



Cardioprotective Effect of *Capsicum annuum* L.VAR. *Grossum* SENDT. on Doxorubicin-Induced Cardiac Oxidative Stress in Rats

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ABSTRACT

The aim of study was to evaluate the cardioprotective activity of *Capsicum annuum* L.var.*grossum* sendt. (CALVGS) in doxorubicin-induced cardiac toxicity in rats. In the current investigation, female/male Wistar rats were divided into five groups, normal, control, CALVGS 200 mg/kg, 400 mg/kg, and 600 mg/kg. Doxorubicin 15 mg/kg was administered in six divided doses to all groups except the normal group for 2 weeks. CALVGS extract was administered to respective groups once daily for total 21 days, on the last day of study(22nd), the animals were anesthetized to record ECG and BP (by cannulating carotid artery), blood was collected from the retroorbital route and SGOT, LDH and CK-MB were estimated, animals were sacrificed to isolate heart and preparation of tissue homogenate. The antioxidant status is evaluated by measuring MDA level, SOD, CAT, GSH enzyme activity. The Heart tissue sample is also preserved for histological investigation. Doxorubicin causes cardiac damage which was manifested by alteration in serum cardiac markers, antioxidant markers, ECG, and hemodynamic and histological changes. Which were prevented due to treatment with CALVGS extract 600 mg/kg. With data obtained in study, it has been concluded that *Capsicum annuum* L.var.*grossum* sendt. treatment for 21 days shields the heart of rat from cardiotoxicity.

Keywords: Cardioprotective, Doxorubicin, Bell pepper, Antioxidant, Cardiotoxicity.

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INTRODUCTION

Today, cancer is a household word, with each of us having at least one close or dear one, a family member or a friend, a neighbour, who has been diagnosed with cancer. In India, there is also a perception that cancer rates are increasing and a hope that perhaps with the advances in technology, cancer is diagnosed more frequently, may be a change in our attitude and approach, the myths associated with cancer are vanishing and we are more open to accepting cancer diagnosis and discussing cancer more openly.¹

On a global scale, cancer is the leading cause of morbidity and mortality. It is the world's second-biggest cause of death, with 10 million deaths in 2020, and it is expected to rise by 70% in the next 20 years. Cancer is responsible for one out of every six deaths worldwide. Lung, liver, colorectal, stomach, and breast cancers are the most prevalent causes of mortality from cancer, yet many tumours can be cured if detected early and treated successfully.²

Anthracycline derivative Doxorubicin is a well-established anticancer medicine that is routinely used to treat a wide range of human neoplastic disorders and solid tumours, including breast cancer, Hodgkin, and non-Hodgkin lymphoma, acute leukaemia's, lung, thyroid, and ovarian cancer.³ The serious adverse effect of doxorubicin on cardiotoxicity limits its use for clinical purposes.⁴

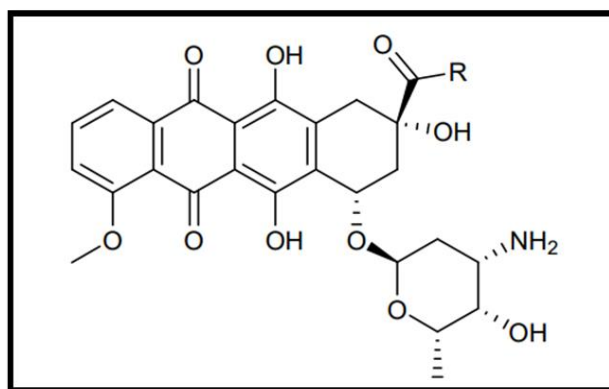


Figure 1: Chemical structure of Doxorubicin

The cardiotoxicity caused by doxorubicin is mediated by various mechanisms including, the generation of free radicals, mitochondrial damage, membrane lipid peroxidation and iron-dependent oxidative damage to macromolecules. Other processes hypothesized include disrupted calcium homeostasis, the release of vasoactive amines, decreased adrenergic stimulation, inhibition of muscle-specific genes, protein cross-linking, and DNA

damage, all of which lead to cardiac dysfunction, apoptosis, and cardiomyopathy development.⁵⁻⁸

Although the mechanisms underlying doxorubicin cardiotoxicity are unknown, there is evidence that cardiotoxicity may result from the formation of free radicals and subsequent redox cycles with O₂, resulting in the formation of reactive oxygen species (ROS) such as Superoxide anion, Hydrogen peroxide and Hydroxyl radicals.⁹ Tissues with less developed activities of antioxidant enzymes (superoxide dismutase, glutathione reductase and catalase) have been reported in doxorubicin-induced cardiotoxicity in animals and the heart is particularly liable to injury by free radicals.¹⁰

Capsicum annuum L.var.grossum sendt. (Bell pepper) is a popular fresh vegetable in the Solanaceae family.¹¹ The consumption of bell pepper gaining interest to a large extent due to the colour, taste, and nutritional value of its bioactive constituents which are dietary antioxidants.^{12,13}

It is high in Vitamin C, and its attractive red colour is due to several carotenoid pigments, including β-carotene, which has pro-vitamin A activity, and oxygenated carotenoids such as capsanthin, capsorubin, and cryptocapsin, which are unique to these fruits and have been shown to be effective in removing free radicals. It also contains a lot of flavonoids like luteolin, quercetin, and capsaicinoids, which are neutral phenolic substances.¹⁴

The present plant is reported for its anti-inflammatory, analgesic, anticancer, antiviral, antibacterial, antifungal, and antidiabetic activity & also used in Neurodegenerative disorders.^{12,15,16}

Hence, in the proposed study, we plan to use the *Capsicum annuum* L.var.grossum sendt. fruit extract to treat the cardiotoxicity produced by doxorubicin.

MATERIALS AND METHODS

Plant material

The *Capsicum annuum* L.var.grossum sendt (Bell Pepper) fruits were collected from the local market in Sangli. The fruits were authenticated by Mr. Waghmare sir, HOD of botany department, Kasturba Walchand College, Sangli.

Drugs and Chemicals

Doxorubicin was a gift sample from Cipla Goa. Urethane (Himedia India), DTNB, heparin, n-butanol, Pyridine, Sodium dodecyl sulphate, Conc. HCL, Hydrogen peroxide, Ammonium acetate, Potassium hydrogen phosphate, Formalin, Potassium dihydrogen phosphate, Trichloro Acetic Acid (all from Research-lab Fine Chem Industries Mumbai), Pyrogallol (Sigma Aldrich, Pvt. Ltd., Bangalore) Thiobarbituric acid (Loba chemicals (Mumbai). All chemicals were of analytical grade, the diagnostic kits from Corals clinical systems were used for serum SGOT, LDH and CK-MB analysis.

Preparation of extract

The Bell pepper fruits were dried in a shade. The dried material was powdered coarsely using a mixer grinder and coarsely powdered dried material was passed through sieve no. 40. Powdered material was extracted by using Soxhlet apparatus with 90% ethanol as a solvent for 48 hours at 600°C. The extract was cooled at room temperature and evaporated to dryness under reduced pressure in Rotary Vacuum Evaporator. Just before oral administration, the extract was dissolved in water.

Experimental Animals

Adult albino male Wistar rats initially weighing between 150-200 g were procured from the animal house of Appasaheb Birnale college of pharmacy, Sangli. All the animals were housed in a group of six under an environmentally controlled room with a 12 h light and dark cycle in polypropylene cages and maintained at a controlled room temperature of 25 ± 2 °C, with humidity of 50±5%, with free access to standard pellet diet and water. After one week of acclimatization period, they were divided into Five groups.

All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Appasaheb Birnale College of Pharmacy, Sangli Maharashtra (Registered no.843/PO/Re/S/04/CPCSEA), India.

Preparation of Drug Solution

Doxorubicin solution was prepared in Normal saline solution.

Dose of Doxorubicin-15 mg /Kg (Divided into six equal doses)

Experimental Design and Protocol

Animal Grouping and Procedure:

A total of 30 rats were randomly selected and allotted to five groups each group containing six animals (n=6). The groups and respective treatments are as follows,

Group I: Served as normal, receive water 5 ml/kg of body weight p.o. and

Group II: Control animal were treated with doxorubicin 2.5 mg/kg of body weight i.p. for six times in 2 weeks on alternate days (Water up to 7 days + Doxorubicin i.e., DOX of 2.5 mg/kg i.p. Injected on 8th, 10th, 14th, 16th, 18th and 21st day to reach total cumulative dose of 15 mg/kg).

Group III: Received Ethanolic extract of CALVGS fruit 200 mg/kg up to 21 days + Doxorubicin i.e., DOX of 2.5 mg/kg i.p. Injected on 8th, 10th, 14th, 16th, 18th and 21st day to reach total cumulative dose of 15 mg/kg).

Group IV: Received Ethanolic extract of CALVGS fruit 400 mg/kg up to 21 days + Doxorubicin i.e., DOX of 2.5 mg/kg i.p. Injected on 8th, 10th, 14th, 16th, 18th and 21st day to reach total cumulative dose of 15 mg/kg).



Group V: Received Ethanolic extract of CALVGS fruit 600 mg/kg up to 21 days + Doxorubicin i.e., DOX of 2.5 mg/kg i.p. Injected on 8 th, 10th, 14th, 16th, 18th and 21st day to reach total cumulative dose of 15 mg/kg).

All animals were observed for whole study period for appearance, behaviour, occurrence of necrosis at the site of administration, and mortality. Before and after completion of experimental period body weights were recorded. Heart/body weight ratio was also calculated using formula heart weight/body weight X1000.

Electrocardiogram:

ECG was recorded after the last dosing of doxorubicin i.e. On the 22nd day. ECG tracings were recorded and monitored using Biopac MP-35 equipment. Rats from each group were anesthetized with Urethane (1.25g/kg), and a needle electrode was inserted under the skin for the limb lead at position II. For each ECG tracing P wave, QRS complex, RR interval, QT interval, and Cardiac Cycle were measured.

Hemodynamic Parameters:

The BIOPAC MP-35 machine was used to measure blood pressure by the Invasive method (carotid artery cannulation). The carotid artery was cannulated by using PE – 50 tube which connected to the pressure transducer that had been previously loaded with heparinized saline. Here, Various parameters such as systolic, diastolic, mean blood pressure, and heart rate were measured in this study.

Serum Parameters:

Blood was collected by Retroorbital route at the end of the study and serum was separated using a centrifuge (REMI, Mumbai) at 3000 rpm for 10 min. The serum samples were examined to determine levels of SGOT, CK-MB, and LDH, Using standard kits. All the kit procedures were performed according to the manufacturer's instructions by using a semiautoanalyzer (Mispa Plus).

Tissue Antioxidant Biomarkers

Animals were euthanized and heart tissue was removed immediately and washed in ice-cold phosphate buffer and dried on filter paper and instantly weighed. A 10% w/v tissue homogenate is made in ice-cold 0.05 M phosphate buffer pH 7.4 by using a tissue homogenizer. The tissue homogenate was used for the evaluation of levels of CAT, SOD, GSH, and MDA.

Histopathological study

At the end of the study (22nd day), the heart was isolated and washed with ice-cold saline. The tissue was fixed in a 10% buffered neutral formalin solution. After fixation tissues were embedded in the paraffin wax and thin sections were cut and stained with eosin and hematoxylin stains. The slides were observed under a light microscope (10 x).

Statistical analysis

The data were expressed as mean \pm SEM. For obtaining this data, the biochemical parameters were statistically analyzed using one-way ANOVA followed by Bonferroni's multiple comparison test.

RESULTS

The effect of ethanolic extract of *capsicum annum* L var *grossum*. sendt. fruits and the chronic administration of Doxorubicin-induced cardiac toxicity were determined by analyzing the results of Physical, hemodynamic, electrocardiographic, biochemical, and histological examinations.

General observation

The common aspects of all the animals were recorded throughout the study. In the later days, doxorubicin-treated (Control group) animals developed a pink tinge, scruffy fur, alopecia, and necrosis at the injected site. They also had red exudates surrounding the eyes and nose, and soft watery feces as compared to the normal group. These changes were less prominent in the groups treated with ethanolic extract of CALVGS fruit 200 mg/kg, 400mg/kg and 600 mg/kg, as compared with the control group of animals.

Effect on body weight

As seen in Table 1, The body weight was significantly decreased (####P<0.0001) in the doxorubicin-treated group (Control group) as compared to the Normal group. The decrease in the % Relative Body weight Change was 5.92% in the control group of animals.

However. The % Relative Body weight Change of groups pre-treated with the ethanolic extract of CALVGS 200,400 and 600 mg/kg extract were increased significantly (****P<0.0001) and found 2.95%, 3.23% and 1.75% respectively as compared with Dox control group.

Heart weight to body weight ratio

As seen in table 2, Treatment with doxorubicin (alone) causes a significant decrease(####P<0.0001) in Heart weight and Heart to body weight ratio as compared with the normal group. The decrease in the Heart weight was 39.83% and decrease in the Heart to body weight ratio was 38.57%.

However, The Significant increase (****P<0.0001) in Heart weight and Heart weight to body weight ratio was observed in all the groups treated with DOX +CALVGS extract 200,400,600 mg/kg as compared with Control group.

Electrocardiographic Changes and Hemodynamic Changes

Table 3 shows the effect of CALVGS fruits on electrocardiographic changes in different groups. The Control group showed a significant increase in QRS duration, elevation of ST segment and QT interval prolongation when compared with the normal group.



Treatment with ethanolic extract of CALVGS fruits 200 mg/g, 400 mg/kg, and 600 mg /kg showed significant modification in the values. Fig.2(C), Fig.2(D) and 2 (E) reflect mild changes in ST elevation caused due to the doxorubicin treatment.

As seen in Table no.3, The Control group showed a significant decrease (####P<0.0001) in systolic arterial BP,

diastolic arterial BP, and heart rate when compared with the normal group. The decrease in systolic blood pressure was 15.76%, diastolic blood pressure was 26.42 %, and the Heart Rate was 8.28%. While other groups pre-treated with 200 mg/kg, 400 mg/kg, and 600 mg/kg CALVGS fruit extract cause significantly increase level of systolic and diastolic BP.

Table 1: Effect of Ethanolic extracts of CALVGS on Body weight in DOX-induced cardiac toxicity.

Groups	Initial Body weight (gm)	Final Body weight (gm)	% Relative Body weight Change
Normal	265.8 ± 5.192	277 ± 5.053	11.17 ± 1.833
Control (Dox)	252.3 ± 3.639	238 ± 3.688	-13.50±1.803#### (↓5.92%)
Dox +CALVGS Extract 200 mg	260.8 ± 7.120	268.5 ± 6.365	7.667±1.647**** (↑2.95%)
Dox +CALVGS Extract 400 mg	253.3 ± 5.426	261.5 ± 5.271	8.167±0.7491**** (↑3.23%)
Dox +CALVGS Extract 600 mg	278.8±2.949	283.7±2.894	4.833±0.6540**** (↑1.75%)

Data were expressed as Mean ±SEM and n = 6, using one-way ANOVA followed by “Bonferroni’s multiple comparison test”. **P<0.01, ****P<0.0001 is considered as significant. # Sign Indicates the control group compared with the normal group (####P<0.0001). and * indicates all treatment groups compared with the control group. Values in the brackets indicate a % ↑ increase or ↓ decrease.

Table 2: Effect of Ethanolic extract of CALVGS on Heart weight and Heart/body weight ratio in DOX-induced cardiac toxicity.

Sr. No	Groups	Heart weight	Heart/ Body weight ratio × 10 ³
1	Normal	1.002 ± 0.01249	3.882 ± 0.1256
2	Control (Dox)	0.6017 ± 0.02315 (↓39.83%)	2.385 ± 0.1205#### (↓38.57%)
3	Dox +CALVGS Extract 200 mg	0.7750 ± 0.01025 (↑28.80%)	2.978 ± 0.08448** (↑24.86%)
4	Dox +CALVGS Extract 400 mg	0.8517 ± 0.01579 (↑41.54%)	3.327 ± 0.08973**** (↑39.49%)
5	Dox+CALVGS Extract 600 mg	0.9367 ± 0.01726 (↑55.67%)	3.353 ± 0.08628**** (↑40.58%)

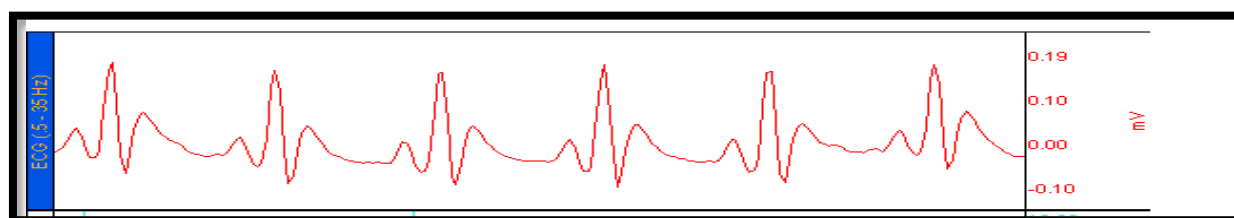
Data were expressed as Mean ±SEM and n = 6, using one-way ANOVA followed by “Bonferroni’s multiple comparison test”. **P<0.01, ****P<0.0001 is considered as significant. # Sign Indicates the control group compared with the normal group (####P<0.0001). and * indicates all treatment groups compared with the control group. Values in the brackets indicate a % ↑ increase or ↓ decrease.

Table 3: Effect of Ethanolic extracts of CALVGS on ECG and Blood pressure in DOX-induced cardiac toxicity.

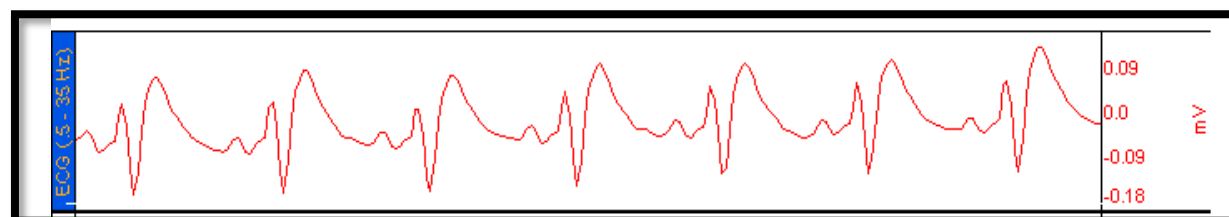
Groups	QT interval (sec.)	ST segment (mv)	QRS Complex (sec.)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Heart rate (bpm)
Normal	0.06000 ± 0.002145	0.1723 ± 0.002124	0.03133 ± 0.001441	125.3 ± 1.085	88.33±1.174	353 ± 0.9458
Control	0.1048 ± 0.001352#### (↑74.66%)	0.2782 ± 0.01350#### (↑61.46%)	0.04217 ± 0.001787#### (↑34.59%)	104.3 ± 1.542#### (↓15.76%)	65.00± 1.633#### (↓26.42%)	323.8 ± 1.352#### (↓8.28%)
DOX +CALVGS Extract 200 mg	0.09367 ± 0.002906** (↓10.63%)	0.2455 ± 0.003998** (↓11.76%)	0.03672 ± 0.0005718* (↓12.93%)	110.7 ± 0.8433** (↑6.13%)	72.83±1.400* (↑12.04%)	329.3 ± 0.9189** (↑1.69%)
DOX +CALVGS Extract 400 mg	0.08317 ± 0.002120**** (↓20.64%)	0.2300 ± 0.003890**** (↓17.33%)	0.03533 ± 0.001085** (↓16.33%)	111.2 ± 0.7923*** (↑6.61%)	78.50± 0.8466**** (↑20.76%)	334.8 ± 0.4773**** (↑3.39%)
DOX +CALVGS Extract 600 mg	0.06800 ± 0.002436**** (↓35.12%)	0.2135 ± 0.0006708**** (↓23.26%)	0.03272 ± 0.001085**** (↓22.41%)	114.8 ± 1.014**** (↑10.06%)	79.83± 2.522**** (↑22.81%)	348.8 ± 0.8724**** (↑7.72%)

Data were expressed as Mean ±SEM and n = 6, using one-way ANOVA followed by “Bonferroni’s multiple comparison test”. * P<0.05, **P<0.01, ***p<0.001, ****P<0.0001 is considered as significant. # Sign indicates control group compared with normal (####P<0.0001)). and * sign indicates treatment groups compared with the control group. Values in the brackets indicate a % ↑ increase or ↓ decrease.

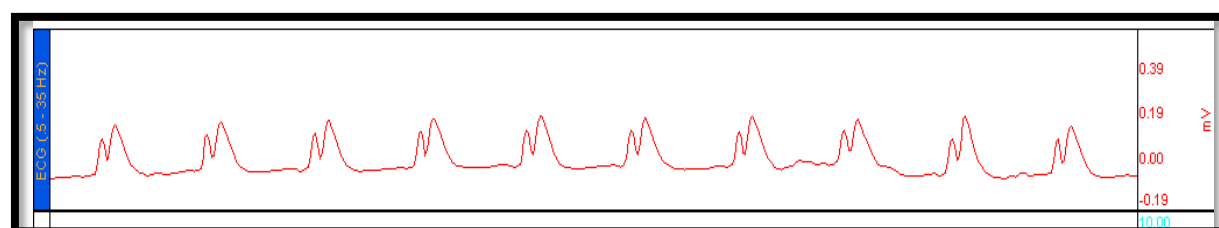




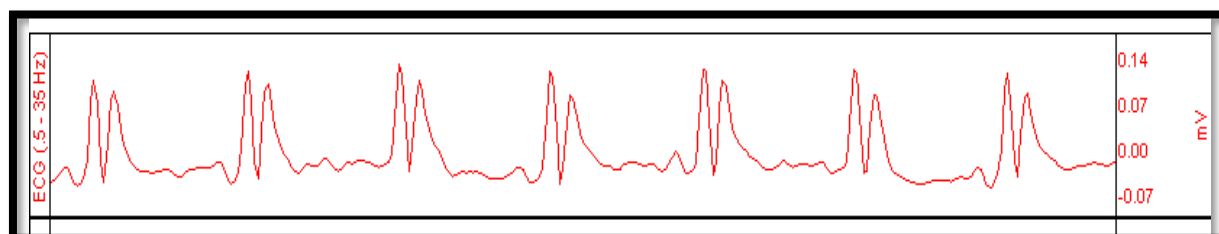
A. Normal (Normal saline)



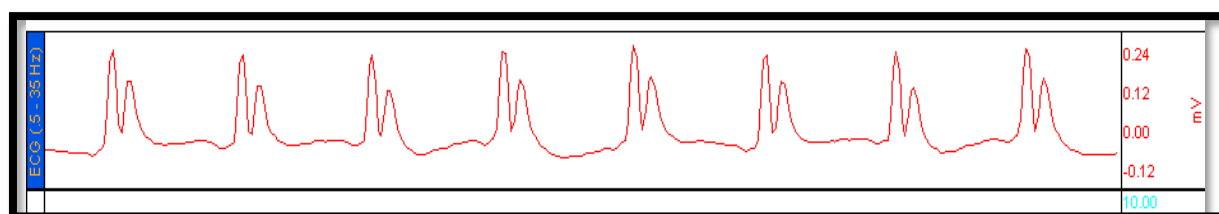
B. Control (DOX)



C. DOX+CALVGS 200mg/kg



D. DOX+CALVGS 400mg/kg



E. DOX + CALVGS 600mg/kg

Figure 2: Graphical representation of Effect of Ethanolic extracts of *Capsicum annum* L.var.*grossum* Sendt. Fruits on ECG in DOX- induced cardiotoxicity

Serum Cardiac Markers

Table 4 shows the effect of CALVGS fruit extract on Serum cardiac markers. The Control group animal shows a significant increment in the level of CK-MB, LDH and SGOT enzymes as compared to the normal group. The Pre-treatment with CALVGS fruit extract 200 mg/kg, 400 mg/kg and 600 mg/kg significantly reduce (**** $P < 0.0001$) the levels of serum cardiac markers.

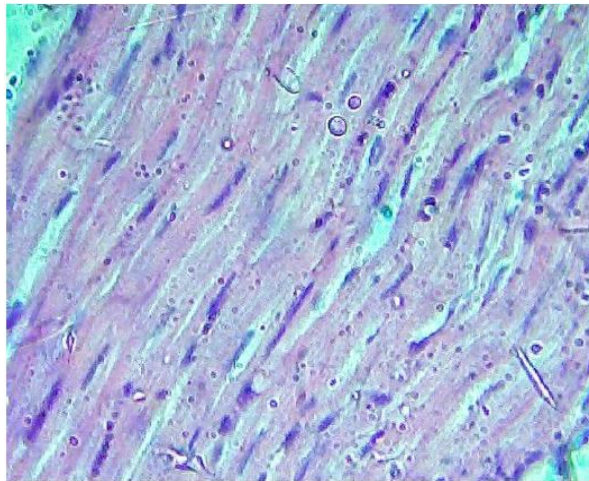
Antioxidants Markers

Control group animals showed decreases in the levels of antioxidant enzymes such as SOD, GSH, and CAT as compared to the Normal group. Whereas animals pre-treated with ethanolic extract of Bell pepper showed an increase in the level of, SOD GSH and CAT as compared with the Control group. Doxorubicin-treated rats showed an increase in MDA (Lipid peroxidation product) levels as compared to the Normal group. Whereas animals pre-treated with ethanolic extract of CALVGS decreases the increased MDA level as compared to the control group.

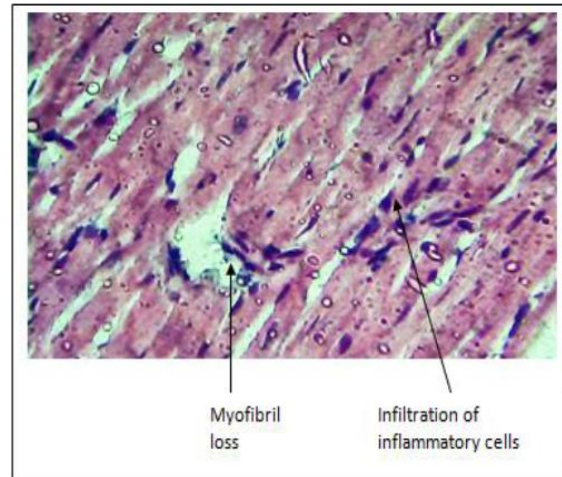
Histopathological Study

Histopathological analysis of myocardial tissue obtained from normal animals showed clear integrity of the myocardial membrane and tightly bound cardiac muscle fibers. Normal animals displayed normal cardiac fibers without any infarction. The cardiac tissue taken from DOX-

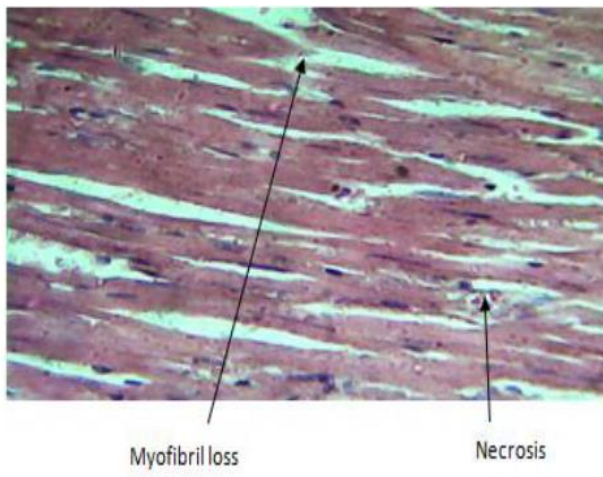
treated animals showed several regions of necrosis and inflammatory cell aggregation and damaged vascular muscle fiber. Animals pretreated with CALVGS 200 mg/kg, 400 mg/kg and 600 mg/kg demonstrated improved cell integrity evidenced, absence of necrosis, marked decrease in the infiltration of inflammatory cells, and maintenance of normal cardiac integrity.



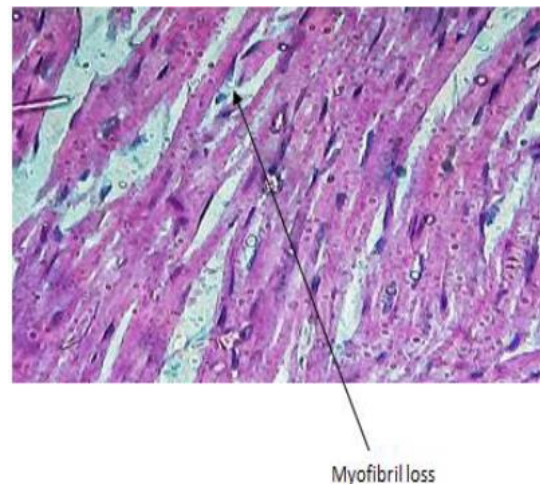
A) Normal



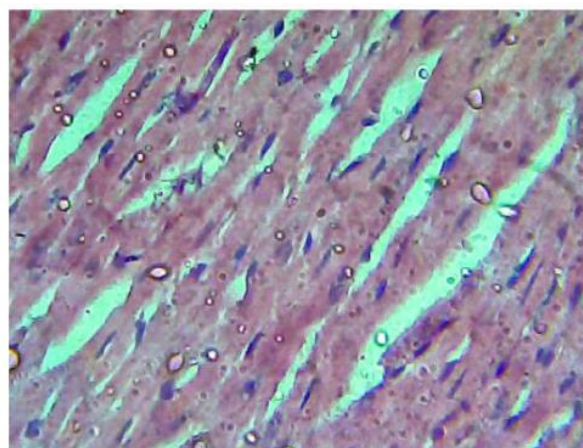
B) Control



C) DOX+ CALVGS 200mg/kg



D) DOX+ CALVGS 400mg/kg



E) DOX+ CALVGS 600mg/kg

Figure 3: Histopathological images of heart pre-treated with CALVGS doxorubicin- induced cardiac toxicity.

Table 4: Effect of Ethanolic extracts of CALVGS on cardiac serum markers in DOX-induced cardiac toxicity

Sr. No	Groups	CK-MB(IU/L)	LDH(IU/L)	SGOT (U/L)
1	Normal	91.17 ± 2.120	109.7 ± 6.709	90.53 ± 2.646
2	Control	229.0 ± 8.095#### (↑151.17%)	211 ± 15.88#### (↑92.34%)	199.1 ± 23.00#### (↑119.92%)
3	DOX +CALVGS Extract 200 mg	174.3 ± 3.921**** (↓23.89%)	172.8 ± 9.499* (↓18.11%)	151.8 ± 7.068* (↓23.76%)
4	DOX +CALVGS Extract 400 mg	153.3 ± 4.410**** (↓33.06%)	166.5 ± 6.526** (↓21.10%)	129 ± 5.848*** (↓35.21%)
5	DOX +CALVGS Extract 600 mg	130.5 ± 3.490**** (↓56.98%)	122.3 ± 2.418**** (↓42.04%)	107.1 ± 4.239**** (↓46.21%)

Data were expressed as Mean ± SEM and n = 6, using one-way ANOVA followed by “Bonferroni’s multiple comparison test”. * P < 0.05, **P < 0.01, ***p < 0.001, ****p < 0.0001 is considered as significant. # Sign indicates control group compared with normal (####P < 0.0001)). and * sign indicates treatment groups compared with the control group. Values in the brackets indicate a % ↑ increase or ↓ decrease.

Table 5: Effect of Ethanolic extracts of CALVGS on Antioxidant markers in DOX-induced cardiac toxicity.

Sr. No	Groups	CAT (µg/mg protein)	SOD (µg/mg protein)	GSH (µmoles/L)	MDA (µM/mg protein)
1	Normal	33.39 ± 0.4181	2.287 ± 0.04047	32.91 ± 0.5640	1.282 ± 0.1438
2	Control (Dox)	15.01 ± 0.3339### (↓55.05%)	0.6117 ± 0.0144#### (↓73.25%)	17.43 ± 0.5404#### (↓47.1%)	5.275 ± 0.08902#### (↑311.4%)
3	DOX +CALVGS Extract 200 mg	17.67 ± 0.4216** (↑17.72%)	0.8333 ± 0.03827*** (↑36.33%)	19.82 ± 0.3427** (↑13.71%)	4.487 ± 0.06766*** (↓15.2%)
4	DOX +CALVGS Extract 400 mg	22.53 ± 0.6824**** (↑50.09%)	1.352 ± 0.05388**** (↑121%)	25.07 ± 0.2369**** (↑43.48%)	3.433 ± 0.1687**** (↓34.92%)
5	DOX +CALVGS Extract 600 mg	27.97 ± 0.2461**** (↑86.34%)	1.887 ± 0.01116**** (↑190%)	27.50 ± 0.2227**** (↑57.77%)	2.578 ± 0.08561**** (↓47.72%)

Data were expressed as Mean ± SEM and n = 6, using one-way ANOVA followed by “Bonferroni’s multiple comparison test”. * P < 0.05, **P < 0.01, ***p < 0.001, ****p < 0.0001 is considered as significant. # Sign indicates control group compared with normal (####P < 0.0001)). and * sign indicates treatment groups compared with the control group. Values in the brackets indicate a % ↑ increase or ↓ decrease.

DISCUSSION

Doxorubicin is a widely used anticancer antibiotic and is supportive in numerous chemotherapy regimens to treat solid tumors and other types of hematological tumors. Intercalation of DNA, preventing replication and protein synthesis as well as inhibition of topoisomerase-II, is the mechanism of doxorubicin for its anticancer activity. Although it is an effective anticancer drug, dose-dependent cardiac toxicity, which is characterized by acute or late-onset chronic progressive cardiomyopathy is a common side effect and hence its therapeutic use is limited. Doxorubicin starts to accumulate in cardiac tissue over a period of time dose-dependently and finally causes cardiac dysfunction⁵⁻⁸

The generation of free radicals by doxorubicin involves more than one mechanism. Doxorubicin is reduced by one electron by the mitochondrial reductase enzyme, which could result in the generation of anthracycline semiquinone free radicals. These are unstable in aerobic conditions and

quickly convert molecular oxygen to reactive oxygen species like superoxide anion and hydrogen peroxide. Increased production of free radicals in cardiomyocytes causes oxidative stress, which can have a number of adverse effects, including changes in mitochondria, energy imbalance, the build-up of p53, activation of p38 and JNK, and finally cell death.

Products of plant origins with flavonoids, polyphenolic, and Carotenoids contents are in high demand in recent times due to their potent antioxidant properties. *Capsicum annum* L.var.grossum sendt. fruits contain a complex mixture of carotenoids like β-carotene, capsantine, capsorubin, cryptocapsin, and flavonoids like luteolin, and quercetin which are unique to these fruits and have been shown to be effective in removing free radicals. These constituents are of flavonoid and phenolic origin and have antioxidant properties.¹¹⁻¹⁴

Hence, In the present research work, we studied a possible potent cardioprotective role of the CALVGS extract against



Doxorubicin-induced cardiotoxicity in experimental animals. Cardiotoxicity was induced by giving 2.5 mg/kg of doxorubicin alternative day by i.p. route for two weeks. The characteristic features of cardiotoxicity like, a decrease in food and water consumption, and body weight, alterations in ECG pattern, increase in serum cardiac markers level in the blood (LDH, CK-MB, SGOT), decreases in anti-oxidant enzyme levels (SOD, CAT, GSH), increase in Lipid peroxidation levels (MDA) and changes in histopathology of heart were recorded at the end of experimental period.

In this study, we observed that rats who received doxorubicin alone (Control group) had scruffy fur, alopecia, and necrosis at the site of injection. They also had red exudates surrounding the eyes and nose, and soft watery feces, in addition, there was a significant reduction in the body weight, heart weight ($\downarrow 39.83\%$), and heart to body weight ratio ($\downarrow 38.57\%$). This is consistent with earlier findings made by. L.Bhatt.et al.¹⁷ The body weight was gradually reduced in doxorubicin-treated rats because of the outcomes of both direct toxic effects on the intestinal mucosa, which manifest as mucositis, and additional indirect action on the GIT tract resulting from decreased food intake, which cause a decrease in enteral hormone secretions and result in decreased trophic effects to the mucosa. Doxorubicin also caused a significant decrease in the heart weight as well as heart weight to body weight ratio which may be because of the vacuolization in the myocytes, myocardial necrosis, and myofibrils loss.¹⁸

Our study demonstrated that all other groups treated with ethanolic extract of CALVGS 200,400 and 600 mg/kg, p.o showed increase in Body weight, Heart weight, and Heart to body weight ratio when compared with the control group. However more significant results observed in 600mg/kg dose of ethanolic extract of CALVGS and the percentage increase in body weight, Heart weight, and Heart to body weight ratio were 3.23 %, 55.67% and 40.58% respectively. The less reduction in body weight change may be due to the beneficial effect of extract on GI tract or improvement in the feeding behaviour.

As per table no.03, DOX-treated rats showed a decrease in systolic ($\downarrow 15.76\%$), diastolic ($\downarrow 26.42\%$), mean blood pressure ($\downarrow 30.45\%$), and heart rate ($\downarrow 8.28\%$). This is likely due to DOX's influence on the myofibrils, which disrupts them and causes a decrease in systolic, diastolic, and mean blood pressure. Doxorubicin was administered for a prolonged period of time this resulted in a decrease in the heart rate. This was in line with earlier studies that supported the generation of reactive oxygen species that disturb calcium homeostasis. which could have resulted in a reduction in the heart rate because the reduction in the intracellular calcium induced excitability of the SA node and other cells in the conducting system.¹⁹

Our study demonstrated that rats treated with ethanolic extract of CALVGS 200,400 and 600 mg/kg, p.o showed an increase in the systolic ($\uparrow 6.13\%$, $\uparrow 6.61\%$, $\uparrow 10.06\%$), diastolic ($\uparrow 12.04\%$, $\uparrow 20.76\%$, $\uparrow 22.81\%$), Mean BP ($\uparrow 16.65\%$, $\uparrow 21.59\%$, $\uparrow 29.14\%$), and heart rate ($\uparrow 1.69\%$,

$\uparrow 3.39\%$, $\uparrow 7.72\%$) in treatment groups compared to control group. The group received CALVGS extract at a dose of 600 mg/kg restoring hemodynamic changes near to normal. The CALVGS extract may be responsible for the stabilization of the myocardium resulting in a reduction in myofibril disruption, which may help keep blood pressure close to normal.

ECG irregularities are the primary criteria applied for the proper diagnosis of myocardial damage. Additionally, ECG changes are the display of the severity of doxorubicin-induced-myocardial damage.²⁰ Treatment with doxorubicin causes mechanical dysfunction of the heart and inhomogeneity of ventricular depolarization and repolarization which is reflected in the occurrence of alterations in the ECG.¹⁸ When doxorubicin is administered in a cumulative dose, the following changes in the electrocardiogram (ECG) are reliably seen: QT prolongation, increment in QRS complex, ST interval elevation, p wave prolongation, and decrease in the heart rate. Although, the precise causes of these acute ECG alterations are unknown.²¹ All of these ECG alterations are due to the prolongation of the action potential. However, it is believed that DOX has the greatest impact on the recovery phase of the transmembrane action potential, which primarily affects Ca^{2+} movements across cellular membranes.²²

Treatment with various doses of 200,400 and 600 mg/kg, p.o of ethanolic extract of CALVGS decreases QT prolongation ($\downarrow 10.63\%$, $\downarrow 20.64\%$, $\downarrow 35.12\%$ respectively) as compared with the control group. It also decreases the duration of p-wave ($\downarrow 28.64\%$, $\downarrow 37.66\%$, $\downarrow 41.11\%$), duration of QRS Complex ($\downarrow 12.93\%$, $\downarrow 16.33\%$, $\downarrow 22.41\%$), as well as ST-segment elevation ($\downarrow 11.76\%$, $\downarrow 17.33\%$, $\downarrow 23.26$). The ST-segment elevation is due to oxidative stress-related cellular membrane damage.³¹ Pre-treatment with CALVGS extract showed a protective effect against altered ECG patterns and may eliminate fatal complications by protecting the cell membrane damage.

Due to the destruction of the myocardium, doxorubicin causes cardiac toxicity. As a result, biochemical and cardiac markers such as creatine kinase-MB, Lactate dehydrogenase (LDH), and Serum Glutamate Oxaloacetate Transaminase (SGOT) were released into the bloodstream and serve as diagnostic indicators of myocardial tissue damage. Serum LDH and CK-MB are measured frequently in regular clinical practice as markers for the diagnosis of cardiac necrosis and toxicity.^{23,24}

In doxorubicin-treated rats, the specific cardiac enzyme levels are significantly elevated due to an inadequate supply of glucose or oxygen, which damages myocytes and causes the myocardial membrane to become more permeable and ruptures resulting in the leakage of these enzymes in the blood.²⁷ According to Monnet E and Balachandar AV, the higher levels of enzymes in the serum may be caused by the damaged myocardium and the rapid cell enlargement of sub-sarcolemma bulbs, which could ease the loss of intracellular enzymes after doxorubicin treatment.^{25,26} The



findings for the control group were similar to previous research reported by N. R. Barman. et.al.²⁷

An elevated level of CK-MB, LDH, and SGOT enzymes in the DOX-treated group was significantly ($****P<0.0001$) reduced by pre-treatment with CALVGS extract. Among the group treated with CALVGS dose of 600 mg/kg bring down the cardiac biomarker level maximum in rats. This reduction suggests that treatment with CALVGS extract may be responsible for maintaining the common structural and architectural integrity of myocardial cells, thereby preventing the escapes of enzymes, which could be due to the protective / membrane stabilizing assets of CALVGS extract on the heart.

The existing data suggest that Dox-induced oxidative stress is due to the generation of free radicals in the heart tissue. Doxorubicin is reduced by one electron by the mitochondrial reductase enzyme, which could result in the generation of anthracycline semiquinone free radical's complex. This complex is unstable and quickly converts molecular oxygen to reactive oxygen species like superoxide anion and hydrogen peroxide, which have the potential to cause damage to various intracellular components. Cardiac tissue is particularly vulnerable to free-radical induced damage because it has low quantities of antioxidant enzymes such as superoxide dismutase, catalase, and GSH.²⁸

Antioxidant enzymes play an important role in scavenging ROS and inhibiting damage to cells from oxidative stress. Antioxidant substances and antioxidant enzymes like GSH, SOD, CAT, GP, and GPx protect the body against oxidative stress caused by tissue damage.

GSH is considered the most significant intracellular hydrophilic antioxidant that saves the cells from free radical damage. As doxorubicin causes an overproduction of superoxide radicals, which leads to an overproduction of hydrogen peroxide. This hydrogen peroxide is neutralized by GPx using hydrogen from GSH molecules resulting in the production of water and oxidized glutathione. In the presence of GR, oxidized glutathione is reduced to regenerate GSH.²⁸

Pretreatment with ethanolic extract of CALVGS showed a significant rise in the level of total antioxidant status. However, pretreatment with CALVGS extracts significantly increase the level of GSH (57.77%). It suggests the antioxidant property of phytoconstituents present in the CALVGS fruits.

The main antioxidant enzymes in the first line of defense are SOD, GSH, and catalase. which dismutates the superoxide radical and converts H₂O₂ and hydroperoxide into safe molecules like H₂O/alcohol and O₂. SOD is a metalloenzyme that catalyzes the conversion reaction of superoxide radicals to hydrogen peroxide. This hydrogen peroxide is converted to water and molecular oxygen by the catalase enzyme. Therefore, increased ROS production in doxorubicin treatment reduces the capacity of cardiomyocytes to eliminate ROS as it decreases the level of

CAT and SOD. However, the increase in levels of SOD and CAT in all groups treated with CALVGS extract suggests antioxidant and free radical scavenging activity. Not only the current study but also other earlier studies on the antioxidant property-based protection against doxorubicin-induced cardiotoxicity have revealed a similar effect on SOD and catalase activity.

Superoxide anions and their derivatives particularly the highly reactive and harmful hydroxyl radicals produced during the metabolism of doxorubicin and causes the lipid peroxidation of the cell membrane. In this study, doxorubicin-treated animals (Control group) showed a significant increment in the malondialdehyde level (\uparrow 311.4%) indicating enhanced lipid peroxidation. MDA is a byproduct of the lipid peroxidation process and MDA is regarded as a reliable indicator for the estimation of both lipid peroxidation and ROS-induced tissue damage.^{29,30}

The overall restoration of antioxidant enzymes and decrease in MDA in homogenate suggest the antioxidant potential of the plant. The previous report of the phytochemical investigation of plant indicated that CALVGS fruit have constituents like flavonoids, polyphenolic compounds, and carotenoids which are known to have antioxidant potential. The cardioprotective activity of CALVGS may be attributed by polyphenolic compounds, flavonoids and carotenoids antioxidant properties of these phytoconstituents present in extract.

Histological features of cardiac tissue were evidence of the therapeutic activity of CALVGS extract against DOX-induced cardiotoxicity. In the current investigation, hematoxylin and eosin staining were used to evaluate collagen/inflammatory cell accumulation in the cardiac tissue. The cardiac muscle fibers of the normal group of animals (Vehicle treated) in the histopathologic investigation were consistent in size, shape, and orientation with no inflammatory-cell infiltrates. Cardiac damage was present in the group that received doxorubicin treatment, as seen by the appearance of swollen mitochondria, infiltration of inflammatory cells, edema, patchy necrosis, myofibrillar loss, and vacuolization of cytoplasm. which was similar to previous research reported by A. Viswanatha Swamy. et.al. and many others.³¹

In the present study, Pretreatment with ethanolic extract CALVGS 200,400 and 600 mg/kg demonstrated less vacuolization, myofibrils loss, reduced edema, and less occurrence of inflammatory cell activity and necrosis. The histopathological evaluation suggests CALVGS extract pre-treated group decreases the doxorubicin-induced cardiotoxicity.

In summary, this study suggests that Pre-treatment with ethanolic extract CALVGS could alleviate the cardiotoxicity and morphological changes induced by a cumulative dose of doxorubicin. All these attenuation effects may be attributed by antioxidative and cardioprotective effect of CALVGS extract.



CONCLUSION

The overall protective effect of ethanolic extract of *Capsicum annuum* L.var.grossum. Sendt extract may be due to the counter action with free radicals by its antioxidant nature which suggests that pre-treatment with CALVGS extract may replenish the cardiomyocytes with antioxidants that are needed for the defense against oxidative stress induced by doxorubicin. However, the exact molecular mechanism CALVGS fruit extract which exerts its protective action against oxidative damage remains to be investigated. If the protective function will be confirmed in cancer patients, CALVGS fruit extract may be used along with doxorubicin.

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