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Cardioprotective effect of *Marsdenia tenacissima* and *Sansevieria roxburghiana* in Doxorubicin induced cardiotoxicity in rats *in vivo* : The role of Dresgenin and Lupeol

Sub title : Cardioprotecive activity of Murva

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Abstract
Objectives: Doxorubicin (DOX) causes cardiac toxicity is the major adverse effect in cancer treatment. Murva is a controversial plant used in Ayurvedic system, it consist of more than 12 medicinal plant roots and were found in different parts of India but *Marsdenia tenacissima* is an acceptable source in Murva whereas *Sansevieria roxburghiana* Schult and Schult.f. (S. zeylanica Roxb.) also considered as Murva in west Bengal, India. The present study was focused on the evaluation of cardioprotective mechanism by *in silico* methods as well as *in vivo* cardioprotective potential of methanol extracts of *Marsdenia tenacissima* and *Sansevieria roxburghiana* on rats.
Materials and methods: Total 48 rats were divided into 8 groups six in each. Doxorubicin 20mg/kg, i.p was administered to all rats on 13th day except the group I. Group II was disease control, group III was standard drug Propronolol treated and from group IV to VII treated with lower two doses of methanol extract of *M. tenacissima* (MEMT) & methanol extract of *S. roxburghiana* (MESR) whereas group VIII received higher doses combination of both above extracts for continuous 14 days. Blood and tissue antioxidant level as well as cardiac enzymes were measured at the end of the study. Damage of cellular functional units were analysed by histopathological study. Dresgenin from *M. tenacissima* similarly Lupeol from *S. roxburghiana* were taken as ligands for the target PPARα protein to find out the mechanism of action. HPTLC fingerprint was taken to know the number of phytoconstituents present in both extracts.
Results: The combination showed the most significant ($p<0.001$) effect on altered cardiac enzymes and antioxidant enzymes levels in both blood and tissues and it also corrected the extreme damage in cellular functional units. Dresgenin and Lupeol showed binding score of -8.2(Kcal/mol), -9 (Kcal/mol) with PPARα. HPTLC reports revealed that 17 &12 peaks were found at 254 nm.

Conclusion: The study results concluded that combination of MESR & MEMT, MESR and MEMT possessed the cardioprotective activity by binding of Dresgenin and Lupeol with PPARα. The order of efficacy was combination of extracts > MESR> MEMT.
Keywords: Doxorubicin, cardioprotective, molecular docking, cardiac enzyme, HPTLC

INTRODUCTION

Doxorubicin (DOX) an anthracycline antibiotic used for the treatment of various neoplastic disorders but when it is used clinically, shows severe organ toxicity. Cardiotoxicity is the major fatal event happened in pediatric and adult patients at normal therapeutic dose which is characterized by an irreversible damage of cardiac muscle leads to a major cause of morbidity and mortality of the treatment related to chemotherapy. Even though, several less toxic derivatives of DOX are available for chemotherapy, induction of cardiotoxicity is taken as the major concern and preferred to use traditional anticancer drugs. Cardioprotective adjuvants such as leucovorin, mesna, angiotensin receptor blockers and beta blockers are available and are administered along with DOX to reduce cardiotoxicity. However, these adjuvants exhibited marked cardioprotection and did not compromise the anticancer activity of DOX[1]. Hence a new strategy was developed in pharmaceutical industries to establish a formulation with extended action of cardioprotection from DOX without compromising the efficacy of cancer chemotherapy. 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 herbal preparation, Ayurvedic formulations have been placed at the first position for more than thousands of years due to their less toxicity and wide acceptability[2]. One of the such Ayurvedic plant is Murva. It is a controversial drug, contain more than 12 medicinal plants roots and were found in different parts of India. In which, *Marsdenia tenacissima* is an acceptable source of Murva whereas *Sansevieria roxburghiana* Schult and Schult.f. (S. zeylanica Roxb.) was consider as Murva in west Bengal, India[3]. Rest of the plants in Murva, their scientific names and the place where we can find are listed below,

Botanical Name	Family	Place
Marsdenia tenacissima	Asclepiadaceae	An accepted botanical source of Murva
Helicteres isora L	Sterculiaceae	Punjab
Maerua arneria	Capparaceae	Bihar
Chonomorpha fragrans	Apocynaceae	Kerala
Clematis triloba	Ranunculaceae	West Bengal
Bauhinia tomentosa	Leguminoseae	West Bengal
Sanseviera roxburghiana Schult and Schult.f. (S. zeylanica Roxb.)	Agavaceae	West Bengal
Wattakaka volubilis(Linn. f.) Stapf	Asclepidaceae	South India
Salvadora persica L	Salvadoracae	South India
Argyreia nerova, *Bojervar.speciosa*),	Convolvulaceae	Other source of Murva
Maerua oblongifolia	Capparaceae	

Dregea volubilis (L.f.) Benth. ex Hook.f	Apocynaceae

Traditionally Murva is used for treatment of anemia, diabetes, stomach disorder, typhoid, cough, fever and urinary tract infection[4]. Based on review of literature, *Marsdenia tenacissima* is traditionally used for heart diseases[3, 5], similarly *Sansevieria roxburghiana* is used as a cardiotonic[6]. But none of them are experimentally proven the effect of these drugs on heart of animals. Therefore, this study was aimed to evaluate the cardioprotective effect of extracts of *Sansevieria roxburghiana* and *Marsdenia tenacissima* on Doxorubicin induced cardiotoxicity in rats.

MATERIALS AND METHODS

Experimental animals

Wistar albino rats (200g- 250g) were taken from the animal house of St. Joseph's College of Pharmacy, Cherthala, Kerala, India, then they were acclimatised for a week under standard controlled condition (12 h light/12 h darkness, at 25^0C). The study protocol (**SJCP/IAEC/2018-4/35**) was approved by Institutional Animal Ethics Committee (IAEC), St. Joseph's College of Pharmacy Cherthala, Kerala, India.

Plant Materials, Drugs and chemicals

Roots and rhizomes of *Sansevieria roxburghiana* (SR) and *Marsdenia tenacissima* (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. The herbarium specimen was deposited at 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 Department of Botany, Santana Dharma College, Alappuzha, Kerala, India.

Doxorubicin (DOX) was procured from Dabur Pharmaceuticals Ltd., New Delhi, India. Propranolol from Cipla Ltd. India. LDH, CK, AST 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 *Marsdenia tenacissima* (MT) Rhizome & roots of *Sansevieria Roxburghiana* (SR) were cleaned and dried at room temperature (shade dry). About 300g of defated coarse powered drug was successively extracted in a Soxhlet apparatus with methanol (70-80ºC for 48 hours). Methanol extract of MT (MEMT), SR (MESR) and aqueous extract of MT (AEMT), SR (AESR) were collected by rotary evaporator followed by dried and stored in a well tight container for experimental purposes.

Molecular Docking

The protein selected for the cardioprotective study was PPARα with PDB ID: 1K7L. 3D structures were downloaded from protein data bank (www.rcsb.org). Protein was prepared by eliminating water and small molecules by using Pymol software (9). The chemical constituents such as Tenasogenin, Cissogenin, Tenacigenin-C, Tenacigenoside and Dresgenin from MT. 6-methyl-1-octanol, Diethyl phthalate, Methyl Hexadecanoate, 3,3-Dimethylhexanal and Lupeol from SR were selected as the ligand. The pubchem was used to retrieve the 3D structure of ligand in SDF format. Openbabel 2.3.2 is used to convert in to PDB format. Ligand an important chemical constituent and target in PDB format were loaded in autodock vina PyRx. The binding energy with least RMSD (upper and lower) are selected and expressed in Kcal/mol. At the first dock the pdb .qt files for protein and ligand were prepared[7, 8]. The pdb.qt format of ligand and target were loaded in Pymol for visualisation.

From the visualisation, number of hydrogen bond and sequence of amino acid in which ligand bounded were obtained.

Cardioprotective activity study design

The total 48 rats of both sex of with Wistar rats (180-220g) were divided into VIII groups containing 6 animals in each. Standard and test drugs were administered to concern group of animals for once daily for 14 consecutive days[9, 10 &11]. Group I: Normal control treated with distilled water orally, Group II served as diseases control received Doxorubicin 20mg/kg i.p only. Group III received the standard Propranolol 10mg/kg, orally and Doxorubicin 20 mg/kg, i.p on 13th day. Group IV and V received 100 mg/kg and 200 mg/kg of MEMT, orally and Doxorubicin 20 mg/kg, i.p on 13th day. Group VI and VII received the 50 mg/kg and 100 mg/kg MESR, orally and Doxorubicin 20mg/kg, i.p on 13th day. Group VIII received 100 mg/kg of MESR and 200 mg/kg of MESR& MEMT and doxorubicin 20 mg/kg, i.p on 13th day. All the animals were challenged by using single dose administration of Doxorubicin 20 mg/kg i.p on 13th day except group I animals. After 48 hours of Doxorubicin administration, blood was collected and animals were sacrificed to isolate the vital organs like liver, kidney and heart for histopathological studies. Blood and liver antioxidant enzymes such as SOD, GSH and MDA level and cardiac enzymes such as CK-MB, and LDH 1 were estimated with Accurex biomedical Pvt., Ltd India using semi autoanalyser.

Histopathology study

The organs such as hearts, livers and kidneys were isolated immediately after sacrificing the animal and washed with ice cold normal saline, trimmed and placed in 10% formaldehyde. The organs were sectioned and stained with hematoxylin and eosin (H&E). The structures were examined under light microscope10X and 40X by a pathologist blinded to the groups under study[12].

Estimation of tissue antioxidant level

Isolated hearts were divided in to two portions to prepare homogenates like 10%w/v homogenate in potassium chloride(0.15M) and 10%w/v homogenate in 0.25% w/v sucrose in phosphate buffer (5M pH7.4) . Both the homogenate were centrifuged at 8000 rpm for 10 minute the supernatant from first homogenate were used for the estimation of MDA and supernatant from second homogenate were used for estimation of SOD, GSH[13]. All the estimation were done according to the manufacture manual of reagent Accurex biomedical Pvt., Ltd India.

HPTLC analysis

HPTLC fingerprinting analysis was done with CAMAG LINOMAT 5 where 2 µL of sample was applied by using Hamilton syringe on $60F_{254}$ TLC plate as band length of 5 mm using. Later it was kept in TLC developing chamber which was saturated with solvent vapour (mobile phase) Toluene: Ethyl acetate: Methanol (7:3:1). Then it was dried by hot air and kept in photo documentation chamber followed by scanned at 254 nm 366 nm, and 550 nm after derivetizing with anisaldehyde-sulphuric acid reagent[14].

Statistical evaluation

Invivo data were expressed as the Mean ±SEM of six values. The difference between experimental groups was compared to negative control and normal control by One Way Analysis Of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where, $p< 0.05$ implied significance.

RESULTS

Docking scores, binding energy, hydrogen bonds and binding sites were obtained from the various isolated chemical constituents of MT and SR was used as ligand for the PPARα receptor which are presented on Table 1&2. In which, 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 PPARα receptor shown in Figure 1, in which three hydrogen bonds were found at 213ALA, 231GLY and 216LYS position. Similarly, visualization of Lupeol on PPARα receptor shown in Figure 2, in which two hydrogen bonds were seen at 214 TYR and 213 ALA position.

Table1 Docking score of various ligands on 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

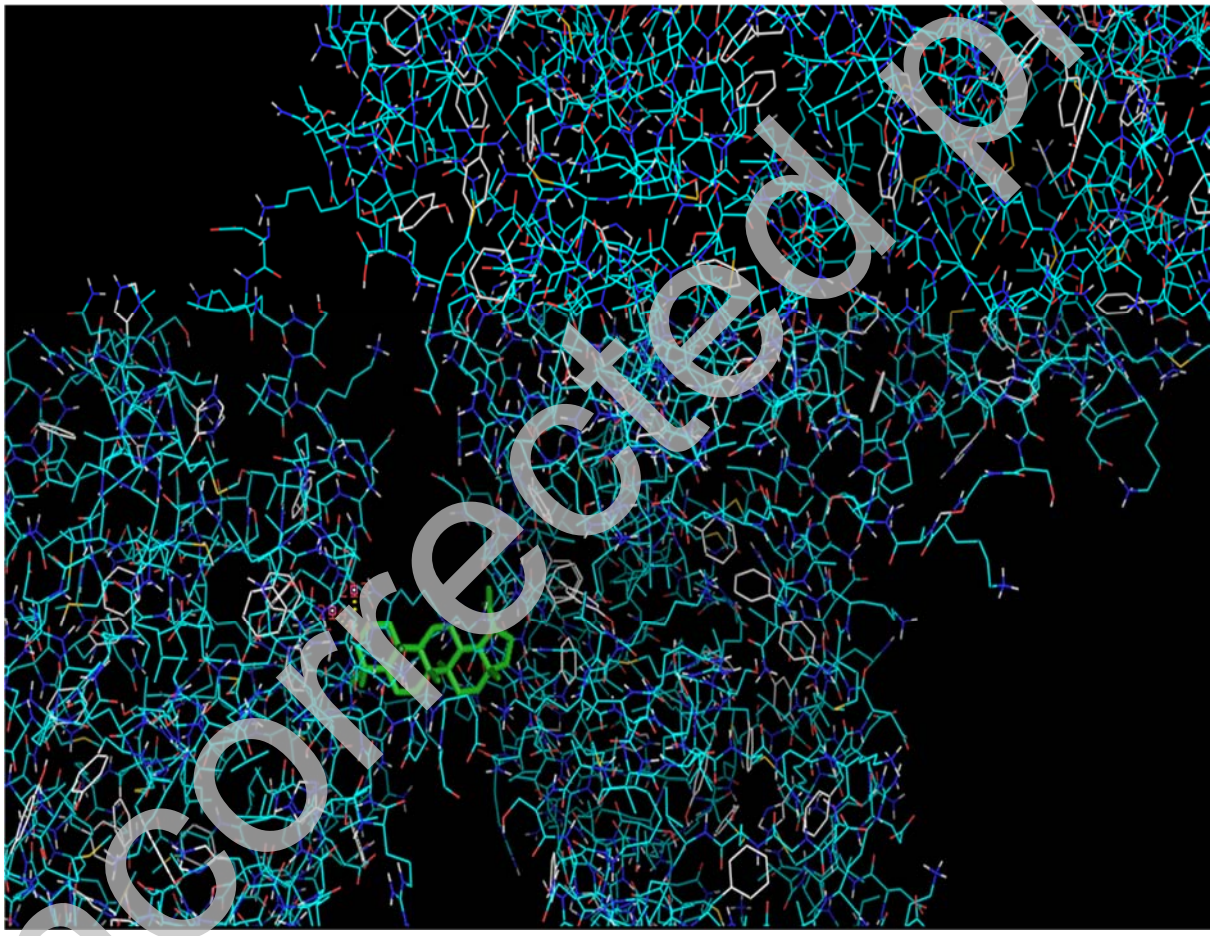


Figure 1 Visualisation of docking in Pymol: PPARα with Lupeol

Figure 2 Visualisation of docking in Pymol: PPARα with Dresgenin

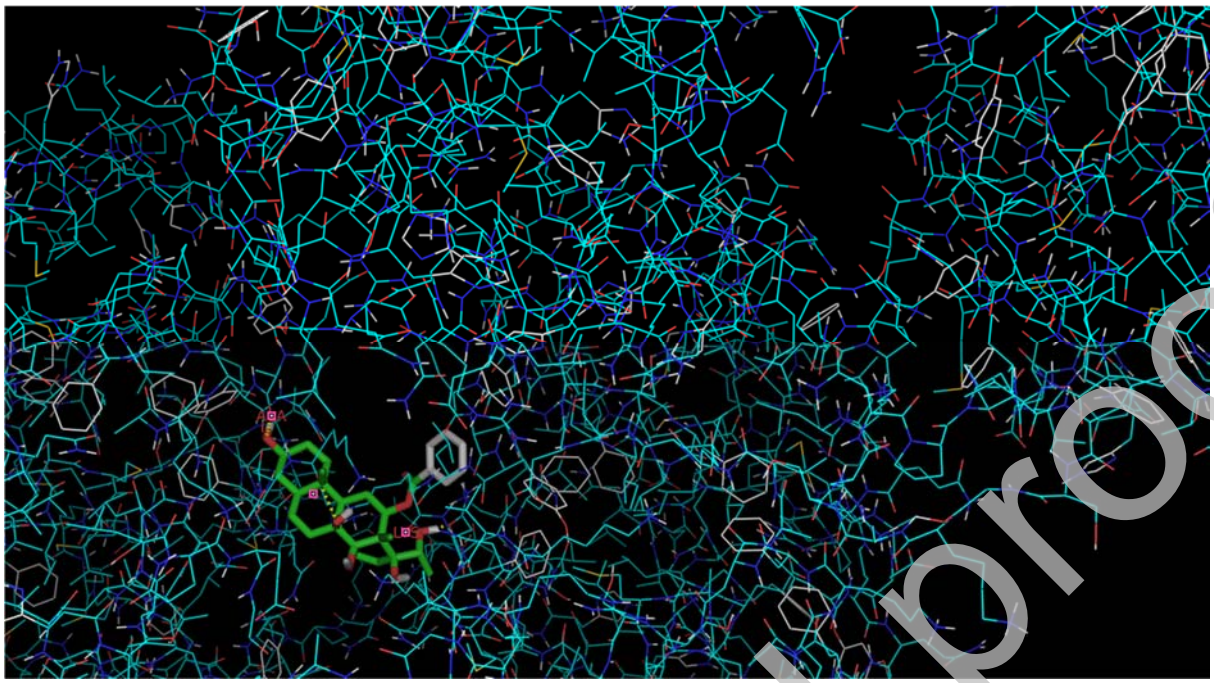


Table 2 Binding energy, hydrogen bonds and binding sites of the targets

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

Table 3 illustrated the effect of MEMT, MESR & and combination of MEMT & MESR on cardiac enzymes of Doxorubicin induced cardiotoxicity in rats. CK-MB and LDH 1were increased in Doxorubicin control group rats but the rats, which were treated with the combination of 100mg/kg of MESR and 200mg/kg of MEMT significantly ($p<0.001$) reduced the level 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 100mg/kg MEMT ($p<0.01$) and 50mg/kg of MESR ($p<0.05$) showed less significant effect on reduction of CK-MB and LDH 1 when compared with disease control animals. The standard drug Propranolol showed highly significant ($p<0.001$) effect on reduction of the elevated cardiac enzymes in Doxorubicin induced cardiotoxic rats.

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±13.56
DOX (20mg/Kg)	1920.17±5.78	3592.67±5.07
Propranolol (10mg/Kg)	1033±6.39	1633.83±14.11
MEMT (100mg/Kg)	1893±4.95 ^{c}	3556.83±3.03^{c}
MEMT (200mg/Kg)	1878.33±3.16^{b}	3539.5± 3.04^{b}
MESR (50mg/Kg)	1803.83±13.87 ^{c}	2892.17±14.72^{c}
MESR (100mg/Kg)	1505.33±11.33^{c}	2309±8.83^{c}

MEMT (200mg/Kg)+ MESR (100mg/Kg)	1104.5±6.49^{c}	1961.83±12.01^{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 -**$p < 0.01$ and c-*** $p < 0.001$ as compared to doxorubicin (DOX) group.

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 (20mg/Kg)	26.24±0.21	138.84±0.23	5.47±0.09
Propranolol (10mg/Kg)	37.36±0.38	45.20±0.64	9.99±0.30
MEMT (100mg/Kg)	27.24±0.18^{b}	137.78±0.34^{d}	6.00±0.07^{d}
MEMT (200mg/Kg)	29.75±0.17^{c}	136.87±0.23^{b}	6.22±0.02^{a}
MESR (50mg/Kg)	29.996±0.13^{c}	80.4±0.297^{c}	6.51±0.02^{b}
MESR(100mg/Kg)	32.92±0.18^{c}	68.77±0.74^{c}	7.52±0.08^{c}
MESR(100mg/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 to doxorubicin (DOX) group.

The effect of MEMT and MESR on tissue (heart) antioxidant enzymes of cardiotoxicity induced rats were shown in Table 4. There was an increased level of MDA and decreased level of SOD and GSH were found with only 20 mg/Kg doxorubicin treated disease control group. Both the doses of orally administered MESR and the combination of MESR+MEMT showed highly significant ($p<0.001$) effect on the reduction of MDA as well as enhancement of SOD and GSH found with 14 days treatment. In case of MEMT, 100mg/Kg dose showed less significant ($p < 0.01$) effect on raise of SOD but it was non-significant ($p > 0.05$) on reduction of MDA and raise of GSH level in heart whereas 200mg/Kg of MEMT showed significant alteration on the reestablishment of cardiac antioxidant enzyme level as that of normal rats.

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 (20mg/Kg)	1.04±0.21	38.84±0.23	25.44±0.19
Propranolol (10mg/Kg)	12.3±0.31	15.20±0.64	69.95±1.30
MEMT (100mg/Kg)	7.21±0.18^{b}	17.78±0.34^{d}	56.11±0.08^{d}
MEMT (200mg/Kg)	9.55±0.27^{c}	16.87±0.23^{b}	65.4±0.52^{a}
MESR (50mg/Kg)	10.1±0.15^{c}	13±0.297^{c}	67.55±0.12^{b}
MESR(100mg/Kg)	12.12±0.08^{c}	11±0.74^{c}	69.58±2.08^{c}
MESR(100mg/Kg) +MEMT (200 mg/Kg)	14.69±0.23^{c}	8.54±0.51^{c}	99.79±1.38^{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 to doxorubicin (DOX) group.

Fourteen days, single dose oral feeding of MEMT and MESR on blood antioxidant enzymes of cardiotoxicity induced rats were shown in Table 5. The levels of GSH and SOD of disease control group were lower than normal controls ($p<0.001$). The MDA levels in the blood was higher than those of the normal control group ($p<0.001$). Treatment with 100, 200 mg/kg of MEMT and 50mg/kg, 100MG/Kg of MESR significantly ($p<0.001$) altered and brought close to that of normal where the combination of both extracts showed marked reversal of MDA , SOD and GSH level in DOX induced cardiotoxicity in rats.

Figure 3 HPTLC fingerprint of MESR at 254 nm

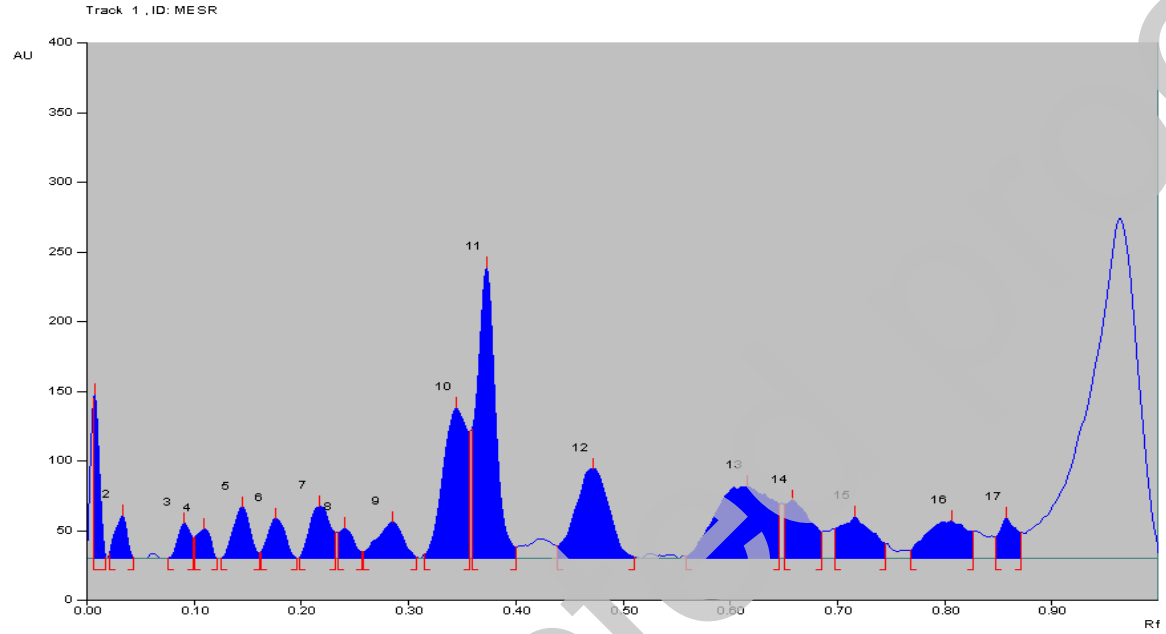


Table 6. R_f values of MESR at 254 nm

Track 1, ID: MESR

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.01 Rf	114.4 AU	0.01 Rf	117.8 AU	2.99 %	0.02 Rf	1.1 AU	614.2 AU	3.63 %	unknown *
2	0.02 Rf	2.9 AU	0.03 Rf	31.1 AU	3.43 %	0.04 Rf	0.6 AU	314.0 AU	1.85 %	unknown *
3	0.07 Rf	0.1 AU	0.09 Rf	25.5 AU	2.81 %	0.10 Rf	15.0 AU	279.1 AU	1.65 %	unknown *
4	0.10 Rf	15.2 AU	0.11 Rf	20.9 AU	2.31 %	0.12 Rf	0.0 AU	259.4 AU	1.53 %	unknown *
5	0.13 Rf	0.2 AU	0.14 Rf	36.8 AU	4.06 %	0.16 Rf	3.9 AU	529.4 AU	3.13 %	unknown *
6	0.16 Rf	4.3 AU	0.18 Rf	28.8 AU	3.17 %	0.20 Rf	0.2 AU	426.4 AU	2.52 %	unknown *
7	0.20 Rf	0.4 AU	0.22 Rf	37.5 AU	4.13 %	0.23 Rf	18.9 AU	630.1 AU	3.72 %	unknown *
8	0.23 Rf	18.8 AU	0.24 Rf	21.7 AU	2.39 %	0.26 Rf	4.6 AU	283.8 AU	1.68 %	unknown *
9	0.26 Rf	4.9 AU	0.28 Rf	26.3 AU	2.90 %	0.31 Rf	1.1 AU	572.5 AU	3.38 %	unknown *
10	0.32 Rf	2.9 AU	0.34 Rf	108.1 AU	11.91 %	0.36 Rf	30.8 AU	2262.4 AU	13.36 %	unknown *
11	0.36 Rf	92.4 AU	0.37 Rf	208.7 AU	23.01 %	0.40 Rf	7.9 AU	3269.0 AU	19.30 %	unknown *
12	0.44 Rf	8.7 AU	0.47 Rf	64.6 AU	7.12 %	0.51 Rf	1.1 AU	1842.0 AU	10.87 %	unknown *
13	0.56 Rf	1.3 AU	0.62 Rf	51.6 AU	5.69 %	0.65 Rf	39.4 AU	2486.2 AU	14.68 %	unknown *
14	0.65 Rf	39.1 AU	0.68 Rf	41.6 AU	4.58 %	0.69 Rf	18.8 AU	938.5 AU	5.54 %	unknown *
15	0.70 Rf	21.2 AU	0.72 Rf	29.7 AU	3.27 %	0.75 Rf	10.7 AU	844.4 AU	4.99 %	unknown *
16	0.77 Rf	5.8 AU	0.81 Rf	26.9 AU	2.96 %	0.83 Rf	19.2 AU	943.9 AU	5.57 %	unknown *
17	0.85 Rf	15.1 AU	0.86 Rf	29.6 AU	3.26 %	0.87 Rf	18.7 AU	443.7 AU	2.62 %	unknown *

Figure 3 and Table 6 represented the HPTLC fingerprint of MESR which was applied in track 1 and was viewed at 254nm. Total seventeen peak were found with R_f value ranging from 0.01 to 0.86.

Figure 6 HPTLC of MEMT at 254 nm.

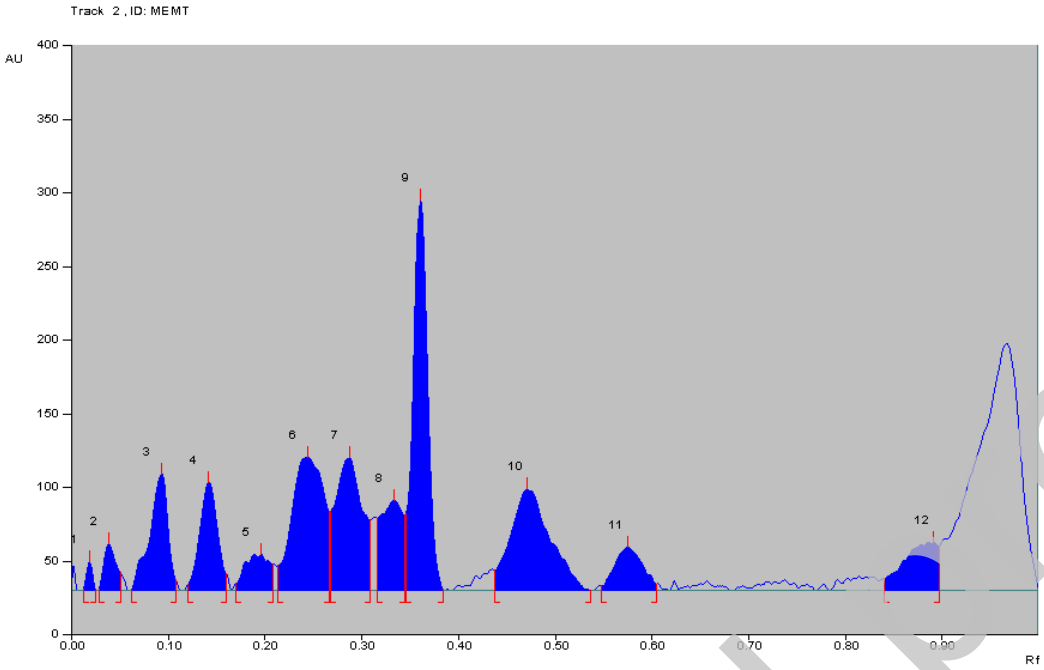


Table 7 R_f value of MEMT at 254 nm

Track 2, ID: MEMT

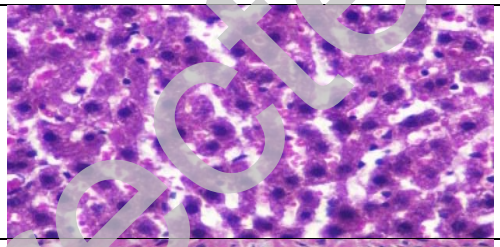
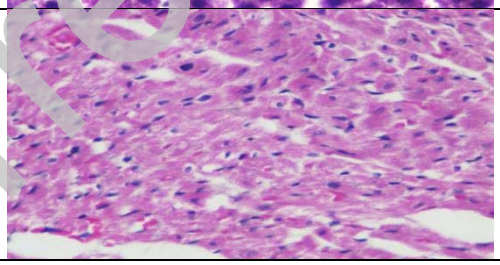
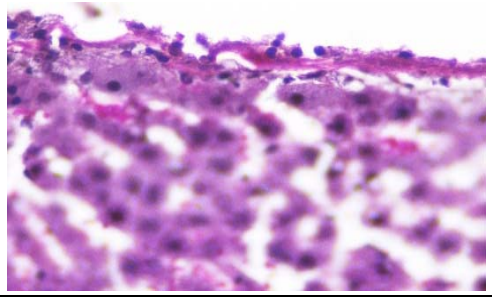
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.01 Rf	0.5 AU	0.02 Rf	19.3 AU	2.23 %	0.03 Rf	0.3 AU	108.0 AU	0.83 %	unknown *
2	0.03 Rf	1.0 AU	0.04 Rf	31.7 AU	3.66 %	0.05 Rf	12.1 AU	363.1 AU	2.04 %	unknown *
3	0.06 Rf	0.4 AU	0.09 Rf	79.0 AU	9.13 %	0.11 Rf	6.9 AU	1360.0 AU	7.55 %	unknown *
4	0.12 Rf	4.0 AU	0.14 Rf	73.5 AU	8.49 %	0.16 Rf	11.7 AU	1246.6 AU	6.92 %	unknown *
5	0.17 Rf	4.8 AU	0.20 Rf	24.3 AU	2.81 %	0.21 Rf	17.7 AU	595.2 AU	3.25 %	unknown *
6	0.21 Rf	17.1 AU	0.24 Rf	90.6 AU	10.47 %	0.27 Rf	53.3 AU	2768.6 AU	15.37 %	unknown *
7	0.27 Rf	53.5 AU	0.29 Rf	89.9 AU	10.39 %	0.31 Rf	47.8 AU	2322.6 AU	12.89 %	unknown *
8	0.32 Rf	49.2 AU	0.33 Rf	61.4 AU	7.09 %	0.34 Rf	50.9 AU	1321.5 AU	7.34 %	unknown *
9	0.35 Rf	52.0 AU	0.36 Rf	265.1 AU	30.64 %	0.38 Rf	0.8 AU	3466.0 AU	19.24 %	unknown *
10	0.44 Rf	13.6 AU	0.47 Rf	68.7 AU	7.94 %	0.54 Rf	0.0 AU	2589.6 AU	14.38 %	unknown *
11	0.55 Rf	2.9 AU	0.57 Rf	29.2 AU	3.38 %	0.60 Rf	4.4 AU	793.5 AU	4.41 %	unknown *
12	0.84 Rf	8.1 AU	0.89 Rf	32.5 AU	3.76 %	0.90 Rf	29.8 AU	1082.6 AU	6.01 %	unknown *

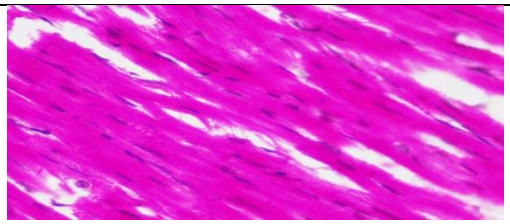
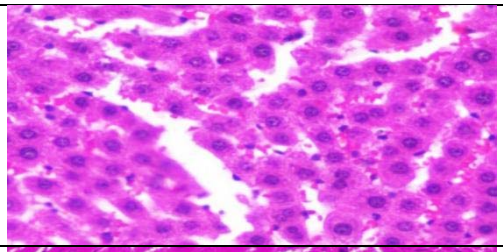
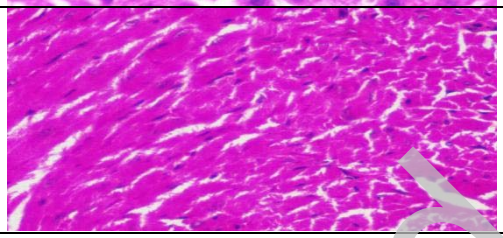
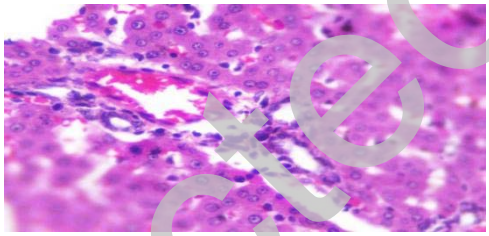
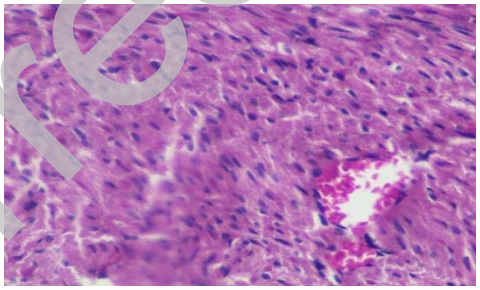
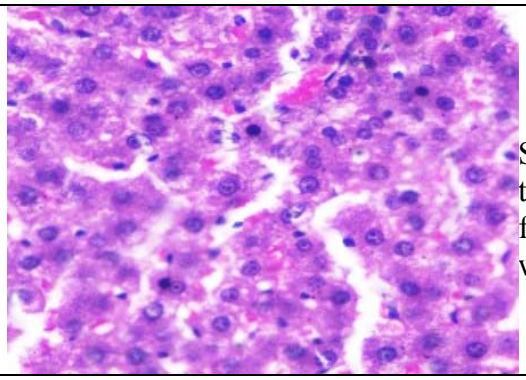
Figure 4 and Table 7 represented the HPTLC fingerprint of MESR which was applied in track 2 and was viewed at 254nm. Total twelve peak were found with R_f value ranging from 0.01 to 0.84.

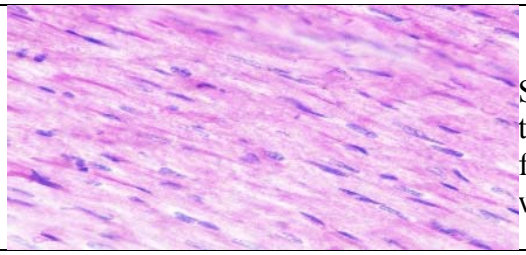
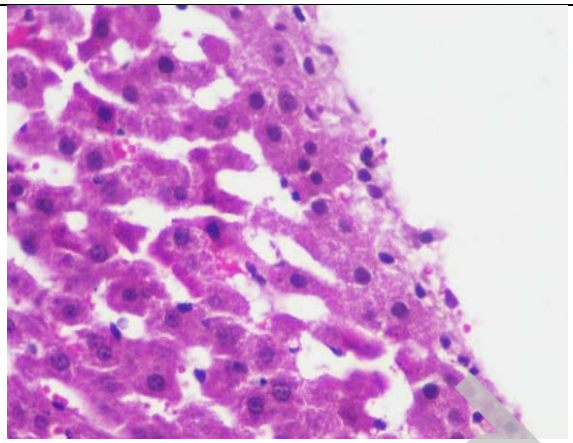
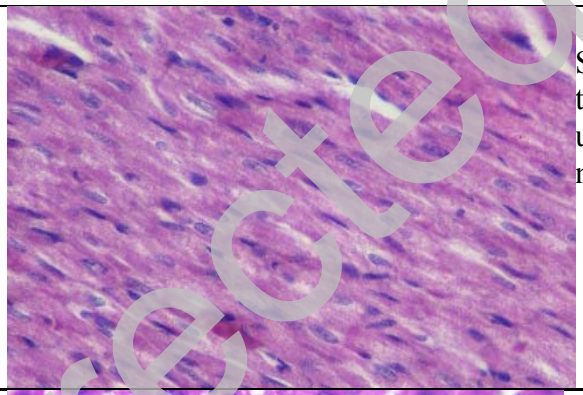
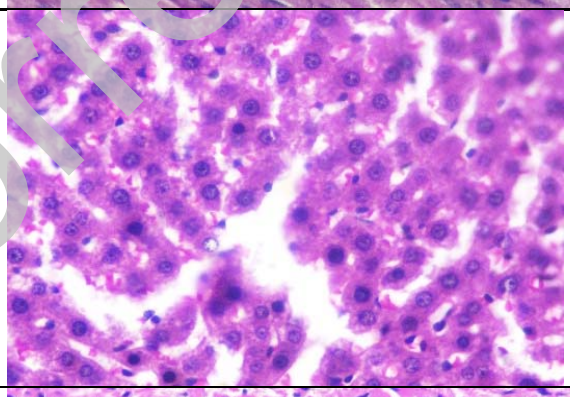
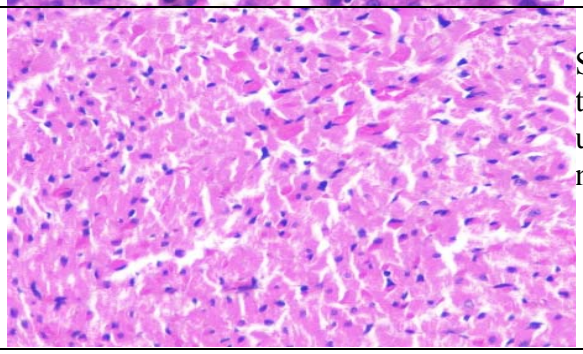
Figure 5 TLC plate photograph of MEMT AND MESR at 254nm

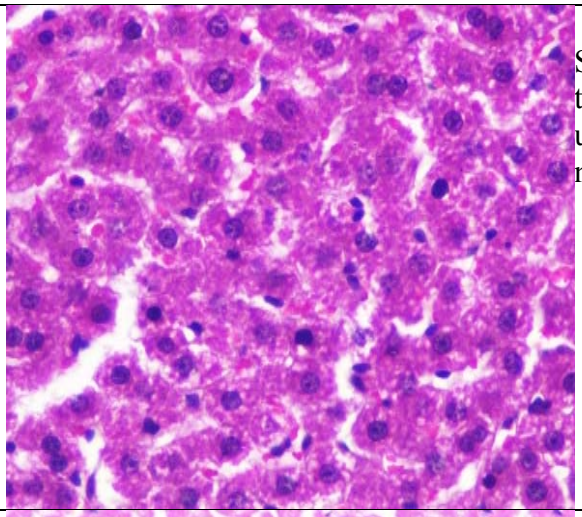
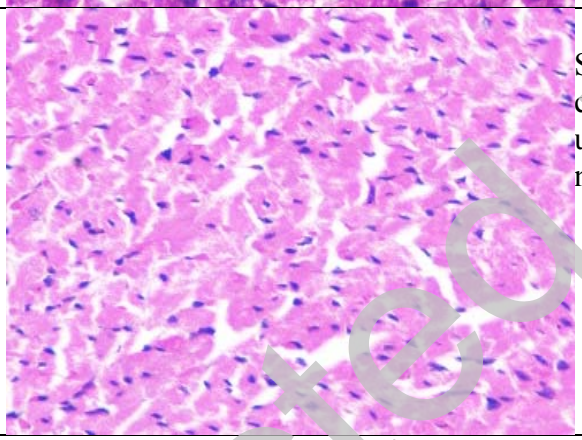
A photograph of TLC plate with methanol extracts of SR and MT shown in the figure Fig. 5. The bands were formed with respect to the track where the sample applied.

Table 8 Histopathology reports of 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 (10mg/kg) (liver)		Section showed that the cellular functional units were within normal limits.
PH	Propranolol (10mg/kg) (heart)		Section showed that the cellular functional units were within normal limits.
MTLL	MEMT (100mg/kg) (liver)		Section showed that infiltrates with mononuclear, capsular, diffuse and mild alteration in cellular functional units.
MTLH	MEMT (100mg/kg) heart		Section showed that the cellular functional units were within normal limits.
MTHL	MEMT (200mg/kg) (liver)		Section showed that the cellular functional units were within normal limits.

MTHH	MEMT (200mg/kg) (Heart)		Section showed that the cellular functional units were within normal limits.
SRLL	MESR (50mg/kg) (liver)		Section showed the infiltrates with mononuclear, capsular, diffuse and minimal damage to the cellular functional units.
SRLH	MESR (50mg/kg) (Heart)		Section showed that the cellular functional units were within normal limits.
SRHL	MESR (100mg/kg) (liver)		Section showed that the cellular functional units were within normal limits.
SRHH	MESR (100mg/kg) (Heart)		Section showed that the cellular functional units were within normal limits.

CML	100mg/kg of MESR &200mg/kg of MEMT (liver)		Section showed that the cellular functional units were within normal limits.
CMH	100mg/kg of MESR &200mg/kg of MEMT(Heart)		Section showed the cellular functional units were within normal limits.

NCL- Normal control liver, NCH- Normal control heart, DCL- Disease control liver, DCH- Disease control heart, PL- Propranolol control liver, PH- Propranolol control heart, MTLL- MT lower dose liver, MTLH- MT lower dose heart, MTHL- MT higher dose liver, MTHH- MT higher dose heart, SRLL- SR lower dose liver, SRLH- SR lower dose heart, SRHL- SR higher dose liver, SRHH- SR higher dose heart, CML- combination of MT & SR liver, CMH- combination of MT & SR heart.

Histopathology reports of vital organs such as liver and heart of mice were presented in Table 8. The organ damage with various cellular functional units were reported in the disease control as well as the lower dose treatment of MEMT and MESR group animals organ samples but it was normalized in higher dose treatment groups and combination of 100mg/kg of MESR &200mg/kg of MEMT treated mice. Since it was a comparative study, combination of MEMT & MESR showed the maximum protection of tissues than the damages caused by individual extract treatment on DOX induced cardiotoxicity in rats.

DISCUSSION

Doxorubicin is an anticancer drug which belongs to Anthracycline antibiotics and widely used for various haematological and solid tumors. Cardiotoxicity is a major adverse effect caused by Doxorubicin via free radical production, calcium overloading, mitochondrial dysfunction, and peroxynitrite formation. The cumulative effects of these mechanism leads to altered gene and protein expression followed by cardiomyocytes death. This can be assessed by evaluation of an isoezymes CK-MB and LDH 1 in serum. They are the cardiac marker enzymes where LDH activity was found to be high in serum within 10 hours of acute myocardial infarction (AMI) of patients. Similarly, CK-MB also undetectable in normal person or may found in small fraction in the blood but if any myocardial muscle insults comes its level was raised to be high in serum. Therefore both CK-MB and LDH 1 is the reliable cardiac specific marker used for diagnosis of cardiactoxicity symptoms[15]. The present

study results revealed that treatment with MEMT, MESR and the combination of both extract normalized the cardiac marker enzyme changes in rats.

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

Peroxisome proliferator activated receptors (PPARs) is a nuclear receptor exist in three isoforms—α, β/δ. It controls cellular physiology and pathology, also regulate tissue metabolic homeostasis of skeletal muscle, adipose, intestine tissue, cardiovascular system which are frequently involved in many inflammatory processes. α, β/δ form of PPAR are present in the heart, apart from the metabolic function they are involved in regulation of circadian rhythm, remodelling of extracellular matrix, active in oxidative stress and tissue inflammation. Cardiac dysfunction is due to loss of PPARα caused by oxidative stress which affects the myosin molecule. Generally, cardioprotective are the drugs act as an agonist of PPARα and reduce the inflammatory condition, increase adiponectin expression on cardiac muscle and reduced efficiency of heart which may be due to increased expression of cardiac UCP3 mRNA. Researchers found that cardiovascular PPAR-α expression in cardiomyocyte hypertrophy condition reduce the inflammation by activation of inflammatory signalling pathways and also have antioxidative effects. Arrhythmogenic right ventricular dysplasia (ARVD) is due to functional abnormalities of PPAR, a rare genetic disease characterized by a progressive fibro fatty infiltration, decreased PPAR-α, and increased PPAR-γ expression in the right ventricle[17]. In order to the molecular mechanism of the cardioprotective nature of these plant extracts, few isolated plant constituents were tested with the target protein PPAR-α, where Dresgenin from *MT* and Lupeol from *SR* showed the high affinity towards the target protein and these phytoconstituents protect the myocardium from the toxic agents.

HPLTC in fingerprinting of natural drugs may encourage the recognition of natural products and it was suited to deliver the core scaffolds for forthcoming drugs. Hence, there will be further developments in the use of novel analytical techniques in natural products 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 added an additional value of medicinal importance for this Morva species.

Hisopathological reports revealed that there were treatment related microscopic changes observed in liver and heart of rats. There was an infiltrates with mononuclear, capsular, diffuse and minimal damage to the cellular functional units were found with animal received 50mg/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 range of normal in other extracts treated and control animals. It can therefore conclude that all histological changes observed and were brought back to normal by extracts treatment.

CONCLUSION

The cardioprotective study results suggested that a correlation between antioxidant enzyme and the degree of damage caused by Doxorubicin. It was concluded that by increasing antioxidant enzymes that combat free radical damage. *Marsdenia tenacissima* was reported to have an antioxidant property moreover it was used for the treatment of cancer Similarly, ethyl acetate extract of *Sansevieria roxburghiana* possess significant antioxidant as well as anticancer effect.

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