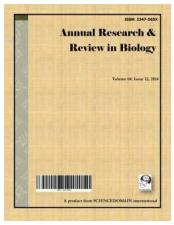

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Cardioprotective Effect of *Scleria lithosperma* on Doxorubicin-induced Cardiotoxicity in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CPK, PP and MM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors CPK, VJR and PP managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study was designed to evaluate the protective effects of ethanolic extract of whole plant of *Scleria lithosperma* (EEWSL) against doxorubicin-induced cardiotoxicity in rats.
Methodology: EEWSL was orally administrated in two different doses (250 mg/kg/day and 500 mg/kg/day) to wistar albino rats for 28 days and then intoxicated with doxorubicin (20 mg/kg) by intraperitoneal injection to induce myocardial toxicity. Lipid profile (Total cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein-cholesterol (LDL-C) and High Density Lipoprotein-cholesterol (HDL-C)), antioxidant marker enzymes (Cardiac superoxide dismutase, Cardiac catalase activity, Glutathione reductase activity) and liver diagnostic marker enzymes (SALT, SAST, Creatine phosphokinase, Lactate dehydrogenase) were measured at the end of experimental period. Histopathological changes of heart were observed with optical microscopy.

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Results: Doxorubicin (DOX) alone injected rats showed altered lipid profile and significant increase in serum markers (Serum glutamate pyruvate transaminase, Serum glutamate oxaloacetate tranaminase, Lactate dehydrogenase and Creatine phosphokinase) of heart injury and lipid peroxidation. Levels of endogenous antioxidant enzymes were also decreased when compared to normal control group. EEWSL pretreatment of DOX-challenged rats significantly reduced the risk of cardiotoxicity by decreasing the levels of liver diagnostic marker enzymes, TC, TG, LDL-C and VLDL-C and increasing the levels of HDL-C and antioxidant enzymes (cardiac superoxide dismutase, Cardiac catalase activity, Glutathione reductase activity) Histopathology of DOX- induced heart of rats pretreated with EEWSL showed a significant recovery from necrosis.
Conclusion: Current findings suggest that EEWSL has protective effects against DOX induced cardiotoxicity and this can be attributed due to its antioxidant properties and inhibition of lipid peroxidation.

Keywords: Doxorubicin; Scleria lithosperma; cardiotoxicity; antioxidants.

1. INTRODUCTION

Cytotoxic chemotherapeutic drugs induced cardiotoxicity is the leading cause of mortality and morbidity in both developing and developed countries. Overall 41% of cancer patients would suffer cardiotoxicity by administration of anti-cancer drugs [1]. Chemotherapeutic drugs being effective in treating various malignancies, particularly anthracycline group of antibiotics namely doxorubicin exhibit potentially toxic degenerative cardiomyopathy, congestive heart failure and dose-dependent side effects on myocardial tissues. The mechanism of doxorubicin-induced cardiotoxicity includes free radical production, membrane lipid peroxidation [2-4], mitochondrial dysfunction calcium overload [5,6], iron-dependent oxidative damage to macromolecule [7], superoxide accumulation and peroxynitrite formation [8]. The heart is particularly susceptible to free radical injury, as it contains less free radical detoxifying enzymes (superoxide dismutase, glutathione and catalases) than the other metabolic organs such as liver or kidney [9]. Oxidative stress is now considered as the cornerstone for cardiomyocyte cell death by apoptosis or cell necrosis [10].

Recently there has been renewed interest in the medicinal plants with antioxidant and free radical scavenging properties, which are known to have preventive roles in the treatment of ischemic heart disease. *Scleria lithosperma*, a medicinal herb of cyperaceae family is also named as Florida Keys Nutrush. In traditional medicine, this plant extracts has been used as antiseptic, antipyretic, hepatoprotective, antinephritic and for enlargement of the stomach in children [11-13]. *S. lithosperma* rhizome paste mixed with water is applied externally to treat eczema, leucoderma and scabies [14]. The cardioprotective effect of *Scleria lithosperma* against doxorubicin-induced myocardial changes have not been previously investigated. In the present study, we examined the efficacy of the ethanolic extract of whole plant of *Scleria lithosperma* against DOX-induced cardiotoxicity in rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Doxorubicin hydrochloride was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals were of the highest grade and commercially available.

2.2 Plant Materials and Extraction

Whole plant of *Scleria lithosperma* was collected from Tirumala forest, Tirupati, India. The plant was identified and authenticated by Prof. K. Madhavachetty, Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, India.

Fresh plant material was washed under running tap water, dried in the shade and then homogenized to fine powder. 300 g of dried plant powder was extracted with 95% ethanol in a soxhlet extractor. Extract was concentrated under reduced pressure using rota flash evaporator and the percentage yield of the ethanolic extract was 7.8%. Since, the ethanolic extract was not soluble in water; it was suspended in 5% gum acacia. The extract was qualitatively tested for the presence of phytochemicals by TLC and test tube reactions [15,16].

2.3 Animals

Wistar albino rats weighing between 150-200 g, 5-6 week old were provided from Sri

Venkateshwara Enterprises, Bangalore, India. The animals were housed under standard environmental conditions (temperature 22±2℃; humidity 60±4%) with 11±1 h light/dark cycle at the animal house in large polypropylene cages. Rats were fed with standard rats chow and water *ad libitum*. All animal experiments were approved by the Institutional Animal Ethical Committee (1521/PO/a/11/CPCSEA) and all procedures were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals."

2.4 Induction of Experimental Myocardial Infarction (MI)

Doxorubicin hydrochloride was dissolved in sterile double distilled water and injected (20 mg/kg, IP) to rats after the last dose of the extract for induction of experimental MI [17].

2.5 Experimental Design

To study the protective effect of EEWSL on DOX-induced cardiotoxicity, rats were randomly assigned to five groups of six animals each and treated as follows: Group I served as normal control and were fed saline (0.75 ml/animal) orally for 28 days. Group II: served as extract control and received EEWSL (500 mg/kg), orally for 28 days. Group III served as Drug control and was treated with Saline (0.75 ml/animal) orally for 28 days + DOX 20 mg/kg body weight i.p after 28^{th} day.

Group IV rats were treated with EEWSL (250 mg/kg) orally for 28 days + DOX (20 mg/kg body weight IP after 28^{th} day). Group V rats were treated with EEWSL (500 mg/kg) orally for 28 days + DOX (20 mg/kg body weight IP after 28th day).

At the end of the experimental period (48 h, after doxorubicin injection or 30^{th} day for normal and extract control groups), all the rats were anaesthetized and blood samples were collected prior to sacrifice from the ophthalmic artery in the orbital rim. The collected blood was centrifuged at 2500 x g for 20 min to separate serum that were preserved for biochemical analysis. The animals were sacrificed and hearts was excised immediately and fixed in 10% formalin saline for histopathological examination.

2.6 Determination of Serum Marker Enzymes

The activities of serum ALT and AST were determined spectrophotometrically by the method of Mohur and Cook [18]. The cardiac enzymes, LDH in serum was determined by the method of King [19] and CPK was determined by the method of Okinaka et al. [20].

2.7 Determination of Serum Lipid Profile

The levels of serum total cholesterol, triglycerides were estimated by the method of Folch et al. [21]. Serum low density lipoproteins (LDL), high density lipoproteins (HDL) and VLDL fraction were determined according to the dual precipitation technique [22].

2.8 Determination of Antioxidant Enzymes

Cardiac superoxide dismutase (SOD) activity was determined by the method of Sun et al., [23] Cardiac catalase activity (CAT) was determined according to the method of Aebi [24]. Glutathione reductase activity was determined according to the method described by Staal et al. [25].

2.9 Determination of Lipid Peroxidation

Lipid peroxidation was measured by the method of Yagi [26].

2.10 Histopathological Examination

At the end of the study, Heart tissues from all groups of rats were cut at 0.5 µm thicknesses, mounted on slides, stained with hematoxylin and eosin (H&E) and examined under light microscope for histoarchitectural changes.

2.11 Statistical Analysis

The results were expressed as the mean ± SD. Statistical evaluation among the groups were performed using One-way ANOVA and Tukey's test. P value less than 0.05 was considered to be statistically significant.

3. RESULTS

3.1 Phytochemical Analysis

Phytochemical results revealed that ethanolic extract of whole plant of *Scleria lithosperma* (EEWSL) contains alkaloids, glycosides, flavonoids, tannins, phenolic and saponins.

3.2 Effect on Serum Marker Enzymes

The serum diagnostic marker enzymes of myocardial toxicity; SAST, SALT, LDH, CPK

were increased in the drug control group (Group III) by 50.67%, 103%, 79%, 47.4% compared to normal control rats (Group I). Pretreatment with different doses of EEWSL (250 mg/kg and 500 mg/kg) significantly reduced the levels of these enzymes when compared with drug control group (P<0.001).

Significant difference was not detected in EEWSL (500 mg/kg) alone-treated animals (Group II) as compared to the normal control rats (Table 1).

3.3 Effect on Lipid Profile

The levels of total cholesterol, triglycerides, LDL and VLDL were significantly increased and HDL was decreased in blood serum of drug control group (Group III) as compared to normal control rats (P<0.01). EEWSL pretreated groups (Group IV& V) significantly prevented these adverse changes and maintained the rats at nearly normal status. There was no significant lipid profile alteration observed in the extract control (Group II) as compared to the normal control rats (Table 2).

3.4 Effect on Antioxidants

Wistar albino rats treated with doxorubicin (Group III) showed significant decrease in SOD, CAT and GR compared to the normal control rats (Group I). Pretreatment with EEWSL (250 mg/kg and 500 mg/kg) significantly increased SOD (8.7% and 28%), CAT (35% and 84%) and GR (53% and 70%) activities as compared to DOX-treated animals (P<0.0001). Animals treated with EEWSL-500 mg/kg alone (Group-II) showed levels of these antioxidant enzymes similar to normal control rats (Group I) (Table 3).

3.5 Effect on Myocardial Lipid Peroxidation

Myocardial lipid peroxidation was significantly increased in doxorubicin-treated animals (Group

Table 1. Effect of EEWSL extract on SAST, SALT, CPK and LDH in rats with doxorubicin - induced cardiotoxicity

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
SAST (IU/L)	31.83±1.738	32.24±2.981^{ns}	47.96±2.97****	37.6±1.347^{a}	30.81±1.683^{b}
SALT (IU/L)	19.15±1.268	24.683±1.817****	38.8±1.654****	23.91±1.13^{a}	20.01±1.731^{a}
CPK (IU/L)	127.8±1.613	131.06±3.028^{ns}	188.41±5.884****	121.9±1.551^{a}	105.53±4.289^{a}
LDH (IU/L)	81.84±2.127	83.05±1.691^{ns}	147.31±4.521****	105.28±3.365^{a}	97.08±3.788^{a}

*Values are expressed as mean ± SD; n= 6; ns =non significant, **** P<0.001 w.r.t normal control, a P<0.0001, b p<0.001 w.r.t 1 DOX-treated group (One way ANOVA followed by Tukey's test)*

Table 2. Effect of DOX and EEWSL extract on serum total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, and VLDL-cholesterol in different groups

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Total cholesterol (mg/dL)	61.08±1.742	56.25±2.852*	101.16±4.383****	95.75±1.709^{a}	86.65±2.563^{d}
Triglycerides (mg/dL)	51.16±2.544	43.4±3.214***	65.83±3.940****	59.6±2.796^{a}	55.5±3.065^{d}
LDL (mg/dL)	42.45±2.468	45.91±3.304^{ns}	65.13±4.257***	57.5±2.736^{b}	45.48±3.013^{d}
HDL (mg/dL)	24.76±2.314	25.78±3.694^{ns}	16.63±3.146***	22.66±1.097^{d}	24.73±2.33^{c}
VLDL (mg/dL)	12.83±3.435	10.21±1.291^{ns}	16.16±3.209^{ns}	14.65±1.084^{ns}	11.2±0.683^{a}

*Values are expressed as mean ± SD; n= 6; a P<0.05, b P<0.01, c P<0.001, d P<0.0001 w.r.t DOX-treated group, * P<0.05, *** P<0.01, **** P<0.0001 w.r.t normal control group (One way ANOVA followed by Tukey's test)*

Table 3. Effect of EEWSL extract and DOX on myocardial superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) in different groups

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
SOD	8.6±0.458	6.73±0.46***	5.23±1.276****	9.35±0.411^{a}	11.05±0.214^{a}
CAT	7.76±0.205	6.4±0.404****	5.5±0.341****	10.55±0.34^{a}	14.28±0.362^{a}
GR	5.75±0.345	5.35±0.390^{ns}	4.78±0.318***	8.2±0.286^{a}	9.8±0.326^{a}

*Values are expressed as mean ± SD; n= 6; *** P<0.001, **** P<0.0001 w.r.t normal control group. a P< 0.0001 w.r.t Dox treated group (One way ANOVA followed by Tukey's test)*

III) as compared to the normal control animals (P<0.0001). Pretreatment with EEWSL (Group IV& V) showed significant protection from lipid peroxidation. TBRS levels in EEWSL-500 mg/kg alone-treated animals remain unchanged as compared to the normal control animals (Table 4).

3.6 Histopathological Results

Histopathological examination of the myocardium of normal rats showed normal myocardial architecture and regular cell distribution (Fig. A). The tissue sections of the extract alone treated rats showed normal myofibrillar structure with mild degenerative changes (Fig. B). Rats treated with DOX (20 mg/kg) revealed degenerative changes with collapsed myocardial fibers, myocytic necrosis, increase in inflammatory cells and infiltration of lymphocytes (Fig. C). Heart tissue sections of EEWSL (250 mg/kg) pretreated and doxorubicin challenged group (Group IV) showed mild infiltration of lymphocytes. Group V (EEWSL-500 mg/kg + Doxorubicin) showed less infiltration of inflammatory cells. Both the treatment groups (EEWSL-250+DOX, EEWSL-500+DOX) revealed histoarchitecture similar to that of normal rats and there was no evidence of focal necrosis (Fig. D, E).

4. DISCUSSION

The current study entails the cardioprotective potential of the *Scleria lithosperma* whole plant extract against doxorubicin induced cardiotoxicity for the first time. Scleria lithosperma, a medicinal herb of Cyperaceae family is considered as a remedy for heart diseases in Indian system of folklore medicine. Doxorubicin continues to be an effective and widely used broad spectrum chemotherapeutic agent. However, its clinical use is limited because of its serious dose-dependent cardiotoxicity [27]. Clinical and experimental evidences suggested that increased oxidative stress plays a critical role in subsequent cardiomyopathy and heart failure associated with DOX treatment [28,29].

Phytochemical investigation of EEWSL revealed the presence of glycosides, tannins, phenolic, saponins, alkaloids and flavonoids which are responsible for potent antioxidant property. Herbal medicines possessing antioxidant and free radical scavenging activities may have a protective role in cardiovascular disease and provide viable alternatives [30].

Myocardium contains plentiful concentrations of diagnostic markers of the myocardial and once metabolically damaged, it releases its contents into the extra cellular fluid infarction [31]. Current investigation showed significant elevation in the levels of diagnostic marker enzymes (SAST, SALT, CPK, and LDH) in the serum of DOX-injected rats. This is due to increased susceptibility of myocardial cell membrane to oxidative damage upon doxorubicin treatment [32]. Pretreatment of EEWSL significantly lowered the serum levels of diagnostic marker enzymes in DOX induced myocardial infarction by maintaining the integrity of myocardial cell membrane. This might be due to free radical scavenging property of antioxidative phytochemicals such as flavonoids present in the plant.

Lipid composition plays an important role in maintaining structural stability of myocardium. Hyperlipidaemia, accumulation of free cholesterol and LDL cholesterol in the myocardial tissue is associated with the development of cardiovascular disease. Results of the present study in doxorubicin treated animals revealed its hyperlipidemic effect by increasing the levels of serum total cholesterol, TG, LDL and decrease in the HDL when compared to normal control animals. Cardiotoxicity of doxorubicin might be due to interference in lipid metabolism and enhanced membrane degradation [1]. Pretreatment with EEWSL showed a significant decline in the levels of serum total cholesterol, TG, LDL, VLDL and increase in HDL and maintained the rats at nearly normal status indicating reduced cholesterol biosynthesis and increased LDL uptake by liver. Protective effects of EEWSL are due to presence of phytochemicals like saponins and flavonoids.

Table 4. Effect of DOX and EEWSL extract on myocardial lipid peroxidation (LP) in different groups

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
LP(μM/g tissue)	143.01±4.074	149±4.423^{ns}	212.45±6.05****	174.3±5.073^{a}	151.86±3.199^{a}

*Values are expressed as mean ± SD; n= 6; ns = non significant, **** P<0.0001 w.r.t normal control group. a P < 0.0001w.r.t Dox-treated group (One way 178 ANOVA followed by Tukey's test)*

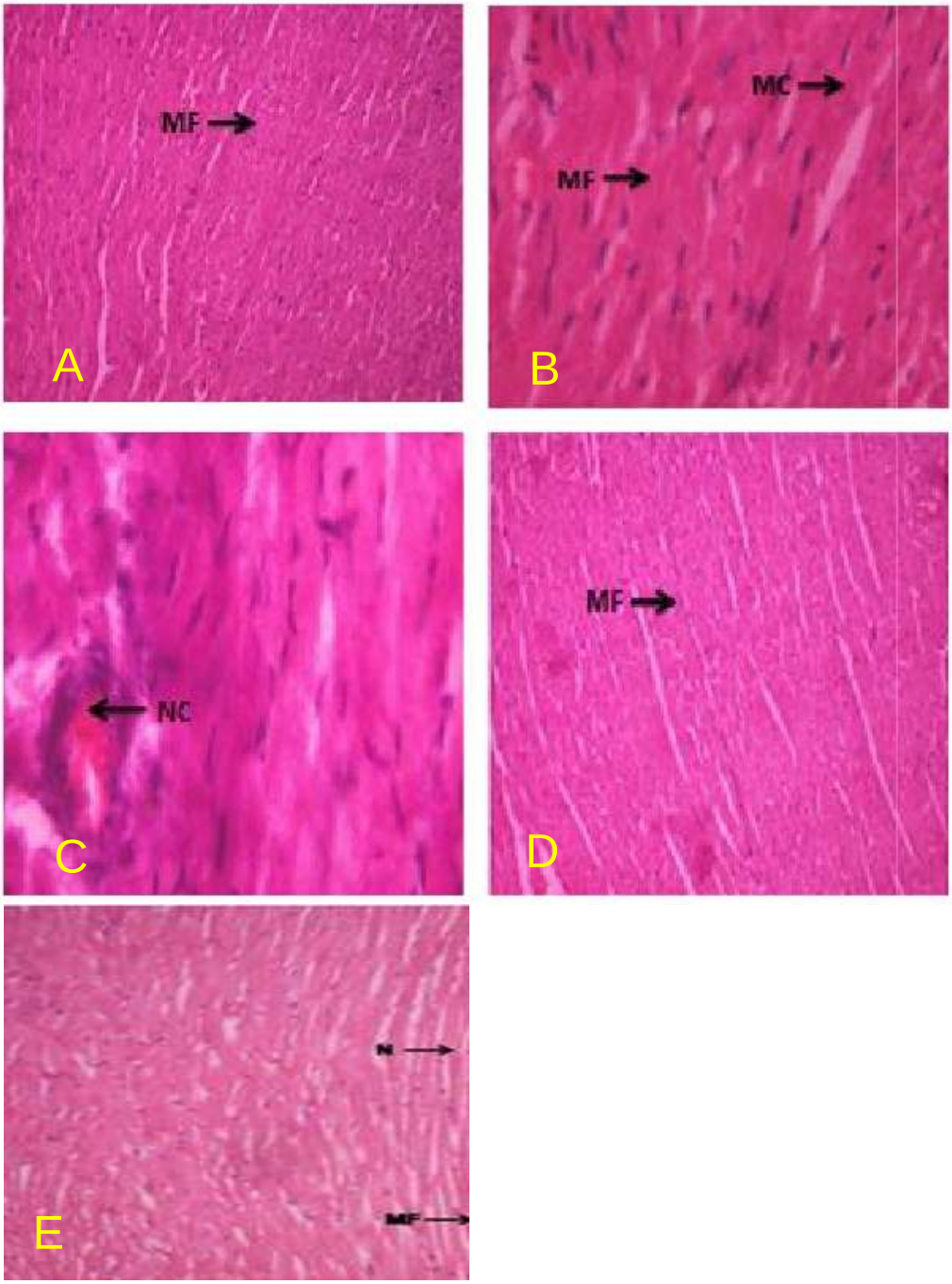

Fig. 1. Photomicrograph of the histopathological changes in heart tissue of the experimental groups. (A) Group-I (normal); (B) Group 201 (Extract control) (C) Group-III (Doxorubicin treated) (D) Group-IV (EEWSL-250mg +DOX)II 202 (E) Group-V (EEWSL-500mg+DOX)

MF: Myocardial fibres, MC: Myocardial cells, NC: Necrosis

High serum levels of HDL were associated with reduced risk for the development of atherosclerotic diseases. HDL particles were believed to be antiatherogenic, secondary to their capacity to drive cholesterol transport and antagonize pathways of inflammation, thrombosis, oxidation [33].

Generation of reactive oxygen species (ROS) is the main side effect of doxorubicin. At cellular level, quinone form of doxorubicin gets reduced to semiquinone by interacting with NADH or NADPH oxidases. Semiquinone undergoes autooxidation in presence of oxygen and generates superoxide anion and then free radicals. Antioxidant acts as a first line of defense against oxidative stress. Antioxidant enzymes such as SOD, CAT and GR are easily inactivated by lipid peroxides or ROS, which results in the decreased activities of these enzymes in doxorubicin induced cardiotoxicity rats [34]. Oral pretreatment with EEWSL improved the activities of SOD, catalase and GR by scavenging superoxide anions and hydrogen peroxides produced by doxorubicin. This might be due to balance between oxidation and antioxidation system and increased antioxidant enzymes.

Cardioprotective activity of EEWSL was further supported by increased myocardial antioxidant enzyme activity and decreased lipid peroxidation extent. One of the principal causes of doxorubicin induced cardiotoxicity was lipid peroxidation, which is reported to cause cellular damage and is primarily responsible for ROS-induced organ damage [35]. The degree of lipid peroxidation was evaluated by estimating thiobarbituric acid reactive substances (TBRS). The current studies have shown a reduction in lipid peroxidation in the group IV and V when compared to DOX-treated rats, which proves stress stabilizing action of Scleria lithosperma, as a result of enhanced antioxidant system.

The histopathological examination of doxorubicin alone treated animals showed severe loss of myofibrils, inflammation, focal necrosis, and oedema. The severity of the histopathological changes were much less in sections from EEWSL pretreated and doxorubicin challenged animals. Thus, the observed maintenance of the myocardial cell membrane integrity would lead to decreased leakage of cardiac enzyme markers.

5. CONCLUSION

The present study provided experimental evidence that EEWSL had shown to reduce doxorubicin-induced cardiotoxicity. EEWSL proved to be effective in reducing the myocardial damage, lipid peroxidation and in maintaining lipid homeostasis. The cardioprotective potential might be due to its antioxidant action. Study of individual phytoconstituents of the *Scleria lithosperma* extract and their mechanisms of action are current under investigation in our laboratory.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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