

## Evaluation of cardioprotective activity of Ethanolic extract of dried leaves of *Cinnamomum tamala* in rats

B. Nagaraju\*, S. Vidhyadhara, Ch. Aruna Kumar, S. Vikas, and D. Suryanarayana Raju

Department of Pharmacology, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur -522019, Andhra Pradesh, India.

### \*Correspondence Info:

Nagaraju Bandaru

Lecturer,

Chebrolu Hanumaiah Institute of Pharmaceutical Sciences,

Chandramoulipuram, Chowdavaram,

Guntur -522019, Andhra Pradesh, India.

E-mail: [bnrajupharma@gmail.com](mailto:bnrajupharma@gmail.com)

### Abstract

The present study was designed to scientifically evaluate the cardio protective potential of Ethanolic Extract of dried leaves of *Cinnamomum tamala* (Family: Lauraceae) (EECT), against doxorubicin induced myocardial infarction in Wistar albino rats. Myocardial infarction was produced in rats with 15mg/kg of doxorubicin administered intraperitoneally (i.p), in six divided doses for two weeks. Effect of oral treatment of EECT at two doses (200 and 400 mg/kg body weight), both in prophylactically and curatively manner was evaluated against doxorubicin (15mg/kg, i.p) induced myocardial infarction. Levels of marker enzymes- Creatinine Phospho Kinase (CPK), Lactate Dehydrogenase (LDH), Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were estimated in both the serum and heart tissues; antioxidant parameters viz., catalase (CAT) and malondialdehyde (MDA) were assayed in heart homogenate. Doxorubicin significantly increases the serum levels of marker enzymes and reduction of endogenous antioxidants when compared with normal rats. EECT elicited a significant cardio protective activity by lowering the levels of serum marker enzymes and lipid peroxidation and elevated the levels of catalase. The study confirms the cardioprotective potential of EECT against doxorubicin-induced cardiotoxicity in rats.

**Keywords:** Cardio protective, lipid peroxide, doxorubicin, marker enzymes, *Cinnamomum tamala*

### 1. Introduction

Epidemiological studies predict an ominous prevalence of cardio vascular diseases globally as well as in India during next decade. Myocardial infarction, a highly prevalent ischemic condition characterized by tissue necrosis develops essentially due to an imbalance between oxygen need and actual supply and results in irreversible histopathological damages and subsequent cardiovascular complications. [1]

The clinical use of doxorubicin (DOX, Adriamycin®), a broad spectrum chemotherapeutic agent, is limited by its dose-dependent cardiotoxicity, which leads to congestive heart failure and death. DOX-induced acute cardiotoxicity may begin with the initiation of chemotherapy and may resolve [2], whereas the chronic cardiotoxicity develops anytime after completion of DOX regimens and occurs in patients given cumulative dose of  $\geq 500$  mg DOX/m<sup>2</sup>. The precise mechanism of DOX-induced cardiotoxicity is incompletely understood. According to the widely recognized hypothesis, DOX undergoes redox cycling by mitochondrial complex I and NADH dehydrogenase, leading to oxidative stress, membrane lipid peroxidation, mitochondrial dysfunction, and altered cardiac gene expression altogether leading to cardio myocyte death. [3]

Spices are dried parts of herbs used as flavouring agents in cooking in oriental countries owing to their taste and aroma. Indian bay leaf (*Cinnamomum tamala* Nees.) is one among them. The dried leaf of this plant is a spice commonly used in Indian homes for seasoning. It belongs to the family *Lauraceae* and is indigenous to the Asian minor and southern Europe.[4]

Until now, the antidiabetic activity, anti-bacterial activity, antioxidant activity, antimicrobial, anti-inflammatory activity, anti-diarrhoeal activity and antihyperlipidemic activity of CT extracts have been evaluated. Based on this information present study was designed to investigate the cardioprotective effect of ethanolic extract of *Cinnamomum tamala* Nees. leaves.[5]

**2. Material and Methods**

**2.1 Plant material**

The dried leaves of *Cinnamomum tamala* were purchased from the local market. The leaves were

authenticated by K. Ravi kanth, Head of the department of Botany, D. N. R. College, Bhimavaram. The leaves were powdered and stored in air tight container for the studies.

## 2.2 Preparation of extract

The coarse powdered material was subjected to sequential soxhlet extraction. The solvents used are petroleum ether, chloroform and ethanol. The dried powder was defatted by macerating the powder for 7 days in petroleum ether with occasional stirring. Then the marc was subjected to soxhlet extraction with chloroform and ethanol respectively. Finally, the resultant marc was subjected to aqueous extraction. The collected extracts were then concentrated using rotary vacuum evaporator and were air dried at room temperature, weighed and percentage yield was calculated[6].

## 2.3 Preliminary Phytochemical investigation

The extract was subjected to chemical tests qualitatively for identification of different phytoconstituents like glycosides, saponins, carbohydrates, sterols, alkaloids, flavonoids, tannins, proteins and triterpenoids[7].

## 2.4 Acute toxicity studies

Healthy young adult rodents were fasted and administered with a simple bolus dose of test substance by oral gavage. The pre-specified fixed doses of the test substances were used i.e., 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg. Then animals were observed upto 15 days and the body weight and mortality were recorded.[8,9]

## 2.5 Experimental animals

Adult Wistar rats of either sex (150–200 gm; obtained from National Institute of Nutrition, Hyderabad) were housed under standard animal house conditions (23 ± 2 °C; LD 12:12 and 45–50% humidity) and provided with pelleted diet and water *ad libitum*. The animals were maintained as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India.No-1529/P0/a/11/CPCSEA.

## 2.6 Experimental design:

The animals were divided into eight groups. Each group had 6 rats.

- 1) Group I: Control rats received 1% Na CMC 2.5ml/kg/day p.o for a period of 30 days.
- 2) Group II: These rats received doxorubicin (2.5 mg/kg body weight, i.p, in six divided doses alternatively for two weeks to a total cumulative dose of 15mg/kg).
- 3) Group III: Rats were treated orally for a period of 30 days with EECT (dose- 200 mg/kg body weight).
- 4) Group IV: Rats were treated orally for a period of 30 days with EECT (dose- 400 mg/kg body weight).
- 5) Group V: Rats were treated orally with 200mg/kg EECT for first 15 days followed by doxorubicin treatment (2.5 mg/kg body weight, i.p) in six divided doses for next 15 days.

6) Group VI: Rats were treated orally with 400mg/kg EECT for first 15 days followed by doxorubicin treatment (2.5 mg/kg body weight, i.p) in six divided doses for next 15 days.

7) Group VII: Rats received doxorubicin (2.5 mg/kg body weight, i.p) in six divided doses for first 15 days, followed by oral treatment with 200mg/kg EECT for next 15 days.

8) Group VIII: Rats received doxorubicin (2.5 mg/kg body weight, i.p) in six divided doses for first 15 days, followed by oral treatment with 200mg/kg EECT for next 15 days [10,11].

## 2.7 Determination of biological parameters

At the end of treatment period, animals were fasted overnight for a period of 12 hr. Blood (0.5 ml) was withdrawn via retro-orbital plexus under mild ether anesthesia and was collected in micro tubes previously filled with 10% EDTA solution (20 µl of 10% EDTA/ ml of blood). The micro tubes were centrifuged at 4000 rpm at 4°C for 20 min to obtain clear plasma. The plasma was then analyzed for CPK, LDH, SGPT and SGOT in the semi auto analyzer using commercially available biochemical kits.

Later the animals were sacrificed under ether anaesthesia and a midline abdominal incision was performed and the heart tissue was quickly dissected out and washed in ice cold saline. A portion of each heart was taken from all the groups and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of biomarkers (CPK, LDH, SGPT and SGOT) and biochemical parameters (CAT and MDA).The remaining portion of the heart tissue was used for histopathological studies. [13,14]

## 2.8 Histopathological studies

The heart tissue sections were fixed in 10% formalin. The specimens were processed by standard procedure and embedded in paraffin wax. The blocks were sectioned from the ventricular portion and stained according to the hematoxylin and eosin method and were examined by light microscopy.

## 2.9 Statistical analysis

The results were expressed as mean S.E.M. The results were analyzed using ANOVA followed by Dunnett's multiple comparison test. Data was computed for statistical analysis by using Graph Pad Prism 5 Software.

## 3. Results

### 3.1 Percentage yield of extracts & Preliminary phytochemical investigation

After complete extraction and drying, the dried mass of the different extracts of *Cinnamomum tamala* leaves was weighed in a digital balance.

**Table 1: Preliminary Phytochemical Analysis**

S. No	Constituents	Pet. Ether Extract	Chloroform Extract	Ethanolic Extract	Aqueous Extract
1.	Alkaloids	-	+	+	+
2.	Carbohydrates	+	+	+	+
3.	Cardiac glycosides	-	-	+	-
4.	Antraquinone glycosides	-	-	+	-
5	Gums and mucilage	-	-	-	-
6.	Proteins and aminoacids	-	+	+	+
7.	Tannins and phenolic compounds	-	+	+	+
8.	Steroids and sterols	-	-	-	-
9.	Triterpenoids	+	-	+	-
10.	Saponins	-	+	+	+
11.	Flavonoids	-	+	+	+

+- Present; -- Absent

From the data of percentage yield and phytochemical screening of different extracts, it was evident that the ethanolic extract was obtained in higher amounts and had more amounts of active components respectively. So the present study was focussed on only the ethanolic extract of *Cinnamomum tamala* leaves (EECT).

### 3.2 Acute toxicity studies

No behavior changes and mortality was observed during toxicity studies.

#### 3.2.1 Experiment

Chronic administration of Dox-induced cardiac toxicity and the effect of ethanolic extract of *Cinnamomum tamala* leaves were established by assessing the biochemical and histopathological studies.

#### 3.2.2 General observation

In Doxorubicin treated group, the animals were lethargic and sick when compared with the normal. These symptoms were markedly reduced in the groups treated with EECT when compared with the doxorubicin control group.

#### 3.2.3 Serum and homogenate markers - CPK, LDH, ALT and AST.

Treatment with doxorubicin causes an elevation in level of these enzymes which are considered as the biomarkers of myocardial damage when compared with the normal. Our study showed decrease in the elevated levels of these enzymes. Pretreatment with EECT 200 and 400mg/kg showed a dose dependent significant decrease in the elevated enzymes when compared with the post treatment.

From the table, it was evident that the pretreatment of animals with the dose of 400mg/kg EECT had significantly decreased the doxorubicin induced biomarkers levels, when compared to other treatments.

#### 3.2.4 Tissue antioxidant marker and lipid peroxidation of heart homogenate:

Doxorubicin causes a decrease in the level of endogenous antioxidant reserve-CAT and shows an increase in the lipid peroxidation of the heart compared with the normal. Pretreatment with EECT 200 and 400mg/kg showed a significant increase in the CAT levels, while significantly decreasing the levels of MDA in a dose dependent manner, when compared with the control.

From the table, it was evident that the pretreatment of animals with the dose of 400mg/kg EECT had significantly increased and decreased the levels of CAT and MDA respectively that are altered due to doxorubicin induction, when compared to other treatments. (CAT:  $15.33 \pm 1.14$  to  $29.17 \pm 1.13$ ; MDA:  $51.17 \pm 2.33$  to  $25.33 \pm 0.76$ ).

#### 3.2.5 Histopathological changes on Dox-induced cardiotoxicity

The sections of heart of normal rats (vehicle treated rats) showed normal morphological appearance Figure 11a. The cardiac muscle fibres were found to be of uniform size, shape, without any inflammation. The sections of heart obtained from the doxorubicin treated animals showed severe congestion of blood vessels. Degenerative changes and areas of necrosis in cardiac muscle fibres were observed along with moderate infiltration of mononuclear cells Figure 11b. The sections of heart of rats treated with only extract showed normal histological architecture in low dose (200 mg/kg) Figure 11c and revealed normal histological appearance and only mild congestion of blood vessels in high dose (400 mg/kg) Figure 11d.

In the rats pre-treated with EECT 200 mg/kg, the sections of heart showed near normal architecture with mild congestion of blood vessels Figure 11e. In the rats pre-treated with EECT 400 mg/kg, sections of heart showed almost normal architecture. No areas of necrosis or infiltration of mononuclear cells were observed Figure 11f.

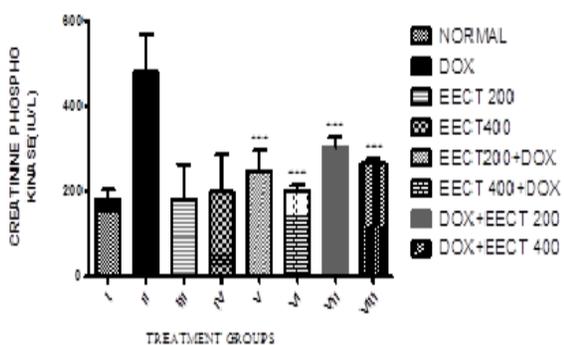
In the rats, which were subjected to post-treatment with low dose (200mg/kg), the heart sections showed congestion of blood vessels with few patchy areas of myocardial degeneration and mononuclear infiltration Figure 11g. In the rats, which were subjected to post-treatment with high dose (400mg/kg), the heart sections showed near normal architecture with few areas of congestion Figure 11h. All the sections were shown below from Figure 11a-11h.

**Table 2: Observation of Biochemical parameters**

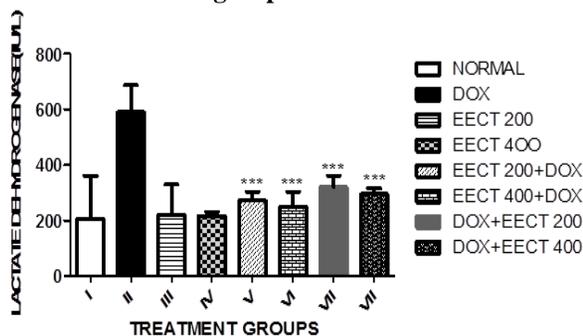
	Groups							
	I Control	II DOX	III EECT200	IV EECT400	V EECT200+ DOX	VI EECT400+ DOX	VII DOX+ EECT200	VIII DOX+ EECT400
<b>Serum biomarkers levels (IN IU/L)</b>								
CPK	180.2±9.806	479.0±35.76	182.7±32.65	198.3±35.91	247.5±19.96 <sup>***</sup>	<b>202.5±5.17<sup>***</sup></b>	286.5±11.79 <sup>***</sup>	265.0±4.35 <sup>***</sup>
LDH	206.5±62.85	589.8±40.06	220.3±44.68	216.7±5.89	272.3±12.61 <sup>***</sup>	<b>231.7±19.66<sup>***</sup></b>	319.5±17.35 <sup>***</sup>	295.0±8.72 <sup>***</sup>
ALT	45.67±2.87	220.05±35.17	55.0±7.58	53.0±2.85	161.03±5.78 <sup>†</sup>	<b>112.5±5.81<sup>**</sup></b>	174.5±3.49 <sup>†</sup>	155.7±3.45 <sup>†</sup>
AST	91.5±6.56	248.0±26.46	99.69±5.12	96.5±3.27	100.0±5.62 <sup>***</sup>	<b>93.67±5.63<sup>***</sup></b>	139.2±6.48 <sup>**</sup>	123.7±8.58 <sup>**</sup>
<b>Heart tissue homogenate markers (IN IU/L)</b>								
sCPK	134.2±11.81	326.0±9.03	136.0±11.83	139.3±5.41	167.0±5.22 <sup>***</sup>	<b>150.8±3.45<sup>***</sup></b>	173.0±2.95 <sup>***</sup>	169.5±4.36 <sup>***</sup>
LDH	132.3±5.45	416±45.9	191.3±5.87	186.8±9.60	234.7±4.01 <sup>***</sup>	<b>215.2±3.93<sup>***</sup></b>	253.8±2.38 <sup>**</sup>	247.0±3.36 <sup>***</sup>
ALT	48.83±6.61	138.2±7.28	50.17±5.44	51.67±7.00	70.5±2.96 <sup>***</sup>	<b>62.67±1.72<sup>***</sup></b>	84.5±3.96 <sup>**</sup>	70.5±3.56 <sup>***</sup>
AST	55.17±8.57	136.2±2.56	57.5±8.29	55.0±3.44	71.5±5.94 <sup>**</sup>	<b>65.67±6.00<sup>***</sup></b>	78.5±4.06 <sup>**</sup>	72.5±5.94 <sup>***</sup>
<b>Tissue antioxidants(CAT in UNITS/mg protein and MDA in nmoles/g tissue)</b>								
CAT	32.0±1.18	15.33±1.14	31.17±1.49	29.5±1.89	23.83±0.6 <sup>***</sup>	29.17±1.13 <sup>***</sup>	21.67±0.88 <sup>**</sup>	22.67±0.76 <sup>***</sup>
MDA	15.67±0.95	51.17±2.33	16.17±1.04	15.67±1.02	29.17±0.79 <sup>***</sup>	25.33±0.76 <sup>***</sup>	34.0±1.00 <sup>***</sup>	33.5±1.17 <sup>***</sup>

All values are expressed as mean±SEM for each group (n= 6/group). Significance was determined by One-Way ANOVA followed by Dunnett comparison test; \*P<0.01 Vs doxorubicin control; \*\*P<0.001 Vs doxorubicin control;\*\*\*P<0.0001 Vs doxorubicin control.

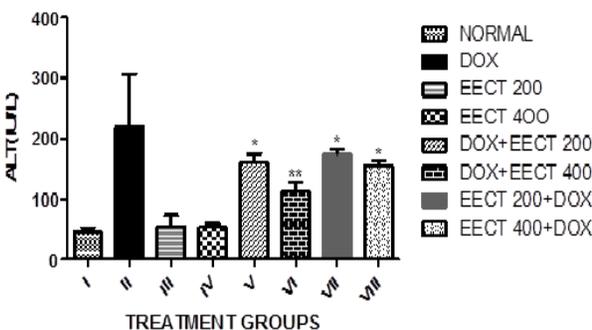
**Serum biomarkers**



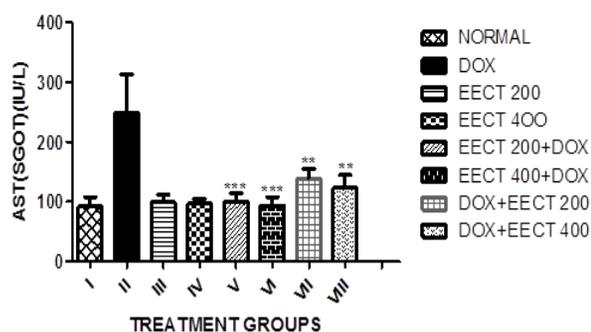
**Figure 1: Effect of EECT on CPK levels in different groups of rats**



**Figure 2: Effect of EECT on LDH levels in different groups of rats**

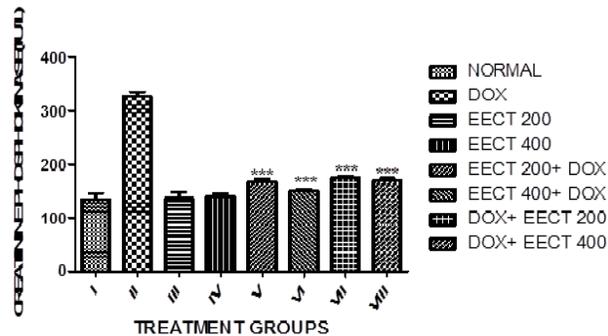


**Figure 3: Effects of EECT on ALT levels in different groups of rats**

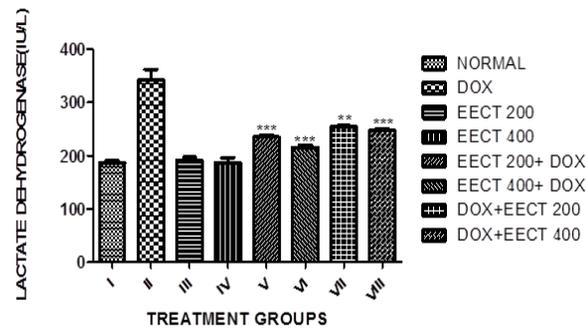


**Figure 4: Effects of EECT on AST levels in different groups of rats**

**Heart tissue homogenate markers (IN IU/L)**



**Figure 5: Effect of EECT on CPK level in different groups of rats**



**Figure 6: Effect of LDH levels in different groups of rats**

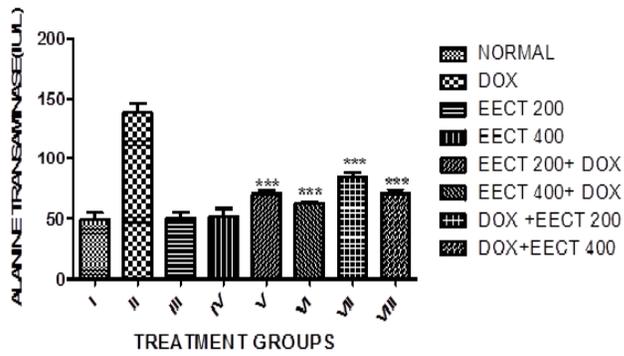


Figure 7: Effect of EECT on ALT levels in different groups of rats

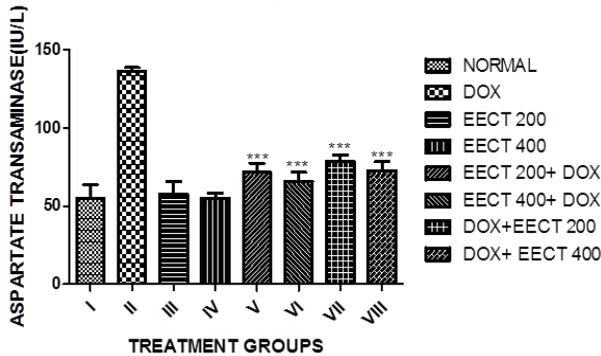


Figure 8: Effect of EECT on AST levels in different groups of rats

Tissue antioxidants (CAT in units/mg protein and MDA in nmoles/g tissue)

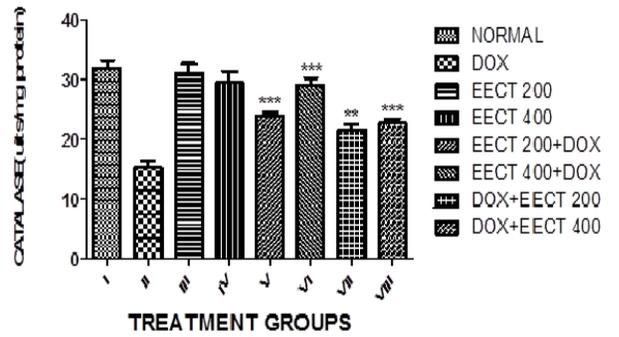


Figure 9: Effect of EECT on catalase levels in different groups of rats

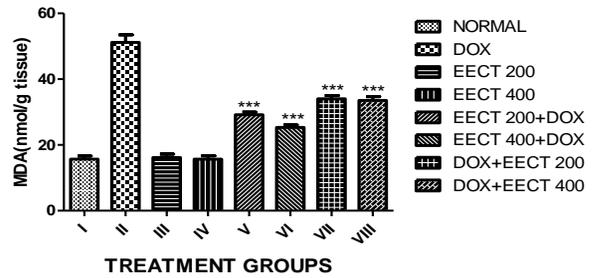


Figure 10: effect of EECT on MDA levels in different groups of rats

Histopathological studies

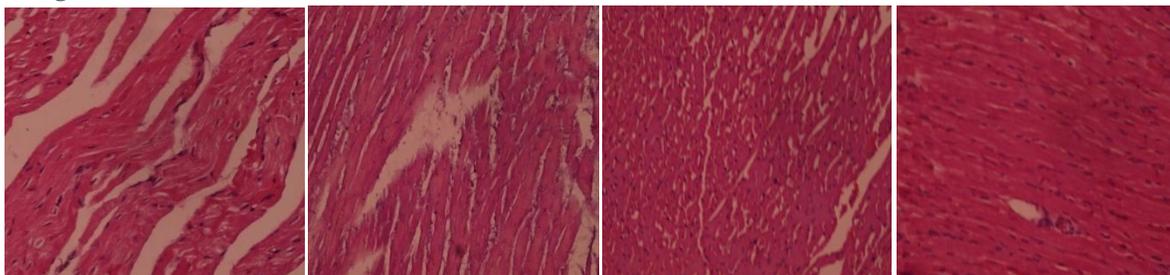


Figure (a)

Figure (b)

Figure (c)

Figure (d)

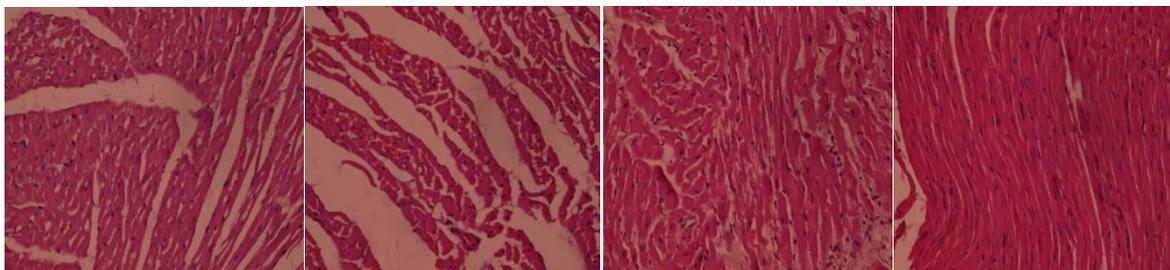


Figure (e)

Figure (f)

Figure (g)

Figure (h)

Figure 11: Histopathological studies

4. Discussion

Doxorubicin is converted to semiquinone by mitochondrial, lysosomal and cytosolic enzymes. Semiquinone is a charged moiety that readily donates an electron to an oxygen molecule, resulting in generation of an oxygen free radical or superoxide ion/hydroxyl radicals. Due to the presence of less developed antioxidant defence

mechanisms of heart, they are particularly vulnerable to apoptosis by anthracycline-induced reactive oxygen species. Therapeutic strategies, designed to augment cellular endogenous defence systems as antioxidants have been identified as a promising approach to combat against DOX toxicity. The present study also evidenced the formation of free radicals which brought about the biochemical and the histopathological changes in rats treated with doxorubicin.

Deficiency of oxygen supply or glucose supply may cause damage to the myocardial cell membrane leading to increased permeability and rupture, so that the enzyme leaks out. These enzymes which are also known as specific biomarkers can be estimated to check the damage. Doxorubicin causes an elevation in levels of CPK, LDH, ALT and AST when compared with the normal. Treatment with EECT leaves, at a dose of 400mg/kg, prophylactically, has shown a significant decrease in the level of these enzymes suggesting the protective or membrane stabilizing effect of the extract on the myocardium, when compared to the dose of 200mg/kg and curative treatment (Doses: 200 and 400mg/kg).

Oxidative stress and mitochondrial dysfunction are associated with disease and toxic process. It results from over production of ROS, often leading to peroxidation of membrane phospholipids and production of reactive aldehydes. Treatment with doxorubicin causes a decrease in the antioxidant stores of the heart viz., catalase while the extent of lipid peroxidation increases when compared with the normal. The present study demonstrates a significant increase in the endogenous antioxidant stores of CAT while the MDA levels were decreased by the prophylactic dose (400mg/kg) when compared with the dox control. These results indicate the protective effect or free radical scavenging effect of the EECT in oxidative damage done by doxorubicin. The presence of tannins, flavonoids and alkaloids might be responsible for the free radical scavenging and antioxidant activity of the extract which in returns provides cardioprotection.

Histopathological examination of myocardial tissue obtained from normal animal exhibited clear integrity of myocardial membrane. The heart sections obtained from Dox-treated animals showed disruption of several subcellular elements including loss of myofibrils, swelling of mitochondria, vacuolization of the cytoplasm, formation of lysosomal bodies and dilation of the sarcotubular system. Treatment with the EECT leaves demonstrated less disruption of the myofibrils and less vacuolization of the cytoplasm. This further confirms the membrane stabilizing effect of the extract.

## 5. Conclusion

Cardiotoxicity induced by doxorubicin is due to the oxidative stress, which can be observed by the elevated levels of biomarkers (CPK, LDH, AKT and AST) and biochemical parameter (MDA); whereas the other biochemical parameter, Catalase (CAT) levels were decreased. The toxicity can also be confirmed through the histopathological changes induced by doxorubicin, which can be treated with the ethanolic extract of *Cinnamomum tamala* leaves. However the ethanolic extract of *Cinnamomum tamala* leaves shows significant protective effect.

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