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Original Article

Protective Activity of *Rumex vesicarius* Leaf Extract on Doxorubicin Induced Cardiotoxicity

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ABSTRACT

Background: Doxorubicin is an important and efficient anticancer medicine that is widely used to treat several types of cancer. However, its clinical use is limited due to dose-dependent cardiotoxicity. Elevated tissue levels of cellular superoxide anion/oxidative stress contribute to doxorubicin-induced cardiotoxicity. **Objectives:** The current study aimed to look into the protective effects of *Rumex vesicarius* leaves against the doxorubicin induced cardiotoxicity. **Materials and Methods:** In treatment schedule, thirty male Wistar albino rats were allocated into five groups of six animals each. Cardiotoxicity was induced by single dose administration of Doxorubicin (20 mg/kg b. wt). The standard group received Amlodipine (5 mg/kg b. wt) and treatment groups received *Rumex vesicarius* leaves extract (200 and 400 mg/kg b. wt). Cardiac serum parameters-LDH, CK-MB, ALT, AST, and calcium and heart tissue LPO, GSH, and CAT levels were estimated. **Results:** Rats administered with doxorubicin have significantly increased cardiac serum parameters, tissue LPO, and Ca^{++}-ATPase while significantly decreasing tissue GSH, CAT, Na^{+}/K^{+}-ATPase and Mg^{++}-ATPase levels. Pretreatment with extract (400 mg/kg) showed significant protective activity against doxorubicin induced cardiotoxicity. Additionally, histopathological research was significantly reinforced the cardio protective effects of *Rumex vesicarius* leaves. **Conclusion:** *Rumex vesicarius* leaf extract showed significant cardioprotective activity against doxorubicin induced toxicity.

Keywords: *Rumex vesicarius*, Doxorubicin, Amlodipine, Oxidative stress, Membrane bound enzymes.

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INTRODUCTION

Cardiovascular Diseases (CVDs) are the most prevalent cause of mortality and other disorders worldwide, including India. The conditions affecting the heart and vasculature include hypertension, arrhythmias, myocardial infarction, coronary heart disease, ischemic heart disease, congestive heart failure, and stroke. According to the global burden of illness study's calculations, India's age-standardized CVD mortality rate (282/1 lakh individuals) is bigger than the global death rate (233/1 lakh individuals).[1]

Medication induced cardiac damage is a severe adverse effect of many conventional chemotherapeutic agents. The chemotherapy induced cardiotoxicity was categorized into type I (Irreversible damage) and type II (Reversible damage). Type I cardiotoxicity was caused by antimetabolites, alkylating agents, topoisomerase inhibitors, taxanes and anthracyclines, and type II cardiotoxicity was caused by trastuzumab, bevacizumab, lapatinib and sunitinib. The typical signs and symptoms of chemotherapy induced myocardial infarction are fatigue, weakness, continual cough, dyspnea, dizziness, and tachycardia.[2,3]

Doxorubicin is an anthracycline anticancer drug more effective and frequently preferred as a chemotherapeutic agent for various malignancies. Cardiomyopathy is the drug's main disadvantage and causes dose-dependent cardiac toxicity ranging from 400 to 700 mg/m^2. Cardiotoxicity develop during or within two to three days after delivery.[4] There is an 11% likelihood of acute cardiotoxicity. Heart palpitations and/or chest discomfort tend to be the symptoms. The electrocardiogram displayed non-specific ST-T alterations, a left axis change, and decreased QRS complex amplitude. Around 9% of the patients treated with doxorubicin showed left ventricular dysfunction. Oxidative stress, depletion of Ca^{2+} in the sarcoplasmic reticulum, altered calmodulin-dependent

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kinase II, mitochondrial biogenesis inhibition, and activation of apoptosis pathways by inhibiting topoisomerase 2β are the significant causes of cardiomyopathy caused by doxorubicin.[5]

Rumex vesicarius, a plant that belongs to the family Polygonaceae, frequently utilized and accessible edible plant called Bladder dock (English) or Chukkakura (Telugu) and is native to Northern Africa, Western Australia and Asia. The HPTLC analysis of *Rumex vesicarius* revealed the following phytoconstituents such as apigenin, ß-carotenes, catechin, emodin, epicatechin, luteolin, chromone, quercetin, rutin, physcion, chrysophanol, vitamin C, proteins, lipids, and organic acids and several minerals (Na, K, Cu, Mg, Fe, Ca and Mn).[6] *Rumex vesicarius* proven for its antioxidant activity,[7] antimicrobial effect,[8] antidiabetic activity,[9] diuretic effect,[10] antipyretic effect,[11] antiemetic effect,[12] spasmogenic and spasmolytic activity,[13] hepatoprotective effect,[14] and nephroprotective effect.[15]

The Adrenaline induced myocardial infarctions in male rabbits were treated with *Rumex vesicarius* aqueous-methanolic leaf extract to evaluate the hypotensive, cardioprotective, anticoagulant, and antioxidant effect.[16] R. vesicarius leaf extract was evaluated for its cardioprotective effect in Isoproterenol induced myocardial infarction in rats.[17]

Scientific data on the cardio protective effect of *Rumex vesicarius* leaves has yet to be reported against doxorubicin. Therefore, the main drive of the current investigation was to evaluate the protective properties of *Rumex vesicarius* leaf against doxorubicin induced cardiotoxicity.

MATERIALS AND METHODS

Drugs and chemicals

Doxorubicin Hydrochloride injection (DOXORUBA IP-50 mg/25 mL; Company: GETWELL Oncology Pvt. Ltd.,) was purchased from GETWELL Oncology Pvt. Ltd., Haryana, India. All chemicals and diagnostic kits used in the study were procured from Sigma Aldrich Pvt. Ltd., and Coral Diagnostics, India, respectively.

Collection and authentication of plant material

Fresh leaves of *Rumex vesicarius* purchased in the month of August at the local market in Medchal village, Medchal district, Telangana state, India, were authenticated by Dr. P.V. Prasanna, Scientist-F, Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana state, India. Voucher specimen number: BSI/DRC/2018-19/Tech./596 and deposited in the Department of Pharmacognosy, CMR College of Pharmacy, Hyderabad, India.

Preparation of the plant extract

Rumex vesicarius leaves were cleaned with distilled water, dried in the shade, and ground with a machine. The powder material was blended in an 80:20 ratios of water and alcohol, then set aside at 25°C for a week while being occasionally shaken. It was then filtered after being agitated for 20 min. The filtrate was dried in a rotary flash evaporator and retained at 4°C for further research.

Preliminary phytochemical screening

The Hydroalcoholic Extract of *Rumex vesicarius* Leaves (HAERVL) was subjected to qualitative phytochemical screening to identify the phytoconstituents.[18]

Acute toxicity studies

According to OECD 425 recommendations, an acute toxicity investigation was conducted.

Experiment Protocol

After 14 days of acclimatization, the adult male Wistar albino rats (170-200 g) were randomly divided into five groups of six animals in each by blocking technique. The male Wistar albino rats were divided into five groups of six animals each (n=6).

Normal group

The rats received vehicle (5 mL/kg b. wt, p. o) for 30 days.

Toxic group

The rats received vehicle (5 mL/kg b. wt, p. o) for 30 days and were administered with doxorubicin (20 mg/kg b. wt i.p) once on the 29th day.

Standard group

The rats were pretreated with Amlodipine (5 mg/kg b. wt, p. o) as a standard drug for 30 days and doxorubicin was administered (20 mg/kg b. wt, i.p) once on the 29th day.

HAERVL 200 mg/kg and HAERVL 400 mg/kg groups: The rats were pretreated with HAERVL (200 and 400 mg/kg b. wt, p. o) for 30 days and on the 29th day a single dose of doxorubicin (20 mg/kg b. wt, i.p) administered.

After completing the treatment schedule, animals were starved in the night before blood was drawn using the retroorbital puncture technique. Blood serum was used to estimate LDH, CK-MB, AST, ALT, and Calcium. Animals were sacrificed by cervical dislocation. Heart tissue was isolated and wet weight was measured. The heart was divided into two portions. The first portion of the heart was used for histopathological study. The remaining amount was used for the estimation of oxidative stress markers such as LPO, GSH, and CAT[19-21] and membrane-bound enzymes (Na^+/K^+-ATPase, Mg^{++}-ATPase, and Ca^{++}-ATPase) levels.[22-24]

Histopathological study

The histopathological study was performed by hematoxylin and eosin staining techniques.

Statistical Analysis

The values were presented as Mean±SEM, and statistical analysis was carried out using one-way ANOVA, using GraphPad Prism 5.0. Error bars represent 95% confidence interval.

RESULTS

Preliminary phytochemical studies

The HAERVL revealed the presence of carbohydrates, proteins, alkaloids, glycosides, flavonoids, phenols, and tannins.

Acute toxicity studies

The HAERVL was well tolerated and showed zero morbidity and mortality. The rats were treated with 2000 mg/kg b. wt, p. o of the extract, and they behaved normally, showing no significant changes in behavior patterns, physiological changes, coma, or death.

Effect on body and heart weights

Administration of doxorubicin in the toxic group on the 29th day has shown a significant decrease in body weight compared to the normal group. A substantial increase in the body weight of rats pretreated with HAERVL at 200 and 400 mg/kg b. wt was noted as compared with a toxic group. An increase in the body weight of the standard and HAERVL 400 mg/kg b. wt pretreatment groups showed similar results compared with the normal group. The toxic group showed increased heart weight and pretreatment with Standard Amlodipine, HAERVL 200 and 400 mg/kg b. wt showed a normalization of the heart weights (Table 1).

Effect of HAERVL on cardiac serum markers

A substantial increase in the serum levels of LDH, CK-MB, ALT, AST, and Calcium was observed in the toxic group, indicating cardiac damage. Pretreatment with HAERVL at 200 and 400 mg/kg b. wt has shown a significant normalization of serum levels of LDH, CK-MB, ALT, AST, and Calcium (Figure 1).

Effect of HAERVL on cardiac oxidative stress markers

There was a significant increase in the LPO level and a significant decrease in the levels of GSH and CAT in the toxic group. The LPO levels in the standard group significantly dropped, while the GSH and CAT levels significantly rose. Pretreatment with HAERVL at doses of 200 and 400 mg/kg b. wt has shown a significant decrease in LPO with a simultaneous increase in the levels of GSH and CAT (Figure 2) when compared with a toxic group.

Effect of HAERVL on cardiac membrane bounded enzymes

In the toxic group of rats administered with doxorubicin, a significant decrease in Na^+/K^+-ATPase and Mg^{++}-ATPase levels with a simultaneous increase in Ca^{++}-ATPase levels was noticed. A significant increase in the Na^+/K^+-ATPase and Mg^{++}-ATPase levels and a significant decline in the levels of Ca^{++}-ATPase were observed in the standard group. Pretreatment with HAERVL at 200 and 400 mg/kg b. wt showed increased levels of Na^+/K^+-ATPase and Mg^{++}-ATPase and moderate recovery from Ca^{++}-ATPase levels (Figure 3) as compared with a toxic group.

Histopathological studies

In histological examinations, the cardiac membrane of normal rats showed long and thick branching muscle fibers, intercalated discs, centrally placed single oval nucleus and normal capillary system. Rats that received doxorubicin showed damaged striations, loss of myofibrils, destruction of intercalated discs, interstitial edema, leucocyte infiltration, and vascular injury. Rats pretreated with the standard group showed mild damage of striations, no edema and no cytoplasmic vacuole formation. Pretreatment with extract at a dose of 200 mg/kg b. wt showed moderate loss of striations and edema as demonstrated by a decreased necrotic region and infiltration of leukocytes in rats. Pretreatment with extract at a dose of 400 mg/kg b. wt has shown significant protection against doxorubicin induced toxicity, which was evident by a marked reduction of necrosis, infiltration of leukocytes with usual striations, and preservation of the normal integrity of the cardiac tissue (Figure 4).

DISCUSSION

Doxorubicin is a highly effective antitumor drug, but its usefulness is limited as the cumulative doses cause cardiotoxicity. Preclinical and clinical studies have demonstrated a relationship

Table 1: Effect of HAERVL on body and heart weights.

Group	Body weight (g)		Heart weight (g)
	1st day	31st day	31st day
Normal group	157± 2.11	194±.3.07 (↑37 g)	0.467±0.01
Toxic group	144±2.71	157±2.11 (↑13 g)	0.571±0.02$^{\$\$\$}$
Standard group	170±2.58	201±1.67 (↑31 g)	0.481±0.01***
HAERVL 200 mg	168±2.14	180±1.29 (↑12 g)	0.530±0.01$^{**\$\$\$}$
HAERVL 400 mg	161±1.67	190±2.47 (↑29 g)	0.493±0.02**

Values are represented as Mean±SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by post hoc Dunnett's test $^{***}p<0.001$, $^{**}p<0.01$,$^{*}p<0.05$ Vs toxic group; $^{\$\$\$}p<0.001$, $^{\$\$}p<0.01$, $^{\$}p<0.05$Vs normal group.

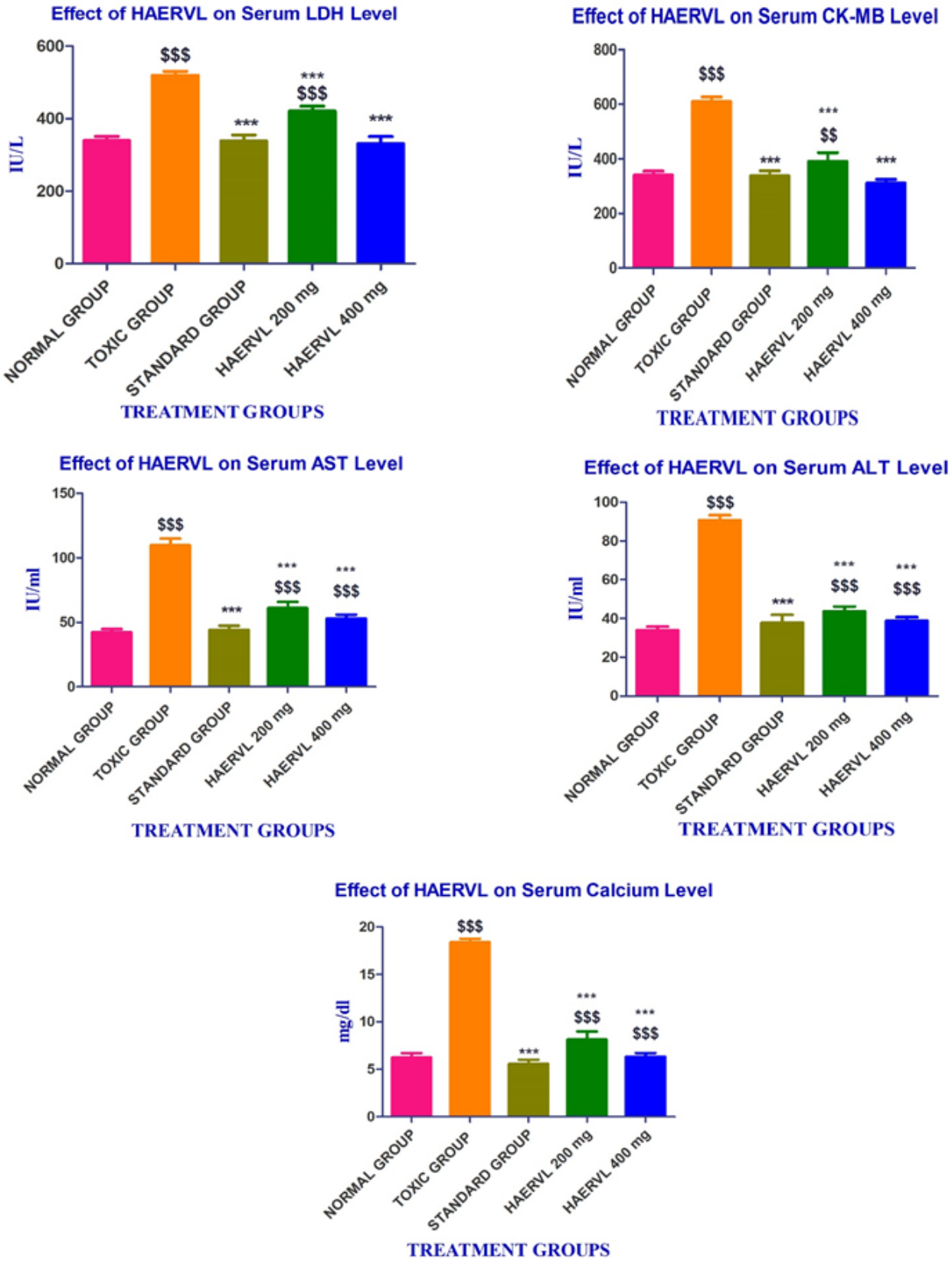

Figure 1: Effect of HAERVL on serum cardioprotective parameters. Values are represented as Mean±SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by post hoc Dunnett's test ***p<0.001, **p<0.01, *p<0.05 Vs toxic group; $^{\$\$\$}p$<0.001, $^{\$\$}p$<0.01, $^{\$}p$<0.05Vs normal group.

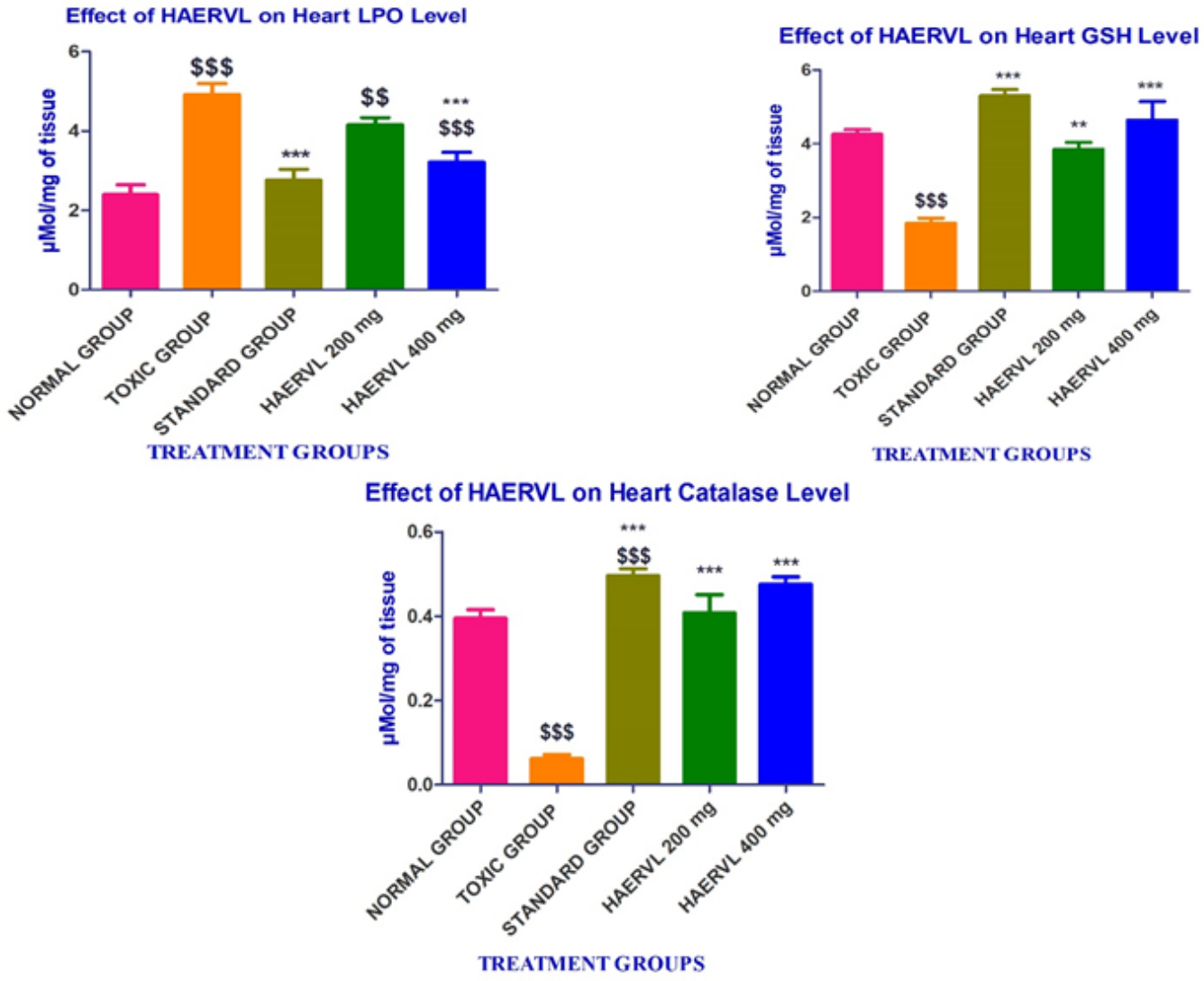

Figure 2: Effect of HAERVL pretreatment on oxidative stress markers. Values are represented as Mean±SEM (*n*=6). Statistical analysis was performed using one-way ANOVA followed by post hoc Dunnett's test ***$p<0.001$, **$p<0.01$, *$p<0.05$ Vs toxic group; $^{\$\$\$}p<0.001$, $^{\$\$}p<0.01$, $^{\$}p<0.05$Vs normal group.

between doxorubicin induced cardiotoxicity and oxidative stress.[25-27] Doxorubicin enhances the formation of free radicals and oxidative stress by mechanisms such as 1) the capacity of flavin-centered, NADPH-dependent reductases to generate a reduced form of anthracycline semiquinone free radicals in complex I of mitochondrial ETC that cause apoptosis in the myocardium.[28,29] 2) Doxorubicin binds with ferric ions to alter iron homeostatic processes associated with aconitase-iron regulatory protein-1, inactivating cytochrome c oxidase, which leads to impairment of cell function and destruction. 3) Doxorubicin also binds with β- glycoprotein to induce caspase production and apoptosome that cause DNA and protein damage and mitochondrial dysfunction.[30-33] Amlodipine significantly inhibits doxorubicin-induced myocyte apoptosis by suppressing the mitochondrial apoptotic pathway. This effect is attributed to the antioxidant properties of amlodipine.[34]

A preliminary phytochemical investigation of *Rumex vesicarius* extract results revealed the presence of carbohydrates, proteins, alkaloids, glycosides, flavonoids, phenols, and tannins. Acute toxicity studies of extract showed tolerance up to a dose of 2000 mg/kg b. wt, and no specific changes were noticed in general appearance. Based on the acute toxicity studies, 1/5th and 1/10th of the test doses were selected for evaluating cardioprotective activity in doxorubicin induced cardiotoxicity.

The significant decreased body weight reported after administration of doxorubicin in experimental animals might be due to a decrease in the intake of food and water by altering the function of the intestinal mucosa, promoting catabolism and muscle wasting, and a significant increase in the circulating concentration of corticosterone/testosterone ratio.[35-37] The decreased heart weight observed in the toxic group indicates the loss of myofibrils and myocardial necrosis. The present study observations were correlated with earlier reports of Shamala,

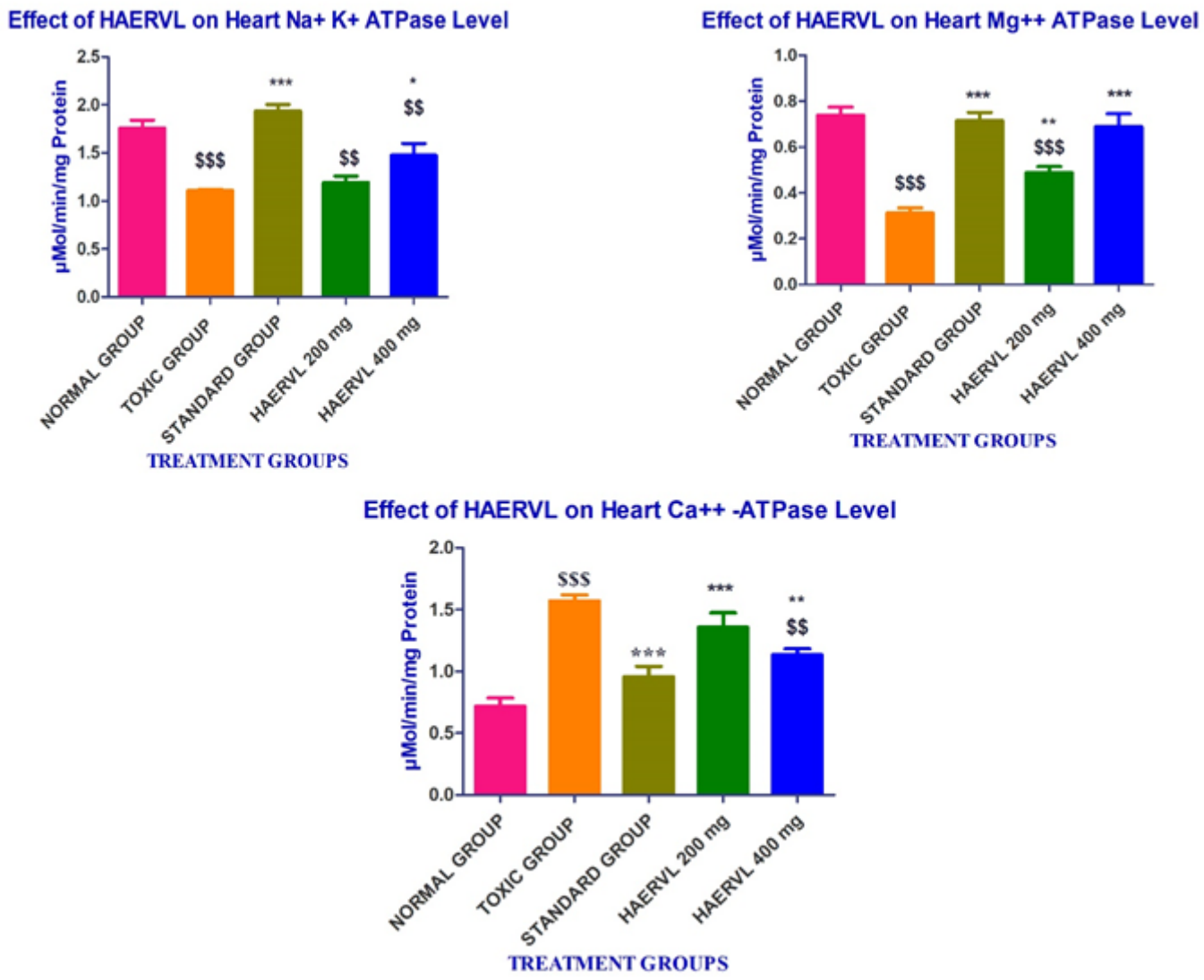

Figure 3: Effect of HAERVL on cardiac membrane bounded enzymes. Values are represented as Mean±SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by post hoc Dunnett's test ***p<0.001, **p<0.01, *p<0.05 Vs toxic group; $^{\$\$\$}p$<0.001, $^{\$\$}p$<0.01, $^{\$}p$<0.05Vs normal group.

Krishna, 2013 and Tamizhselvi *et al*., 2020,[38,39] and pretreatment with *Rumex vesicarius* extract 400 mg/kg b. wt showed a significant increase in the body and heart weights.

The CK-MB, LDH, and AST enzymes are frequently used to determine the presence and prognosis of myocardial injury.[40,41] ALT also serves as a parameter related to endothelium dysfunction induced atherosclerosis and inflammation in the myocardium.[42] Afsar *et al*., 2017, illustrated Acacia hydaspica R. Parker prevents doxorubicin-induced cardiac injury by weakening oxidative stress and structural changes in cardiomyocytes in male Sprague-Dawley rats. At 400 mg/kg dose, Acacia hydaspica R. Parker prevents doxorubicin-induced toxicity by reducing the release of serum CK, CK-MB, AST, and LDH levels and normalizing hematological parameters. In addition, it was noticed that there was a reversal of the levels of tissue antioxidants (CAT, POD, SOD, QR, GSH, GST, GSR, and, GSH-Px) and decreasing levels of oxidative stress biomarkers (LPO, H2O2, and nitrite concentration) in the cardiac tissue.[43]

When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST and ALT are released into the bloodstream. The amount of AST and ALT in the blood is directly related to the extent of the tissue damage.[44] Low cardiac output and arterial hypoperfusion resulted in "Acute Cardiogenic Liver Injury (ACLI)," which was associated with increased levels of AST and ALT in heart failure that was attributed to hepatocellular damage from decreased perfusion.[45] The present study showed an abnormal rise in the serum levels of these enzymes after the administration of doxorubicin in the toxic group due to its lipid peroxidation nature in the myocardium. The present study results coincided with earlier reports.[46,47] Pretreatment with *Rumex vesicarius* extract at 200 and 400 mg/kg b. wt showed significant restoration in the serum levels of CK-MB, LDH, ALT, and AST enzymes.

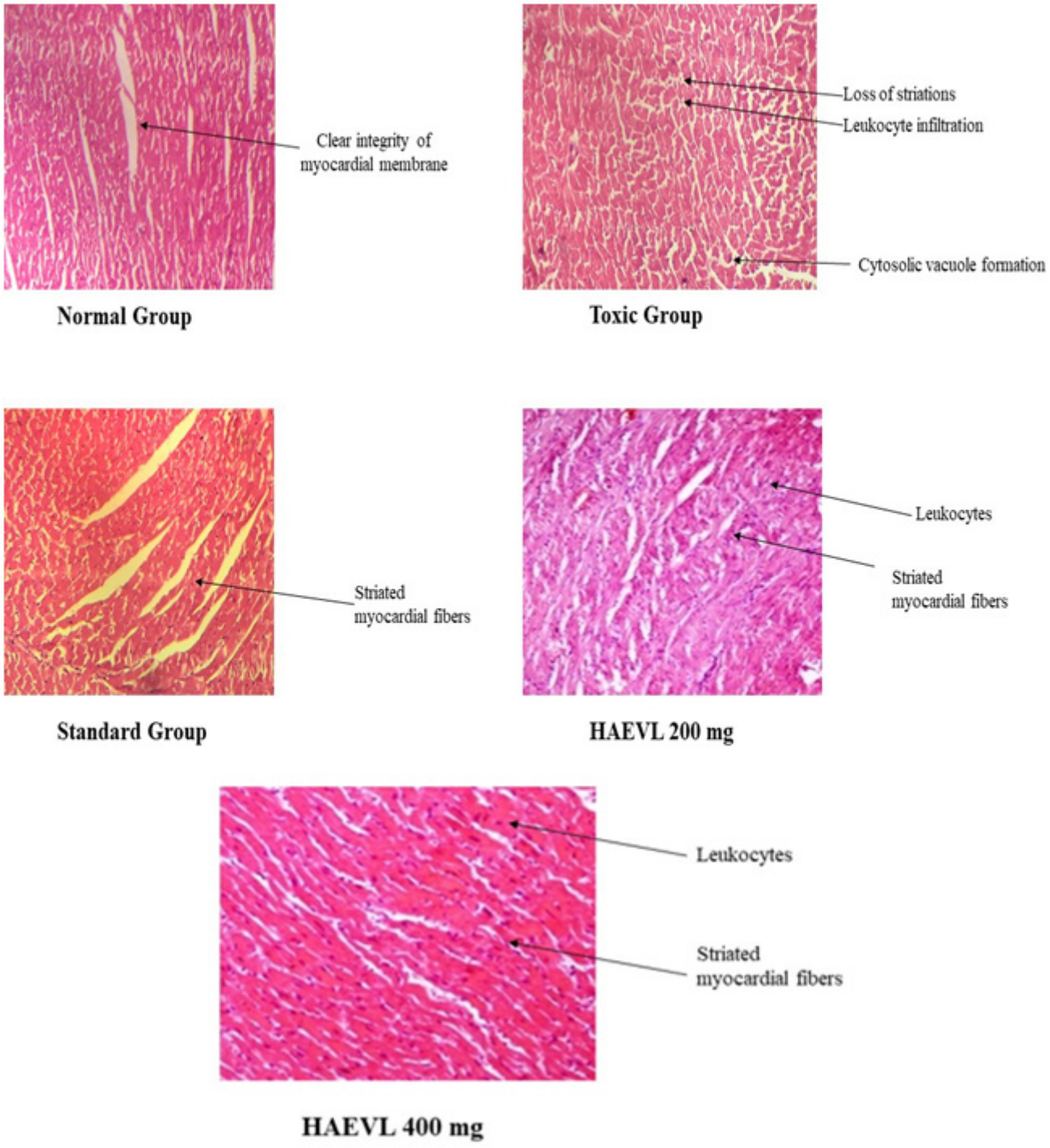

Figure 4: Histopathological studies of Heart (40X).

The calcium ion is one of the essential mediators of cell damage. The cytosolic free calcium concentration was deficient; most of it is temporarily present in the mitochondria and endoplasmic reticulum. Doxorubicin induced cardiomyopathy is accompanied by an abnormal increase in intracellular calcium levels, resulting in increased mitochondrial and endoplasmic reticulum permeability and thus inducing apoptosis. Dysregulation of intracellular calcium concentration leads to an irregular heartbeat rhythm.[48] Administration of doxorubicin in the toxic group has shown a significant increase in serum calcium levels. A significant decrease in calcium levels was seen in animals pretreated with doses of 200 and 400 mg/kg b. wt *Rumex vesicarius* extract.

The experimental result indicates that doxorubicin causes oxidative stress through increased superoxide and hydroxyl radicals within the heart tissue. The generated Reactive Oxygen Species (ROS) can damage intracellular components. The heart

is the organ most susceptible to free radical damage as few detoxifying substances are present. Antioxidants are the first line of defense against oxidative injury and are crucial in reducing ROS-induced myocardial injury. High ROS causes a reduction in the generation and activity of antioxidant enzymes, which in turn causes an elevated creation of free radicals and hydrogen peroxide, eventually creates Hydroxyl radical (OH), which causes many harmful events.[49,50]

There was a considerable rise in the LPO and a fall in the levels of GSH and CAT in the heart tissue as a result of increased oxidative stress and the depletion of antioxidants was reported after single dose administration of doxorubicin.[51] In the present investigation, a considerable increase in LPO levels and a significant drop in GSH and CAT levels were seen in the rats of the toxic group that had received doxorubicin. LPO levels were much lower and decreased glutathione and catalase levels were significantly higher after pretreatment with *Rumex vesicarius* extract at 200 and 400 mg/kg b. wt.

Membrane bounded enzymes such as Na^+/K^+-ATPase, Mg^{++}-ATPase and Ca^{++}-ATPase play a vital role in maintaining of ion levels within the limits necessary to regulate the contraction and relaxation cycles of cardiac muscle.[52] In the present study, the toxic group showed a significant decrease in Na^+/K^+ -ATPase and Mg^{++}-ATPase levels and a significant increase in levels of Ca^{++}-ATPase. The results of the present study coincided with the observations of Kumar and Hiremath, 2016.[53] The *Rumex vesicarius* extract 400 mg/kg b. wt pretreated rats showed a significant increase in the levels of Na^+/K^+-ATPase and Mg^{++}-ATPase and a significant decrease in the Ca^{++}-ATPase levels.

Histopathological examination reported cardiomyopathy by the presence of enlarged, swollen mitochondria, necrosis, infiltration of inflammatory cells, and vacuoles within the cytoplasm in all the rats administered with doxorubicin in the toxic group. The pretreatment with *Rumex vesicarius* extract showed significant protective activity against doxorubicin induced cardiotoxicity.

CONCLUSION

The present study revealed that *Rumex vesicarius* leaves have good protective activity against doxorubicin induced cardiotoxicity and oxidative stress due to the presence of polyphenols. However, the detailed molecular mechanisms involved in the cardioprotective activity of *Rumex vesicarius* leaves have to be studied.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HAERVL: Hydroalcoholic Extract of Rumex vesicarius Leaves; **HPTLC:** High-performance thin layer chromatography; **ETC:** Electron transport chain; **LDH:** Lactate dehydrogenase; **CK-MB:** Creatine kinase-MB; **AST:** Aspartate transaminase; **ALT:** Alanine transaminase, **LPO:** Lipid peroxidation; **GSH:** Reduced glutathione; **CAT:** Catalase; **ANOVA:** Analysis of Variance.

SUMMARY

This research aimed to create a *Rumex vesicarius* to protect against the cardiotoxic effect induced by doxorubicin in Wistar albino rats. The demonstrated *Rumex vesicarius* leaf extract substantially enhanced cardiac function when compared to the group treated with only doxorubicin. The biochemical analysis indicated pretreatment with *Rumex vesicarius* significantly controlled abnormally elevated serum LDH, CK-MB, ALT, AST, and calcium levels after doxorubicin exposure. The decreased cardiac membrane bond enzyme levels, such as Na+/K+-ATPase and Mg++-ATPase, and increased Ca++-ATPase levels were controlled in pretreated groups with *Rumex vesicarius*. Histopathological examination further revealed a reduction in the loss of striations, leukocyte infiltration, and inflammation in pretreatment with *Rumex vesicarius*. Moreover, *Rumex vesicarius* displayed potent antioxidant properties, suggesting its potential to protect against doxorubicin-induced oxidative stress. These results emphasize the viability of these *Rumex vesicarius* leaves as a safeguard against cardiotoxicity in doxorubicin therapy. However, further research is necessary to confirm its effectiveness in clinical applications.

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