

Cardioprotective Activity of *Randia Dumetorum* against Doxorubicin Induced Cardiotoxicity

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<http://dx.doi.org/10.13005/bbra/3023>

(Received: 26 April 2022; accepted: 14 September 2022)

In the present investigation the cardioprotective activity of ethanolic extract of *Randia dumetorum* fruits at the doses of 100, 200 and 400 mg/kg was investigated against doxorubicin induced cardiotoxicity model. In high fat diet induced atherosclerosis several hemodynamic parameters such as systolic and diastolic blood pressure, serum parameters such as lactate dehydrogenase (LDH), tissue parameters such as superoxide dismutase (SOD), reduced glutathione (GSH), and malonaldehyde (MDA) were determined and found to be significantly altered in induction control group treated with doxorubicin. The histopathological studies of cardiac tissue were also performed wherein doxorubicin showed toxic effects on tissue. Ethanolic extract of *Randia dumetorum* fruits showed protection against doxorubicin induced cardiotoxicity by normalizing the altered parameters and producing ameliorating effects against doxorubicin induced cardiac damage. The multistep putative action of ethanolic extract of *Randia dumetorum* fruits may be attributed to the prominent phytoconstituent namely 2-(3,5-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol estimated through HPTLC analysis of the extract. Thus, the study exhibited the protective effect of ethanolic extract of *Randia dumetorum* fruits against doxorubicin induced cardiotoxicity.

Keywords: Cardiotoxicity; Doxorubicin; lactate dehydrogenase (LDH); *Randia dumetorum*; superoxide dismutase (SOD).

The term cardiovascular disease (CVD) represents a broad range of diseases including heart disease, stroke, hypertension, hyperlipidemia, thromboembolism, coronary heart disease, congestive heart failure (CHF), hardening of the arteries, other circulatory system diseases etc. The published reports state that cardiovascular diseases are currently the leading cause of death especially in industrialized countries.¹ In addition to mortality, poorly managed CVD can lead to significant long-term disability from the complications of

heart attacks, strokes, heart failure, and end-stage renal disease. CVD has become serious public health issue and hence requires greater attention to promote adequate awareness and treatment, both to health care providers and to the public. The growth of CVD in India further indicates that certain conditions like complicated hyperlipidemia, drug induced cardiotoxicity, atherosclerosis hasten the progress of disease and can cause various complications.²

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Cardiotoxicity includes a wide range of cardiac effects from small changes in blood pressure and arrhythmias to cardiomyopathy. Drug-induced cardiotoxicity represents one of the most serious and major toxic effects induced by several chronically administered drugs. Cardiotoxicity accounted for 60% of all drugs withdrawn till date, which was mainly due to cardiac ischemia-related and arrhythmogenic side effects. The outcome of therapy is largely governed by precipitation of untoward effects which in turn may obstruct continuation of therapy. Drug-induced cardiotoxicity represents a major adverse effect of some common traditional antineoplastic agents. Doxorubicin (DOX) is one of the most potent anticancer agents, which has been reported to be associated development of cardiotoxicity in the form of cardiomyopathy and congestive heart failure in >50% of patients in last 10 years. Doxorubicin (DOX) has been reported to produce a multimodal cardiotoxic action, including impairment of mitochondrial metabolism, oxidative stress, disruption of calcium modulation and direct DNA damage.^{3,4} This in turn promoted doxorubicin to be used as an inducing agent in experimental pharmacology. Thus, the current scenario advocates a need for novel agents with higher cardioprotective efficacy against doxorubicin induced cardiotoxicity.⁵

Randia dumetorum commonly known as madanphala belongs to the family Rubiaceae.⁶ The fruits of *Randia dumetorum* have been documented for several pharmacological activities like antibacterial, antioxidant, anti-allergic, anti-inflammatory, analgesic, immunomodulatory activities etc.^{7,8} In light of this, present study has been undertaken.

MATERIALS AND METHODS

Plant material collection and authentication

Fruits of *Randia dumetorum* were procured from local market. The plant materials were taxonomically identified and authenticated.

Preparation of extract

The powdered drug (100 gm) was extracted successively using a Soxhlet extractor with 200ml of ethanol. Extracts were filtered, concentrated and after complete solvent evaporation, each of these

solvent extract was weighed and preserved at 5°C in an airtight bottle until further use.

Preliminary phytochemical screening of extract

The ethanolic extract of fruits of *Randia dumetorum* were analyzed for the presence of phytochemical constituents such as terpenoids, alkaloids, quinines, flavonoids, saponins, steroids and phenolic compounds using the standard qualitative phytochemical methods.⁹

Drugs and chemicals

All the drugs and chemicals of AR grade were procured from local vendor.

Animals

Male Swiss albino mice (18-22gm) and Wistar albino rats (180-220gm) of either sex was procured from local vendor and were maintained at $25 \pm 2^\circ$ C and relative humidity of 45 to 55% and under standard environmental conditions (12 h light: 12 h dark cycle) at animal house. The animals had free access to food and water throughout study. Institutional Animal Ethical Committee approved the protocol. All the experiments were carried out between 9:00- 16:00 hour.

Ethical clearance

Institutional Animal Ethical Committee of D. Y. Patil College of Pharmacy, Akurdi, Pune approved the protocol.

Preliminary acute toxicity test

Healthy adult male Swiss albino mice (18-22 g) were subjected to acute toxicity studies of ERD as per guidelines (AOT 425) suggested by the organization for economic cooperation and development (OECD-2000). The mice were observed continuously for 2 h for behavioural and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days.^{10,11}

Cardioprotective activity

Doxorubicin induced cardiotoxicity model

48 preselected rats were divided into 06 groups, each group consisting of 06 rats (Table 1).

These rats were subjected to respective drug treatment for the period of 10 days along with intraperitoneal administration of doxorubicin (20mg/kg) from 8th day.

On 10th day, 60 minutes after dosing, following procedures were carried out.

- Recording of blood pressure (hemodynamic parameters)
- Estimation of serum and tissue parameters

(oxidative biomarkers/antioxidant parameters)

• Histopathological studies¹²⁻¹⁴

Recording of hemodynamic parameters

The systolic and blood pressure was measured using non-invasive blood pressure measurement apparatus.¹²

Estimation of serum parameters

The blood was collected from the retro orbital plexus of each rat. Serum was separated in a centrifuge at 7000 rpm for 15 minutes and lactate dehydrogenase (LDH), creatinine phosphokinase – MB isoenzyme (CKMB) levels were measured using standard kit according to the manufacturer's instruction manual using auto analyzer.^{13,14}

Estimation of tissue parameters (oxidative biomarkers/antioxidant parameters)

All rats were euthanized and hearts were removed and weighed, after squeezing out the blood, 02 hearts of randomly selected rats were sent for histopathological investigation while rest 04 hearts were processed & homogenized with 10% chilled TRIS hydrochloride buffer (10mM, pH 7.4) using tissue homogenizer at 7500 rpm for 15 minutes. The clear supernatant was used for the estimation of superoxide dismutase (SOD), reduced glutathione (GSH), and malonaldehyde (MDA) using standard kit.¹⁴

Histopathological studies

The cardiac tissue was dissected and excised from the isolated heart and washed with the normal saline, and stored in 10% buffered neutral formalin and then subjected to histopathological examination to evaluate the details of architecture in each group microscopically.^{13,14}

Data Analysis

Data obtained was subjected to statistical analysis of suitable parametric or non-parametric test using GraphPad InStat software, USA.

High Performance Thin Layer Chromatography (HPTLC) studies of ethanolic extract of *Randia dumetorum* fruit¹⁵

Instrumentation

HPTLC system of CAMAG, Muttenz, Switzerland, Anchrom Enterprises (I) Pvt. Ltd, Mumbai, consisting of sample applicator (Linomat 5), Twin trough chamber with lid (10×10 cm, CAMAG, Muttenz, Switzerland), UV cabinet (Aetron, Mumbai) with dual wavelength (254/366

nm) and the HPTLC photodocumentation (Aetron, Mumbai) was used for study.

Chromatographic conditions

The sample was spotted in the form of bands of width of 6 mm with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F254 (5 cm × 10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Toluene: Ethyl acetate (7: 3 v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 10 min. The length of chromatogram run was 8 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier.

Sample Preparation

10 mg of ethanolic extract of *Randia dumetorum* fruit was dissolved in 10 ml of ethanol. 10 µl volume of clear supernatant sample was applied on the TLC plate

Calculation of R_f Values

Plate was observed in the daylight, under UV light (254 and 366 nm). Retention factor (R_f) was calculated by following formula (Chatwal and Anand, 2004; Sethi and Charegaonkar, 1999).

$$R_f = A/B$$

A = distance between point of application and central point of spot of material being examined.

B = distance between the point of application and the mobile phase front.¹⁵

RESULTS

Preliminary phytochemical screening

The results of preliminary phytochemical screening of ethanolic extract of fruits of *Randia dumetorum* (ERD) showed the presence of triterpenoids, glycosides, saponins, carbohydrates, flavonoids, tannins and proteins.

Preliminary acute toxicity test

Ethanolic extract of fruits of *Randia dumetorum* (ERD) was found to be safe and no mortality was observed up to 2000 mg/kg.

Doxorubicin (DOX) induced cardiotoxicity model

Recording of hemodynamic parameters

The results of the study (Table No. 2) showed significant ($P < 0.001$) decrease in the systolic as well as diastolic blood pressure in doxorubicin induction control group as compared to normal control groups. ERD was capable of overcome the doxorubicin induced decrease in the blood pressure and showed significant increase in the blood pressure dose dependently at doses 200 mg/kg ($P < 0.01$), 400 mg/kg ($P < 0.001$) and standard FF65 ($P < 0.001$).

Estimation of serum parameters

The results of the study (Table No. 3) showed significant ($P < 0.001$) increase in the LDH as well as CKMB in doxorubicin induction control group as compared to normal control groups. ERD produced significant and dose dependant decrease in both LDH and CKMB at doses 200 mg/kg ($P < 0.01$), 400 mg/kg ($P < 0.001$) and standard FF65 ($P < 0.001$).

Estimation of tissue parameters

The results (Table No. 4) showed that doxorubicin administration caused significant elevation ($P < 0.001$) of tissue malondialdehyd (MDA) levels as well as significant ($P < 0.001$) decrease in tissue levels of superoxide dismutase (SOD) and reduced glutathione (GSH) as compared to normal control group. ERD reverted these alterations significantly and dose dependently at doses 200 mg/kg ($P < 0.01$), 400 mg/kg ($P < 0.001$) and standard FF65 ($P < 0.001$) in comparison with doxorubicin induced control group.

Determination of heart weight, body weight and heart weight to bodyweight ratio

The results of the investigation (Table No. 5) indicated a significant decrease in heart weight as well as body weight in doxorubicin induced control group as compared to normal control group. The groups treated with extract successfully attenuated the reduction in weight produced by the doxorubicin and exhibited significant and dose dependant increase in the body weight at doses 200

Table 1. Experimental design

Experimental Groups	Treatment
Normal Control	Vehicle 1ml/kg
Dox Control	Dox (20 mg/kg), i.p.
ERD 100 + DOX	ERD 100 mg/kg p.o. + DOX (20 mg/kg), i.p.
ERD 200 + DOX	ERD 200 mg/kg p.o. + DOX (20 mg/kg), i.p.
ERD 400 + DOX	ERD 400 mg/kg p.o. + DOX (20 mg/kg), i.p.
FF65 +DOX	FF 65 mg/kg p.o. + DOX (20 mg/kg), i.p.

Table 2. Recording of hemodynamic parameters

Experimental Groups	Systolic blood pressure (mm/Hg)	Diastolic blood pressure (mm/Hg)
Normal Control	186 ± 1.93	91.50±1.76
Dox Control	99.16± 1.01***	62.66±2.88***
ERD 100 + DOX	100.56± 1.45	68.29±1.36
ERD 200 + DOX	115.40±1.81 ^{##}	72.15± 1.63 ^{##}
ERD 400 + DOX	150.11±2.10 ^{###}	75.39±1.41 ^{###}
FF65 +DOX	155.21±2.10 ^{###}	80.57±2.12 ^{###}

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test. *** $P < 0.001$ as compared to normal control. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ as compared to DOX induced control.

mg/kg ($P<0.01$), 400 mg/kg ($P<0.001$) and standard FF65 ($P<0.001$) in comparison with doxorubicin induced control group. There was no significant difference found in the heart weight to body weight ratio in between all the groups.

Histopathological studies of cardiac tissue

Rats exposed to doxorubicin showed interfibrillar hemorrhages, congestion, and focal

areas of disrupted cardiac muscle fibers, hyperemia, cellular infiltration and necrosis, parenchymatous degeneration, eosinophilic degeneration, interstitial edema in the heart tissue. Pre-treatment with extract ERD 400 mg/kg and standard FF65 efficiently ameliorated these alterations showing protective activity against doxorubicin induced cardiotoxicity (Figure 1).

Table 3. Estimation of serum parameters

Experimental Groups	LDH(IU/L)	CKMB(IU/L)
Normal Control	755.83±17.06	96.33±2.98
Dox Control	1288.66±13.90***	160.00±2.74***
ERD 100 + DOX	1277.5±15.71	152.16±2.52
ERD 200 + DOX	1213.00±19.46 [#]	146.50±0.99 ^{###}
ERD 400 + DOX	1191.00±10.62 ^{##}	139.00±1.31 ^{###}
FF65 +DOX	1108±14.55 ^{##}	131.34±1.88 ^{###}

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test. *** $P<0.001$ as compared to normal control. [#] $P<0.05$, ^{##} $P<0.01$, ^{###} $P<0.001$ as compared to DOX induced control

Table 4. Estimation of tissue parameters

Experimental Groups	MDA(mm/mg protein)	SOD(U/gm protein)	GSH(mm/mg protein)
Normal Control	33.50± 0.92	28.25±0.59	10.73±0.24
Dox Control	77.14± 1.66***	15.73±0.40***	5.205±0.11***
ERD 100 + DOX	76.13±1.31	16.11±0.27	4.99± 0.07
ERD 200 + DOX	67.41±1.79 [#]	18.91±0.23 ^{###}	5.89±0.14 ^{##}
ERD 400 + DOX	65.43±2.87 ^{##}	21.56±0.30 ^{###}	6.55±0.21 ^{###}
FF65 +DOX	61.28±1.72 ^{##}	24.66±0.45 ^{###}	7.38±0.19 ^{###}

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test. *** $P<0.001$ as compared to normal control. [#] $P<0.05$, ^{##} $P<0.01$, ^{###} $P<0.001$ as compared to DOX induced control

Table 5. Determination of heart weight, body weight and heart weight to bodyweight ratio

Experimental Groups	Heart weight (mg)	Body weight (gm)	Heart weight /body weight ratio
Normal Control	370.00±5.30	204.83±3.55	1.80±0.042
Dox Control	301.83±5.12***	178.33±2.88***	1.69±0.050
ERD 100 + DOX	302.83±4.67	190.33±3.77	1.57± 0.020
ERD 200 + DOX	323.33±3.46 ^{##}	198.33±2.24 ^{##}	1.63± 0.029
ERD 400 + DOX	329.33±3.34 ^{###}	207.83±2.05 ^{###}	1.58±0.031
FF65 +DOX	335.48±4.12 ^{###}	203.22±2.34 ^{###}	1.55±0.045

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test. *** $P<0.001$ as compared to normal control. [#] $P<0.05$, ^{##} $P<0.01$, ^{###} $P<0.001$ as compared to DOX induced control.

Representative photomicrographs (H & E stain) of liver tissue (A) Normal control (B) Positive control (C) ERD 400 (D) FF65. Rats exposed to doxorubicin showed interfibrillar hemorrhages, congestion, and focal areas of disrupted cardiac muscle fibers, hyperemia, cellular infiltration and necrosis, parenchymatous degeneration, eosinophilic degeneration, interstitial edema in the heart tissue. Pre-treatment with extract ERD 400 mg/kg and standard FF65 efficiently ameliorated these alterations.

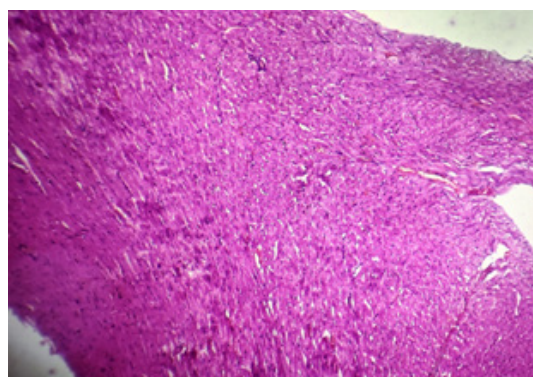
High Performance Thin Layer Chromatography (HPTLC) studies of ethanolic extract of *Randia dumetorum* fruit¹⁵

The band at R_f Value 0.32 was scratched, extracted with ethanol and evaporated to dryness (The process required semipreparative TLC to achieve sufficient amount) for further analysis by IR, NMR and Mass Spectrometry.

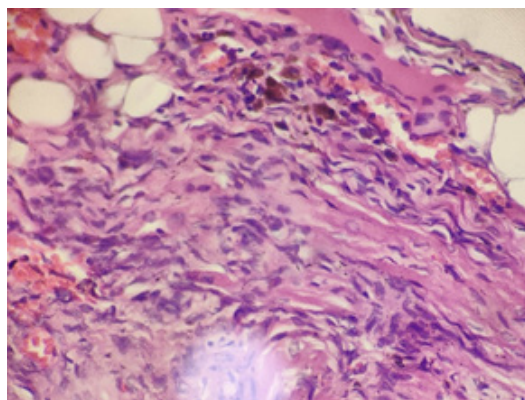
Spot at R_f Value – 0.32

DISCUSSION

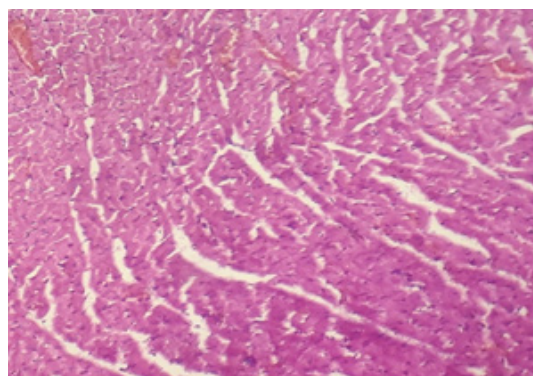
Drug induced cardiotoxicity is another concern which is becoming important day by day due to the development and use of various drugs.¹⁶ Doxorubicin is the most widely used chemotherapeutic agents hence its cardiotoxicity is of paramount concern.¹⁷ This most frequent and serious adverse effect (cardiotoxicity) is commonly observed in doxorubicin-treated cancer surviving patients.¹⁶ This side effect may restrict the use of drugs irrespective of their potential as a therapeutic agent.¹⁸ Considering therapeutic potential of doxorubicin as an anticancer agent, it is not affordable to stop this therapy due to associated to cardiotoxicity, rather complimentary therapy that can selectively reduce or prevent the cardiotoxicity is the most appropriate solution.^{19,17}



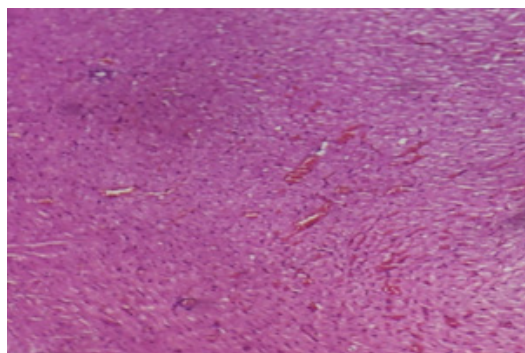
A. Normal Control



B. Positive Control



C. ERD 400



D. FF65

Fig. 1. Effect of Ethanolic extract of fruits of *Randia dumetorum* (ERD) doxorubicin induced changes in heart tissue

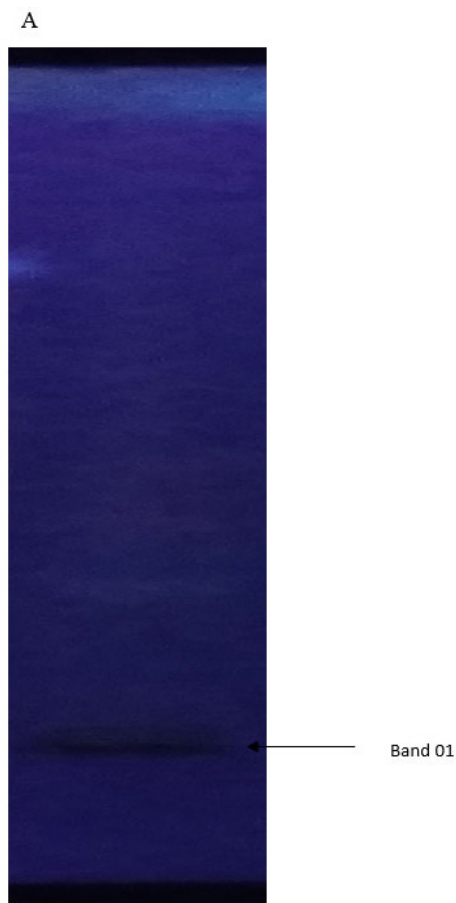


Fig. 2. Ethanolic extract of fruits of *Randia dumetorum* at 366 nm, Volume applied 10 ml

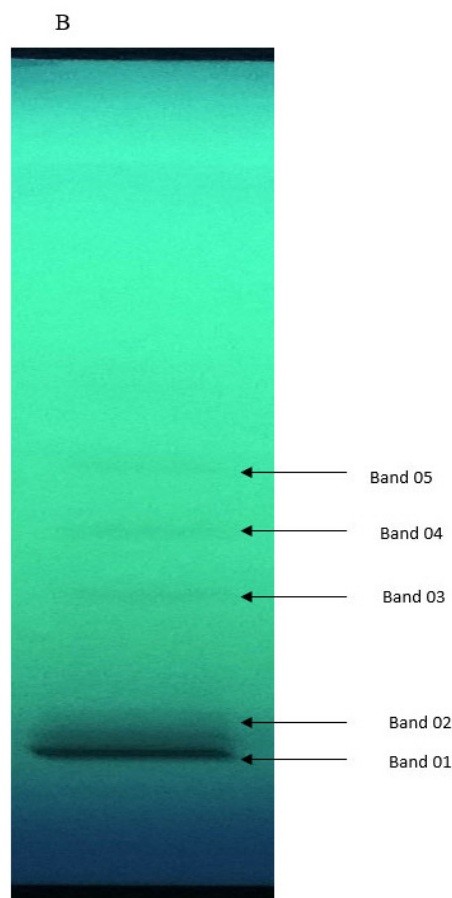


Fig. 3. Ethanolic extract of fruits of *Randia dumetorum* at 254 nm, Volume applied 10 ml

Herbal medicines play considerable role in health care to a large proportion of world's population and have been regarded as component of cultural heritage of various tribes. Since these drugs are from natural origin hence are documented for reducing the risks of cardiac ailments by several mechanisms by virtue of presence of several phytoconstituents.²⁰

Randia dumetorum is such an indigenous medicinal plant which is traditionally claimed to be useful in the treatment of cardiovascular diseases but haven't yet been adequately documented, hence its cardioprotective activity was evaluated against doxorubicin induced cardiotoxicity.²¹

The traditional claim, scientific observations and presence of phytochemicals have close relationship towards the actual therapeutic outcome.^{22,11} In light of this, the preliminary

phytochemical analysis of ethanolic extracts of fruits of *Randia dumetorum* was carried out. The extract showed the prominent presence of triterpenoids, glycosides, saponins, carbohydrates, flavonoids, tannins and proteins. After the confirmation of phytochemical profile and its pharmacological importance, then toxicity profile testing becomes highly essential to confirm its safety before going for actual exploration of its pharmacological activity. Toxicity profile testing is critically important for any therapeutic medication for confirmation of the extent of its therapeutic utilization.¹⁰ Hence acute oral toxicity test was conducted for ERD extract for confirmation of its safety. The results of acute oral toxicity studies of ERD revealed that both these extracts are safe up to 2000 mg/kg which is highest prescribed limit as per this test. Thus, ERD fulfilled the safety criteria

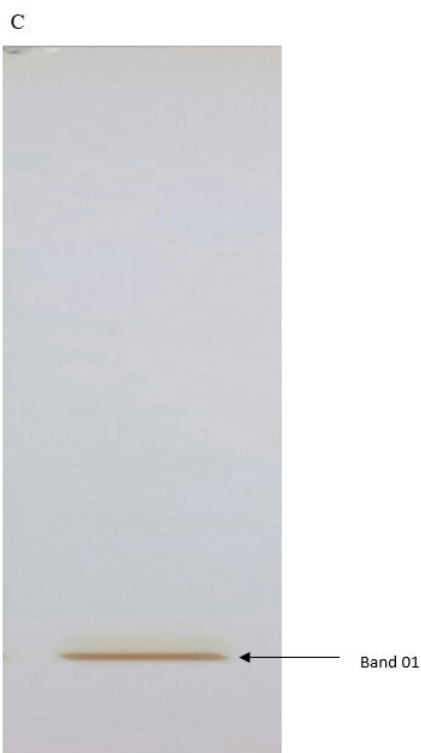


Fig. 4. Ethanolic extract of fruits of *Randia dumetorum* at visible, Volume applied 10 ml

before the assessment of preclinical activity. Based upon these findings and available literature, the three different doses i.e., 100, 200 and 400 mg/kg of extract were evaluated for cardioprotective activity against doxorubicin induced cardiotoxicity.

The results of current study were quite encouraging. The significant restoration of decreased systolic as well as diastolic blood pressure by the extract exhibited its potential from basic step. It has been reported that approximately 60% of patients with cardiotoxicity usually experience fluctuations in blood pressure as first symptom which if attended well then further complications do not occur at all.²³ Moreover, as on date, modern therapy does not have any satisfactory remedy to elevate this lowered blood pressure hence simple fluctuation may result in serious condition.²⁴ Accordingly, ERD was found to be effective which is a primary but important outcome of the study.

The damage to cardiomyocytes indicating anatomical manipulation is the next step of cardiotoxicity. This damage is usually indicated by the elevated levels of LDH and CKMB.²⁵ In this connection, ERD showed significant restoration of elevated levels of serum LDH as well as CKMB.

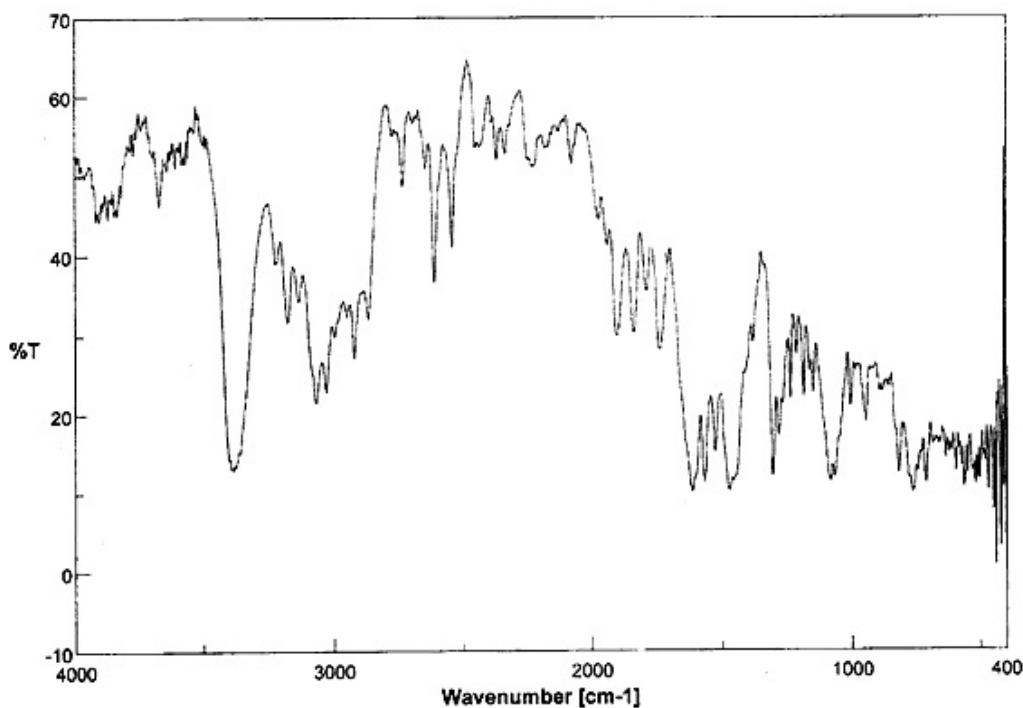


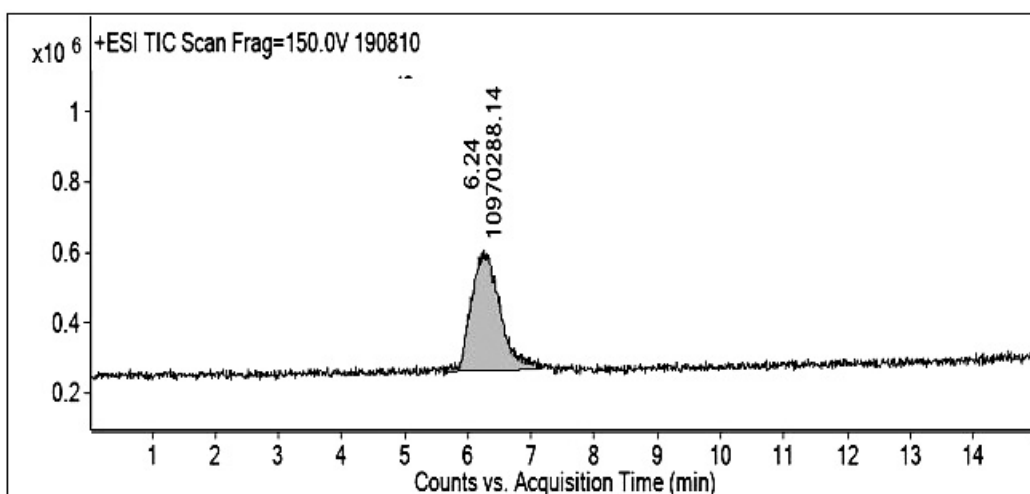
Fig. 5. FT-IR Spectrum of compound at $R_f = 0.32$

ERD showed the presence of triterpenoids and glycosides which are already reported to control CKMB.^{26,27}

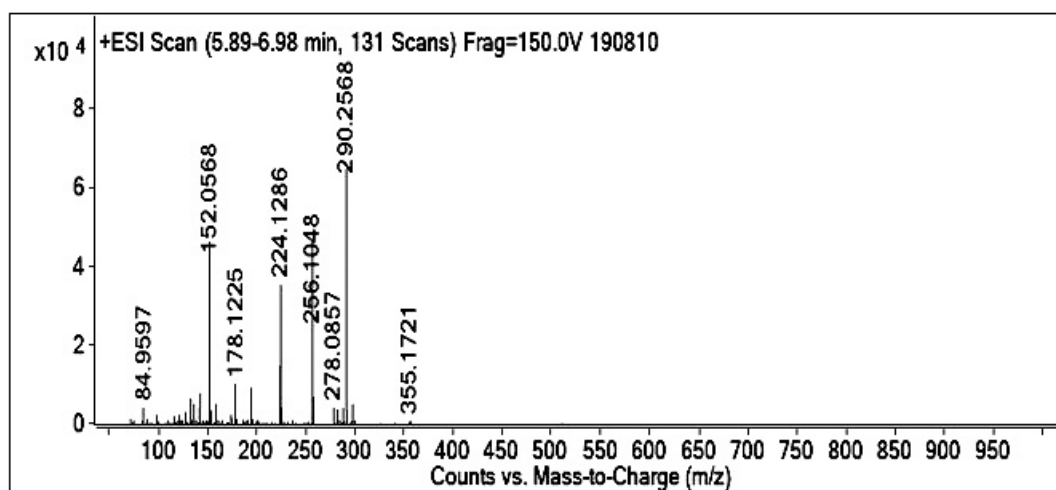
It is well documented that about 85% of patients who are on doxorubicin therapy report alterations in these two markers and out of which almost 25 % of patients need to switch to other therapy.²⁸ In the patients with mild elevation in LDH which do not need to stop or switch from doxorubicin therapy are still under risk zone. The patients upon consumption of aspirin, alcohol, exposure to stress and strenuous exercise may

suddenly lead to easy shoot up of levels.²⁹ Hence the results obtained for ERD are highly valuable to continue doxorubicin therapy with minimum risk.

An increase of free radicals and a decrease in the activity of endogenous antioxidants in the myocardium is believed to play important roles in the pathogenesis of doxorubicin-induced cardiotoxicity and subsequent heart failure.³⁰ In light of this, the significant modulations in antioxidant parameters such as malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione (GSH) by ERD were recorded.



(A)



(B)

Fig. 6. LC-MS A) Chromatograph and B) Spectrum of compound at R_f – 0.32

These promising reports are suggestive of role of antioxidant potential of the ERD. The elevated MDA levels is usually observed in cancer patients which further increases with doxorubicin therapy and can worsen the conditions rapidly. This is one of the best examples of limitations of therapy due to drug induced toxicities.³¹ Hence the significant reduction in the MDA levels by both the extracts which is not only useful to halt doxorubicin induced

toxicity but can speed up the recovery too. This also suggest possible use of the ERD as an adjuvant therapy to doxorubicin in cancer patient. The significant modulation in GSH and SOD which are part of body's antioxidant mechanism further endorse the role of ERD in preventing doxorubicin induced toxicity.³²

It is also documented that GSH imbalance is seen in various diseases including cancer. Further

Table 6. Interpretation of NMR spectrum

Sr. No.	Part of molecule	Vibration	General Range (Cm ⁻¹)	PI 36
1	Ar Rings	a) C=C stretch	1500-1650	1512
		b) C-H stretch	3000-3100	3067
		c) C-H bend	740-762	767
		d) Overtone	1700-2000	1700-2000
2	CH ₂ /CH-(Aliphatic)	a) C-H stretch	2850-3000	2919
		b) C-H bend	1300-1470	1472
3	-O-H	O-H stretch	3200-3600	3386

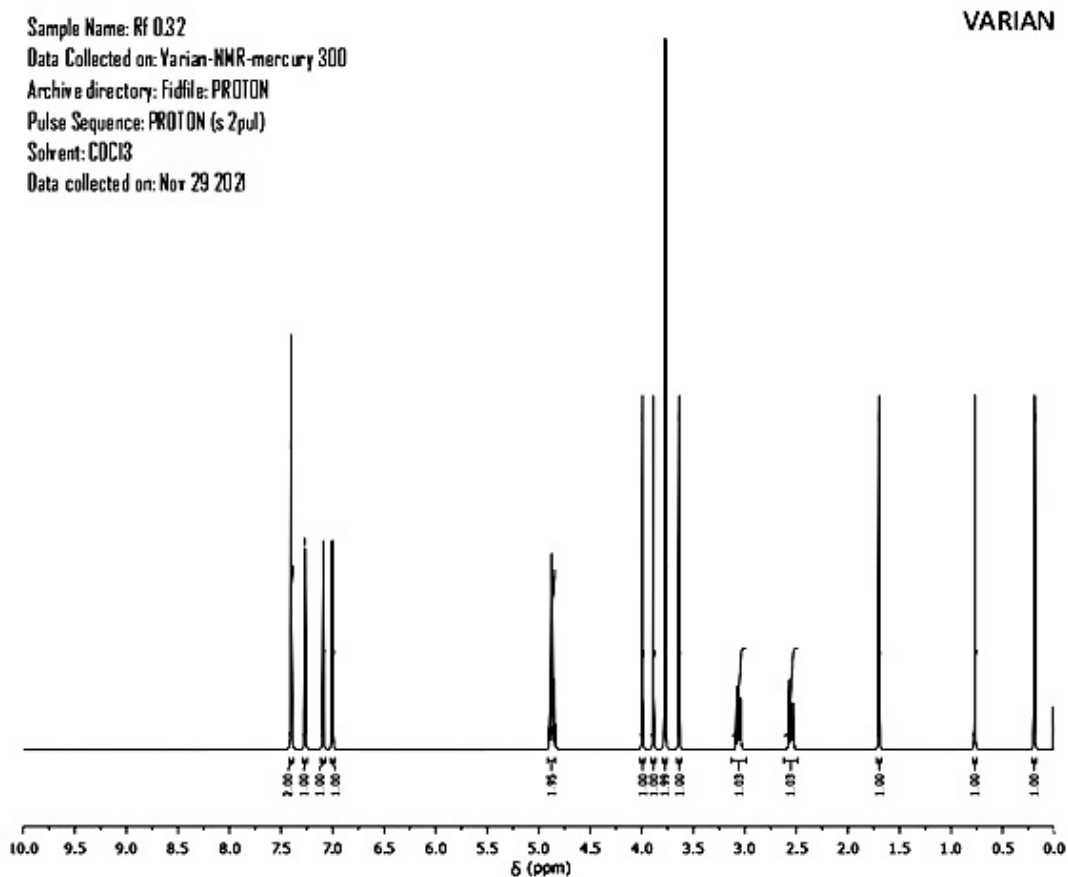
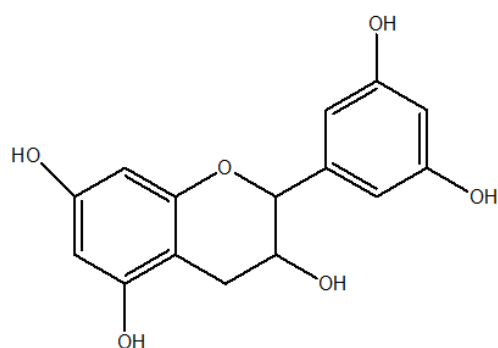


Fig. 7. NMR Spectrum of compound at R_f – 0.32

Table 7. Interpretation of NMR spectrum

No.	δ	No of Protons	Multiplicity	Type
1	2.552	1 H	d	CH ₂ proton of the chromene ring
2	3.064	1 H	d	CH ₂ proton
3	3.686	1 H	s	OH Proton
4	3.771	1 H	s	O2 OH Protons
5	3.854	2 H	s	OH Proton
6	3.925	1 H	s	OH Proton
7	4.862-4.884	2 H	m	CH protons of the chromene ring
8	6.981	1 H	s	O1 Aromatic protons of the chromene ring
9	7.074	1 H	s	O1 Aromatic protons of the chromene ring
10	7.256	1 H	s	O1 Aromatic protons of phenyl ring
11	7.388	2 H	s	O2 Aromatic protons of phenyl ring

**Fig. 8.** 2-(3,5-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol

this imbalance is more intense with increasing age making the conditions more complicated.³³ This further makes doxorubicin therapy more.³⁰ On this background, significant reduction in GSH levels with the pre-treatment of extracts not only useful to prevent doxorubicin induced toxicity but also to slow down the natural deterioration due to the existing cancer. This dual effect is an ideal achievement of this study. Both the extracts have already been reported for its anti-inflammatory activity. On the contrary, continuous inflammation is one of the reasons for occurrence of cancer and aggravation of already existed cancer. Hence, addition of ERD as an adjuvant therapy as proposed above can have an add on benefit to restrict the role of inflammatory mediators in the progress of the cancer.³³ This multistep action is most important advantage over the modern medicine.³⁴

Cardiomyocyte death, decrease in myocyte volume and its number leading to

decrease in heart weight is another toxic effect of doxorubicin. In the current study, this toxic effect could not be modulated by the ERD suggesting non-utility for this type of damage.³⁵ Reducing heart weight can initiate the series of adverse events like reduction in blood pressure followed by difficulty in pumping and then reflex elevation in blood pressure which in due course of usually time results in multiple organ failure.³⁶

Here at initial stage, ERD may provide some relief by restoring reduced dropped blood pressure but cannot offer any protection over subsequent events. Thus, this study also reported the inability of ERD to protect or reverse the critical situation that arises due to the death of cardiomyocytes.³⁷

Histopathology is an important tool to know extent of tissue damage in pathophysiology. As a general rule, when the tissue architecture shows adverse changes, it indicates the progressive step of any diseased condition. Moreover, when these initial reversible changes become irreversible then condition become more critical.³⁸ The drug which prevents or delays the involvement of the tissue, halt process at reversible stage or delays to go into the irreversible stage is found to be effective.³⁹ In this regard, ERD showed a widespread improvement in histopathology suggesting their role even in progressive stage of toxicity. Amelioration of necrosis by ERD is an indication to keep pathological changes to reversible nature which is the most valuable result. This property of the ERD makes it suitable to provide wider protection and ensure patients quality of life for longer period which is one of

the most important expectations in the case of cardiotoxicity.^{40,39}

The HPTLC analysis of ERD was carried out to identify the phytoconstituent responsible for the cardioprotective activity. The results of study revealed the prominent presence of a compound namely 2-(3,5-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol as pharmacologically important phytoconstituent. The isolation of these phytoconstituents is an important concluding result of the study and cardioprotective activity of ERD may be attributed to this phytoconstituent.⁴¹

The overall results thus showed a potent and widespread protective activity of ethanolic extract of fruits of *Randia dumetorum* against doxorubicin induced cardiotoxicity indicating its use in the cancer patients for development of cardioprotective treatment especially for doxorubicin-treated cancer survivors.⁴²

CONCLUSION

The present study documented the cardioprotective activity of ethanolic extract of fruits of *Randia dumetorum* against doxorubicin induced cardiotoxicity. The extract offered wide range of protection through control of hemodynamic parameters, modulation of various markers, imparting antioxidant action and thereby improvement in histopathology as well showing potent cardioprotective activity against doxorubicin induced cardiotoxicity. The multistep putative action of ethanolic extract of fruits of *Randia dumetorum* may be attributed to the prominent phytoconstituents namely 2-(3,5-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol revealed in the study.

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