Turk J Pharm Sci 2021;18(3):271-281
DOI: 10.4274/tjps.galenos.2020.27880

ORIGINAL ARTICLE

Cardioprotective Effect of *Marsdenia tenacissima* and *Sansevieria roxburghiana* in Doxorubicin-induced Cardiotoxicity in Rats *in vivo*: The Role of Dresgenin and Lupeol

Sıçanlarda Doksorubisin Kaynaklı Kardiyotoksisitede *Marsdenia tenacissima* ve *Sansevieria roxburghiana*'nın *in vivo* Kardiyoprotektif Etkisi: Dresgenin ve Lupeol'ün Rolü

Aparna Ann MATHEW, Raju ASIRVATHAM*, Dawn V TOMY

St. Joseph's College of Pharmacy, Department of Pharmacology, Kerala, India

ABSTRACT

Objectives: The major adverse effect of doxorubicin (DOX) in cancer treatment is cardiac toxicity. Murva is a controversial plant used in the Ayurvedic system, which consist of more than 12 medicinal plant roots found in different parts of India. *Marsdenia tenacissima* (MT) is an acceptable source in Murva, whereas *Sansevieria roxburghiana* (SR) Schult & Schult.f. (*S. zeylanica* Roxb.) are also considered as Murva in West Bengal, India. The present study focused on the evaluation of the cardioprotective mechanism as well as the *in vivo* cardioprotective potential of methanol extracts of MT and SR on rats by using *in silico* methods.

Materials and Methods: A total of 48 rats were divided into 8 groups with 6 in each group. DOX 20 mg/kg, intraperitoneally (i.p.) was administered to all rats on the 13th day, with the exception of group 1. Group 2 was the disease control, group 3 was the treated with the standard drug propronolol, and groups 4 to 5 were treated with two lower doses of methanol extract of MT (MEMT) and methanol extract of SR (MESR), whereas group 7 received higher dose combinations of both extracts for 14 continuous days. Blood and tissue antioxidant levels as well as cardiac enzymes were measured at the end of the study. Damage to cellular functional units was analyzed by histopathological study. Dresgenin from MT similarly lupeol from SR were taken as ligands for the target peroxisome proliferator activated receptors (PPARα) protein to find out the mechanism of action. High-performance thin layer chromatography (HPTLC) fingerprinting was performed to determine the number of phytoconstituents present in both extracts.

Results: The combination that showed the most significant ($p<0.001$) effect on altered cardiac enzymes and antioxidant enzyme levels in both blood and tissues also corrected the extreme damage in cellular functional units. Dresgenin and lupeol showed binding scores of -8.2 (kcal/mol) and -9 (kcal/mol), respectively, with PPARα. HPTLC reports revealed that 17 and 12 peaks were found at 254 nm for dresgenin and lupeol, respectively.

Conclusion: The study results concluded that the combination of MESR and MEMT and that of MESR and MEMT exerted cardioprotective activity via binding of dresgenin and lupeol to PPARα. The order of efficacy was the extract combination > MESR > MEMT.

Key words: Doxorubicin, cardioprotective, molecular docking, cardiac enzyme, HPTLC

ÖZ

Amaç: Doksorubisinin (DOX) kanser tedavisinde en önemli yan etkisi kardiyak toksisitedir. Murva, Hindistan'ın farklı bölgelerinde bulunan 12'den fazla tıbbi bitki kökünden oluşan Ayurvedik sistemde kullanılan tartışmalı bir bitkidir. *Marsdenia tenacissima* (MT), Murva'da kabul edilebilir bir kaynak iken, *Sansevieria roxburghiana* (SR) Schult & Schult.f. (*S. zeylanica* Roxb.) da Batı Bengal, Hindistan'da Murva olarak kabul edilmektedir. Bu çalışmada, MT ve SR metanol ekstraktlarının *in siliko* yöntemler kullanılarak *in vivo* kardiyoprotektif potansiyelinin yanı sıra kardiyoprotektif mekanizmanın değerlendirilmesi amaçlanmıştır.

*Correspondence: rajuasirvatham@gmail.com, Phone: +91 9488182049, ORCID-ID: orcid.org/0000-0002-7939-4975

Received: 17.03.2020, Accepted: 01.06.2020

©Turk J Pharm Sci, Published by Galenos Publishing House.

Gereç ve Yöntemler: Her grupta 6 tane olmak üzere toplam 48 sıçan 8 gruba ayrıldı. DOX 20 mg/kg intraperitoneal (i.p.) olarak grup 1 hariç tüm sıçanlara 13. günde uygulandı. Grup 2 hastalık kontrolü, grup 3 standart ilaç propronolol ve 4'ten 5'e kadar olan gruplar, iki farklı düşük dozda MT metanol özütü (MEMT) ve SR metanol özütü (MESR) ile tedavi edilirken, grup 6, 14 ardışık gün boyunca her iki özütün daha yüksek doz kombinasyonlarını aldı. Çalışmanın sonunda kan ve doku antioksidan seviyeleri ile kardiyak enzimler ölçüldü. Hücresel fonksiyonel birimlere verilen hasar histopatolojik çalışma ile değerlendirildi.

MT'den Dresgenin benzer şekilde SR'den elde edilen lupeol, etki mekanizmasını bulmak için hedef peroksizom proliferatör aktive reseptörler (PPARα) proteini için ligand olarak alındı. Her iki ekstraktta bulunan bitki bileşenlerinin sayısını belirlemek için yüksek performanslı ince tabaka kromatografisi (HPTLC) parmak izi yapıldı.

Bulgular: Hem kanda hem de dokularda değişen kardiyak enzimler ve antioksidan enzim düzeyleri üzerinde en önemli etkiyi ($p<0,001$) gösteren kombinasyon, hücresel fonksiyonel birimlerdeki aşırı hasarı da düzeltti. Dresgenin ve lupeol, PPARα ile sırasıyla -8,2 (kcal/mol) ve -9 (kcal/mol) bağlanma skorları gösterdi. HPTLC raporları, sırasıyla dresgenin ve lupeol için 254 nm'de 17 ve 12 pik bulunduğunu gösterdi.

Sonuç: Çalışma sonunda, MESR ve MEMT ile MESR ve MEMT kombinasyonunun, dresgenin ve lupeolün PPARα'ya bağlanması yoluyla kardiyoprotektif aktivite sergilediği sonucuna varmıştır. Etkinlik sırası özüt kombinasyonu > MESR > MEMT idi.

Anahtar kelimeler: Doksorubisin, kardiyoprotektif, moleküler yerleştirme, kardiyak enzim, HPTLC

INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic used for the treatment of various neoplastic disorders, shows severe organ toxicity when used clinically. Cardiotoxicity is the major fatal event that occurs in pediatric and adult patients at the normal therapeutic dose and is characterized by irreversible damage of cardiac muscle leading to a major cause of chemotherapy-associated morbidity and mortality. Even though several less toxic derivatives of DOX are available for chemotherapy, cardiotoxicity induction is taken as the major concern, and the use of traditional anticancer drugs is preferred. Cardioprotective adjuvants such as leucovorin, mesna, angiotensin receptor blockers, and beta blockers are available and are administered along with DOX to reduce cardiotoxicity. These adjuvants exhibit marked cardioprotection and do not compromise the anticancer activity of DOX.[1] Hence, a new strategy was developed in pharmaceutical industries to establish a formulation with extended cardioprotective activity without compromising the cancer chemotherapeutic efficacy of DOX. Under this concept, recently, medicinal herbs and their formulations received greater attention on the treatments of various lives threatening disease because of their efficacy and rapid curative properties. Among the available herbal preparations, Ayurvedic formulations have been placed at the top of the list for many thousands of years due to their low toxicity and wide acceptability.[2] One such Ayurvedic plant is Murva. It is a controversial drug, containing more than 12 medicinal plants roots found in different parts of India. *Marsdenia tenacissima* (MT) is an acceptable source of Murva, whereas *Sansevieria roxburghiana* (SR) Schult and Schult.f. (*S. zeylanica* Roxb.) are considered as Murva in West Bengal, India. The remaining plants in Murva, their scientific names, and their source locations are *Helicteres isora L* (Sterculiaceae) from Punjab, *Maerua arneria* (Capparaceae) from Bihar, *Chonomorpha fragrans* (Apocynaceae) from Kerala, *Clematis triloba* (Ranunculaceae), *Bauhinia tomentosa* (Leguminoseae) and SR Schult & Schult.f. (*S. zeylanica* Roxb.) from West Bengal, *Wattakaka volubilis* (Linn. f.) Stapf (Asclepidaceae) and *Salvadora persica L* (Salvadoraceae) from South India, *Argyreia nerova* (Convolvulaceae), *Maerua oblongifolia* (Capparaceae), and *Dregea volubilis* (Apocynaceae) from other regions of India.[3] Traditionally, Murva is used for the treatment of anemia, diabetes, stomach disorders, typhoid, cough, fever, and urinary tract infections.[4] Based on a review of the literature, MT is traditionally used for heart diseases,[3,5] similarly, SR is used as a cardiotonic.[6] However, the effects of these drugs on the hearts of animals have not been tested experimentally. Therefore, this study aimed to evaluate the cardioprotective effect of extracts of SR and MT on DOX-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Experimental animals

Wistar albino rats (200-250 g) were obtained from the animal housing facility of St. Joseph's College of Pharmacy, Cherthala, Kerala, India and then acclimatized for 1 week under standard controlled conditions (12 hours light/12 hours darkness, at 25°C). The study protocol (SJCP/IAEC/2018-4/35) was approved by the Institutional Animal Ethics Committee (IAEC), St. Joseph's College of Pharmacy Cherthala, Kerala, India.

Plant materials, drugs, and chemicals

Roots and rhizomes of SR and MT were collected from Kerala in the month of October 2018. MT was identified and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University Tirupathi A.P. A herbarium specimen was deposited at the Department of Botany, Sri Venkteswara University (voucher number 1132). SR was identified and authenticated by Dr. Jose Mathew, Assistant Professor, Department of Botany, Santana Dharma College, Alappuzha. The herbarium specimen (no: AAM001) was deposited at the Department of Botany, Santana Dharma College, Alappuzha, Kerala, India.

DOX was procured from Dabur Pharmaceuticals Ltd., New Delhi, India. Propranolol was purchased from Cipla Ltd., India. Lactate dehydrogenase (LDH), choline kinase (CK), aspartate transaminase, and other assay kits were purchased from Accurex biomedical Pvt. Ltd., India. All other chemicals used during the study were of analytical grade.

Extraction

The roots of MT and the rhizome and roots of SR were cleaned and dried at room temperature (shade dried). Approximately 300

g of defatted, coarse powered drug was successively extracted in a Soxhlet apparatus with methanol (70-80˚C for 48 hours). Methanol extract of MT (MEMT) and SR (MESR) and aqueous extract of MT and SR were collected with a rotary evaporator followed by drying and storage in an airtight container for experimental purposes.

Molecular docking

The protein selected for the cardioprotective study was peroxisome proliferator activated receptors (PPARα) with PDB ID: 1K7L. Three-dimensional (3D) structures were downloaded from a protein data bank (www.rcsb.org). Protein was prepared by eliminating water and small molecules by using Pymol software. Chemical constituents, such as tenasogenin, cissogenin, tenacigenin-C, tenacigenoside, and dresgenin from MT and 6-methyl-1-octanol, diethyl phthalate, methyl hexadecanoate, 3, 3-dimethylhexanal, and lupeol from SR, were selected as the ligands. PubChem was used to retrieve the 3D structures of ligands in SDF format, and Openbabel 2.3.2 was used to convert them to PDB format. Ligands, important chemical constituents, and targets in PDB format were loaded into autodock vina PyRx. The binding energy with the least RMSD (upper and lower) were selected and expressed in kcal/mol. At the first dock, the pdb.qt files for protein and ligand were prepared.[7,8] The ligands and targets in pdb.qt format were loaded into Pymol for visualization. From the visualization, the number of hydrogen bonds and sequence of amino acids to which the ligand bound were obtained.

Design of the cardioprotective activity study

A total of 48 Wistar rats of both sexes weighing 180-220 g were divided into eight groups containing six animals in each. Standard and test drugs were administered to the respective groups of animals once daily for 14 consecutive days.[9-11] Group 1, the normal control, was treated with distilled water orally. Group 2 served as the disease control and received DOX 20 mg/kg intraperitoneally (i.p.) only. Group 3 received the standard propranolol 10 mg/kg orally and DOX 20 mg/kg, i.p. on the 13th day. Groups 4 and 5 received 100 mg/kg and 200 mg/kg of MEMT orally and DOX 20 mg/kg i.p. on the 13th day. Groups 6 and 7 received 50 mg/kg and 100 mg/kg MESR orally and DOX 20 mg/kg, i.p. on the 13th day. Group 8 received the combination of 100 mg/kg of MESR and 200 mg/kg MEMT orally and DOX 20 mg/kg i.p. on the 13th day. All animals were challenged by using single-dose administration of DOX 20 mg/kg i.p. on the 13th day, except group 1 animals. After 48 hours of DOX administration, blood was collected, and animals were sacrificed for isolation of the vital organs such as the liver, kidney, and heart for histopathological studies. Blood and liver antioxidant markers such as superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) levels and cardiac enzymes such as CK-MB, and LDH 1 were estimated with Accurex biomedical Pvt. Ltd., India using a semi autoanalyzer.

Histopathology study

Organs such as the heart, liver, and kidneys were isolated immediately after the animal was sacrificed, washed with ice-cold normal saline, trimmed, and placed in 10% formaldehyde. The organs were sectioned and stained with haematoxylin and eosin. The structures were examined under a light microscope at 10X and 40X magnification by a pathologist blinded to the groups under study.[12]

Estimation of tissue antioxidant levels

Isolated hearts were divided in to two portions for the preparation of homogenates: 10% (w/v) homogenate in potassium chloride (0.15 M) and 10% (w/v) homogenate in 0.25% (w/v) sucrose in phosphate buffer (5 M pH 7.4). Both homogenates were centrifuged at 8000x g for 10 minutes. The supernatant from the first homogenate was used for the estimation of MDA, and the supernatant from the second homogenate was used for the estimation of SOD and GSH.[13] All estimations were conducted according to the manufacturer's manual of reagents by Accurex biomedical Pvt. Ltd., India.

High-performance thin layer chromatography HPTLC analysis

HPTLC fingerprinting analysis was performed with CAMAG LINOMAT 5, where 2 µL of sample was applied by using a Hamilton syringe on a $60F_{254}$ TLC plate as a band length of 5 mm. Later, it was kept in a TLC developing chamber, which was saturated with solvent vapor (mobile phase) of toluene: ethyl acetate: methanol (7:3:1). The plate was then dried with hot air, placed in a photo documentation chamber, and scanned at 254 nm, 366 nm, and 550 nm, following derivatization with anisaldehyde-sulfuric acid reagent.[14]

Statistical analysis

In vivo data were expressed as the mean ± standard error of the mean of six values. The difference between experimental groups was compared with the negative control and normal control by One-Way ANOVA followed by Newman-Keul's multiple comparison test, where $p<0.05$ implied significance.

RESULTS

Docking scores, binding energies, hydrogen bonds, and binding sites were obtained from the various isolated chemical constituents of MT and SR used as ligands for the PPARα receptor, and the results are presented in Table 1, 2. Dresgenin from MT and lupeol from SR showed higher docking scores of -8.2 (kcal/mol) and -9.1 (kcal/mol) respectively. Visualization of dresgenin with the PPARα receptor is shown in Figure 1, in which three hydrogen bonds were found at the 213ALA, 231GLY, and 216LYS positions. Similarly, visualization of lupeol on the PPARα receptor is shown in Figure 2, in which two hydrogen bonds were seen at the 214 TYR and 213 ALA positions.

Table 3 illustrates the effect of MEMT, MESR, and the combination of MEMT and MESR on cardiac enzymes of DOX-induced cardiotoxicity in rats. CK-MB and LDH 1 were increased in DOX control group rats, but rats treated with the combination of 100 mg/kg of MESR and 200 mg/kg of MEMT showed significantly ($p<0.001$) reduced levels of cardiac enzymes. Similarly, 50 and 100 mg/kg of MESR also significantly ($p<0.001$) reduced the CK-MB and LDH 1 enzymes, whereas 100 mg/kg MEMT ($p<0.01$) and 50 mg/kg of MESR ($p<0.05$) showed a less significant

Table 1. Docking scores of various ligands on the PPARα receptor

Sl. no	*Marsdenia tenacissima*		*Sansevieria roxburghiana*	
	Ligand	Docking score (kcal/mol)	Ligand	Docking score (kcal/mol)
1	Tenasogenin	-7.2	Lupeol	-9.1
2	Cissogenin	-7.1	6-methyl-1-octanol	-6.8
3	Tenacigenin-C	-6.8	Diethyl phthalate	-5.7
4	Tenacigenoside	-4.1	Methyl hexadecanoate	-5.5
5	Dresgenin	-8.2	3,3-dimethylhexanal	-4.6

PPAR: Peroxisome proliferator activated receptors

Table 2. Binding energy, hydrogen bonds, and binding sites of the targets

Ligands	PPAR alpha receptor		
	Binding score (kcal/mol)	Hydrogen bonds	Binding site
Dresgenin	-8.2	3	213ALA, 231GLY, and 216LYS
Lupeol	-9.1	2	214 TYR and 213 ALA

PPAR: Peroxisome proliferator activated receptors

Table 3. Effect of MEMT and MESR on serum cardiac marker enzymes

Treatment/parameters	CK-MB	LDH 1
Normal control	1035.67±3.91	1545.5± 3.56
DOX (20 mg/kg)	1920.17±5.78	3592.67±5.07
Propranolol (10 mg/kg)	1033±6.39	1633.83±14.11
MEMT (100 mg/kg)	1893±4.95[c]	3556.83±3.03[c]
MEMT (200 mg/kg)	1878.33±3.16[b]	3539.5±3.04[b]
MESR (50 mg/kg)	1803.83±13.87[c]	2892.17±14.72[c]
MESR (100 mg/kg)	1505.33±11.33[c]	2309±8.83[c]
MEMT (200 mg/kg) + MESR (100 mg/kg)	1104.5±6.49[c]	1961.83±12.01[c]

All values are expressed as mean ± SEM (n=6), One-Way ANOVA followed by Newman-Keul's multiple comparison test. [a]p<0.05, [b]p<0.01, [c]p<0.001 as compared with the doxorubicin group
DOX: Doxorubicin, MEMT: Methanol extract *Marsdenia tenacissima*, MESR: Methanol extract *Sansevieria roxburghiana*, CK: Choline kinase, LDH: Lactate dehydrogenase, SEM: Standard error of the mean

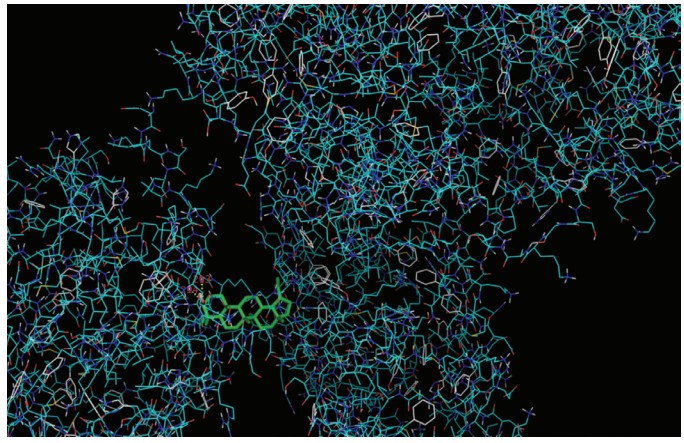


Figure 1. Visualization of docking in Pymol: PPARα with lupeol
PPAR: Peroxisome proliferator activated receptors

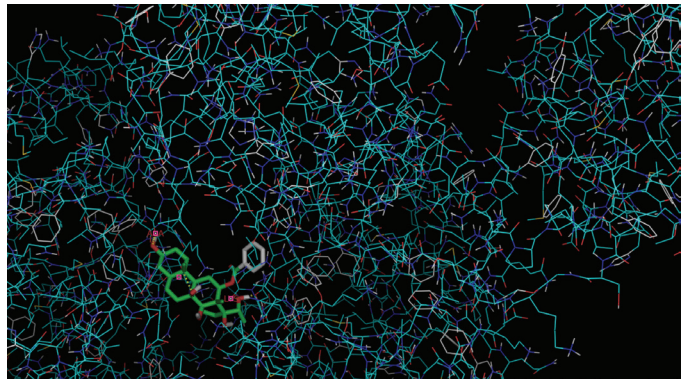


Figure 2. Visualization of docking in Pymol: PPARα with dresgenin
PPAR: Peroxisome proliferator activated receptors

effect on reduction of CK-MB and LDH 1 when compared with disease control animals. The standard drug propranolol showed a highly significant (p<0.001) reducing effect on the elevated levels of cardiac enzymes in DOX-induced cardiotoxicity in rats.

The effect of MEMT and MESR on tissue (heart) antioxidant enzymes of cardiotoxicity-induced rats are shown in Table 4. There was an increased level of MDA and a decreased level of SOD and GSH only in the 20 mg/kg DOX-treated disease control group. Both the doses of orally administered MESR and the combination of MESR + MEMT showed a highly significant (p<0.001) effect on the reduction of MDA as well as enhancement of SOD and GSH found with 14 days of treatment. In the case of MEMT, the 100 mg/kg dose showed a less significant (p<0.01)

effect on the increase in SOD, but the effect on the reduction of MDA and increase in the GSH level in the heart was non-significant (p>0.05), whereas 200 mg/kg of MEMT showed a significant alteration of the re-establishment of cardiac antioxidant enzyme levels with respect to that of normal rats.

The effects of a 14-day, single-dose oral feeding of MEMT and MESR on blood antioxidant enzymes of cardiotoxicity-induced rats are shown in Table 5. The levels of GSH and SOD of the disease control group were lower than those of normal controls (p<0.001). The MDA levels in the blood were higher than those of the normal control group (p<0.001). Treatment with 100 or 200 mg/kg of MEMT and 50 mg/kg or 100 mg/kg of MESR significantly (p<0.001) altered and nearly normalized MDA, SOD, and GSH levels, whereas the combination of both extracts showed a marked reversal of DOX-induced cardiotoxicity in rats.

Figure 3 and Table 6 represent the HPTLC fingerprint of MESR, which was applied in track 1 and was viewed at 254 nm. A total of 17 peaks were found with R_f values ranging from 0.01 to 0.86.

Figure 4 and Table 7 represent the HPTLC fingerprint of MESR, which was applied in track 2 and was viewed at 254 nm. A total of 12 peaks were found with R_f values ranging from 0.01 to 0.84.

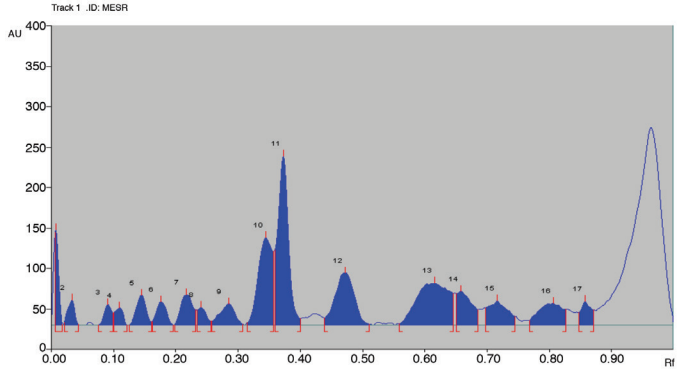


Figure 3. HPTLC fingerprint of MESR at 254 nm

HPTLC: High-performance thin layer chromatography, MESR: Methanol extract *Sansevieria roxburghiana*

Table 4. Effect of MEMT and MESR on tissue antioxidant enzymes of cardiotoxicity- induced rats

Treatment/parameter	SOD (U/mg protein)	MDA (nmol/g of protein)	GSH (mmol/g protein)
Normal control	45.28±0.15	48.47±0.44	10.58±0.39
DOX (20 mg/kg)	26.24±0.21	138.84±0.23	5.47±0.09
Propranolol (10 mg/kg)	37.36±0.38	45.20±0.64	9.99±0.30
MEMT (100 mg/kg)	27.24±0.18[b]	137.78±0.34[d]	6.00±0.07[d]
MEMT (200 mg/kg)	29.75±0.17[c]	136.87±0.23[b]	6.22±0.02[a]
MESR (50 mg/kg)	29.996±0.13[c]	80.4±0.297[c]	6.51±0.02[b]
MESR (100 mg/kg)	32.92±0.18[c]	68.77±0.74[c]	7.52±0.08[c]
MESR (100 mg/kg) + MEMT (200 mg/kg)	36.65±0.09[c]	53.54±0.51[c]	9.79±0.27[c]

All the values were expressed as mean ± SEM (n=6), One-Way ANOVA followed by Newman-Keul's multiple comparison test. [a]p<0.05, [b]p<0.01, [c]p<0.001, and [d]NS p>0.05 as compared with the doxorubicin group
DOX: Doxorubicin, MEMT: Methanol extract *Marsdenia tenacissima*, MESR: Methanol extract *Sansevieria roxburghiana*, SOD: Superoxide dismutase, MDA: Malondialdehyde, GSH: Glutathione, SEM: Standard error of the mean

Table 5. Effect of MEMT and MESR on blood antioxidant enzymes of cardiotoxicity-induced rats

Treatment/Parameter	SOD (U/mL serum)	MDA (nmol/mL)	GSH (U/L)
Normal control	15.21±0.5	8.47±0.44	70.48±0.37
DOX (20 mg/kg)	1.04±0.21	38.84±0.23	25.44±0.19
Propranolol (10 mg/Kg)	12.3±0.31	15.20±0.64	69.95±1.30
MEMT (100 mg/kg)	7.21±0.18[b]	17.78±0.34[d]	56.11±0.08[d]
MEMT (200 mg/kg)	9.55±0.27[c]	16.87±0.23[b]	65.4±0.52[a]
MESR (50 mg/kg)	10.1±0.15[c]	13±0.297[c]	67.55±0.12[b]
MESR (100 mg/kg)	12.12±0.08[c]	11±0.74[c]	69.58±2.08[c]
MESR (100 mg/kg) + MEMT (200 mg/kg)	14.69±0.23[c]	8.54±0.51[c]	99.79±1.38[c]

All values are expressed as mean ± SEM (n=6), One-Way ANOVA followed by Newman-Keul's multiple comparison test. [a]p<0.05, [b]p<0.01, [c]p<0.001, and [d]NS p>0.05 as compared with the doxorubicin group
DOX: Doxorubicin, MEMT: Methanol extract *Marsdenia tenacissima*, MESR: Methanol extract *Sansevieria roxburghiana*, SOD: Superoxide dismutase, MDA: Malondialdehyde, GSH: Glutathione, SEM: Standard error of the mean

A photograph of a TLC plate with methanol extracts of SR and MT is shown in Figure 5. The bands were formed with respect to the track where the sample was applied.

Histopathology reports of vital organs such as the livers and hearts of mice are presented in Table 8. Organ damage to various cellular functional units was reported in organ samples of the disease control as well as the lower-dose treatment of MEMT and MESR group animals, but it was normalized in the higher dose treatment groups and in mice treated with the combination of 100 mg/kg of MESR and 200 mg/kg of

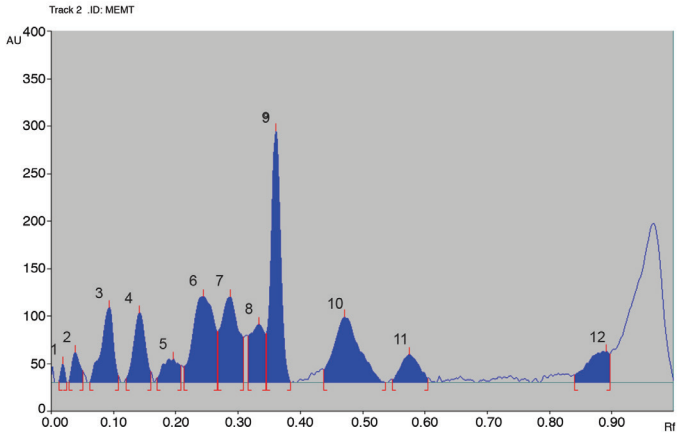


Figure 4. HPTLC of MEMT at 254 nm

HPTLC: High-performance thin layer chromatography, MEMT: Methanol extract *Marsdenia tenacissima*



Figure 5. TLC plate photograph of MEMT and MESR at 254 nm

TLC: Thin layer chromatography, MEMT: Methanol extract *Marsdenia tenacissima*, MESR: Methanol extract *Sansevieria roxburghiana*

Table 6. R_f values of MESR at 254 nm

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %	Assigned substance
1	0.01 R_f	114.4 AU	0.01 R_f	117.8 AU	12.99%	0.02 R_f	1.1 AU	614.2 AU	3.63%	Unknown"
2	0.02 R_f	2.9 AU	0.03 R_f	31.1 AU	3.43%	0.04 R_f	0.6 AU	314.0 AU	1.85%	Unknown"
3	0.07 R_f	0.1 AU	0.09 R_f	25.5 AU	2.81%	0.10 R_f	15.0 AU	279.1 AU	1.65%	Unknown"
4	0.10 R_f	15.2 AU	0.11 R_f	20.9 AU	2.31%	0.12 R_f	0.0 AU	259.4 AU	1.53%	Unknown"
5	0.13 R_f	0.2 AU	0.14 R_f	36.8 AU	4.06%	0.16 R_f	3.9 AU	529.4 AU	3.13%	Unknown"
6	0.16 R_f	4.3 AU	0.18 R_f	28.8 AU	3.17%	0.20 R_f	0.2 AU	426.4 AU	2.52%	Unknown"
7	0.20 R_f	0.4 AU	0.22 R_f	37.5 AU	4.13%	0.23 R_f	18.9 AU	630.1 AU	3.72%	Unknown"
8	0.23 R_f	18.8 AU	0.24 R_f	21.7 AU	2.39%	0.26 R_f	4.6 AU	283.8 AU	1.68%	Unknown"
9	0.26 R_f	4.9 AU	0.28 R_f	26.3 AU	2.90%	0.31 R_f	1.1 AU	572.5 AU	3.38%	Unknown"
10	0.32 R_f	2.9 AU	0.34 R_f	108.1 AU	11.91%	0.36 R_f	30.8 AU	2262.4 AU	13.36%	Unknown"
11	0.36 R_f	92.4 AU	0.37 R_f	208.7 AU	23.01%	0.40 R_f	7.9 AU	3269.0 AU	19.30%	Unknown"
12	0.44 R_f	8.7 AU	0.47 R_f	64.6 AU	7.12%	0.51 R_f	1.1 AU	1842.0 AU	10.87%	Unknown"
13	0.56 R_f	1.3 AU	0.62 R_f	51.6 AU	5.69%	0.65 R_f	39.4 AU	2486.2 AU	14.68%	Unknown"
14	0.65 R_f	39.1 AU	0.66 R_f	41.6 AU	4.58%	0.69 R_f	18.8 AU	938.5 AU	5.54%	Unknown"
15	0.70 R_f	21.2 AU	0.72 R_f	29.7 AU	3.27%	0.75 R_f	10.7 AU	844.4 AU	4.99%	Unknown"
16	0.77 R_f	5.8 AU	0.81 R_f	26.9 AU	2.96%	0.83 R_f	19.2 AU	943.9 AU	5.57%	Unknown"
17	0.85 R_f	15.1 AU	0.86 R_f	29.6 AU	3.26%	0.87 R_f	18.7 AU	443.7 AU	2.62%	Unknown"

MESR: Methanol extract *Sansevieria roxburghiana*

MEMT. The combination of MEMT and MESR showed greater protection of tissues than individual extract treatment against DOX-induced cardiotoxicity in rats.

DISCUSSION

DOX is an anticancer drug belonging to the anthracycline antibiotics and widely used for various hematological and solid tumors. Cardiotoxicity is a major adverse effect caused by DOX via free-radical production, calcium overloading, mitochondrial dysfunction, and peroxynitrite formation. The cumulative effects of these mechanism lead to altered gene and protein expression followed by cardiomyocyte death. This can be assessed by evaluation of the isoenzymes CK-MB and LDH 1 in serum. They are the cardiac marker enzymes where LDH activity was found to be high in patients' serum within 10 hours of acute myocardial infarction. Similarly, CK-MB may also be undetectable in normal people or may be found in a small fraction in the blood, but if any myocardial muscle insults occur, its level will be elevated in the serum. Therefore, both CK-MB and LDH 1 are reliable cardiac-specific markers used for diagnosis of cardiotoxicity symptoms.[15] The present study results revealed that treatment with MEMT, MESR, and the combination of both extracts normalized cardiac marker enzyme changes in rats.

Oxidative stress (OS) is the most commonly reported adverse effect of a few anticancer drugs such as anthracyclines, cisplatin, and cyclophosphamide. It may occur either directly or indirectly during chemotherapy, but by this mechanism, only few chemotherapeutic agents are cytotoxic to cancerous cells. The generated OS acts on non-targeted normal tissue, leading to tissue injury. DOX also causes OS by the proposed mechanism, the formation of reactive oxygen species (ROS), when the drug accumulates in cellular mitochondria, which leads to the production of redox imbalance followed by sequential generation of superoxide radicals that result in oxidative tissue injury. The second proposed mechanism is that the developed ROS attenuate cardiotoxicity via deletion of Topoisomerase 2β from cardiomyocytes. However, an anticancer drug with antioxidant properties can prevent OS-induced cellular damage and indirectly block ROS and the interaction of the drug with "Top2β".[16] The present study also maintained the tissue antioxidant enzyme level in DOX-induced cardiotoxicity in rats.

PPARs are nuclear receptors that exist in three isoforms: PPARα, PPARγ and PPARβ. They control cellular physiology and pathology and also regulate tissue metabolic homeostasis of skeletal muscle, adipose tissue, intestinal tissue, and the cardiovascular system, which are frequently involved in many inflammatory processes. The α, β/δ forms of PPAR are present in the heart. Apart from their metabolic functions, they are involved in the regulation of circadian rhythms, extracellular matrix remodeling, OS, and tissue inflammation. Cardiac dysfunction is due to loss of PPARα caused by OS, which affects the myosin molecule. Generally, cardioprotective drugs act as agonists of PPARα and reduce the inflammatory condition, increase adiponectin expression on cardiac muscle, and reduce the efficiency of the heart which may be due to increased expression of cardiac UCP3 mRNA. Researchers found that cardiovascular PPARα expression in conditions of cardiomyocyte hypertrophy reduce inflammation by activating inflammatory signaling pathways and also have antioxidative effects. Arrhythmogenic right ventricular dysplasia is due to functional abnormalities of PPAR, a rare genetic disease characterized by a progressive fibro fatty infiltration, decreased PPARα, and increased PPARγ expression in the right ventricle.[17] In order to elucidate the molecular mechanism of the cardioprotective nature of these plant extracts, a few isolated plant constituents were tested with the target protein PPARα, where dresgenin from MT and lupeol from SR showed the high

Table 7. R_f value of MEMT at 254 nm

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %	Assigned substance
1	0.01 R_f	0.5 AU	0.02 R_f	19.3 AU	2.23%	0.03 R_f	0.3 AU	108.0 AU	0.60%	Unknown"
2	0.03 R_f	1.0 AU	0.04 R_f	31.7 AU	3.66%	0.05 R_f	12.1 AU	368.1 AU	2.04%	Unknown"
3	0.06 R_f	0.4 AU	0.09 R_f	79.0 AU	9.13%	0.11 R_f	6.9 AU	1360.0 AU	7.55%	Unknown"
4	0.12 R_f	4.0 AU	0.14 R_f	73.5 AU	8.49%	0.16 R_f	11.7 AU	1246.6 AU	6.92%	Unknown"
5	0.17 R_f	4.8 AU	0.20 R_f	24.3 AU	2.81%	0.21 R_f	17.7 AU	585.2 AU	3.25%	Unknown"
6	0.21 R_f	17.1 AU	0.24 R_f	90.6 AU	10.47%	0.27 R_f	53.3 AU	2768.6 AU	15.37%	Unknown"
7	0.27 R_f	53.5 AU	0.29 R_f	89.9 AU	10.39%	0.31 R_f	47.8 AU	2322.6 AU	12.89%	Unknown"
8	0.32 R_f	49.2 AU	0.33 R_f	61.4 AU	7.09%	0.34 R_f	50.9 AU	1321.5 AU	7.34%	Unknown"
9	0.35 R_f	52.0 AU	0.36 R_f	265.1 AU	30.64%	0.38 R_f	0.8 AU	3466.0 AU	19.24%	Unknown"
10	0.44 R_f	13.6 AU	0.47 R_f	68.7 AU	7.94%	0.54 R_f	0.0 AU	2589.6 AU	14.38%	Unknown"
11	0.55 R_f	2.9 AU	0.57 R_f	29.2 AU	3.38%	0.60 R_f	4.4 AU	793.5 AU	4.41%	Unknown"
12	0.84 R_f	8.1 AU	0.89 R_f	32.5 AU	3.76%	0.90 R_f	29.8 AU	1082.6 AU	6.01%	Unknown"

MEMT: Methanol extract *Marsdenia tenacissima*

Table 8. Histopathology reports of the cardioprotective study

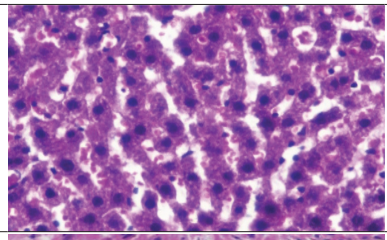
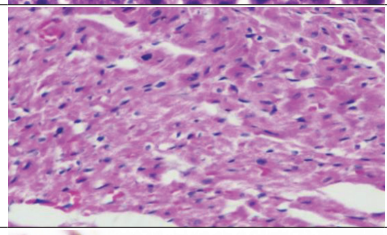
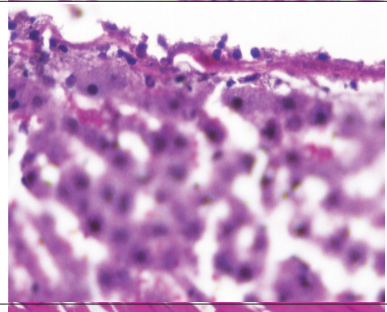
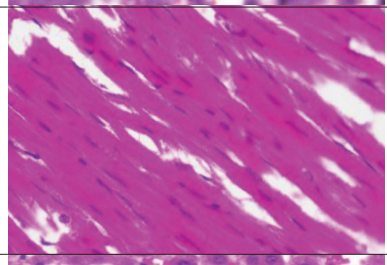
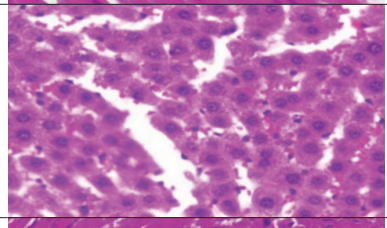
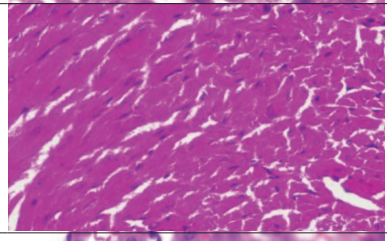
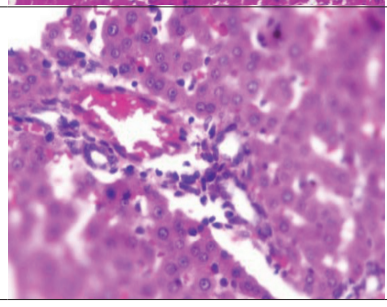
Sample code	Group details	Image	Histopathology report
NCL	Normal control (liver)		Section showed that all the cellular functional units were within normal limits
NCH	Normal control (heart)		Section showed that all the cellular functional units within normal limits
DCL	Disease control (liver)		Section showed the infiltrates with mononuclear, capsular, diffuse and mild alteration in cellular functional units
DCH	Disease control (heart)		Section showed the infiltrates with mononuclear, myocardial, multifocal and minimal damage to the cellular units
PL	Propranolol (10 mg/kg) (liver)		Section showed that the cellular functional units were within normal limits
PH	Propranolol (10 mg/kg) (heart)		Section showed that the cellular functional units were within normal limits
MTLL	MEMT (100 mg/kg) (liver)		Section showed that infiltrates with mononuclear, capsular, diffuse and mild alteration in cellular functional units

Table 8. Continued

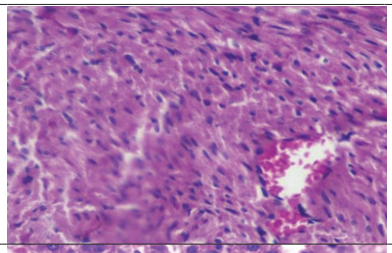
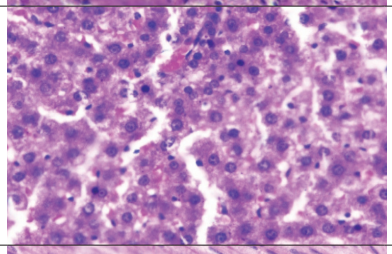
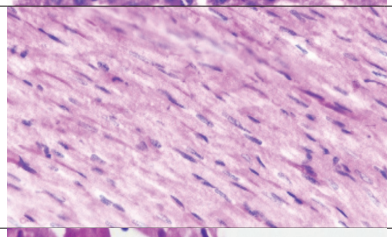
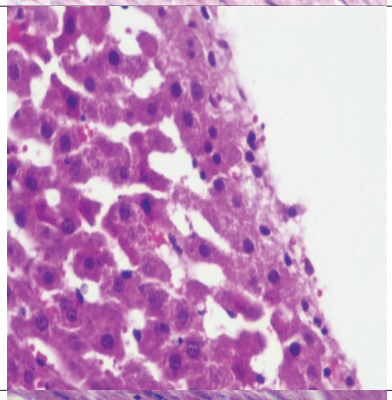
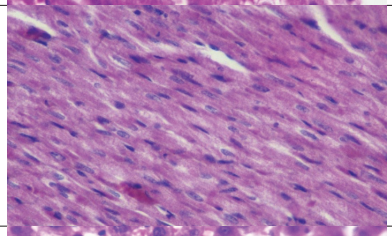
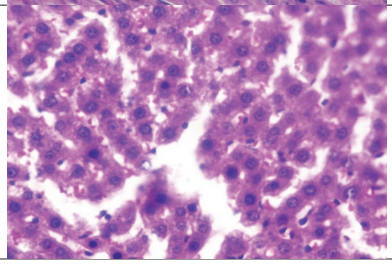
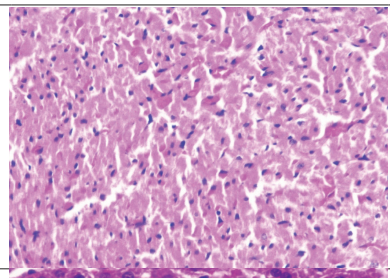
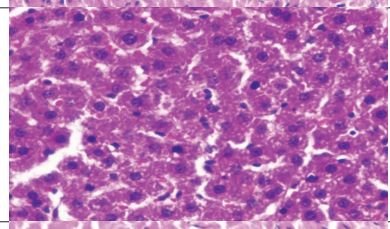
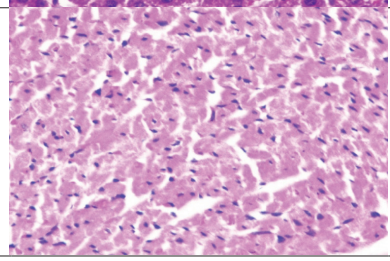
Sample code	Group details	Image	Histopathology report
MTLH	MEMT (100 mg/kg) (heart)		Section showed that the cellular functional units were within normal limits
MTHL	MEMT (200 mg/kg) (liver)		Section showed that the cellular functional units were within normal limits
MTHH	MEMT (200 mg/kg) (heart)		Section showed that the cellular functional units were within normal limits
SRLL	MESR (50 mg/kg) (liver)		Section showed the infiltrates with mononuclear, capsular, diffuse and minimal damage to the cellular functional units
SRLH	MESR (50 mg/kg) (heart)		Section showed that the cellular functional units were within normal limits
SRHL	MESR (100 mg/kg) (liver)		Section showed that the cellular functional units were within normal limits

Table 8. Continued

Sample code	Group details	Image	Histopathology report
SRHH	MESR (100 mg/kg) (heart)		Section showed that the cellular functional units were within normal limits
CML	100 mg/kg of MESR & 200 mg/kg of MEMT (liver)		Section showed that the cellular functional units were within normal limits
CMH	100 mg/kg of MESR & 200 mg/kg of MEMT (heart)		Section showed the cellular functional units were within normal limits

MEMT: Methanol extract *Marsdenia tenacissima*, MESR: Methanol extract *Sansevieria roxburghiana*

affinity toward the target protein, and these phytoconstituents protected the myocardium from toxic agents.

HPTLC fingerprinting of natural drugs may encourage the recognition of natural products, and it is suited to the delivery of core scaffolds for forthcoming drugs. Hence, there will be further developments in the use of novel analytical techniques in natural product drug discovery campaigns.[14] The qualitative analysis of MEMT and MESR through HPTLC confirmed the existence of many secondary metabolites. Traditional therapeutic uses of this species are due to the pre-existences of these metabolites. Therefore, the present study adds value to the medicinal importance of this Morva species.

Histopathological reports revealed treatment-related microscopic changes in the livers and hearts of rats. Infiltrates with mononuclear, capsular, diffuse, and minimal damage to the cellular functional units were found in animals that received 50 mg/kg of MESR, whereas the infiltrates with mononuclear, myocardial, multifocal, and minimal damage to the cellular units were found in disease control animals. All the incidences were within the normal range in other extract-treated and control animals. It can, therefore, be concluded that all histological changes observed were normalized by extract treatment.

CONCLUSION

The cardioprotective study results suggest a correlation between antioxidant enzymes and the degree of damage caused by DOX. By increasing the activities of antioxidant enzymes that combat free-radical damage, MT exerts antioxidant effects that can be useful in the treatment of cancer. Similarly, ethyl acetate extract of SR possess significant antioxidant as well as anticancer properties.

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Lyu YL, Liu LF. Doxorubicin cardiotoxicity revisited: ROS versus Top 2. Recent Advances in Cancer Research and Therapy. 2012;351-369.
2. Huang MY, Zhang LL, Ding J, Lu JJ. Anticancer drug discovery from Chinese medicinal herbs. Chine Med. 2018;13:35.
3. Tiwari V, Singh A, Tiwari A. Phytopharmacological Overview on Controversial Drug: Murva. Traditional and Folk Herbal Medicine: Recent Researches. 2018;2:475-526.
4. Hatapakki BC, Hukkeri V. Antipyretic activity of Root of *Marsdenia tenacissima* in Rats. J Nat Remedies. 2011;11:98-102.
5. Bharath KM, Rohini A, Laxmi SP, Samatha Y. Antinociceptive, antiinflammatory and antiarthritic activity of *Marsdenia tenacissima* stem extract. Int J Phytopharmacol. 2014;5:411-415.
6. Dulla O. *Sansevieria roxburghiana* Schult. & Schult. F., Agavaceae: phytochemistry, traditional uses and its pharmacological activities - a review. World Sci News. 2016;59:24-34.
7. Forli S, Huey R, Pique ME, Sanner MF, Goodsell D, Olson AJ. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc. 2016;11:905-919.