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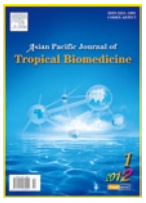

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Cardioprotective effect of aqueous, ethanol and aqueous ethanol extract of *Aerva lanata* (Linn.) against doxorubicin induced cardiomyopathy in rats

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ABSTRACT

Objective: To evaluate the cardioprotective effect of aqueous, ethanol and aqueous ethanol extract of *Aerva lanata* (*A. lanata*) against doxorubicin induced cardiomyopathy in Wistar rats. **Methods:** In this study, cardioprotective effect of aqueous, ethanol and aqueous ethanol extract of *A. lanata* against doxorubicin induced cardiomyopathy rats at the dosage of 200 mg/kg body weight for 28 days and also compared with standard allopathic drug aspirin. At the end of 28 days, enzymatic antioxidants, non-enzymatic antioxidants, lipid peroxidation, membrane bound enzymes were estimated in tissue homogenate. Cardiac biomarkers such as LDH, AST, ALT and lipid profiles such as cholesterol, triglycerides, HDL, LDL and VLDL were estimated in serum. Histopathology of heart samples was also performed. **Results:** It was shown that all the three extracts of *A. lanata* has cardioprotective activity ($P<0.05$) similar to that of aspirin. There are no significant changes between groups VI, VII and VIII with group I. These results implied that *A. lanata* does not contain toxic substances. **Conclusions:** By all these findings *A. lanata* has showed significant cardioprotective activity, which provides scientific proof of these traditional values.

1. Introduction

Cardiomyopathy is a main side effect of cancer. Overall 41% of cancer patients would suffer cardiotoxicity by administration of anticancer drugs. Doxorubicin anthracycline is an anticancer drug used against solid tumors, leukemia, breast cancer, small cell carcinoma of lung and esophageal carcinoma[1]. But it has specific toxicity to cardiac tissue[2]. Due to high intake of doxorubicin that leads to form cardiac myopathy and also it create stress, sustained hypertrophic stimulation become maladaptive, worsening morbidity and mortality risks because of congestive heart failure[3].

The mechanisms proposed that doxorubicin bound with ferric iron to induce the production of reactive oxygen species that leads to causing impairment of cell functioning and cytolysis and also bound β- glycoprotein induce the production of caspase[3,8] and apoptosome that cause DNA damage. DNA damage in proliferative cells activates a pathway that arrest cell division to allow either DNA repair or the induction of cell death by apoptosis[4].

Antioxidant compounds are present high content in plants. It has shown protective effects against diseases without reducing their therapeutic efficacy. More over, there is a growing interest in the usage of natural antioxidants as a protective strategy against cardiovascular related problems such as ischemia reperfusion[5]. In the past, the development of herbal anti-osteoporosis formulas was mainly presumed by scientists in Asian countries[6,7]. *A. lanata* one of the medicinal herbs have a therapeutic effects. The plant has been reported to possess hypoglycemic and antihyperlipedimic[8]. *A. lanata* (L) contains the source of chemicals of immense medicinal and pharmaceutical importance such as O-acyl glycosides, β -sitosterol, daucosterol, syringic acid, vanillic acid, feruloyl tyramine, feruloyl homovanillylamine, narcissin and aervitrine. Hence, considering the importance of *A. lanata*

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an attempt is made to present an overview of phytochemical and pharmacological activities of this plant[9].

In this study, to evaluate the cardio protective effect of *A. lanata* against cardio toxicity induced by doxorubicin.

2. Materials and methods

2.1. Plant collection

Fresh plants parts were collected from Coimbatore, Tamil Nadu, India. The plant was authenticated by Dr. G.V.S Moorthy, Botanical survey of India, TNAU Campus, Coimbatore. The voucher No. BSI/SC/5/23/10-11/Tech/[22]. Fresh plant material was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles.

2.2. Extraction

100 g of dried plant powder was extracted in 500 mL of ethanol, aqueous and aqueous ethanol (1:1) for 24 hr in occasional shaker at room temperature. The supernatant was collected and evaporated to make the final volume one-fifth of the original volume. It was stored at 4 ^{o}C in airtight bottles for further studies.

2.5. Experimental design

The wistar strain of female albino rats weighing between 140-160 g were obtained from Animal House of Karpagam University, Coimbatore. The animals were housed in large spacious cages and were given food and water ad libitum during the course of the experiment. The animal room was well ventilated and the animals had a (10±1) hour night schedule, throughout the experimental period. The study was approved by Institutional Animal Ethical Committee (IEAC) constituted for the purpose of CPCSEA, Government of India. The animals were divided into nine groups. Each groups had 6 rats.

Group I: Control rats.

Group II: Doxorubicin (2.5 mg/kg body weight IP on days 1, 7, 14, 21, 28).

Group III: Doxorubicin (2.5 mg/kg body weight IP on days 1, 7, 14, 21, 28) + aqueous extract of *A. lanata* (200 mg/kg body weight for 28 days).

Group IV: Doxorubicin induced (2.5 mg/kg body weight IP on days 1, 7, 14, 21, 28) + Ethanol extract of *A. lanata* (200 mg/kg body weight for 28 days).

Group V: Doxorubicin induced (2.5 mg/kg body weight IP on days 1, 7, 14, 21, 28) + aqueous ethanol extract of *A. lanata* (200 mg/kg body weight for 28 days).

Group VI: Aqueous extract of *A. lanata* alone (200 mg/kg body weight for 28 days)

Group VII: Ethanol extract of *A. lanata* alone (200 mg/kg body weight for 28 days)

Group VIII: Aqueous ethanol extract of *A. lanata* alone (200 mg/kg body weight for 28 days) .

Group IX: Doxorubicin (2.5 mg/kg body weight IP on days 1, 7, 14, 21, 28) + Aspirin (1.2 mg/kg body weight for 28 days).

After the experimental period, the animals were sacrificed under light chloroform anesthesia. Blood was drawn from the para-orbital venous complexes, blood, serum and heart was separated. The heart was excised immediately, cleaned free of extraneous material and perfused with ice cold saline (0.9%) which are used for biochemical estimations and also stored in 10% formalin, which are used for histopathological studies respectively.

2.6. Determination of serum lipid profile

Lipid profiles contain total cholesterol, triglycerides, HDL, LDL and VLDL were analyzed by kit method.

2.7. Determination of serum biomarkers

Serum biomarkers like AST, ALT and LDH were analyzed by kit method.

2.8. Determination of lipid peroxidation

Lipid peroxidation was analyzed by Ohkawa *et al.*, method[10].

2.9. Determination of enzymatic antioxidants

Superoxide dismutase activity assayed by Misra and Fridovich method[11]. Catalase activity assayed by Sinha method[12]. Glutathione peroxidase activity assayed by Rotruck *et al* method[13]. Glutathione reductase and Glutathione S transferase activity assayed by Moron *et al* method[14].

2.10. Determination of non-enzymatic antioxidants

Reduced glutathione was analysed by Ellman method[15] . Vitamin C was analysed by Omaye method[16]. Vitamin E was analysed by Emmerie and Engel method[17] .

2.11. Determination of membrane bound enzymes

Na^{+}/K^{+} dependent ATPase was determined with the concentration of sodium by Bonting method[18]. Ca^{2+} dependent ATPase was determined with the concentration of calcium by Hjerken method[19]. Mg^{2+} dependent ATPase was determined with the concentration of magnesium by Ohinishi *et al* method[20].

2.12. Statistical analysis

The results obtained were expressed as Mean ±SD. The Statistical comparison among the groups were performed with one way ANOVA and DMRT using a statistical package

program (SPSS 10.0) at $P<0.05$.

3. Results

3.1. Effect of A. lanata on serum lipid levels

Animals treated with doxorubicin produced significant increase in the levels of cholesterol, triglycerides, LDL and VLDL and significant decrease in HDL level when compared with group I shown in table 1. In drug treated groups III, IV and V, significant decrease in cholesterol, triglycerides, LDL and VLDL and significant increase in HDL compared with doxorubicin treated group. There is no significance difference between control and plant treated groups (Group VI, VII and VIII) and also standard drug treated group (Group IX).

3.2. Effect of A. lanata on serum biomarkers

Animals treated with doxorubicin produced significant increase in the levels of AST, ALT and LDH when compared with group I in table 2. In drug treated groups III, IV and V, significant decrease in AST, ALT and LDH compared with doxorubicin treated group. There is no significance difference between control and plant treated groups (Group VI, VII and VIII) and also standard drug treated group (Group IX).

3.3. Effect of A.lanata on Lipid peroxidation

Effect of doxorubicin on lipid peroxidation shown in table 3. The level of MDA would increase in Group II when compared with Group I. In drug treated groups III, IV and V, MDA level would decrease when compared with doxorubicin treated group. There is no significance difference between control and plant treated groups (Group VI, VII and VIII) and also standard drug treated group (Group IX).

3.4. Effect of A.lanata on Enzymatic antioxidants

Table 4 shows the level of enzymatic antioxidants. In group II, the level of all enzymatic antioxidants would decrease when compared with Group I. In drug treated groups III, IV and V, antioxidants level would increase when compared with doxorubicin treated group. There is no significance difference between control and plant treated groups (Group VI, VII and VIII) and also standard drug treated group (Group IX).

3.5. Effect of A.lanata on Non – enzymatic antioxidants

Table 1
Effect of aqueous, ethanol and aqueous ethanol extract of *A. lanata* on the activities of lipid profiles in serum of control and experimental group.

Groups	Cholesterol	Triglycerides	HDL	LDL	VLDL
Group I	73.12±1.81^{a}	64.63±3.57^{ab}	24.62±0.363^{d}	55.54±1.33^{b}	18.27±0.453^{a}
Group II	216.39±19.49^{c}	196.54±10.31^{e}	9.28±3.89^{a}	166.27±17.53^{d}	54.09±4.87^{c}
Group III	99.46±11.01^{b}	92.47±1.37^{d}	15.89±2.20^{b}	75.99±10.21^{c}	24.86±2.75^{b}
Group IV	79.83±9.43^{a}	71.14±2.69^{bc}	14.96±1.88^{b}	61.61±8.69^{b}	19.95±2.36^{a}
Group V	73.65±3.00^{a}	70.12±7.73^{bc}	18.73±0.600^{c}	55.95±2.51^{ab}	18.41±0.751^{a}
Group VI	71.23±2.32^{a}	60.97±5.45^{a}	22.24±0.459^{d}	54.35±6.03^{a}	17.81±0.579^{a}
Group VII	72.31±1.11^{a}	68.08±5.83^{bc}	24.46±0.220^{d}	55.07±2.17^{ab}	18.07±0.275^{a}
Group VIII	73.65±2.08^{a}	71.34±1.97^{bc}	23.73±0.415^{d}	55.70±1.60^{ab}	18.41±0.521^{a}
Group IX	73.38±3.81^{a}	71.75±1.13^{c}	21.67±0.763^{d}	55.37±3.83^{ab}	18.35±0.954^{a}

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a–f) differ significantly at $P< 0.05$ (DMRT).
Units: Cholesterol, Triglycerides, HDL, LDL, VLDL – mg/dL.

Table 2
Effect of aqueous, ethanol and aqueous ethanol extract of *A. lanata* on the activities of marker enzymes in serum of control and experimental group.

Groups	LDH	ALT	AST
Group I	174.94±10.10^{a}	28.34±3.19^{a}	18.14±1.91^{a}
Group II	275.51±9.18^{e}	50.03±2.39^{d}	33.09±3.08^{e}
Group III	198.77±5.30^{d}	37.51±0.86^{c}	21.05±0.95^{d}
Group IV	184.92±7.53^{c}	33.47±0.73^{b}	20.91±1.58^{cd}
Group V	174.94±6.41^{b}	33.80±2.41^{b}	20.49±1.86^{bcd}
Group VI	178.43±2.92^{a}	28.88±2.31^{a}	18.27±1.64^{ab}
Group VII	143.48±6.61^{a}	29.77±1.50^{a}	19.06±1.25^{abcd}
Group VIII	143.98±4.15^{a}	29.63±0.76^{a}	18.65±1.79^{abc}
Group IX	151.69±13.70^{a}	30.59±0.37^{a}	20.12±0.51^{abcd}

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a–f) differ significantly at $P< 0.05$ (DMRT).
Units: AST, ALT, LDH – nmoles of pyruvate liberated/min/mg protein.

Table 3
Effect of Aqueous, Ethanol and Aqueous ethanol extract of *A. lanata* on the LPO level of heart in doxorubicin induced cardio toxicity in rats.

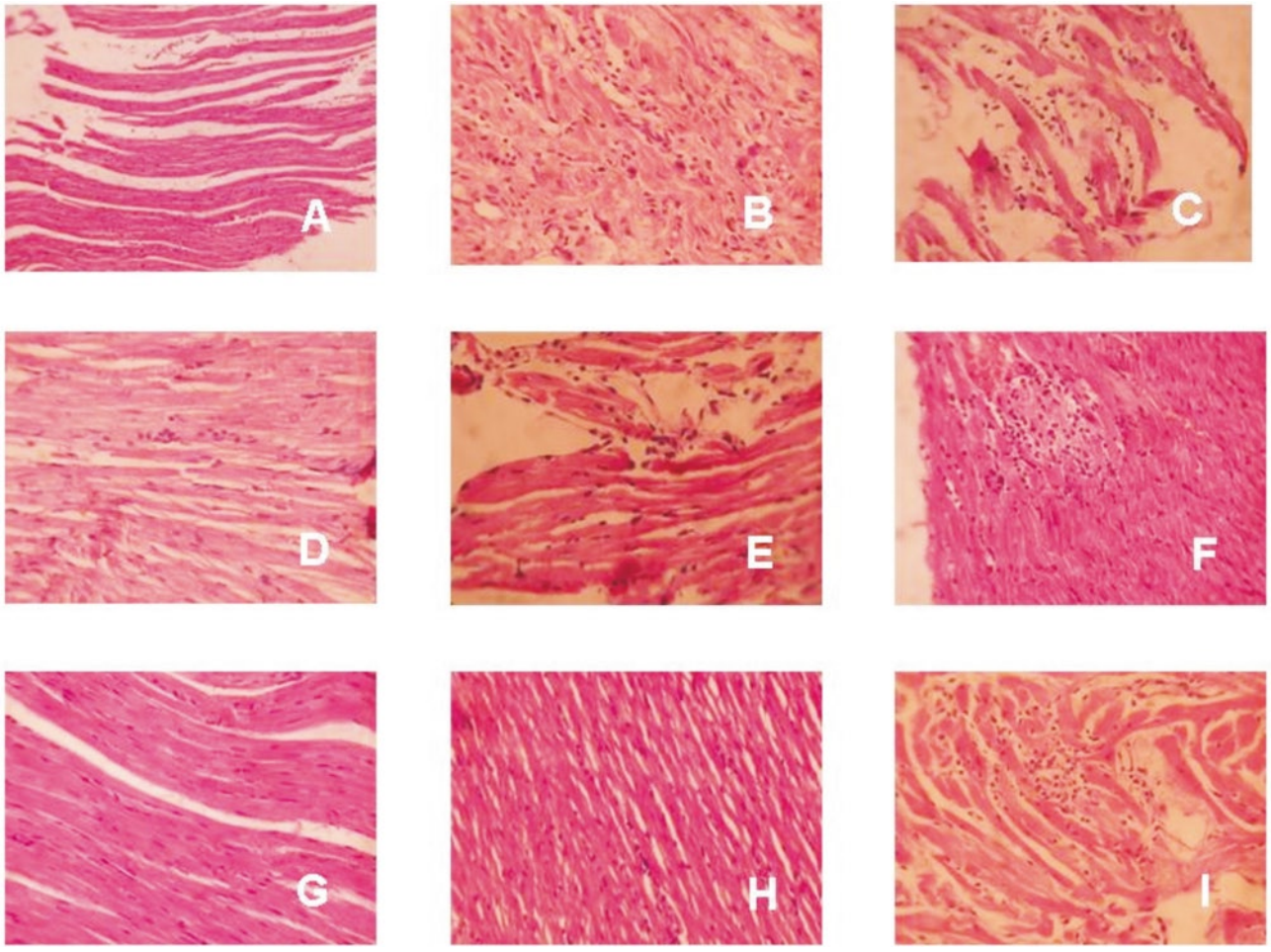
Groups	LPO
Group I	1.856 ± 0.148^{a}
Group II	4.502 ± 0.316^{d}
Group III	2.861 ± 0.069^{c}
Group IV	2.267 ± 0.146^{b}
Group V	2.016 ± 0.100^{a}
Group VI	2.006 ± 0.023^{a}
Group VII	1.893 ± 0.034^{a}
Group VIII	1.948 ± 0.182^{a}
Group IX	1.821 ± 0.145^{a}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a–f) differ significantly at $P< 0.05$ (DMRT).
Unit: LPO – nM of MDA formed / min/ mg protein.

Table 5 shows the level of non-enzymatic antioxidants. In group II, the level of all non-enzymatic antioxidants would decrease when compared with group I. In treatment groups III, IV and V, non-antioxidants level would increase when compared with doxorubicin treated group. There is no significance difference between control and plant treated groups (Group VI, VII and VIII) and also standard drug treated group (Group IX).

3.6. Effect of A. lanata on membrane bound enzymes

Animals treated with doxorubicin (Group II) produced significant increase in Na^{+}-K^{+} ATPase and Mg^{2+}- ATPase, significantly decrease in Ca^{2+}- ATPase when compared with Group I are shown in table 6. In drug treated groups III, IV and V, antioxidants level would reversible and similar to Group I. There is no significance difference between control and plant treated groups (Group VI, VII and VIII) and also standard drug treated group (Group IX).



Figue 1. Histology of heart tissue from control and *A. lanata* treated animals.
A: Normal heart shows no obvious abnormality; B: Doxorubicin induced cardiotoxicity heart shows more number of lymphocytic populations, non specific chronic myocarditis; C: Doxorubin induced and treated with aqueous extract of *A. lanata*. Less number of lymphocytic populations when compared with group II. D: Doxorubin induced and treated with ethanol extract of *A. lanata*. No number of lymphocytic population and it similar to normal.E: Doxorubin induced and treated with aqueous ethanol extract of *A. lanata* les number of lymphocytic population when compared with groupII. F: Aqueous extract of *A. lanata* trated alone. No lymphocytic population and no myocarditis similar to that of normal; G:Ethanol extract of *A. lanata* trated alone.No lymphocytic population and no myocarditis similar to that of normal.H: Aqueous ethanolic extract of *A. lanata* trated alone. No lymphocytic population and no myocarditis similar to that of normal. I: Doxorubin induced and treated with Aspirin standard drug. Less number of lymphocytic populations when compared with group II.

Table 4
Effect of aqueous, ethanol and aqueous ethanol extract of *A. lanata* on the activities of enzymatic antioxidants in heart of control and experimental group.

Groups	SOD	Catalase	Gpx	GR	GST
Group I	1.06±0.031^{e}	1.70±0.011^{d}	3.28±0.052^{f}	10.43±0.825^{ef}	76.64±3.05^{de}
Group II	0.63±0.178^{a}	1.05±0.074^{a}	2.34±0.305^{a}	5.44±0.648^{a}	44.27±4.03^{a}
Group III	0.94±0.051^{b}	1.14±0.106^{b}	2.87±0.153^{b}	7.25±0.864^{b}	55.55±2.68^{b}
Group IV	1.04±0.011^{bc}	1.58±0.108^{c}	2.97±0.041^{bc}	7.60±1.36^{bc}	58.39±2.44^{b}
Group V	0.989±0.051^{bc}	1.55±0.061^{c}	3.07±0.061^{cd}	8.24±0.752^{c}	61.89±2.33^{c}
Group VI	1.03±0.077^{bc}	1.67±0.036^{d}	3.19±0.005^{def}	9.67±0.694^{e}	75.12±2.05^{d}
Group VII	1.08±0.067^{c}	1.62±0.019^{cd}	3.12±0.007^{de}	9.71±0.206^{e}	75.37±1.24^{d}
Group VIII	1.06±0.045^{c}	1.68±0.051^{d}	3.30±0.024^{f}	10.31±0.197^{ef}	78.80±1.85^{e}
Group IX	1.07±0.045^{c}	1.67±0.05^{d}	3.25±0.021^{ef}	10.67±0.251^{f}	75.23±2.24^{d}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a–f) differ significantly at $P< 0.05$ (DMRT). Units : SOD – inhibition of 50% nitrite formation/min/mg protein; CAT – μ mole of H2O2 consumed/min/mg protein; GPx – μ g of glutathione oxidized/min/mg protein; GR – μ mole of glutathione utilized/min/mg protein; GST – μ moles of CDNB – GSH conjugate formed/min/mg protein.

Table 5
Effect of aqueous, ethanol and aqueous ethanol extract of *A. lanata* on the activities of non enzymatic antioxidants in heart of control and experimental group.

Groups	Vitamin C	GSH	Vitamin E
Group I	1.102±0.051^{d}	10.94±0.491^{d}	1.569±0.106^{c}
Group II	0.711±0.032^{a}	6.81±0.606^{a}	0.661±0.125^{a}
Group III	1.039±0.048^{c}	8.86±0.813^{b}	1.154±0.072^{b}
Group IV	0.979±0.044^{b}	9.75±0.625^{c}	1.257±0.106^{c}
Group V	1.041±0.066^{c}	8.86±0.738^{b}	1.362±0.035^{d}
Group VI	1.115±0.021^{d}	11.14±0.488^{d}	1.594±0.035^{e}
Group VII	1.109±0.037^{d}	10.74±0.207^{d}	1.620±0.020^{e}
Group VIII	1.138±0.054^{d}	10.877±0.145^{d}	1.737±0.112^{f}
Group IX	1.095±0.049^{cd}	10.831±0.218^{d}	1.517±0.069^{e}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a–f) differ significantly at $P< 0.05$ (DMRT). Units: GSH, Vitamin C, Vitamin E (μ g/mg protein).

Table 6
Effect of Aqueous, Ethanol and Aqueous ethanol extract of *A. lanata* on the activities of membrane bound enzymes in heart of control and experimental group.

Groups	Na^{+}–K^{+} ATPase	Ca^{2+}– ATPase	Mg^{2+}– ATPase
Group I	3.99±0.086^{df}	1.79±0.038^{a}	0.384±0.026^{c}
Group II	2.76±0.097^{a}	2.95±0.041^{f}	0.184±0.011^{a}
Group III	3.23±0.240^{c}	2.26±0.087^{d}	0.293±0.015^{b}
Group IV	2.91±0.072^{b}	1.97±0.021^{cd}	0.367±0.034^{c}
Group V	2.93±0.152^{b}	1.96±0.032^{cd}	0.375±0.014^{c}
Group VI	4.08±0.067^{f}	1.98±0.324^{d}	0.379±0.027^{c}
Group VII	3.81±0.032^{d}	1.92±0.021^{bc}	0.429±0.031^{d}
Group VIII	3.98±0.032^{df}	1.94±0.077^{bcd}	0.478±0.032^{d}
Group IX	3.93±0.096^{d}	1.88±0.018^{b}	0.475±0.025^{d}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a–f) differ significantly at $P< 0.05$ (DMRT). Units : Na^{+}–K^{+} ATPase, Ca^{2+}– ATPase, Mg^{2+}– ATPase – μ moles of phosphorus liberated/mg protein.

3.7. Histopathological observation

The histology of heart tissue from control and *A. lanata* treated animals (Group VI, VII and VIII) and standard drug treated group (Group IX) showed normal morphological appearances, whereas in group II shows the chronic, severe loss of myofibrils and non specific myocarditis seen in atrial wall. The histology of heart tissues from doxorubicin treated with *A.lanata* shows less myofibril and less myocarditis and it similar to that of normal.

4. Discussion

The study entails the cardio protective effect of *A. lanata* against doxorubicin induced cardiotoxicity. *A.lanata* is a medicinal plant to treat against various disorders. This present study is aimed to explore the cardio protective effects of oral administration of *A. lanata* against doxorubicin induced cardiotoxicity in rats.

In previous study suggests that doxorubicin induced oxidative stress is due to generation of free radicals in heart tissue[21]. The doxorubicin induced mitochondrial injury

is critical to the heart because it would presumably have extremely adverse effects on the contractile functioning of the cardiac myocyte by alterations in energy metabolism[22].

Increased in lipid profile like cholesterol, triglycerides, LDL, VLDL and decreased HDL in doxorubicin treated groups indicate doxorubicin may be interfering with metabolism or biosynthesis of lipids. Pretreatment with *A.lanata* shows reduction in total cholesterol, triglycerides, LDL, VLDL and increase in HDL was observed Decreased lipid profiles and increase HDL in *A. lanata* treated groups may be due to presence of some bioactive compounds in plants. Lipid lowering effect of *A. lanata* is due to inhibition of hepatic cholesterol biosynthesis, increased fetal bile acid secretion and stimulation of receptor mediated catabolism of LDL cholesterol and increase in the uptake of LDL from blood by liver.

Heart tissue injury induced by doxorubicin in rats was indicated by elevated level of serum biomarker enzymes such as AST, ALT and LDH. LDH abundant in red blood cells and can function as a marker of hemolysis. Due to tissue breakdown LDH level is elevated. AST is defined as a biochemical marker for diagnosis of acute myocardial infarction. However the use of AST for a such a diagnosis is now redundant and has been suppressed by cardiac troponin[23].The increase the levels of marker enzymes in serum suggests an increased leakage of this enzymes from mitochondria as a result of toxicity induced by treatment with doxorubicin. This index has been recently used in other studies to test for cardiotoxicity[24]. *A. lanata* was found to inhibit the elevation of serum biomarkers. *A. lanata* pretreatment led to inhibit of AST, ALT and LDH release into serum. The present study is in good agreement with those Andreadou *et al*[25].

Elevated lipid peroxidation and poor antioxidant systems have suggested that a lack of antioxidant defense is responsible for the elevated lipid peroxidation in erythrocytes. MDA level is widely used as marker of free radical mediated lipid peroxidation injury. Maximum induction of LPO was observed in group II when compared with group I. The significant dose dependent of *A. lanata* decrease in cardiac MDA concentrations that confirms that concomitant administration with *A. lanata* extract could effectively protect against doxorubicin induced cardiotoxicity. Our study was in accordance with earlier reports3, that lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbations in lipid fluidity. It has been hypothesized that one of the principal causes of doxorubicin induced cardiotoxicity.

Cardioprotective effect of *A. lanata* was further supported by increased myocardial enzymatic and non-enzymatic antioxidant activity. Antioxidants play a vital role in scavenging reactive oxygen species and protect cells from oxidative damage. Antioxidant enzymes such as SOD, Catalase, Glutathione peroxidase, Glutathione reductase, Glutathione S transferase and non - enzymatic antioxidants Vitamin C, Vitamin E, Reduced glutathione are easily inactivated by lipid peroxides or reactive oxygen species, which results in decreased activities of these enzymes in doxorubicin induced cardiotoxicity rats[26]. Both enzymatic and non-enzymatic antioxidants were significantly elevated by administration of *A. lanata* extract to doxorubicin induced cardiomyopathy rats, suggests that it has ability to restore these enzymes activity in doxorubicin damaged heart.

The current investigation revealed that doxorubicin showed significantly fall in activity of membrane bound enzymes. Membrane bound ATPases of cardiac cells play a significant role in contraction and relaxation cycles of cardiac muscle by maintaining normal ion levels within the myocytes. Changes in the properties of this ion pumps affect cardiac function. It was reported that inhibition of Ca^{2+} ATPase induced pertubation of cell calcium homeostatis and this effect may associated with increase Ca^{2+} permeability27. In the heart cytosolic calcium accumulation may be a relevant mechanism leading to cell death and has been proposed to play an important role in pathogenesis of lethal myocardial cellular injury. The restoration of membrane bound enzymes like Na^{+}/ K^{+} ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase in *A. lanata* treated rats is indicative of membrane stabilizing protective effect of *A. lanata*.

All the three extracts of *A. lanata* do not contain any toxic substances. For this, there is no significant difference of enzymatic antioxidants, non-enzymatic antioxidants, serum lipid peroxidation, serum biomarkers, lipid profiles, membrane bound enzymes between groups VI, VII, VIII with group I. This present study is good agreed with functional group analysis through FTIR spectrum in aqueous, ethanol and aqueous ethanol extract of *A. lanata*[28].

Group I shows that normal. While group II has more number of lymphocytic population and chronic myocarditis in focal area because of the action of doxorubicin. Doxorubicin produced a lot of free radicals that damage heart to produce myocarditis. In *A. lanata* pretreated groups (Group III,IV,V) shows the very less number of lymphocytic population when compared with group II and also Group IX, doxorubicin treated with aspirin shows no obvious abnormality and similar to that of normal control. Phytochemical study of *A. lanata* suggests that presence of saponins, tannins, flavonoids, terpenoids and phenols. These bioactive compounds scavenge the free radicals produced and protect cardiac from damage. In groups VI,VII and VIII are treated with *A. lanata* alone shows similar to that of normal control. These results implicated that *A.lanata* does not contain toxic substances.

In conclusion, the present result suggests that *A. lanata* prevented the doxorubicin induced myocardial toxicity by boosting the enzymatic and non-enzymatic antioxidant activity. The cardio protective effect of *A. lanata* could be due to lipid lowering and decrease the level of serum biomarkers. Further studies are needed to elucidate the exact mechanisms of action of *A. lanata* and its clinical application. In future, It could be used for pharmacotherapeutic agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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