Clinical Phytoscience

ORIGINAL CONTRIBUTION

Open Access

Cardioprotective effects of *Rhododendron arboreum* leaf extract against Doxorubicin-induced cardiotoxicity in Wistar rats by modulating electrocardiographic and cardiac biomarkers

Adersikha Pradhan[1*], Manodeep Chakraborty[1], Oonglim Lepcha[1], Ananya Bhattacharjee[1], Devid Chutia[1] and Nihar Ranjan Bhuyan[1]

Abstract

Background Cardiotoxicity and related complications are well-known adverse effects of anticancer drugs like doxorubicin (DOX). A medicinal plant called *Rhododendron arboreum* is used by traditional healers of Sikkim in the treatment of heart ailments and has also been reported for widespread therapeutic effects in many clinical studies. Thus the present study has been designed to evaluate the protective effects of *Rhododendron arboreum* leaf extract (RALE) against DOX-induced cardiotoxicities.

Methods Commencement of research with the collection of the *Rhododendron arboreum* leaves and drying it in the shade, the extraction was performed using the Soxhlet method with an ethanolic solvent. The phytoconstituents of the RALE were then quantified and qualitatively evaluated. Doxorubicin-induced cardiotoxicity was carried out using four groups consisting of six animals each. Doxorubicin was administered with a dose of 3 mg/kg injected intraperitoneally (i.p.) on the 1st,7th,14th,21st and 28th day of cumulative dose of 15 mg/kg throughout the experimental period with RALE treatment (250 mg/kg and 100 mg/kg) orally for 28 days. The influence of the treatment was analyzed by quantification of cardiac biomarkers and electrocardiographic method.

Results The serum levels of cardiac biomarkers such as Lactate Dehydrogenase (LDH), Creatine kinase-N-acetyltransferase (CK-NAC), Creatine kinase-MB (CK-MB), Aspartate Transaminase (AST), Alanine Transaminase (ALT), which were elevated due to DOX-induced cardiotoxicity were significantly reduced in all RALE (250 mg/kg and 100 mg/kg) treated groups. Similarly, the electrocardiographic changes like prolonged QT interval, widening of QRS complex amplitude, undefined ST segment, arrhythmias and increased heart rate were also restored close to normal in all treated groups compared to the DOX control group.

Conclusion Following the data observed during the study, results reported that *R. arboreum* possesses the free radical scavenging property, improved cardiotoxic laboratory parameters and restored reversible cellular injury due to existing of the principle constituent's impact on proinflammatory mediators.

Keywords Cardiotoxicity, *Rhododendron arboreum*, Doxorubicin

*Correspondence:
Adersikha Pradhan
adersikha25@gmail.com
Full list of author information is available at the end of the article

Springer Open

© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Introduction

Across the world, cardiovascular diseases (CVDs) are among the main causes of mortality. In particular, they are the largest source of disease burden, predicted to account for 523 million cases and 18.6 million fatalities in 2019 [1]. Cardiovascular diseases referred to instances in which blood vessels are constrained or impeded, and that may result in heart failure, chest pain, or an attack. Various cardiac abnormalities, including those which impair the heart's rhythm, valves, or function, are also regarded as various types of cardiovascular disease [2] such as coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, pulmonary embolism and other conditions [1].

The generation of reactive free radicals within the human body is the primary cause of CVDs or the destruction of cellular biomolecules. Unless the delicate balance between free radical and antioxidant activity changes under any pathophysiological circumstances, it may result in oxidative stress and increased tissue damage, which is a significant determinant of the development of cardiovascular disease [3]. Arrhythmias, thrombosis, hypertension, Heart failure with reduced Ejection Fraction (HFrEF), ischemia/myocardial infarction, and a diversity of other significant cardiac problems have all been attributed to cardiotoxic effects of chemotherapy [4].

The significant improvement in cancer survival rates over the past few years may be attributed to advancements in oncology in the areas of screening, diagnostic imaging, and new therapies. Nevertheless, this increased cancer survival rate is typically accompanied by a wide range of therapeutic adverse effects, especially heart-related, which have a detrimental effect on the patient's health and quality of life [5]. Cardio-oncology rose to prominence as a specialization in the prevention or treatment of cardiac issues due to cancer treatment, resulting in an enhanced understanding of the molecular mechanisms of chemotherapeutic drugs, especially Anthracycline-induced cardiotoxicity (AIC) [6].

Doxorubicin (DOX), a precursor of anthracyclines, is the most extensively used, beneficial, and successful antineoplastic agent. It is derived from a gram-positive bacteria called *Streptomyces peucetius* [7–9] to treat a variety of malignancies, including breast cancer and lymphoma. However, its therapeutic application is restricted due to its cardiotoxic effects [10].

Reactive oxygen species (ROS) production, mitochondrial DNA damage, inflammation, apoptosis, the inhibition of protein synthesis or abnormal protein processing, lysosomal modifications, and cardiomyocyte death are mostly a couple of the potential mechanisms that could be held responsible for DOX-induced cardiotoxicity [11, 12]. The acute type DOX toxicity often emerges 2–3 days after the end of the chemotherapy and is characterized by tachycardia, electrocardiographic abnormalities, premature beats, myopericarditis, and occasionally abrupt left ventricular failure [13].

It is now universally acknowledged that chemotherapy-induced cardiotoxicity increases the risk of mortality and morbidity for the treated patient. Nevertheless, there are yet no efficient therapeutic options or prophylactic interventions for DOX-induced cardiotoxicity [11].

In recent years, an increasing number of people have appealed to herbal medications or products to enhance their health, individually or in combination with traditional remedies. Indeed, from the beginning of civilization, the usage of herbs/plants has provided an effective therapy for the treatment of ailments. Furthermore, many conventional/pharmaceutical treatments are derived primarily from nature as well as traditional effective treatments that are prevalent worldwide [14].

Rhododendron arboreum is a high-altitude plant, that belongs to the family Ericaceae. This plant is having ethnopharmacological utilization across the world [15]. Since the plant contains a variety of bioactive phytoconstituents such as phenolic compounds, flavonoids (Quercetin, Rutin), Urosolic acid, saponins and tannins, which has been discovered to have a diversity of pharmacological activities, including anti-inflammatory, cardioprotective, anti-oxidative, anti-fungal, anti-allergic, analgesic, diuretic, hepatoprotective, alleviate headache and fever, for the treatment of gout and rheumatic disorders, relieve cold, cough, asthma, bronchitis, nasal bleeding and lung infections also beneficial in case of indigestion and post-delivery complications [16]. There is currently no study that has found *R. arboreum* leaves to have a cardioprotective potency against the anticancer drug Doxorubicin-induced cardiotoxicity. As a result, the proposed investigation seeks to evaluate the effect of *R. arboreum* at various concentrations on Doxorubicin-induced cardiotoxicity in the Wistar albino rats.

Methods and materials

Collection and authentification of plant material

R. arboreum leaves were collected from Sombaria, West Sikkim India. After being collected, a herbarium of *R. arboreum* leaves was prepared and authenticated with the specimen (No. 21HMPL01) at the Botanical Survey of India, Gangtok, Sikkim. Further, the leaves were

preserved in the shade to avoid direct sunlight. To aid in the extraction process.

Extraction process

The extraction of *Rhododendron arboreum* leaves was carried out by using the Soxhlet apparatus by taking 300 ml ethanol as the solvent. The coarse powder of 50 g was extracted. Therefore, the percentage yield was calculated by using the formula. Furthermore, the obtained extract was subjected to solvent evaporation using a rotary evaporator to enhance solvent recovery and drying.

Qualitative and quantitative phytochemical analysis

Identification and Quantification for secondary metabolites of ethanolic extract of *R. arboreum* leaf (RALE) were performed by screening both qualitative and quantitative analysis.

To detect the presence of alkaloids, proteins, amino acids, anthraquinones, flavonoids, carbohydrates, saponins, tannins, steroids, reducing sugars, triterpenoids, and cardiac glycosides qualitative analysis test procedure was performed [17]. Additionally, for quantification total phenolic and flavonoid content was determined by Folin Ciocalteu's method and aluminium chloride colorimetric assay respectively [18].

Experimental animals welfare

In separate polypropylene cages, 24 healthy male Wistar rats (body weight: 150-200 g, age: 8–10 weeks) were raised at the Himalayan Pharmacy Institute, Majhitar. Animals were kept at temperatures (22–24 °C), 12-h light/12-h dark cycle, and 40%–60% relative air humidity according to conventional preferences. Rats were given unlimited access to clean drinking water and a calorie-dense diet of rat pellets. The rats were randomly allocated to different experimental groups and then given a week to acclimatize. Following the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), the Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was acquired from the Institutional Animal Ethics Committee for conducting the study [5]. The IAEC approval for the research work (No. HPI/2023/60/IAEC/PP-0194).

The rationale for dose selection of *R. arboreum* leaf extract

Two dosages were considered for this research study from an earlier literature review; which are 100 mg/kg and 250 mg/kg, which represent the low and high doses respectively [19].

Animal model protocol

The experimental animal groups were categorized into 4 groups of 6 experimental rats in each group.

Group I: Normal control group received saline through oral route for 4 weeks.
Group II: DOX control group (cardiotoxicity-induced rat) doxorubicin was subjected at a dose of 3 mg/kg i.p. for 28 days on 1, 7, 15, 21 and 28.
Group III: low dose treatment group was treated with ethanolic extract of RALE(100 mg/kg) orally for 28 days.
Group IV: high-dose treatment group was treated with an ethanolic extract of RALE (250 mg/kg) orally for 28 days.

The oral route was used for all treatment groups for 28 days. A dose of 3 mg/kg i.p. of DOX was administered to each group apart from Group I to induce DOX toxicity. On Days 1, 7, 14, 21, and 28, a cumulative percentage of 15 mg/kg was administered [10]. Twenty-four hours after the last treatments blood samples were collected and serum was prepared by centrifugation. The isolated serum samples were subjected to analysis of biomarkers and also forty-eight hours after the last treatments the electrocardiographic estimation was performed [10].

Serum preparation for cardiac biomarker assay

Twenty-four hours after the last session of the experiment's treatment period. A tail vein method was used to collect blood samples from each treatment group under anaesthesia. Whole blood was drawn into tubes and stabilised for between 15–30 min to ensure it was able to clot at room temperature. The serum was prepared from the blood using a centrifugation (ifuge-C4000 centrifuge, Neuation, India) at 1000 rpm for 10 min at 37 °C temperature [10]. Lastly, the serum is used to estimate cardiac serum biomarkers parameters including aspartate aminotransferase (AST), alanine transaminase (ALT), Creatine kinase-MB (CK-MB), Creatine kinase-N-acetyltransferase (CK-NAC) and lactate dehydrogenase (LDH) [12, 20].

ECG parameter assessment

The ECG signal illustrates patterns with different frequency ranges that cycle from beat to beat. In the process of delineating an ECG, the algorithm usually starts with the QRS complex, the most pronounced wave. The delineator also marks the starting point, peak, and completion of the P-wave, QRS complex, and T-wave [21].

Forty-eight hours after the last phase of treatment. Each group of animals were anaesthetized using a

combination of ketamine (45 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). Rats were restrained to the backs with their front legs semi-flexed and their hind legs slightly extended by a thread to restrict their ability to move and to make it easier to attach the metallic leads (I, II, II). The chest lead was subcutaneously attached near the heart towards the left ventricle after that the electrodes were mounted subcutaneously on the front and hind legs. According to the ECG recording procedure. To measure and record different ECG waves, leads were connected to the digital physiograph (AD instruments) with the help of lab chart software, the recording was observed and analyzed at an intensity of 0.5 mV and a graph speed of 100 mm/sec [22]. The following ECG parameters were monitored for changes:

Heart Rate, QRS Interval, RR Interval, QT Interval, PR Interval.

Statistical analysis

One-way analysis of variance (ANOVA), followed by the Tukey-Karmer multiple comparison tests, was the method used to analyse the data. At $p < 0.05$, differences were identified as statistically significant [10].

Results

Phytochemical analysis

The extracted yield of the plant was found to be 11.7 g. Preliminary phytochemical analysis revealed the presence of reducing sugar, carbohydrates, flavonoids, phenols, glycosides, saponins, and tannins. The total phenolic content and total flavonoid content were further determined to be 577.44 g/mg of extract and 65.83 g/mg of extract, respectively (Table 1).

Table 1 Results of preliminary phytochemical estimation

Phytoconstituents	Ethanolic extract of *Rhododendron arboreum*
Alkaloids	–
Carbohydrates	+
Flavonoids	+
Glycosides	–
Saponins	+
Steroids	–
Tannins	+
Proteins	–
Reducing sugars	+
Anthraquinones	–
Phenols	+

(+) indicates the presence of phytoconstituents

(-) indicates the absence of phytoconstituents

Clinical signs and symptoms of rats for all the animal groups

Male Wistar albino rat's appearance was observed throughout 28 days of the research treatment for all animal groups.

A severe red nasal discharge, exudates on the area of the hind legs, scruffy fur, and fur loss over the upper part of the torso and abdomen region were symptoms of all the wistar rats within the DOX control group, which weighed 150 g and 110 g, respectively (Fig. 1). Similarly, red nasal discharge, scruffy fur, and lesser fur loss on the back have been identified in the RALE-treated low-dosage group. RALE treatment in the high dosage group, however, only produced red nasal discharge.

Effect of *Rhododendron arboreum* leaf extract on various serum biomarker levels

❖ Outcome of ALT against Doxorubicin-induced cardiotoxicity:

As shown in (Fig. 2), serum ALT level was demonstrated to be an extremely significant increase (p<0.001) in the DOX control group (toxic control group) as compared to the normal control.

Apparently, in comparison to the DOX control group, the high-dose group (250mg/kg) and the low-dose group (100mg/kg) both treated with RALE determined extremely significant decrease (p<0.001) in serum ALT level, which is exhibited in Table 2 and Fig. 2.

❖ Outcome of AST against Doxorubicin-induced cardiotoxicity

AST, an enzyme involved in amino acid metabolism, was demonstrated to be an extremely significant increase (p<0.001) in the DOX control group as compared to the normal control group.

In comparison to the DOX control group, the high-dose group (250mg/kg) and low-dose group (100mg/kg) both treated with RALE demonstrated extremely significant decrease (p<0.001) in serum AST level. The values and the outcomes are shown in Table 2 and Fig. 2.

❖ Outcome of CK-MB against Doxorubicin-induced cardiotoxicity

The clinical significance of the presence of CK-MB enzyme is witnessed by increased due to heart injury in rats. The DOX control group showed an extremely significant increase (p<0.001) as compared to the normal control group in serum CK-MB level.

In comparison to the DOX control group, the high-dose group (250mg/kg) and low-dose group (100mg/kg) both treated with RALE demonstrated extremely

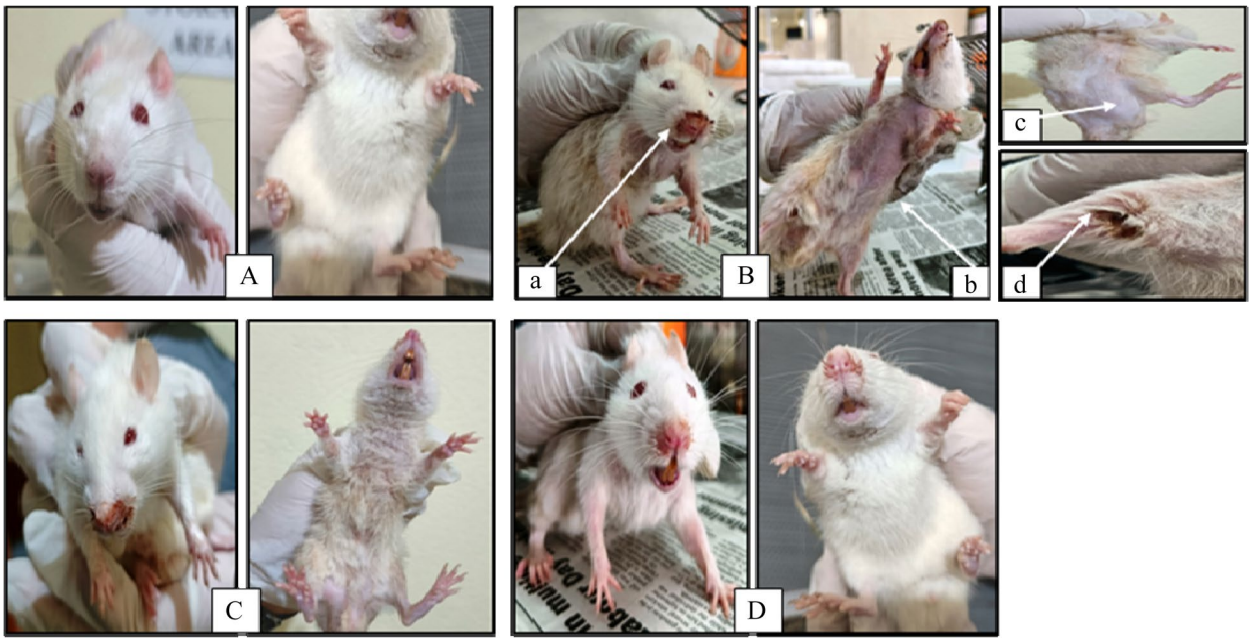

Fig. 1 Clinical signs and symptoms of rat for all the animal groups. **A** Appearance of normal control group, **B** Appearance of DOX control group a. severe red nasal discharge b. Fur loss over the upper part of the torso and abdomen region c. Fur loss on back region, d. Exudates on hind legs, **C** Appearance of low dose group, a. severe red nasal discharge b. lesser fur loss, **D** Appearance of high dose group a. Red nasal discharge b. No fur loss

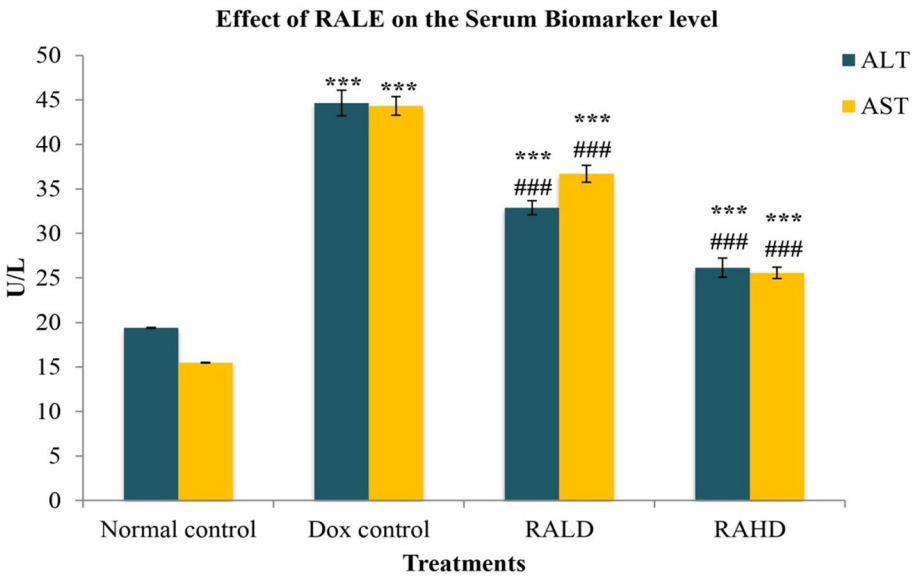

Fig. 2 Effect on the serum biomarker level (ALT, AST) of Wistar rats (N-6)

significant decrease (p<0.001) in serum CK-MB level. Comparisons of all values are exhibited in Table 2 and Fig. 3.

❖ Outcome of CK-NAC against Doxorubicin-induced cardiotoxicity

CK-NAC is one of the isoenzymes that provides a definitive diagnosis of cardiac diseases. It was demonstrated to be an extremely significant increase (p<0.001) in the DOX control group as compared to the normal control group.

Table 2 Effect of *Rhododendron arboreum* leaf extract on various serum biomarker levels of Wistar rats (N-6)

SL.NO	Treatment groups	AST (U/L)	ALT (U/L)	CK-MB (U/L)	CK-NAC (U/L)	LDH (U/L)
1.	Normal control	15.49 ± 0.04	19.41 ± 0.04	76.60 ± 0.03	44.76 ± 0.11	121.4 ± 0.01
2.	DOX control	44.33 ± 1.05***	44.65 ± 1.44***	210.2 ± 0.32***	143.2 ± 2.03***	205.7 ± 1.28***
3.	RALD-100 mg/kg	36.70 ± 0.95$^{***###}$	32.88 ± 0.78$^{***###}$	180.0 ± 0.45$^{***###}$	79.85 ± 2.45$^{***###}$	165.9 ± 0.25$^{***###}$
4.	RAHD-250 mg/kg	25.56 ± 0.64$^{***###}$	26.14 ± 1.08$^{***###}$	146.9 ± 0.51$^{***###}$	65.97 ± 1.16$^{***###}$	134.2 ± 4.35$^{**###}$

Values are expressed as mean ± SEM, $n = 6$, $^{***}P < 0.001$, $^{**}P < 0.01$when compared to a normal group; $^{###}P < 0.001$ compared to a DOX control group

DOX Doxorubicin, *RALD Rhododendron arboreum* low dose, *RAHD Rhododendron arboreum* high dose, *ALT* Alanine aminotransferase, *AST* Aspartate aminotransferase, *CK-MB* Creatine kinase-MB, *CK-NAC* Creatine kinase-N-acetyltransferase, *LDH* Lactate dehydrogenase

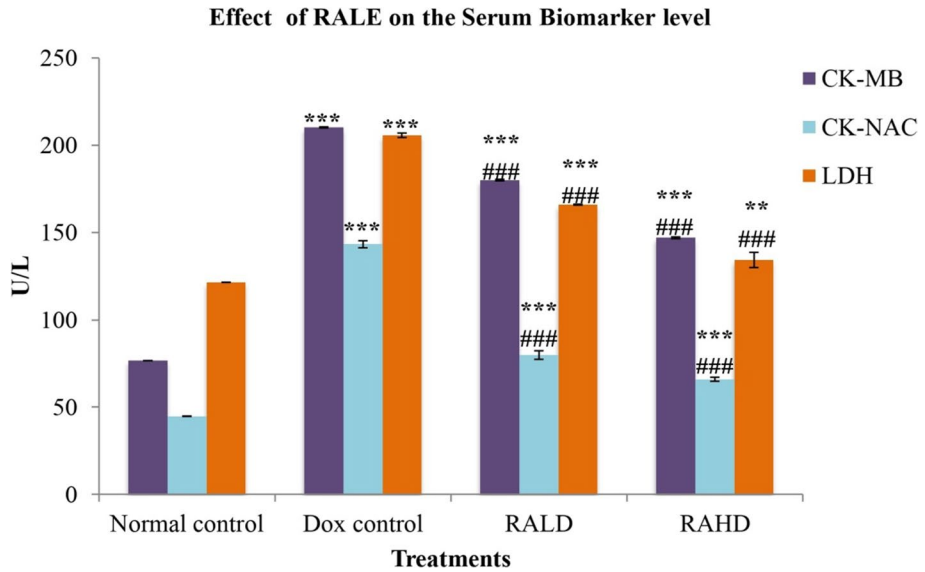

Fig. 3 Effect on the serum biomarker level (CK-MB,CK-NAC,LDH) of Wistar rats(N-6). The values for both the graph are expressed as mean ± SEM, n=6, $^{***}P < 0.001$, $^{**}P < 0.01$ when compared to a normal group; $^{###}P < 0.001$ compared to a DOX control group. RALD: *Rhododendron arboreum* low dose; RAHD: *Rhododendron arboreum* high dose; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CK-MB: Creatine kinase-MB; CK-NAC: Creatine kinase-N-acetyltransferase; LDH: Lactate dehydrogenase

In comparison DOX control group, the high-dose group (250mg/kg) and the low-dose group (100mg/kg) both treated with RALE demonstrated extremely significant decrease (p<0.001) in serum CK-NAC level. Comparisons of all values are exhibited in Table 2 and Fig. 3.

❖ Outcome of LDH against Doxorubicin-induced cardiotoxicity

The enzyme LDH is distributed in tissues, particularly the heart. Clinical significance is determined by elevated levels of LDH in serum in contrast to myocardial infarction.

Serum for LDH level of the DOX control group was extremely significant increase (p<0.001) when compared to the normal control group.

In comparison to the DOX control group, the high-dose group (250mg/kg) and low-dose group (100mg/kg) both treated with RALE demonstrated extremely significant decrease (p<0.001) in serum LDH level. Comparisons of all values are exhibited in Table 2 and Fig. 3.

Effect of *Rhododendron arboreum* leaf extract on various parameters of ECG.

❖ Significance of heart rate against Doxorubicin-induced cardiotoxicity.

The rate of heartbeat is easily determined by Electrocardiogram because the heart rate is the reciprocal of the time interval between two successive heartbeats. There was an extremely significant increase (p<0.001) in the heart rate of the DOX control group in comparison with a normal control group.

The heart rate of the high-dose group (250mg/kg) and low-dose group (100mg/kg) both treated with RALE demonstrated an extremely significant decrease

Table 3 Effect of *Rhododendron arboreum* leaf extract on various parameters of ECG of Wistar rats (N-6)

SL.NO	Treatments groups	Heart Rate (BPM)	QRS Interval (ms)	RR Interval (ms)	QT Interval (ms)	PR Interval (ms)
1.	**Normal control**	336.6 ± 1.65	13.82 ± 0.16	177.7 ± 0.14	44.74 ± 0.10	32.49 ± 0.11
2.	**DOX control**	$458.4 \pm 4.18^{***}$	$27.91 \pm 0.62^{***}$	$134.8 \pm 1.01^{***}$	$82.29 \pm 3.05^{***}$	$49.17 \pm 0.68^{***}$
3.	**RALD- 100 mg/kg**	$407.4 \pm 1.79^{***\#\#\#}$	$22.51 \pm 1.60^{***\#\#}$	$148.1 \pm 0.70^{***\#\#\#}$	$71.08 \pm 2.06^{***\#\#}$	$41.16 \pm 0.26^{***\#\#\#}$
4.	**RAHD- 250 mg/kg**	$383.1 \pm 1.66^{***\#\#\#}$	$17.60 \pm 0.17^{*\#\#\#}$	$153.5 \pm 0.76^{***\#\#\#}$	$56.34 \pm 1.39^{**\#\#\#}$	$37.49 \pm 0.78^{***\#\#\#}$

Values are expressed as mean ± SEM, $n = 6$, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, when compared to normal group; $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, when compared to DOX control group
DOX Doxorubicin, *RALD Rhododendron arboreum* low dose, *RAHD Rhododendron arboreum* high dose

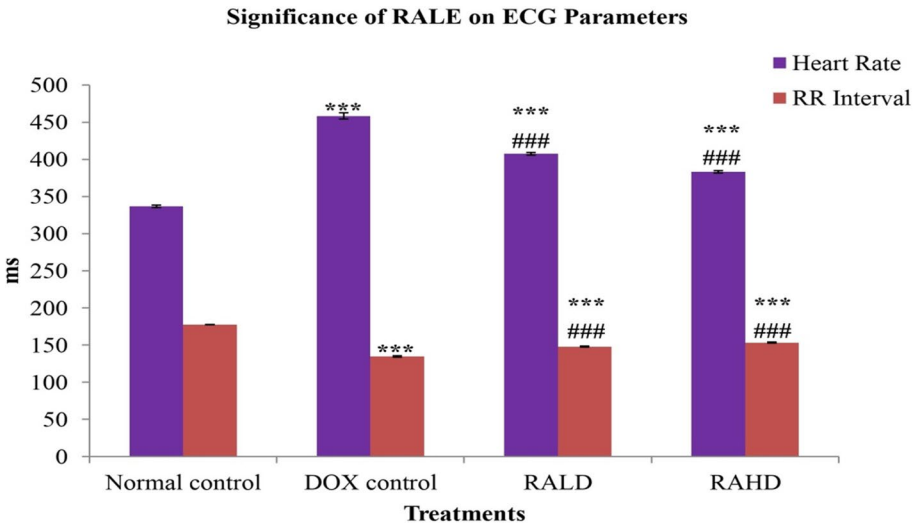

Fig. 4 Changes of ECG parameters of (Heart rate and RR interval) of Wistar rats (N-6)

(p<0.001) in contrast with the DOX control group. All the values are described in Table 3 and Fig. 4.

❖ Significance of RR interval against Doxorubicin-induced cardiotoxicity.

There was an extremely significant decrease (p<0.001) in the RR interval amplitude of the DOX control group in comparison with a normal control group.

RR interval of the high-dose group (250mg/kg) and low-dose group (100mg/kg) both treated with RALE demonstrated an extremely significant increase (p<0.001) in contrast with the DOX control group. All the values are described in Table 3 and Fig. 4.

❖ Significance of QRS interval against Doxorubicin-induced cardiotoxicity.

There was an extremely significant increase (p<0.001) in the QRS interval amplitude of the DOX control group in comparison with a normal control group.

QRS interval amplitude of the low-dose group (100mg/kg) treated with RALE demonstrated a moderately significant decrease (p<0.001), Whereas the QRS interval amplitude of the high-dose group treated with RALE was extremely significant decrease (p<0.01) in contrast with the DOX control group. All the values are described in Table 3 and Fig. 5.

❖ Significance of QT interval against Doxorubicin-induced cardiotoxicity.

Contraction of the ventricles lasts almost from the beginning of the Q wave to the end of the T wave. This interval is called the QT interval. There was an extremely significant increase (p<0.001) in the QT interval amplitudes of the DOX control group in comparison with a normal control group.

QT interval amplitude of the low-dose group (100mg/kg) treated with RALE demonstrated a moderately significant decrease (p<0.001), Whereas the QT interval amplitude of the high-dose group treated with RALE demonstrated extremely significant decrease (p<0.01) in contrast with the DOX control group. All the values are described in Table 3 and Fig. 5.

❖ Significance of PR interval against Doxorubicin-induced cardiotoxicity.

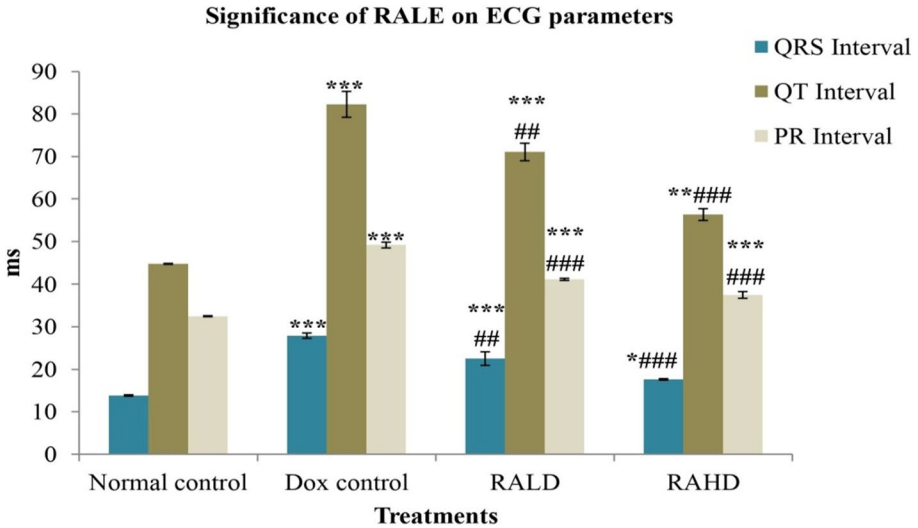

Fig. 5 Changes of ECG parameters of (QRS interval, QT interval and PR interval) of Wistar rats (N-6). The values for both the graph are expressed as mean ± SEM, n=6, $^{***}P < 0.001$, $^{**}P < 0.0$, $^{*}P < 0.05$, when compared to a normal group; $^{\#\#\#}P < 0.001$ compared to a DOX control group. DOX: Doxorubicin, RALD: *Rhododendron arboreum* low dose; RAHD: *Rhododendron arboreum* high dose

The PR interval is important because it reflects the conduction from the atria to the ventricles through the AV junction and His Purkinje system. It also includes the time between the beginning of the P wave and the beginning of the QRS complex is the interval between the beginning of the electrical excitation of the atria and the beginning of the excitation of the ventricles (atrial depolarization). This period is called the PQ interval. An extremely significant increase (p<0.001) in the PR interval amplitude of the DOX control group in comparison with a normal control group.

The high-dose group (250mg/kg) and low-dose group (100mg/kg) both treated with RALE demonstrated an extremely significant decrease (p<0.001) in contrast with the DOX control group. All the values are described in Table 3 and Figs. 5 and 6.

Discussion

The primary underlying cause of morbidity and mortality worldwide is chemotherapy-induced cardiovascular complications [23]. The purpose of this research scrutiny was to determine whether the ethanolic extract of *Rhododendron arboreum* leaves is capable of preventing cardiotoxicity induced by doxorubicin in Wistar rats.

In this study model Doxorubicin-induced cardiotoxicity, toxicities in the cardiovascular system was induced by doxorubicin in Wistar rats with a cumulative dose of 15 mg/kg divided into five doses each given 3 mg/kg injected intraperitoneal route in rats for 28 days [10]. Doxorubicin-induced cardiotoxicity has been associated with a broad range of mechanisms according to numerous investigations [12] including an inability of mitochondria to function properly, alteration in an iron regulatory protein, the expulsion of nitric oxide, mediators of inflammatory reactions, calcium imbalance, autophagy, and destruction of cells have been speculated to play a significant role, the proliferation of reactive oxygen species (ROS), which results in lipid peroxidation and the gradual depletion of antioxidant enzymes, is reported to be the primary one [20]. Contrarily in this current research study symptoms for all the animal groups were observed throughout the experiment, which showed evidence of a severe red nasal discharge, exudates on the area of the hind legs, scruffy fur, fur loss over the upper part of the torso and abdomen region compared to the normal group. Fur loss is due to holocrine secretion which is considered a form of apoptosis associated with nonphysiological increases in sebocyte cell death disrupting the sebaceous glands [24] and whereas red nasal discharge induced by doxorubicin is associated with nasal inflammation (rhinitis), weakened immune system leading to infections, or direct damage to blood vessels causing bleeding [25]. The High dose of RALE (250 mg/kg) treated reduced red nasal discharge and no fur loss when compared with the DOX control group. Whereas ethanolic extract of *R. arboreum* leaf (RALE) treated low dose group (100 mg/kg) demonstrated red nasal discharge, scruffy fur and lesser fur loss on the back compared to the DOX control group. This observation accounts for the effective cell-protecting property of *R. arboreum* with anti-inflammatory, antioxidant and antimicrobial effects [26].

As the *R. arboreum* ethanolic extract of leaf contains phenolic and flavonoid compounds, which are known to have antioxidant properties. Phenolic compounds

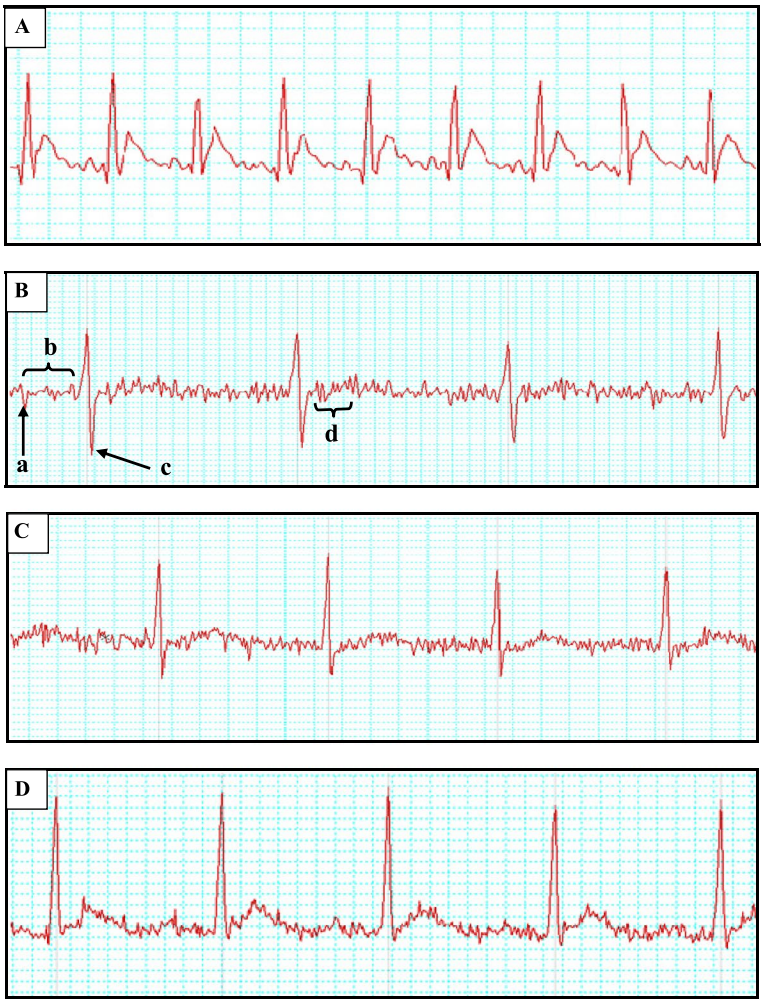

Fig. 6 Qualitative analysis of ECG of all the experimental groups (N-6). **A** Normal control group, **B** DOX control group a. inverted P wave, b. increased PR interval c. deepening of QRS complex amplitude, and d: prolongation of the QT interval, **C** RALD-100 mg/kg control group, **D** RAHD- 250 mg/kg control group. DOX: Doxorubicin, RALD: *Rhododendron arboreum* low dose; RAHD: *Rhododendron arboreum* high dose

illustrated their efficacy in this research investigation by preventing the synthesis of many reactive oxygen species. They further exerted positive regulatory effects on signaling pathways such as upregulation of the phosphoinositide 3-kinase (PI3K)/Akt pathway resulting in the inhibition of apoptosis and the augmentation of cell survival in cardiomyocytes, reducing rise of cellular Ca^{2+} and retaining matrix metalloproteinases (MMP) resulting in restoration of mitochondrial integrity, and inhibition of nuclear factor kappa-B (NF-κB)by disabling the activation of FasL, Fas, c-Myc, and p53 genes as well as proinflammatory cytokines. Although there are a variety of mechanisms for the antioxidant activity of phenolic acids (because of the reactivity of the phenol moiety), radical scavenging via hydrogen atom donation is considered to be the primary mechanism [27, 28]. Moreover, antioxidant, anti-inflammatory, anti-apoptotic, anti-calcium overload, and iron scavenging characteristics are a few of the diverse pharmacological actions of flavonoids. Dietary supplements that include flavonoids, such as quercetin, rutin, and luteolin, are crucial in the defense against cardiac toxicity because they reduce ROS, lipid peroxidation, mitochondrial permeability, and apoptosis by various mechanisms. In this current research investigation Phlpp1 and p-Akt protein expression is elevated by flavonoids. It is known that phlpp1 modulates the AKT protein to promote cell survival and significantly reduce the apoptosis brought on by doxorubicin. Furthermore, it increased the expression of the protein 14–3-3γ, which facilitates the prevention of myocardial damage. Remarkably it has characteristics that avert calcium overload by significantly increasing SERCA2a expression and preventing contractile dysfunction. In addition, it inhibited caspase-3 activity, which prevents mitochondrial permeability transition pore (mPTP)

from opening, which leads to mitochondrial swelling and excessive release of reactive oxygen species [29].

In accordance with earlier research reports, doxorubicin substantially raised all the cardiac serum biomarkers including AST, ALT, CK-MB, CK-NAC and LDH abilities in the present research study analysis [20]. The current research data analysis of the RALE with antioxidant properties restored all the cardiac serum biomarker levels which were elevated by the DOX control group probably flavonoid compounds minimized the leaking of these enzymes through inhibition of the mPTP pathway and release of cytochrome c, which restored the myocardial cellular damage, membrane integrity and protecting the cardiac membrane from being damaged. In addition, phenolic compounds may also have been accomplished by reducing oxidative stress by hampering the function of various signaling pathways, including mitogen-activated protein kinase (MAPK) (such as extracellular signal-regulated kinase 1/2 (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK) and nuclear factor kappa light chain enhancer of activated B cells (NF-κB) pathways, which contribute to cellular damage, apoptosis, and inflammation [12, 20].

In previous research investigations, it was noted that DOX-treated exhibited prolonged QT intervals, much longer PR intervals, widened QRS complex amplitudes, non-specific ST segments and increased heart rate [30]. According to the results of the current study's analysis of all these ECG alterations, the P wave, QRS complex, QT interval, heart rate, arrhythmias, and undefined ST segment were significantly improved after treatment with RALE, which has antioxidant and anti-inflammatory properties, substantially via maintaining ion channel function through scavenging ROS and minimizing oxidative damage which promotes normal electrical transmission. Subsequently, it enhances ion channel function and suppresses the inflammatory response triggered by doxorubicin-induced cardiotoxicity, which might minimize inflammation-related electrical remodeling and arrhythmias by preventing the release of pro-inflammatory cytokines and immune cell infiltration. It modulates the intracellular calcium levels in cardiomyocytes and helps stabilize the aberrant calcium interactions which might help ECG outcomes revert to normal and reduce arrhythmias. It modifies the ion channels involved in cardiac repolarization, notably potassium channels, via changing ion channel activity. The aforementioned might be the result of normalizing the amplitude of the QRS complex and restoring prolonged QT intervals. Furthermore, by maintaining the health and functionality of cardiomyocytes, it improves cellular survival mechanisms, inhibits apoptosis, and encourages the regeneration of cardiac tissue. This decreases arrhythmogenic episodes and improves normal ECG rhythms. The RALE ECG alterations may have a preserving or cell membrane stabilizing impact on the myocardium. Therefore, the outcomes of the present investigation indicated that both RALE dosages (100 mg/kg and 250 mg/kg) significantly reduced these acute modifications in the ECG [21, 30–32].

Conclusion

In this present research study, the observed data against DOX-induced cardiotoxicity reports that both the high dose (250 mg/kg) and low dose (100 mg/kg) of ethanolic extract of *R. arboreum* leaf extract (RALE) which acted as a cardioprotective agent by showing significant impacts such as preventing the generation of ROS, hampering function of various signaling pathways related to the development of cardiotoxicity and regeneration of cardiac tissues.

Therefore, the observed data results of this research demonstrated that the ethanolic extract of RALE has a myocardial potency against DOX-induced cardiotoxicity in Wistar rats.

Abbreviations

DOX	Doxorubicin
RALE	Ethanolic extract of Rhododendron arboreum leaf
AST	Aspartate aminotransferase
ALT	Alanine transaminase
CK-MB	Creatine kinase-MB
CK-NAC	Creatine kinase-N-acetyltransferase
LDH	Lactate dehydrogenase

Acknowledgements

All authors would like to express their gratitude to Himalayan Pharmacy Institutes for providing us with laboratory facilities for this research study.

Authors' contributions

The final manuscript was reviewed and approved by all authors.

Declarations

Ethics of approval and consent to participate

Following the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), the Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was acquired from the Institutional Animal Ethics Committee for conducting the animal model study. The IAEC approval for the research work (No. HPI/2023/60/IAEC/PP-0194).

Competing interests

The authors affirm that they have no interest in conflict.

Author details

[1]Himalayan Pharmacy Institute, Majhitar, East Sikkim-737136 Sikkim, India.

Received: 20 June 2023 Accepted: 23 October 2023
Published online: 22 November 2023

References

1. Hassen HY, Ndejjo R, Van Geertruyden J-P, Musinguzi G, Abrams S, Bastiaens H. Type and effectiveness of community-based interventions in improving knowledge related to cardiovascular diseases and risk factors: A systematic review. Am J Prev Cardiol. 2022;10:100341. https://doi.org/10.1016/j.ajpc.2022.100341.
2. Ornato JP, Hand MM. Warning signs of a heart attack. Circulation. 2014;129. https://doi.org/10.1161/CIRCULATIONAHA.113.006126.
3. Parcha V, Yadav N, Sati A, Dobhal Y, Sethi N. Cardioprotective effect of various extract of *Rhododendron arborium* Sm flower on Albino rats. J Pharmacogn Phytochem. 2017;6:1703–7.
4. Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J Mol Cell Cardiol. 2012;52(6):1213–25. https://doi.org/10.1016/j.yjmcc.2012.03.006.
5. Ahmed AZ, Satyam SM, Shetty P, D'Souza MR. Methyl gallate attenuates Doxorubicin-induced cardiotoxicity in rats by suppressing oxidative stress. Scientifica (Cairo). 2021;2021:10–2. https://doi.org/10.1155/2021/6694340.
6. Cubbon RM, Lyon AR. Cardio-oncology: Concepts and practice. Indian Heart J. 2016;68:S77-85. https://doi.org/10.1016/j.ihj.2016.01.022.
7. Megías-Vericat JE, Martínez-Cuadrón D, Sanz MÁ, Poveda JL, Montesinos P. Daunorubicin and cytarabine for certain types of poor-prognosis acute myeloid leukemia: a systematic literature review. Expert Rev Clin Pharmacol. 2019;12:197–218. https://doi.org/10.1080/17512433.2019.1573668.
8. Antolín S, Acea B, Albaina L, Concha Á, Santiago P, García-Caballero T, et al. Primary systemic therapy in HER2-positive operable breast cancer using trastuzumab and chemotherapy: Efficacy data, cardiotoxicity and long-term follow-up in 142 patients diagnosed from 2005 to 2016 at a single institution. Breast Cancer Targets Ther. 2019;11:29–42. https://doi.org/10.2147/BCTT.S179750.
9. Meyer M, Seetharam M. First-Line Therapy for Metastatic Soft Tissue Sarcoma. Curr Treat Options Oncol. 2019;20:6. https://doi.org/10.1007/s11864-019-0606-9.
10. Chakraborty M, Kamath JV, Bhattacharjee A. Potential interaction of green tea extract with hydrochlorothiazide against doxorubicin-induced myocardial damage. J Ayurveda Integr Med. 2015;6:187–93. https://doi.org/10.4103/0975-9476.146555.
11. Yu P, Wang J, Xu GE, Zhao X, Cui X, Feng J, Sun J, Wang T, Spanos M, Lehmann HI, Li G. RNA m6A-regulated circ-ZNF609 suppression ameliorates doxorubicin-induced cardiotoxicity by upregulating FTO. JACC. 2023. https://doi.org/10.1016/j.jacbts.2022.12.005
12. Ikewuchi CC, Ikewuchi JC, Ifeanacho MO. Aqueous leaf extracts of *Chromolaenaodorata* and *Tridaxprocumbens* attenuated doxorubicin-induced pulmonary toxicity in Wistar rats. Biotechnologia. 2021;102(4):387. https://doi.org/10.5114/BTA.2021.111096.
13. Schirone L, D'ambrosio L, Forte M, Genovese R, Schiavon S, Spinosa G, et al. Mitochondria and Doxorubicin-Induced Cardiomyopathy: A Complex Interplay. Cells. 2022;11:1–16. https://doi.org/10.3390/cells11132000.
14. Pan SY, Litscher G, Gao SH, Zhou SF, Yu ZL, Chen HQ, et al. Historical perspective of traditional indigenous medical practices: The current renaissance and conservation of herbal resources. Evid Based Complement Altern Med. 2014;2014:525340. https://doi.org/10.1155/2014/525340.
15. Bhatt N. Cardio protective property of *Rhododendron arboreum*. Can J Clin Nutr. 2018:186–94. https://doi.org/10.14206/canad.j.clin.nutr.2018.01.12.
16. Verma N, Singh AP, Amresh G, Sahu PK, Rao C V. Protective effect of ethyl acetate fraction of *Rhododendron arboreum* flowers against carbon tetrachloride-induced hepatotoxicity in experimental models. 2011;43:291–6. https://doi.org/10.4103/0253-7613.81518
17. Nortjie E, Basitere M, Moyo D, Nyamukamba P. Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: a review. Plants. 2022;11(15):2011. https://doi.org/10.3390/plants11152011.
18. Mahmood T, Bagga P, Siddiqui MH, Fareed S. Determination of total phenol & flavonoid content in seeds of *Psoraleacorylifolia*. Nat Prod Commun. 2011;7:171–3.
19. Mudagal MP, Karia S, Goli D. Preventive effect of *Rhododendron arboreum* on cardiac markers, lipid peroxides and antioxidants in normal and isoproterenol-induced myocardial necrosis in rats. AJPP. 2011;5(6):755–63. https://doi.org/10.5897/AJPP11.007.
20. Sandamali JA, Hewawasam RP, Jayatilaka KA, Mudduwa LK. Cardioprotective potential of Murraya koenigii (L) Spreng leaf extract against doxorubicin-induced cardiotoxicity in rats. Evid Based Complement Alternat Med. 2020;2020:6023737. https://doi.org/10.1155/2020/6023737.
21. Kinoshita T, Yuzawa H, Natori K, Wada R, Yao S, Yano K, Akitsu K, Koike H, Shinohara M, Fujino T, Shimada H. Early electrocardiographic indices for predicting chronic doxorubicin-induced cardiotoxicity. J Cardiol. 2021;77(4):388–94. https://doi.org/10.1016/j.jjcc.2020.10.007.
22. Arini PD, Liberczuk S, Mendieta JG, Santa María M, Bertrán GC. Electrocardiogram delineation in a Wistar rat experimental model. Comput Math Methods Med. 2018;8:2018. https://doi.org/10.1155/2018/2185378.
23. Jew S, AbuMweis SS, Jones PJ. Evolution of the human diet: linking our ancestral diet to modern functional foods as a means of chronic disease prevention. J Med Food. 2009;12(5):925–34. https://doi.org/10.1089/jmf.2008.0268.
24. Selleri S, Seltmann H, Gariboldi S, Shirai YF, Balsari A, Zouboulis CC, et al. Doxorubicin-induced alopecia is associated with sebaceous gland degeneration. 2006:711–20. https://doi.org/10.1038/sj.jid.5700175.
25. Stolarz AJ, Sarimollaoglu M, Marecki JC, Fletcher TW, Galanzha EI, Rhee SW, Zharov VP, Klimberg VS, Rusch NJ. Doxorubicin activates ryanodine receptors in rat lymphatic muscle cells to attenuate rhythmic contractions and lymph flow. J Pharmacol Exp Ther. 2019;371(2):278–89. https://doi.org/10.1124/jpet.119.257592.
26. Koti BC, Vishwanathswamy AHM, Wagawade J, Thippeswamy AHM. Cardioprotective effect of lipistat against doxorubicin induced myocardial toxicity in albino rats. Indian J Exp Biol. 2009;47:41–6. https://doi.org/10.4103/2349-5006.158219.
27. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology reports. 2019;1(24):e00370. https://doi.org/10.1016/j.btre.2019.e00370.
28. Razavi-Azarkhiavi K, Iranshahy M, Sahebkar A, Shirani K, Karimi G. The protective role of phenolic compounds against doxorubicin-induced cardiotoxicity: a comprehensive review. Nutr Cancer. 2016;68(6):892–917. https://doi.org/10.1080/01635581.2016.1187280.
29. Syahputra RA, Harahap U, Dalimunthe A, Nasution MP, Satria D. The role of flavonoids as a cardioprotective strategy against doxorubicin-induced cardiotoxicity: a review. Molecules. 2022;27(4):1320. https://doi.org/10.3390/molecules27041320.
30. Ahmed AZ, Mumbrekar KD, Satyam SM, Shetty P, D'Souza MR, Singh VK. Chia seed oil ameliorates doxorubicin-induced cardiotoxicity in female wistar rats: an electrocardiographic, biochemical and histopathological approach. Cardiovasc Toxicol. 2021;21:533–42. https://doi.org/10.1007/s12012-021-09644-3.
31. Villani F, Monti E, Piccinini F, Favalli L, Lanza E, Dionigi AR, Poggi P. Relationship between doxorubicin-induced ECG changes and myocardial alterations in rats. Tumori J. 1986;72(3):323–9. https://doi.org/10.1177/030089168607200315.
32. Warhol A, George SA, Obaid SN, Efimova T, Efimov IR. Differential cardiotoxic electrocardiographic response to doxorubicin treatment in conscious versus anesthetized mice. Physiol Rep. 2021;9(15):e14987. https://doi.org/10.14814/phy2.14987.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.