An Elsevier Indexed Journal ISSN-2230-7346

*Journal of Global Trends in Pharmaceutical Sciences*

# CARDIOPROTECTIVE EFFECT OF *MOMORDICA DIOICA* ROXB. FRUIT UPON STRESS AND CLOZAPINE INDUCED CARDIOTOXICITY IN RAT MODEL.

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**ARTICLE INFO**

**Key Words**

*Momordica dioica*, Stress, Cardioprotective, cardiac injury markers, stress markers

**ABSTRACT**

**Objective:** Cardiovascular diseases hold the position of being a major cause of morality round the globe. The objective of the study was to evaluate the cardioprotective effect of *Momordica dioica* Roxb. fruit extract on stress and clozapine induced cardiotoxicity in Wistar Albino Rats. **Methodology:** The hydroalcoholic (HAEMD) and aqueous (AQEMD) extract of *Momordica dioica* Roxb. were prepared, standardized and screened for various *in vitro* free radicals scavenging activity. *In vivo* cardioprotective activity was evaluated against stress and clozapine induced cardiotoxicity. The HAEMD & AQEMD were assessed for cardioprotection at two dose levels and activity was compared with standard Vit E. Animals of all groups received treatments for 15 days. Evaluation was done by ECG, cardiac toxicity markers (CK-MB, LDH and AST), stress markers and histopathalogical changes. **Results:** Extracts of AQEMD and HAEMD at dose of 200 mg/kg showed significant activity which is evident from the significant decrease in cardiac injury markers (CK-MB, LDH and AST) as well as stress markers levels when compared with that of control group. The activity was found to be dose dependent and comparable with that of standard. **Conclusion:** The AQEMD and HAEMD of *Momordica dioica* Roxb. have been found to contain several flavonoids and cardioprotective phytochemicals which have the potential to scavenge the free radicals as evident from the *in-vitro* assays. The cardioprotective activity of *Momordica Dioica* is exhibited by free radical scavenging. However further studies are required to assess the cardioprotective activity and validate its use in higher animal models with mechanistic studies.

## INTRODUCTION

Cardiovascular diseases hold the position of being a major cause of morality round the globe. The WHO report 16.7 million deaths due to CVD across the world, per year[2]. About 14 million casualties have been reported due to cardiovascular diseases every year with an expected growth of up to 25 million individuals by the year 2020[1]. Stress-induced cardiomyopathy (ballooning syndrome, broken heart syndrome and cardiomyopathy) is an increasingly reported syndrome generally characterized by transient systolic dysfunction of the apical and/or mid segments of the left ventricle that mimics myocardial infarction (MI)[6]. Postulated mechanisms include catecholamine excess, coronary artery spasm and microvascular dysfunction[7]. Clozapine is an atypical antipsychotic drug that is a tricyclic dibenzodiazepine derivative. It is

one of the effective anti-psychotic agents for the treatment of resistant schizophrenia. Several studies indicated that clozapine-induced myocarditis appears to be modulated by the ß-adrenergic system and its interaction with pro-inflammatory cytokines. The murine model of clozapine-induced myocarditis, therefore, may be helpful to study aetiology, treatment and prevention of clozapine cardiac toxicity in human[8]. *Momordica dioica* Roxb. (MDR) belonging to the family Curcurbitaceae, is generally found in the tropical countries like India, Sri Lanka, Burma, China and Malaya [9]. Traditionally it is used in the treatment of various ailments. However, there were no scientific evidences till date claiming the cardioprotective effect of this plant. Therefore, the current study was aimed to investigate the cardio protective effect of *Momordica dioica* on stress and clozapine induced cardiotoxicity in albino rats.

## Materials and Methods:

### Animal

Wistar Albino rats of either sex weighing 150-200 g were used and the animals were procured from JSS Medical College, animal facility Centre, Mysore. The studies conducted were approved by the Institutional animal Ethical Committee, JSS College of Pharmacy, Mysore, Karnataka (Approval no: 124/2012).

### Plant material

The fresh fruits of MDR were collected and identified and authenticated, a specimen sample (SAMDR032) is deposited in the Dept. of Pharmacognosy of JSS College of Pharmacy, Mysore.

### Preparation of extract

The fruits of MDR were, chopped into small pieces and dried under shade at room temperature for seven days. The dried fruits were pulverized by mechanical means and used for the preparation of hydroalcoholic (HAEMD) and aqueous extract (AQEMD).

### Preliminary phytochemical screening of MDR extracts:

The HAEMD and AQEMD was subjected to the phytochemical analysis using conventional protocol like sterols, tri-terpenes alkaloids, carbohydrates, tannins, flavonoids, reducing sugar, anthraquinone glycosides and cardiac glycosides were done [10-15].

***In vitro* antioxidant Assay:** The antioxidant activity of plant extracts was determined by different *in vitro* methods such as superoxide anion radical scavenging (SO) assay[17], metal chelating assay[18], ferric reducing antioxidant power assay (FRAP)[19] and DPPH radical scavenging assay [20- 21].

***In-vivo* cardio-protective activity [8, 22]:**

**Induction of cardiotoxicity in rats:** Cardiotoxicity was induced in rats by the administration of clozapine (dissolved in 0.1 M HCl and pH is balanced in phosphate buffered saline (PBS) for 7 days (25mg/kg body wt), i. p. Restraint stress was induced on 14$^{th}$ and 15$^{th}$ day the treatment by restrained in a 50ml conical centrifuge tube with multiple punctures and immersed vertically to the level of the xiphoid process into a 24±1$^0$C water bath for 1.

**Grouping and treatment:** Albino rats (160-250g) were randomly divided into seven groups (six in each group) and kept in the cages for one week prior dosing for acclimatisation. Animals receiving 0.5% Na CMC (vehicle) served as normal. Animals of all groups except normal received 25 mg/kg of clozapine in PBS for 7 days i.p followed by stress on 14$^{th}$ and 15$^{th}$ day. The treatment and evaluation was done as shown in the (table 1).

**Statistical analysis:** The values were expressed as Mean±Standard Error of Mean (SEM) of the indicated number of experiments animals. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukeys multiple comparison test. Values were significant if *p value <0.05.*

## RESULTS

**Percentage yield:** The percentage yield of AQEMD and HAEMD were 25.42% w/w and 18.70% w/w respectively.

**Preliminary phytochemical screening of MDR extracts:** Preliminary phytochemical analysis of various extracts of MDR revealed the presence of following

phytochemicals: AQEMD: sterols, triterpenes, saponins, alkaloids, carbohydrates, reducing sugars, tannins, flavonoids and cardiac glycosides. HAEMD: sterols, triterpenes, alkaloids, carbohydrates, reducing sugars. Tannins, flavonoids, anthraquinone derivatives and cardiac glycosides.

***In vitro* antioxidant activity**

**Superoxide anion radical scavenging (SO) assay:** Results of free radical scavenging activity by superoxide anion radical scavenging (SO) method is shown in (Table 2). The scavenging activity was found to be dose dependent and AQEMD ($IC_{50}$ 86.67±1.69 µg/ml) exhibited better SO scavenging potential when compared to HAEMD ($IC_{50}$ 187.46±1.02 µg/ml).

**Ferric reducing antioxidant power assay (FRAP):** Results of free radical scavenging activity by ferric reducing antioxidant assay (FRAP) method is shown in (Fig 1). In the current study, the scavenging activity was found to be dose dependent and AQMD ($IC_{50}$ 119±1.69 µg/ml) exhibited better ascorbic acid scavenging potential when compared to HAEMD ($IC_{50}$ 139±0.53 µg/ml)

**Metal chelating activity:** Results of free radical scavenging activity by metal chelating activity method are shown in (Table 3). The chelating activity was found to be dose dependent and AQMD ($IC_{50}$ 151.98±3.80 µg/ml) exhibited better EDTA scavenging potential when compared to HAEMD ($IC_{50}$196.54±3.3 µg/ml)

**Statistical analysis:** The values were expressed as Mean±Standard Error of Mean (SEM) of the indicated number of experiments animals. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukeys multiple comparison test. Values were significant if *p value <0.05.*

**RESULTS: Percentage yield:** The percentage yield of AQEMD and HAEMD were 25.42% w/w and 18.70% w/w respectively.

**Preliminary phytochemical screening of MDR extracts:** Preliminary phytochemical analysis of various extracts of MDR revealed the presence of following phytochemicals: AQEMD: sterols, triterpenes, saponins, alkaloids, carbohydrates, reducing sugars, tannins, flavonoids and cardiac glycosides. HAEMD: sterols, triterpenes, alkaloids, carbohydrates, reducing sugars. Tannins, flavonoids, anthraquinone derivatives and cardiac glycosides.

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**DPPH radical scavenging activity:** Free radical scavenging activity of AQEMD and HAEMD extracts by DPPH method is represented in (Table 4). The scavenging activity was found to be dose dependent and HAEMD ($IC_{50}$ 258±0.429 µg/ml) exhibited better scavenging potential when compared to AQEMD ($IC_{50}$ 240±0.739.µg/ml).

***In-vivo* cardioprotective activity**

**Morphological parameters**

**Body weight:** No significant body weight changes were observed among all the groups during the study period (Table 5)

**Table 1: Treatment schedule and evaluation protocol employed**

| Group | Treatment | Evaluation |
|---|---|---|
| Normal | Na CMC (vehicle) 1 ml/kg body weight, p.o for 15 days | **Body weight** |
| Control | Vehicle for 15 days and daily 25 mg/kg of clozapine in PBS was given for 7 days through i.p. along with stress on 14th and 15th day. | **ECG** After 3hrs of stress induction, on the 15th day, by using Niviqure inco polygraph system |
| Vitamin –E | 10 mg/kg p.o for 15 days and daily 25 mg/kg of clozapine in PBS was given for 7 days through i.p. along with stress on 14th and 15th day. | **Serum parameters** CK-MB, LDH and AST |
| HAEMD 100mg/kg | 100 mg/kg p.o for 15 days and daily 25 mg/kg of clozapine in PBS was given for 7 days through i.p. along with stress on 14th and 15th day. | **Endogenous antioxidant enzymes** SOD , Catalase, GSH, lipid peroxidation [23-27] |
| HAEMD 200mg/kg | 200 mg/kg p.o for 15 days and daily 25 mg/kg of clozapine in PBS was given for 7 days through i.p. along with stress on 14th and 15th day. | **Histopathalogical studies of heart** |
| AQEMD 100mg/kg | 100 mg/kg p.o for 15 days and daily 25 mg/kg of clozapine in PBS was given for 7 days through i.p. along with stress on 14th and 15th day. | |
| AQEMD 200mg/kg | 200 mg/kg p.o for 15 days and daily 25 mg/kg of clozapine in PBS was given for 7 days through i.p. along with stress on 14th and 15th day. | |

**Table 2: Percentage free radical scavenging of MDR extracts and ascorbic acid by superoxide anion radical scavenging assay method**

| % Scavenging | | | | |
|---|---|---|---|---|
| **Con (µg/ml)** | **AQEMD** | **HAEMD** | **Con (µg/ml)** | **Ascorbic acid** |
| 100 | 56.30±0.111 | 47.37±0.088 | 2 | 36.10±0.06 |
| 150 | 62.63±0.133 | 49.73±0.066 | 4 | 42.73±0.09 |
| 200 | 71.20±0.115 | 61.93±0.233 | 6 | 48.10±0.06 |
| 250 | 80.27±0.088 | 74.00±0.208 | 8 | 59.73±0.09 |
| 300 | 83.70±0.152 | 75.73±0.240 | 10 | 65.63±0.09 |
| **$IC_{50}$** | **86.67±1.69** | **187.46±1.02** | **$IC_{50}$** | **7.601±0.50** |

*Values are given as Mean±SEM, n=3*

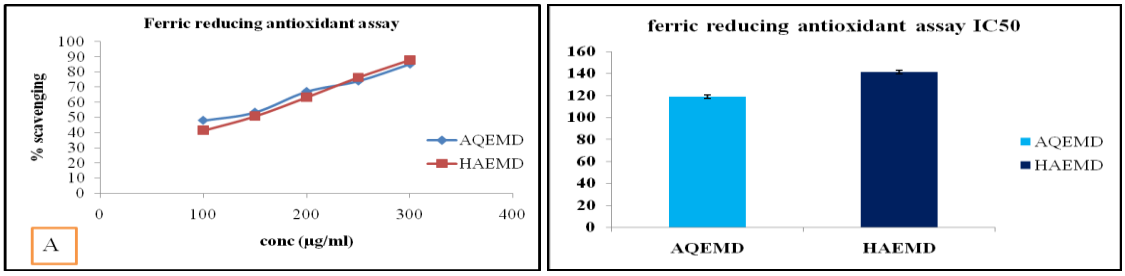


**Fig 1. Free radical scavenging activity of MDR by ferric reducing antioxidant power assay method (A) Percentage inhibition and (B) $IC_{50}$ of MDR**

**Table 3:** Percentage free radical scavenging of MDR extracts by metal chelating activity method.

| %Scavenging | | | | |
|---|---|---|---|---|
| **Con (µg/ml)** | **AQEMD** | **HAEMD** | **Con (µg/ml)** | **EDTA** |
| 100 | 34.4±0.23 | 29.7±0.24 | 10 | 38.4±0.18 |
| 150 | 48.6±0.17 | 36.5±0.12 | 20 | 49.6±0.05 |
| 200 | 69.3±0.11 | 50.5±0.16 | 30 | 60.9±0.05 |
| 250 | 74.1±0.14 | 67.2±0.18 | 40 | 74.7±0.12 |
| 300 | 86.1±0.06 | 85.4±0.25 | 50 | 87.3±0.07 |
| **$IC_{50}$** | **151.98±3.80** | **196.54±3.30** | **$IC_{50}$** | **20.09±0.19** |

*Values are given as Mean±SEM, n=3*

**Table 4.** Percentage free radical scavenging of MDR extracts and ascorbic acid by DPPH method

| %Scavenging | | | | |
|---|---|---|---|---|
| **Con (µg/ml)** | **AQEMD** | **HAEMD** | **Con (µg/ml)** | **Ascorbic acid** |
| 100 | 17.7±0.52 | 23.23±0.92 | 2.0 | 21.62±0.25 |
| 150 | 21.08±0.8 | 31.66±1.06 | 2.5 | 27.43±0.26 |
| 200 | 32.93±1.21 | 40.43±0.75 | 3.0 | 39.38±0.58 |
| 250 | 38.7±0.54 | 53.11±0.69 | 3.5 | 64.28±1.25 |
| 300 | 52.03±1.09 | 63.21±0.57 | 4.0 | 70.62±0.60 |
| **$IC_{50}$** | **258±0.42** | **240±0.73** | **$IC_{50}$** | **3.15±0.85** |

*Values are given as Mean±SEM, n=3*

**Table 5:** Cardioprotective activity of MDR extracts on Stress and clozapine induced cardiotoxicity on normal rats. (Body weight of rats)

| **Group** | **Body weight (g)** | | **% reduction in body weight** |
|---|---|---|---|
| | **On $0^{th}$ day** | **On $16^{th}$ day** | |
| Normal | 173.3±1.67 | 177.5±1.71 | 0.00% |
| Control | 171.7±2.11 | 161.7±1.67[a] | 9.52% |
| Std Vitamin E | 177.5±1.12 | 180.5±1.12 | 0.00% |
| AQEMD 100mg | 175.0±1.29 | 166.7±2.47[b] | 5.69% |
| AQEMD 200mg | 170.5±.1.67 | 177.5±1.71[b] | 0.00%[b] |
| HAEMD 100mg | 174.2±1.54 | 170.3±1.56[b] | 1.06% |
| HAEMD 200mg | 176.7±1.67 | 178.5±2.0[b] | 0.00%[b] |

*Values are in Mean±SEM, n=6 [a]Significant when compared to normal (P<0.05)*
*[b]Significant when compared to control (P<0.05) [c]Significant when compared to standard (P<0.05)*

**Table 6:** Effect of extracts of MDR on non-serum parameters on Stress and clozapine induced cardiotoxicity in rats (Non-serum parameters: ECG)

| **Group** | **ST interval (m Sec)** | **QT interval (m Sec)** | **Heart rate (Beats/min)** |
|---|---|---|---|
| Normal | 36.23±1.56 | 66.88±1.56 | 349.50±16.17 |
| Control | 52.16±2.86[a] | 76.86±1.99[a] | 298.31±12.47[a] |
| Std Vitamin E | 37.34±1.03 | 65.89±1.55 | 372.52±17.93 |
| HAEMD 100mg/kg | 41.14±1.65[b] | 69.66±1.85[b] | 380.84±14.64 |
| HAEMD 200mg/kg | 39.96±1.95[b] | 67.44±1.63[b] | 376.40±18.69[b] |
| AQEMD 100mg/kg | 38.23±1.20[b] | 68.40±1.78 | 366.3±18.96[b, c] |
| AQEMD 200mg/kg | 37.98±1.43[b] | 67.48±1.83[b] | 356.42±19.13[b, c] |

*Values are in Mean±SEM, n=6*
*[a]Significant when compared to normal (P<0.05)[b]Significant when compared to control (P<0.05)*
*[c]Significant when compared to standard (P<0.05)*

**Table 7:** Cardioprotective activity of MDR extracts on Stress and clozapine induced cardiotoxicity on normal rats. (Serum parameters)

| Group | CK MB(U/L) | LDH(U/L) | SGOT(U/L) |
|---|---|---|---|
| Normal | 30.59±1.87 | 161.80±5.30 | 20.34±1.36 |
| Control | 87.71±1.72[a] | 299.20±5.77[a] | 73.58±4.58[a] |
| Std Vitamin E | 51.78±6.57 | 169.80±7.14 | 23.93±2.31 |
| AQEMD 100mg | 64.15±5.03 | 201.10±8.10[b] | 40.89±5.89[b] |
| AQEMD 200mg | 44.40±2.43[b, c] | 177.70±4.01[b] | 27.49±2.47[b] |
| HAEMD 100mg | 59.23±1.61[b] | 173.50±2.01[b] | 28.45±3.17[b] |
| HAEMD 200mg | 36.49±3.92[b, c] | 172.40±3.87[b] | 22.10±2.17[b, c] |

*Values are in Mean±SEM, n=6*
*[a]Significant when compared to normal (P<0.05)*
*[b]Significant when compared to control (P<0.05)[c]Significant when compared to standard (P<0.05)*

**Table 8:** Cardioprotective activity of MDR extracts on Stress and Clozapine induced cardiotoxicity on normal rats. (Endogenous antioxidant enzymes)

| Group | SOD (U/L) | Catalase (U/L) | GSH (U/L) | Lipid peroxidation (U mol/g) |
|---|---|---|---|---|
| Normal | 16.95±0.72 | 6.36±0.32 | 15.08±0.54 | 0.33±0.03 |
| Control | 9.66±0.44[a] | 4.12±0.33[a] | 9.853±0.32[a] | 1.15±0.10[a] |
| Std Vitamin E | 15.8±1.13 | 6.46±0.47 | 13.18±0.57 | 0.27±0.05 |
| AQEMD100mg | 11.8±0.88[b,c] | 5.39±0.45[b] | 12.55±0.98 | 0.46±0.11[b] |
| AQEMD200mg | 12.3±1.09[b,c] | 5.97±0.64[a] | 13.46±1.40[b] | 0.45±0.09[b] |
| HAEMD100mg | 11.4±0.84[b,c] | 5.04±0.27[b,c] | 12.70±1.22[b,c] | 0.68±0.10[b] |
| HAEMD200mg | 15.7±1.15[b,c] | 6.14±0.33[b] | 14.10±0.96[b] | 0.51±0.09[b] |

*Values are in Mean±SEM, n=6 [a]Significant when compared to normal (P<0.05)[b]Significant when compared to control (P<0.05)[c]Significant when compared to standard (P<0.05)*

**Histopathology**

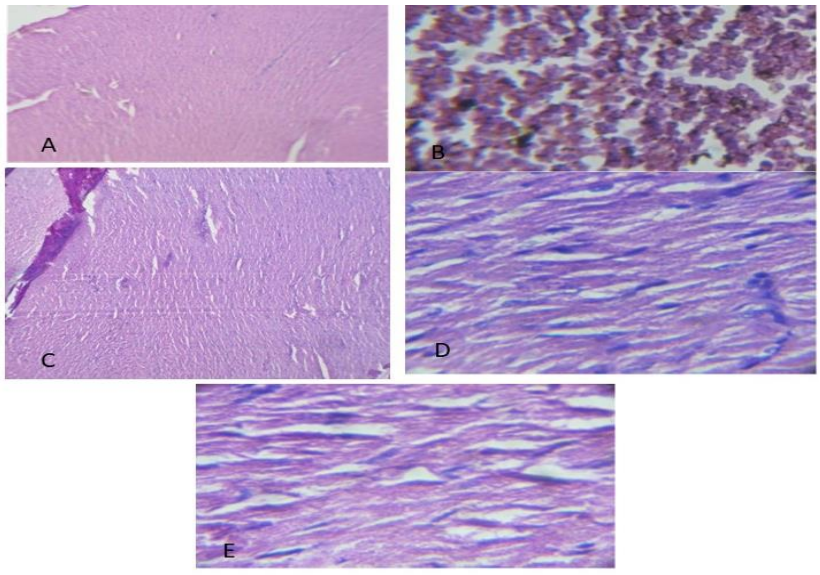


**Fig 4:** Effect of MDR extracts on heart histopathology

(A) Histology of normal heart tissue treated with vehicle exhibited normal myocardial cells each with well-defined myoplasm, prominent nucleus and nucleolus.(B) Histology of heart section treated with clozapine showed damage of myocardial architecture with myocardial necrosis, fatty changes and inflammation.(C) Histology of heart tissue treated with standard vitamin E clearly

showed potential recovery of normal myocyte when compared to clozapine treated group.(D) Histology of heart tissue treated with AQEMD (200 mg/kg) group returned the injured heart to quite normal when compared to clozapine treated group.(E) Histology of heart tissue treated with HAEMD (200 mg/kg) group also showed activity in protecting the heart myocardium as compared to clozapine treated group.

**ECG changes:** Control animals showed significant increase in QT and ST ($p<0.05$) intervals when compared to normal group. Treatment with AQEMD 200mg/kg, HAEMD 200mg/kg administration significantly decreased QT interval ($p<0.05$) and ST interval. (Table 6).

**Serum Parameters (CKMB, LDH, SGOT):** Effect of extracts on the serum CKMB, LDH and SGOT levels were shown in (Table 7). serum CKMB, LDH and SGOT levels were found to be significantly higher (*P<0.05*) in stress and clozapine induced rats, when compared to that of normal rats. Treatment with Vitamin E have lowered the serum levels of CKMB, LDH and SGOT significantly (*P<0.05*) compared to clozapine control rats. Both the extracts (AQEMD and HAEMD) of MDR have shown dose dependent cardioprotective activity, the extract possess least activity at the lower dose (100mg/kg), but both the extracts of dose (200mg/kg) were found to be significant (*P<0.05*) in lowering the levels of CKMB, LDH and SGOT when compared to standard vitamin-E treated animals.

**Evaluation of anti-oxidant markers (SOD, Catalase, GSH and MDA):** Effect of extracts of MDR on SOD, Catalase and Glutathione (GSH) was shown in Table 8. Control animals treated with clozapine have shown significantly lower levels of **SOD**, **Catalase** and **Glutathione (GSH)** on $16^{th}$ day (*P<0.05*) when compared to that in normal group. Group treated with vitamin-E have shown significantly higher (*P<0.05*) levels of the mentioned anti-oxidant markers when compared to the clozapine control rats. Both the extracts of MDR have shown dose dependent cardioprotective activity, whereas both the extracts possess least activity at the lower dose (100mg/kg), but both the extracts at dose (200mg/kg) were found to be significant (*P<0.05*) in increasing the mentioned anti-oxidant marker levels when compared to standard vitamin-E treated group.

**Lipid peroxidises (MDA):** The MDA levels were found to be significantly higher (*P<0.05*) in control group, when compared to that of normal rats. Group receiving Vitamin E have lowered the MDA levels significantly (*P<0.05*) compared to clozapine control group. Both the extracts (AQEMD and HAEMD) of MDR have shown dose dependent cardioprotective activity, where both the extracts possess least activity at the lower dose (100mg/kg), but both the extracts of dose (200mg/kg) were found to be significant (*P<0.05*) in lowering the levels of MDA levels when compared to standard vitamin-E treated animals (Table 8).

## DISCUSSION

A vast population across the globe look forward for alternative therapeutic approaches to synthetic medicines. The drugs from the natural sources majorly plants, have been provided a valuable status and are readily available for healthcare. Their safe and rational use has been disciplined by the WHO. Cardiovascular diseases relate to oxidative stress sharing a common molecular mechanism. Using the cardiotoxicity models, several medicinal plants have been screened for their efficacy against cardiovascular diseases in India, and globally.[27] Investigations on the phytochemical screening of the extracts of MDR revealed the presence of triterpenes, tannins, carbohydrates, reducing sugars, flavonoids, alkaloid, cardiac glycosides. The phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the AQEMD as well as in HAEMD. It is well documented and reported that several such compounds were known to possess potent antioxidant activity. Hence,

the observed antioxidant activity may be due to the presence of any of these constituents. Both the extract shown dose dependent free radical scavenging activity and hence the activity was found to be potent when compare to ascorbic acid. Clozapine model was used as a model for induction of cardiotoxicity. The rats treated with clozapine caused marked increase in the level of SGOT, CKMB, LDH, MDA (malondialdehyde) and decrease in SOD, Catalase, and GSH levels [25-27]. Clozapine treatment increases levels of the catecholamines, norepinephrine and epinephrine. Hyper-catecholaminergic states can causes myocarditis in animals and patients. Clozapine-induced myocarditis has been associated with an increased release of inflammatory cytokines. In stress and clozapine induced cardiotoxicity, the extracts have shown the effect even on the ECG of the rats which was treated with clozapine control. The ECG in control group animals have showed elongation of the ST interval and QT interval 82.4% and 85.01% and respectively. The extracts AQEMD (200mg/kg) and HAEMD (200mg/kg) reduced the ST interval to 94.32% and 96.31% and QT interval to 94.04% and 97.66% respectively. The result showed the reduction of myocardial damage in the treated group AQEMD and HAEMD. The activity was found to be dose dependent and produced non-significant effect at lower dose tested the extract at higher doses produced reversal of clozapine cardiotoxicity and it was found to be significant when compared to standard vitamin-E treated animals. The extract at higher doses produced reversal of clozapine cardiotoxicity and it was found to be significant when compared to standard vitamin-E treated animals. Cardiotoxicity is evidenced by significant increase in serum enzyme levels like CKMB, LDH and SGOT. The elevation of CK-MB isoenzymes is considerably specific for myocardial damage. Total LDH estimation lacks specific since this enzyme is present in various tissues besides myocardium such as in skeletal muscle, kidney, liver, lungs, and red blood cells. However LDH-1 is a myocardial-specific. SGOT lacks specific since this enzyme is present in various tissues besides myocardium such as in skeletal muscle, kidney, liver, lungs, and red blood cells, elevated levels are seen in myocardium damage. In the present study extracts AQEMD and HAEMD of dose 200mg/kg showed significant decrease in CK-MB, LDH and SGOT levels compared to the control group. Tissue endogenous antioxidant enzymes Stress and Clozapine therapy induces oxidative stress and oxidative stress is caused by various free-oxygen radicals including superoxide anion, hydroxyl radical [14], Interaction of free radicals with damage to DNA, proteins and lipids. The activity of the extracts (AQEMD and HAEMD) were very much nearer to the standard drug Vitamin – E and was found to be significant at higher level of extract tested. The activity of the extract was very much nearer to the standard drug Vitamin – E and was found to be significant at higher level of extract tested. Stress and clozapine induced glutathione upregulation is due to enhancement of *de novo* GSH synthesis under conditions of oxidative stress or glutathione depletion[16]. In the present study extracts AQEMD and HAEMD at dose of 100mg/kg showed significant increase in SOD level compared to the control group. In the control group SOD levels were decreased up to 75.24% whereas the animals treated with different extracts AQEMD (100mg/kg) 82.11%, AQEMD (200mg/kg) 82.12% and HAEMD (100mg/kg) 82.07%, HAEMD (200mg/kg) 95.45% the GSH levels were increase. MDA levels were significantly increased up to 69.24% whereas animals treated with different extracts AQEMD (100mg/kg) 59.23 and, AQEMD (200mg/kg) 89.21% and HAEMD (100mg/kg) 57.43%, HAEMD (200mg/kg) 87.54% decrease in the MDA levels significantly. This support the hypothesis that the Mechanism of cardiotoxicity is related to the involvement of reactive oxygen species (ROS) in Clozapine induced lipid peroxidation.

## CONCLUSION

Research indicates that there is a relationship between the risk of developing

coronary heart disease and stress. This is because stress releases certain chemicals like catecholamine's which can increase heart rate and raise blood pressure. Stress also contributes indirectly to cardiovascular diseases. The direct effects of stress are increase in heart rate, concentrations of hormones and catecholamine's, and changes in the hypothalamic–pituitary–adrenal axis, artery spasm, microvascular dysfunction leading to cardiomyopathy [3]. The present study the extract of MDR shown cardioprotective effect in stress and clozapine induced cardiotoxicity in normal animals. Based on the observation and results obtained it is evident that MDR extracts demonstrate promising antioxidant and cardioprotective activity when tested *in-vitro* and *in-vivo* model. The cardioprotective property may be attributed to its free radical scavenging and antioxidant activity, which may be due to the presence of flavonoids and phenolic compounds in the extracts. But further studies are required to support the present assumption and to elucidate detailed cardioprotective mechanism.

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