatory cells. F-Group pre-treated with n-Butanol fraction of *H. auriculata* (200mg/kg) + DOX showing mild degree of necrosis and normalization of cardiac cells architecture.

**CONCLUSION**

Preliminary phytochemical investigation of *Hygrophila auriculata* leaves methanolic extracts shows presence of flavonoids, tannins, glycosides. Thus the strong antioxidant and cardioprotective effect of the extract could be attributed to the presence of bioactive constituents present in the extract. Taking into consideration the reported activities and the various active chemical constituents, in the present study, it is proposed that *Hygrophila auriculata* leaves are beneficial to protect myocardial infarction. Histological observations shows that pre-treated rats with *Hygrophila auriculata* leaves exhibited significant improvement protection of cardiomyocytes against Doxorubicin induced cardiotoxicity. Histological observations supported the results obtained from the biochemical investigations. The protective effect of *Hygrophila auriculata* leaves methanolic extract & its fraction may partly or solely due to the possible antioxidant property of the plant. *Hygrophila auriculata* leaves stands as a potential source for pharmaceutical exploitation. Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of *Hygrophila auriculata* leaves.

**REFERENCES**


