Cardioprotective Effect of Ethanolic Flower Extract of Clitoria Ternatea on Doxorubicin Induced Cardiotoxicity in Rats

Tamizhselvi A1, Durairaj S2, Mohamed Sadiq A3, Gopinath G1, Rajeshkumar S4

1 Department of Bio-Chemistry, Indo-American College, Perungalathur 604407, Cheyyar, Tamil Nadu, India
2 Department of Zoology, Aringnar Anna Government Arts College, 604407, Cheyyar, Tamil Nadu, India
3 Department of Bio-Chemistry, Adhiprasakthi College of Arts & Science, G.B Nagar, Kalavai 632506, Tamil Nadu, India
4 Department of Pharmacology, Nanobiomedicine Lab, Saveetha Dental college, SIMATS, Chennai, Tamil Nadu, India

ABSTRACT

In this study, Doxorubicin is used as an effectual induction of cardiotoxicity and heart malfunction in the taken experimental rats which is an anthra-cycline chemotherapeutic mediator used to the treat of a wide variety of malignancies. To examine the shielding property of ethanolic flower extract of clitoraternatea (EECT) scheduled doxorubicin(DOX) persuaded cardiotoxicity via albino wistar rats, through inspect the enzymatic, non-enzymatic antioxidant position, serum enzyme and Histopathology tissue. Experimental rats been provoked cardiotoxicity by means of a generous single dose of doxorubicin (15mg /body weight ) and treated orally through an ethano-lic extract of clitoraternatea (EECT) for 14 days (100 mg/kg bw, 200 mg/kg bw and 300mg/kg bw,n=5). On 15 days, the rats are sacrificed through treated through Ketamine –Xylazine, Biochemical as well as histological remarks of the heart tissues had carry out. Cardiotoxicity had been calculated throughout to establish the cardio marker enzyme levels such as (LDH) Lactic acid dehydrogenase,(CK-MB) Creatinin kinase and (AST) Aspar-tate transaminase at the finish of the study. Management through EECT of 200mg/kg and 300mg/kg considerably reduced the levels of cardiac marker enzymes(p<0.05) and as well minimize the decrease of weight of the heart of DOX treated group. In heart tissue superoxide dismutase, glutathione and catalase heights be significantly greater than before and lipid oxide peroxide (LPO) significantly decreased in the extract (200 &300 mg /kg bw).

INTRODUCTION

Doxorubicin- an anti-cancer candidate drug, which furthermore provoke myocardial damage in addition to that it is generally used as a chemother-apeutical mediator for a broad spectrum of hard cancer and malignancies, Doxorubicin having iron-anthracycline complex produces oxidative stress, which in turn, causes ruthless injure to the membrane layer, and hinder with the morphology of cytoskeleton (Billingham et al., 1977). The iron-anthracycline complex provoke oxidative stress
and produce free radicals by doxorubicin (van Acker et al., 1996) which aim the heart tissue to lipid peroxidation cause permanent damage to myofibrils, cytoplasmatic cidalation with sarcoplasmic reticulum vacuolization, increased quantity of lysosomes, myocyte necrosis also inflammation of mitochondria, (Minotti et al., 2004), biosynthesis of protein and nucleic acid also hindered (Monti et al., 1995), adrenergic alteration, Na+K+ATPase also shrink (Geetha and Devi, 1992), modification in calcium transfer of sarcoplasmic along with the difference in electrolytes balance of heart muscles of doxorubicin induction (Sveski-Illiskovic et al., 1994). The cell transformed doxorubicin into semiquinone by enzymes present in mitochondrial and lysosomal. The electric Semiquinone voluntarily contributesxygen to an electron, resulting in the making of oxidative stress to cause free radicals liberation. Superoxide dismutase catalyzed the configuration of hydrogen hydroxide from Superoxide (Jain, 2000). CVD is a group of disorders or diseases of the heart and blood vessels, which also include atherothrombotic diseases such as myocardial and cerebral infarction commonly called heart attack and stroke in that order. The events like atherosclerotic plaque creation, endothelial dysfunction (Virmani et al., 2000). Atherosclerosis is an inflammatory disease of the large arteries describes atherosclerotic plaques formation. The possible foundation of endothelial dysfunction leading to atherosclerosis contains enhanced free radicals, elevated and modified low mass lipoprotein-cholesterol, hypertension, diabetes mellitus, genetic alterations and prominent plasma homocystein absorption (Singh et al., 2002). In the majority of cases, atherosclerosis-related clinical events, such as MI or ischemic stroke, are caused by the burst of a vulnerable atherosclerotic lesion (Badimon et al., 2012). Butterfle pea (Clitoraternatea Linn) is used as folk medication. The family tree and germ are use as the stress tonic and laxative. Flowers of butterfle pea restrains anthocyanins. The red violet-blue color of plant and flower is because of Anthocyanins (Harborne, 1998). (A2, A1,B2,B1,D2 and D1) are the main anthocyanin sterigmatins (Abernethy, 1960). The diversity of shades of the flower is owed to a very small digit of a different tincture. These tincture contain the matching carbon skeleton, with diverse only in the character of the substituent grouping (Abernethy, 1960). The current job be accept to revision the cardioprotective role of EECT on cardiac toxicity induced by doxorubicin.

MATERIALS AND METHODS

**Plant**

Clitoraternatea flowers be purchased from koyambedumarket, Chennai, Tamilnadu and were authenticated Dr.V.Gangadevi, Assistant Professor, Botany Department, Aringnar Anna Government Arts College, Cheyyar, Tamilnadu. The herbarium have be present on behalf of auxiliary indication. The flower were shade dried and fine pulverized using pulverizer and maintained in an airtight container at 4º C and used for further study.

**Preparation of extract**

The finely powdered dried flowers of clitoraternatea convert to crude powder. The crude powder was extracted with solvent ethanol through soxhlet until them colorless. Then it was pressure evaporated by rotary evaporator below 45ºC wait the formation of syrupy of the extract and transferred it in a clean dish at room temperature. 17.3% w/v was the yield originated.

**Preliminary phytochemical investigation**

The originated syrupy extract was taken for the following qualitative analysis of Triterpenoids, Flavonoids, Tannins, Saponins, glycosides, sterols, carbohydrates, alkaloids and proteins, (Khandelwal, 2004).

**Animals and chemicals**

Thirty animals of either sex Wister albino rats through an common weight of 221.54 ± 2.56 gms taken for study acquired Biochemistry Department animal House of Adhiparasakthi College of Arts And Science .G.B.Nagar, Kalavai, Vellore-Dist,Tamilnadu. Normal food with libitum water was given to the experimental rats for the entire period of period. Animal husbandry, tests and toxicology were carried out with doctrine guideline regularly. Prior to experiments, animals were familiarized for two days to adopt the laboratory condition to minimize unwanted stress. The animals are sustained under normal laboratory conditions of 25± 1ºC through a 12 h light-dark cycle. Care was full to evade any sort of tense provision. All experimental procedure was executed between 9 and 11 a.m. The entire experiments done with proper obtained from Institutional Animal Ethical Committee, Adhiparasakthi College of Arts And Science, G.B.Nagar, Kalavai, Tamilnadu (IEACAP- CAS/01/2017/02) Doxorubicin (Fresenius Kabi) was purchased from local market. The reagents, Glasswares and water used in this reading were of systematic score and standard.

**Safety evaluation / Toxicity studies**

Acute oral toxicity readings in taken animals were approved with guidelines of OECD-423. Five doses (50, 300, 500, 1000, 2000mg /kg body weight)
### Table 1: Mass of Body, Heart and its Ratio

<table>
<thead>
<tr>
<th>Separations of Groups</th>
<th>Body mass (g)</th>
<th>Heart mass (g)</th>
<th>Body/Heart mass ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Day-1</td>
<td>Final Day-14</td>
<td>Day-14</td>
</tr>
<tr>
<td>Group I (Control)</td>
<td>220.56±2.23</td>
<td>225.16±2.58</td>
<td>1.06±0.04</td>
</tr>
<tr>
<td>Group II (DOX treated)</td>
<td>217.44±2.09</td>
<td>196.89±2.65</td>
<td>0.71±0.02</td>
</tr>
<tr>
<td>Group III (DOX + 100mg extract)</td>
<td>224.66±2.10</td>
<td>209.74±2.88</td>
<td>0.80±0.02</td>
</tr>
<tr>
<td>Group IV (DOX + 200mg Extract)</td>
<td>222.87±2.85</td>
<td>223.66±2.69</td>
<td>0.94±0.03</td>
</tr>
<tr>
<td>Group V (DOX + 300mg Extract)</td>
<td>221.66±2.92</td>
<td>223.18±3.01</td>
<td>1.01±0.03</td>
</tr>
<tr>
<td>Group VI (DOX + 10mg Metoprolol)</td>
<td>216.54±2.14</td>
<td>214.10±3.10</td>
<td>0.95±0.04</td>
</tr>
</tbody>
</table>

DOX-Doxorubicin; Data are mean (SEM); n=5; p<0.05; Anova coupled with Dunnett's test

### Table 2: Effect of EECT on CK-MB, LDH and AST levels in rats on DOX-induced cardiotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK-MB</th>
<th>LDH</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>42.89±1.25</td>
<td>71.85±1.22</td>
<td>50.80±2.596</td>
</tr>
<tr>
<td>Group II (DOX treated)</td>
<td>154.57±2.22</td>
<td>145.45±2.19</td>
<td>207.00±7.516</td>
</tr>
<tr>
<td>Group III (DOX + 100mg Extract)</td>
<td>162.89±2.19</td>
<td>144.55±2.18</td>
<td>179.36±3.411</td>
</tr>
<tr>
<td>Group IV (DOX + 200mg Extract)</td>
<td>151.87±2.05</td>
<td>142.98±2.28</td>
<td>168.60±2.204</td>
</tr>
<tr>
<td>Group V (DOX + 300mg Extract)</td>
<td>122.43±1.83</td>
<td>126.25±2.26</td>
<td>67.80±1.562</td>
</tr>
<tr>
<td>Group VI (DOX + 10mg Metoprolol)</td>
<td>114.21±0.53</td>
<td>116.51±2.55</td>
<td>62.625±1.254</td>
</tr>
</tbody>
</table>

Data are mean (SEM); n=5; p<0.05; Anova coupled with Dunnetts test

### Table 3: Effect of EECT on SOD, CAT, GSH, LPO and tissue protein levels in rats on DOX-induced cardiotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD</th>
<th>LPO</th>
<th>CAT</th>
<th>GSH</th>
<th>Proteing/dL (tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>20.42±1.25</td>
<td>10.46±1.66</td>
<td>210.72±1.68</td>
<td>5.42±1.36</td>
<td>7.45±0.22</td>
</tr>
<tr>
<td>Group II (DOX treated)</td>
<td>6.96±2.05</td>
<td>22.62±2.08</td>
<td>64.82±1.98</td>
<td>1.24±2.01</td>
<td>3.66±0.23</td>
</tr>
<tr>
<td>Group III (DOX + 100mg Extract)</td>
<td>6.12±1.36</td>
<td>20.94±1.66</td>
<td>84.96±1.55</td>
<td>1.41±1.25</td>
<td>4.96±0.22</td>
</tr>
<tr>
<td>Group IV (DOX + 200mg Extract)</td>
<td>9.74±1.38</td>
<td>17.69±1.55</td>
<td>126.46±1.62</td>
<td>2.92±1.65</td>
<td>6.92±0.40</td>
</tr>
<tr>
<td>Group V (DOX + 300mg Extract)</td>
<td>16.12±1.33</td>
<td>14.13±1.62</td>
<td>180.12±1.35</td>
<td>4.09±1.24</td>
<td>7.42±0.55</td>
</tr>
<tr>
<td>Group VI (DOX + 10mg Metoprolol)</td>
<td>17.89±1.25</td>
<td>12.25±1.68</td>
<td>171.84±1.98</td>
<td>4.01±1.11</td>
<td>7.44±0.65</td>
</tr>
</tbody>
</table>

Data are mean (SEM); n=5; p<0.05; Anova coupled with Dunnetts test
RESULTS AND DISCUSSION

Figure 1: The outcome of clitoriaternatea extract treated on doxorubicin-induced animal on body mass

Figure 2: The outcome of clitoriaternatea extract-treated on doxorubicin-induced animal. Values are mean ± SEM, ANOVA followed Dunnetts test, p<0.05. heart mass. Values are mean ± SEM, ANOVA followed Dunnetts test, p<0.05.

of ethanol extract of clitoraternata were vocally directed to groups of similar age and weight. The taken rats were supervised for 60 minutes continuously and after that hourly for 4 hour and finally for every 24 hours until the final protocol for some warning sign of toxicity and transience (Chandan et al., 2007). The indication of embarrassment to the animals were originate at a dosage of the 2000mg/kg bw EECT. Therefore the dose of 1000mg/kg bwup to 1,000 mg/kg was chosen to be safe also the dose of 100, 200 & 300 mg/kgbw of EECT taken to study the cardioprotective commotion.

Experimental Protocol

The experimental protocol applies for assessment of cardioprotective effect of ethanolic extract clitoraternata against DOX-induced cardiotoxicity is as follows. The rats were erratically allocated into six groups of five each.

Group I: Control group received distilled water for 14 days.

Group II: DOX-induced group received a single dose of DOX 15mg/kg I.P on 1st day only.

Group III: Standard Drug treated group received a single dose of DOX 15mg/kg I.P on 1st day and Metoprolol (10 mg/kg bw) for the other 13 days.

Group IV: Treated Group 1 received DOX 15mg/kg I.P on the 1st day and 100mg EECT for other 13 days.

Group V: Treated Group 2 received DOX 15mg/kg I.P on the 1st day and 200mg EECT for other 13 days.

Group VI: Treated Group 3 received DOX 15mg/kg I.P on the 1st day and 300mg EECT for other 13 days.

At the end of the 14th day, the animals were anaesthetized Ketamine – Xylazine and then sacrificed by a high dose of ketamine. Blood samples were collected immediately for enzyme assays. The serum was prepared by centrifuging the blood samples at 3000 rpm for 5 min. The heart of the rats were rapidly dissected and washed in isotonic saline and homogenized quickly with ice-cold 0.1M Tris HCl buffer (pH-7.5) and stored.

Cardiac biochemical markers

The LDH, AST and CK-MB actions were dogged from serum according to normal technique using standard kits.

Lipid peroxide content

Lipid peroxide enzyme levels was determined by thiobarituricacid (TBA) reaction with Malondialdehyde (MDA), a creation create downing to the peroxidation of lipid membranes (Ohkawa et al., 1979).

Catalase activity

The CAT activity of cardiac tissue was resolute according to the method described by Aebi (Aebi, 1984) the method based on the determination of the H2O2 decomposition rate at 240 nm. The values are expressed as U/mg protein.

Glutathione content

Reduced Glutathione was estimated by the method of Ellman (Ellman, 1959) (Ellman Glet al., 1959) About 1.0 ml of 10% TCA was added to 1.0 ml of homogenate and centrifuged. The supernatant was treated with 0.5 ml of Ellmans reagent and 3 ml of phosphate buffer (pH-8.0). The color developed was measured at 412 nm.

Superoxide dismutase activity
The SOD was determined by the ability of the enzyme to inhibit the oxidation of adrenaline to adrenochrome (Saggu et al., 1989). The 0.05 ml supernatant was added to 2.0 ml of carbonate buffer as well as 0.5 ml of 0.01M EDTA solution. 0.5 ml of epinephrine was added for the initiation of reaction. The auto-oxidation of adrenochrome was measured by following change in OD at 480 nm. The results are expressed at U/mg protein.

**Assay of total protein**

To 0.3 ml of solution of tissue homogenate to that alkaline copper sulphate, 2ml kept for 10 min at RT then 02. ml for folinic acid added and kept for 30 min incubation and read at 660nm with standard BSA. The assay of total protein carried out by the (Lowry et al., 1951).

**Histopathology of Heart tissue**

On the 15th Day, the heart is cut off, clean with isotonic saline also homogenized among 1 M Tris HCL buffer. Followed via the tissues be presented in 10% buffer unbiased formalin solution. Following complex tissues be fixed in paraffin –wax and segments be hacked and stain by hematoxylin and eosin. The stained tissue coated slides were examined with microscopy.

**Statistics**

The collected data were articulated as the mean ± SD for analysis of statistical data, group were balanced by ANOVA, tag alonged by Dunnett’s test. The p values < 0.05 was measured considerable.
Figure 6: Histopath images of EECT on DOX-induced Cardio toxicity on heart tissue. A –Control, B –DOX induced, C –extract: 100 mg/kg, D -extract: 200 mg/kg, E -extract: 300 mg/kg

standard. This protocol illustrated reduce in a superior amount of the above oxidative enzymes. Treatment among the EECT 300 mg/Kg demonstrated considerable diminish in (p<0.05) AST, LDH and CK-MB (Figure 3).

Doxorubicin induces endogenous oxidative stress decreased level of SOD, CAT and GSH had shown hanced LPO in the heart tissue(Figures 4 and 5). Treatment of EECT 200 and 300 mg/kg illustrate a significant increase (p<0.05) in SOD, CAT and GSH, the level of LPO will decreased significantly (p<0.05), while compared with Control group. SOD, CAT and GSH be there a vital antioxidant biomolecules in the tissue adjacent to oxidant stress particularly in cardiac tissue (Table 3).

The protein content in the DOX-induced rats demonstrated a decrease in the protein levels when compared to control. Animals treated at a dose of 200 7 300 mg/kg bw of EECT illustrate a considerable high in the levels of protein in contrast to DOX-induced group.

Histopathological Studies

Histopathological assessments of heart tissue achieved as standard control animals established an understandable steady fastness of myocardial covering. Standard control animals demonstrated standard cardiac fiberssmart of which even harmed. Cardiac muscle section ofDOX-induced ratsdemonstrate sufficient region of necrosis with aggregations,cells with severe inflammation and vascular spaces also injured. Rats treated with EECT of 200 & 300mg/kg bwshown an enlargement of the cells reliability established by be short of necrosis, inflammatory cells are noticeable diminish in size and protect the usual morphology of the heart tissue(Figure 6).

The ethanolic flower extract of ClitoriaTernatea-have exposed momentous cardio protection through Doxorubicin-induced cardiac toxicity. Single admin of Doxorubicin(15mg/kg bw) will demonstrate as a source of cardio toxicity in rats (Somashekar, 2015). In this experiment, we developed a Cardiotoxic rat model by injecting a single dose of 15mg/kg bw of DOX and it has been shown to as significantly diminished body, heart and ratio of their masses. The diminish in the mass of the body in this study is in agreement by means of additional studies (Herman et al., 2000)and this is because of food eaten by the animals and it may diminish protein production due to Doxorubicin. The ethanolic extract will demonstrate a amplification in the mass of the body, heart and ratio.

DOX induction created a considerable increase of Creatinine kinase (Somashekar, 2015), LDH (Garba and G, 2005). An elevated level of this oxidative enzymes will back to normal by EECT treatment.
significantly. This mitochondrial dysfunction and oxidative stress is because of the DOX induction causing sick to the rats. DOX will cause membrane lipid peroxidation, production of ROS and also the reactive aldehyde. (Morin et al., 2001). SOD and CAT enzymes shown a significant decrease but an increase in LPO on DOX-induced group when compare to the Normal control group. Treating EECT on DOX-induced group considerably get the level of these enzymes back to normal. This provides evidence that the avoidance of oxidative injury by EECT. The cardiac tissue Histopathology observation of the groups shown a significant changes in EECT treated when compared to DOX-induced group. The heart sections acquired since Doxorubicin-induced animals demonstrated commotion of numerous subcellular feature which includes myofibrils loss, mitochondria swelling, cytoplasmic vacuolization, sarcotubudialation and lysosomal formation (Olson et al., 1974). The architecture of the heart close to normal with control group while treating with 200mg/kg and 300mg/kg bw of EECT. The presence of phytochemical constituents like Triterpenoids, Flavonoids, Tannins, Saponins, glycosides, sterols, carbohydrates, alkaloids, proteins and carbohydrates will strength the cardio protectively of EECT in DOX-induced Cardiotoxicity of the taken experimental animals.

CONCLUSIONS

EECT demonstrated a persuasive anti-cardiotoxic outcome in the taken experimental animal by DOX persuaded cardiotoxicity / myocardial necrosis. Treatment with EECT may effect LDH, CK-MB and AST activity. This put forward that EECT might be the reason in the reduction and avoidance of the severe cardiac complications encountered by Doxorubicin. EECT extract is a novel strategy with the aim for defending against Doxorubicin-induced cardiotoxicity. In conclusion the over statistics put forward to facilitate EECT has the potential antioxidant movement in avoiding the cardiotoxic possesses on persuade by Doxorubicin.

REFERENCES


Morin, D., Barthélémy, S., Zini, R., Labidalle, S., Tillement, J.-P. 2001. Curcumin induces the mito-


