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RESEARCH ARTICLE

Cardio Protective Activity of Calophyllolide on Doxorubicin-induced Cardiac Toxicity

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Abstract

Background: Doxorubicin, a recognized anti-cancer medication, induces cardiotoxicity, thereby restricting its acceptability and application. The toxic effects stem from the generation of free radicals and damage to cardiomyocytes. **Aim**: This study examined the potential of Calophyllolide in mitigating doxorubicin-induced cardiotoxicity, given its noteworthy *in vitro* antioxidant properties. **Methods:** Rats in group I, serving as the normal control, were provided with a standard diet for 7 days. Animals in group II (DOX) were administered Doxorubicin (15 mg/kg body weight/day) subcutaneously, dissolved in physiological saline, on the 8th and 9th days, with a 24-hour interval, inducing myocardial infarction. In groups III and IV, animals received calophyllolide at doses of 200 mg/kg and 400 mg/kg, respectively, for 7 days before being injected with the aforementioned dose of DOX. Upon concluding the study, blood samples were collected via retroorbital puncture for serum, utilized in estimating lipid and liver profile tests such as ALT, AST, LDL, HDL, cholesterol, etc. Additionally, the hearts were isolated and homogenized with tris buffer, and the supernatant layer was used for assessing cardiac biomarkers. **Results:** Dox-treated animals showed abnormalities in ECG and also elevated the liver profile parameters, lipid parameters, and cardiac biomarkers. Whereas Calophyllolide 400 mg/kg treated animals restored the ECG waves to normal and also reduced the biomarkers. The potential cardioprotective benefits of Calophyllolide may be associated with its impact on the ST interval, mitigating myocardial pathological damage. Calophyllolide demonstrates protective effects on H9c2 cells against Doxorubicin-induced cardiac injury by enhancing cell viability, reducing LDH and ROS leakage, and lowering levels of troponin and inflammatory mediators. **Conclusion:** This study results endorse the positive contribution of Calophyllolide in safeguarding against ischemic cardiomyopathy.

Major Findings: Calophyllolide demonstrates significant cardioprotective effects against DOX-induced toxicity by restoring cardiac function parameters such as R wave amplitude, ST interval, LVEDP, and LVSP. It effectively reduced markers of cardiac injury and inflammation, including Tn-I, CK-MB, LDH, TNF-α, and IL-6. Additionally, it improves the liver enzymes and lipid profiles by normalizing SGOT, SGPT, ALP, LDL, TC, and HDL levels. Histopathological analysis further confirms the cardioprotective efficiency of calophyllolide.

Keywords: Biomarkers, Cardioprotective, Doxorubicin, ECG

1. Introduction

A mutant strain of *Streptomyces peucetius var. caesius* produces doxorubicin (Dox), a secondary metabolite that is a member of the anthracycline family. Dox is an efficient antineoplastic agent for a variety of cancer forms. Nevertheless, several systemic side effects limit its widespread application in cancer chemotherapy. It is one of the well-known and widely used antineoplastic drugs for treating various malignancies, such as breast cancer, leukaemia, and paediatric cancer. Doxorubicin exerts its cytotoxic effects primarily by inhibiting DNA topoisomerase II, leading to DNA damage and apoptosis[1]. If anthracyclines are taken more than the 400 mg/m^2 - 700 mg/m^2 dosing range, they can cause dose-dependent cardiac toxicity. Nine percent of individuals treated with anthracycline chemotherapy experienced decreased left ventricular ejection fraction

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after a year of treatment[2]. Dox-induced cardiomyopathy is thought to be primarily caused by oxidative stress and the death of cardiomyocytes mediated by apoptosis. In Dox-induced cardiomyopathy, one of the early phase events is the depletion of sarcoplasmic reticulum Ca^{2+}. Elevations of Ca^{2+} and calmodulin-dependent kinase II are positively linked with heart failure and cardiac cell apoptosis[3]. Furthermore, it is believed that Dox-mediated cardiotoxicity is caused by inhibition of mitochondrial biogenesis, which primarily activates cell death pathways by blocking topoisomerase 2β. Additionally, a number of other mechanisms, including mitochondrial dysfunction, an alteration in iron regulatory protein, the release of nitric oxide, inflammatory mediators, calcium dysregulation, autophagy, and cell death, have been reported to play significant roles in Dox-induced cardiotoxicity[4]. Reactive Oxygen Species (ROS) also play a pivotal role in this process. Several medications are used to treat Dox-induced cardiotoxicities. One such medication is dexrazoxane concurrent with Dox, which binds at topoisomerase 2β to lessen heart failure and cardiotoxicity in people with sensitive hearts. Numerous strategies, such as β-blockers, phytochemicals, and angiotensin inhibitors, exhibit protective activity against Dox-induced cardiotoxicity and prevent cardiovascular dysfunction[4].

Recent research has demonstrated that key cardiovascular disease risk factors like oxidative stress and inflammatory mediators are successfully prevented, controlled, or blocked by natural substances[5]. The features of the bioactive chemicals that medicinal plants contain give them their therapeutic qualities[6]. Numerous plant bioactive substances have been shown to protect CVD, including diosgenin, flavonoids, sulforaphane, catechin, quercetin, resveratrol, carotenoids, tocotrienols, and polyphenols[7].

The bioactive compound calophyllolide was extracted from seeds of *Calophyllum inophyllum* which is a tropical tree commonly known as Alexandrian laurel or tamanu tree. This naturally occurring coumarin is renowned for its diverse pharmacological properties. Calophyllolide exhibits significant anti-inflammatory effects, making it useful in treating various inflammatory conditions. Its strong antioxidant activity helps neutralize free radicals and reduce oxidative stress, protecting cells and tissues from damage. Additionally, calophyllolide promotes wound healing and has been traditionally used to treat skin ailments due to its regenerative properties. It also demonstrates antimicrobial activity against a range of pathogens, including bacteria and fungi, which is beneficial in combating infections[8]. Emerging research suggests that calophyllolide may have cardioprotective effects, particularly in mitigating drug-induced cardiotoxicity, such as that caused by the chemotherapeutic agent doxorubicin. In light of this, the study aimed to investigate the potential cardioprotective effects of calophyllolide. Further, the histological changes in cardiac tissue following treatment with doxorubicin and the potential protective effects of calophyllolide were investigated in this study.

2. Materials and Methods

2.1 Animals

In this investigation, Wistar rats weighing 155–180 g was employed. The techniques and animal tests were carried out in compliance with national standards for the use and handling of laboratory animals with ethical committee approval number ANUCPS/IAEC/AH/P/11/2024 dated 23-07-2024. The rats were acquired from Hyderabad's Mahaveer Enterprises. They were housed in a typical laboratory with an all-natural light and dark cycle.

2.2 Experimental Protocols of Doxorubicininduced MI

The experiment was conducted in between December 2023 to February 2024. After a week of acclimatization[9], the experimental animals were divided into four groups, (comprising six rats each) as follows:

- Normal control
- Doxorubicin (15mg/kg) control
- DOX + calophyllolide 200mg/kg
- DOX + calophyllolide 400mg/kg

Rats in group I (normal control) received a standard diet for a period of 7 days. Group II animals (DOX) were injected with doxorubicin (15 mg/kg body weight/day) dissolved in physiological saline subcutaneously (on the 8^{th} and 9^{th}) for 2 days at 24-h intervals for the induction of myocardial infarction. In groups III and

IV, animals (calophyllolide 200mg/kg and 400 mg/kg) were received, respectively, for 7 days and then injected with DOX at the dose mentioned above.

2.3 Detection of Electrocardiogram (ECG)

Rats underwent a conventional limb leads II ECG after the trial. Rats' ECG recordings were made utilizing the BL-420S experimental equipment. After the last injection of DOX, anesthetized rats had their left ventricle (LV) catheterized. The right carotid artery was used to introduce a tiny pressure transducer into the aorta, which then progressed into the left ventricle while the pressure waveform was continuously monitored. After 10 minutes of stabilization, LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and ±dp/dtmax were recorded and evaluated. These parameters were continually monitored[10].

2.4 Biochemical Analysis

By using a retroorbital puncture, blood was obtained at the end of the study, and serum was used to estimate lipid and liver profile tests[11]. The heart was also extracted and homogenised using tris buffer, and the supernatant layer was used to measure cardiac biomarkers.

Heart tissue homogenate's supernatant layer was utilized to estimate inflammatory mediators like IL6 and TNF alpha as well as cardiac biomarkers like LDH, CK, and troponin[12]. Histopathological investigations were carried out on cardiac tissue, and LDH, CK, and troponin were measured using an autoanalyzer. Inflammatory mediators were measured using an ELISA kit in accordance with the manufacturer's instructions[13].

2.5 Statistical Analysis

The results are expressed as mean ± S.E.M. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. Data were analysed using GraphPad Prism 5 software.

3. Results

3.1 Electrophysiology

Every rat had a needle electrode placed subcutaneously between its paw pads, and the ECG was continually recorded. In the DOX-induced group, the amplitude of the R wave dropped and the ST interval increased in comparison to the normal group. The ST interval and R wave amplitude recovered in the DOX + calophyllolide 200 mg/kg and DOX + calophyllolide 400 mg/kg groups. LVEDP increased significantly after receiving DOX treatment in comparison to the control group. Conversely, when compared to the CON group, DOX significantly reduced LVSP and ± dp/dtmax. Pretreatment with DOX + calophyllolide 400 mg/kg groups brought both parameters back to almost baseline levels.

3.2 Effect of Calophyllolide on Heart Biomarkers

The level of Tn-I and the enzymatic indicators of myocardial damage, CK-MB and LDH, were both markedly elevated after doxorubicin treatment. LDH, CK-MB, and Tn-I levels did not change significantly when treated with 200 mg/kg of calophyllolide; however, LDH, CK-MB, and Tn-I levels did considerably drop when treated with 400 mg/kg of calophyllolide. It suggests that calophyllolide shields the heart from cardiac damage brought on by doxorubicin (Table 1 and Figure 1).

3.2 Effect of Calophyllolide on Inflammatory Mediators

Using immunohistochemistry, the expression levels of TNF-α and IL-6 in cardiac tissue were evaluated. The administration of doxorubicin markedly enhanced the activity of TNF-α and IL-6. Treatment with 200 mg/kg of calophyllolide did not significantly alter TNF-α and IL-6 levels; however, the 400 mg/kg dose of calophyllolide markedly reduced TNF-α and IL-6. It suggests that calophyllolide shields the heart from inflammation brought on by doxorubicin (Table 2).

3.3 Effect of Calophyllolide on Liver Profile

An autoanalyzer was used to measure the levels of the liver biomarkers SGOT, SGPT, and ALP in the blood sample. The administration of doxorubicin led to a considerable increase in SGOT, SGPT, and ALP levels. SGOT, SGPT, and ALP did not significantly change after treatment with 200 mg/kg of calophyllolide; however, SGOT, SGPT, and ALP did considerably decrease after treatment with 400 mg/kg of calophyllolide. It suggests that calophyllolide shields the heart from cardiac damage brought on by doxorubicin (Table 3).

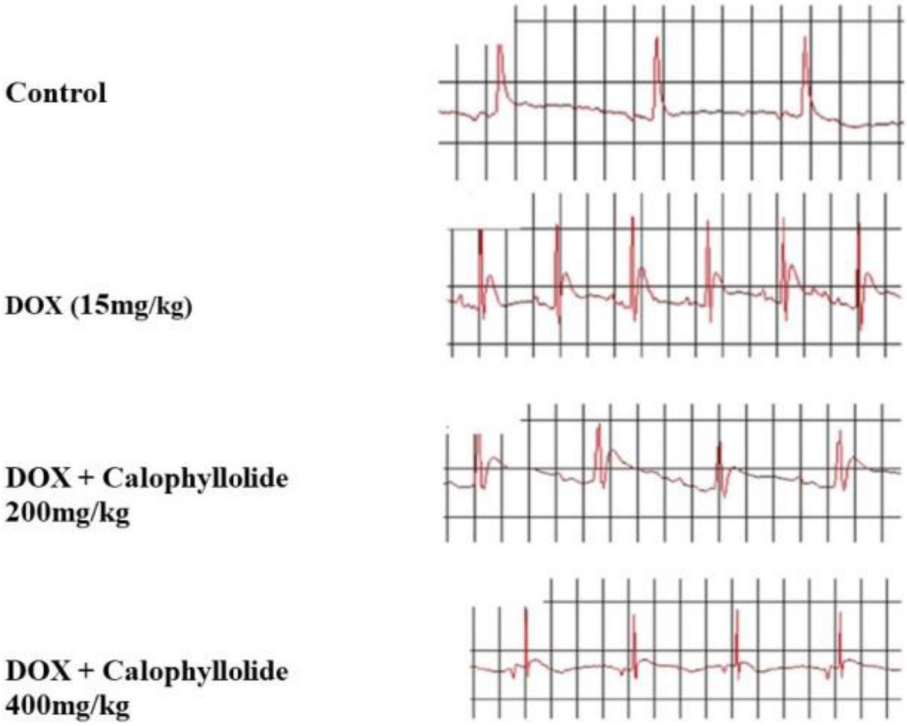


Figure 1. Effect of calophyllolide on ECG.

Table 1. Effect of calophyllolide on LDH (IU/L)

S. No.	Test	LDH(IU/L) Mean ±SEM	Cardiac troponin(pg/mol) Mean ±SEM	CK(IU/L) Mean ±SEM
1	Control	110±0.82*	54.8±1.63*	501±015*
2	DOX (15mg/kg)	320±0.74**	110.2±0.47**	1650±0.78**
3	DOX + calophyllolide 200mg/kg	201±0.68	90.8±0.47	1100±0.24
4	DOX + calophyllolide 400mg/kg	132±0.74***	64.2±0.39***	642±0.40***

The values (n=6) are the means ± SEM. When compared to the control, the administration of doxorubicin considerably raised the LDH level (**p < 0.001). When compared to the doxorubicin group, treatment with 400 mg/kg of calophyllolide dramatically lowered the LDH (***p < 0.001).

Table 2. Effect of calophyllolide on inflammatory mediators

S. No.	Test	TNF-alpha (pg/mg of protein) Mean ±SEM	IL6 (pg/mol) Mean ±SEM
1	Control	40.2±0.45*	50.2±1.45*
2	DOX (15mg/kg)	142.4±0.35**	162.3±0.45**
3	DOX + calophyllolide 200mg/kg	120.5±0.96	139.5±0.21
4	DOX + calophyllolide 400mg/kg	73.2±0.74***	92.7±2.35***

The values are the means ± SEM(n=6) doxorubicin administration significantly increased the TNF-alpha (**p < 0.001) when compared to control. Treatment with calophyllolide 400 mg/kg dose of calophyllolide significantly decreased the TNF-alpha (***p < 0.001) when compared to doxorubicin group.

3.4 Effect of Calophyllolide on Lipid Profile

With the aid of an autoanalyzer, the blood sample's lipid profile values of LDL, TC, and HDL were evaluated. Administration of doxorubicin markedly raised LDL and TC levels while lowering HDL levels. LDL, TC, and HDL levels did not significantly change after treatment with 200 mg/kg of calophyllolide; however, LDL, TC, and HDL levels were dramatically reduced and elevated

with a 400 mg/kg dose of calophyllolide. It suggests that calophyllolide shields the heart from cardiac damage brought on by doxorubicin (Table 4).

3.5 Histopathology

The histopathological study showed that the healthy group's connective tissue patches and muscle fibers appeared normal. The connective tissue connecting extremely deteriorated muscle fibers and inflammatory cells with polymorph nuclei was discovered in the DOX Group. The DOX + calophyllolide 200mg/kg group showed moderately deteriorated muscle fibers but no inflammatory cells. There were no indications of inflammation in the DOX + calophyllolide 400 mg/

Table 3. Effect of calophyllolide on liver profile

S. No.	Test	SGOT(IU/L) Mean ±SEM	SGPT(IU/L) Mean ±SEM	ALP(KA/100ml) Mean ±SEM
1	Control	24.2±0.45*	23.2±0.69*	4.7±0.73*
2	DOX (15mg/kg)	52.6±0.32**	68.8±1.58**	9.4±0.91**
3	DOX + calophyllolide 200mg/kg	38.2±0.14	40.1±0.42	5.9±0.86
4	DOX + calophyllolide 400mg/kg	27.6±0.75***	26.2±0.37***	2.6±0.14***

The values are the means ± SEM(n=6) doxorubicin administration significantly increased the SGOT level (**p < 0.001) when compared to control. Treatment with calophyllolide 400 mg/kg dose of calophyllolide significantly decreased the SGOT (***p < 0.001) when compared to the doxorubicin group.

Table 4. Effect of calophyllolide on lipid profile

S. No.	Test	LDL (mg/dl)	Total cholesterol(mg/dl) Mean ±SEM	HDL (mg/dl) Mean ±SEM
1	Control	16.7±0.12*	40.2±0.25*	30.2±0.56*
2	DOX (15mg/kg)	24.8±0.28**	62.8±0.89**	16.3±0.69**
3	DOX + calophyllolide 200mg/kg	21.8±0.78	51.8±0.45	20.3±0.47
4	DOX + calophyllolide 400mg/kg	19.2±0.03***	46.8±0.89***	24.8±0.16***

The values (n = 6) are the means ± SEM. Administration of doxorubicin raised the LDL level significantly (**p < 0.001) in comparison to the control. The LDL was significantly reduced (***p < 0.001) in the calophyllolide 400 mg/kg group as compared to the doxorubicin group.

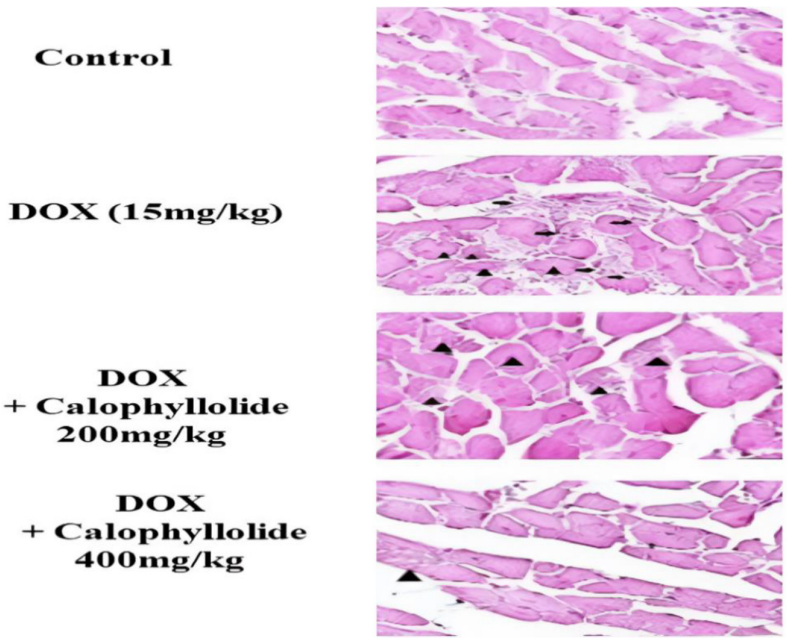


Figure 2. Histopathology.

kg group, while there were novelized muscle fibres that were somewhat degenerate (Figure 2).

4. Conclusion

This study demonstrates that Calophyllolide, particularly at a 400 mg/kg dose, offers significant cardioprotective benefits against Doxorubicin (DOX)-induced cardiac toxicity. The electrophysiological improvements observed, including restored R wave amplitude, ST interval, and LV function parameters, highlight its role in maintaining heart function. Additionally, its ability to reduce key cardiac biomarkers (Tn-I, CK-MB, LDH) and inflammatory mediators (TNF-α, IL-6) suggests potent anti-inflammatory and cardioprotective effects. The normalization of liver enzymes (SGOT, SGPT, ALP) and lipid profile markers (LDL, TC, HDL) further indicates its protective role in mitigating DOX-induced hepatic and metabolic disturbances. Histopathological findings reinforce these protective effects, showing reduced muscle fiber damage and inflammation. These results collectively support calophyllolide as a promising natural compound for preventing DOX-induced cardiotoxicity, offering advantages such as reduced oxidative stress, enhanced cardiac cell viability, and minimal adverse effects. Its efficacy in restoring physiological and biochemical parameters suggests potential applications in cardioprotective therapy, warranting further investigation for clinical translation.

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7. Author Contribution

Both authors JN and RP are equally contributed the manuscript. Both authors have read and agreed to the published version of the manuscript.

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