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TO EVALUATE PROTECTIVE EFFECT OF *MOMORDICA DIOICA* (ROXB) AGAINST STRESS AND CLOZAPINE INDUCED CARDIOTOXICITY IN STZ INDUCED HYPOGLYCAEMIC RATS

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ABSTRACT

The cardiotoxicity is a serious dose limiting side effects of the antipsychotic drugs. These cardiotoxic effects of clozapine can overcome by using combination of herbal extracts. The present study has explored cardioprotective effects of extracts of fruits of *Momordica dioica*Roxb in rats. The extraction was submitted to the *in vivo* cardioprotective activity by stress and clozapine induced cardiotoxicity by using hypoglycaemic rats. The hypoglycaemic group three doses of 100,200 and 400 mg/kg of each extract and Vit-E (10mg/kg) was used as standard along with the glibenclamide (1.5mg/kg) as a standard antidiabetic drug and treated with the extracts for 15days. The clozapine (25mg/kg) was administered through i.p route and on 14th and 15th day water restrain stress was induced to animals and activity was evaluated by the non-serum and biochemical parameters on the 16th day. The MDR extracts showed protective effects on weight of the heart than the control group, the rats treated with stress and clozapine caused marked increases in the level of CK-MB,LDH and SGOT indicating the tissue damages in the toxic conditions and the extracts of MDR dose 200mg/kg showed significant decreases CK-MB, LDH, and SGOT levels compared to the control group. The histopathological studies treated with extracts at high dose (400mg/kg) protected heart from toxic damage of myocardium and showed predominantly normal. The results from in vitro studies and both the extracts have potent antioxidant and free radical scavenging properties. The study results conclude MDR attenuated the cardioprotective activity in diabetic rats, however, further molecular levels studies are required to support the data.

INTRODUCTION

Cardiovascular disease remains a leading cause of death in most industrialized countries. The world health organization estimates approximately 14 million individuals died of cardiovascular disease in 1990, and this is projected to rise to about 25 million by 2030[1]. The current projections suggest that by the year 2030 India will have Largest CVS burden in the world. The deaths from the non-communicable disease and injury are expected to rise from 33 million to 58 million annually [2]. The drug-induced cardiotoxicity is one major cause of cardiovascular disease. The cardiotoxicity is defined by the National Cancer Institute as the 'toxicity that affects the heart', this definition includes a direct effect of the drug on the heart

and an indirect effect due to the enhancement of hemodynamic flow alterations or due to thrombotic events[3]. The stress is also a contributing factor, research indicates that there is a relationship between the risk of developing coronary heart disease and stress. This is because stress releases catecholamine's which can increase heart rate and raise blood pressure. Stress also contributes indirectly to CVD, as people under stress may turned towards the substance use like smoking and drink more than these who lead stress-free lives [3, 4]. The stress-induced cardiomyopathy, the onset of the disease is frequently but not always triggered by the cute medical illness or by intense emotional or physical stress. The postulated mechanism includes excesses of catecholamines, coronary artery spasm, and microvascular dysfunction. Alternately, there may be dynamic mid-cavity or left ventricular outflow tract obstruction, which may contribute to apical dysfunction. The clozapine is an atypical antipsychotics drug that is a tricyclic dibenzodiazepine derivative, these drugs used as the last resort in patients that have not responded to other antipsychotics treatments due to its agranulocytosis and the continues plasma level monitoring are required during the treatment. However, one of the very effective antipsychotics treatment choices. The mechanism by which clozapine causes myocardial toxicity is unclear. The hypothesis suggested that myocardial toxicity is an immunoglobulin e- mediated hypersensitivity reactions, however, the type of reactions rarely occur in the clozapine therapy and another theory postulates these reactions are due to a hyper eosinophil syndrome induced by clozapine myocarditis[4]. The *Momordica dioica*Roxb (MDR), perennial climbing creeper, is generally found throughout subcontinent and cultivated in the Deccan. The methanolic extracts, aqueous, flavonoid and non-flavonoid fractions of the *Momordica dioica*exhibited moderate and concentration-dependent cardioprotective activity[5, 6].

MATERIALS AND METHODS:

Animals: Albino rats of either sex weighing 150-200 g were used in the experiment. Animals used in the study 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).

Chemicals: The chemicals which were used for the present study were procured from Sigma and Himedia, Merck and Rankem. All other chemicals and reagents used were of analytical grade.

Experimental design: The albino rats were randomly divided in to seven groups of six animals each and kept in their cages for 1-week prior to dosing for acclimatization. Streptozotocin (STZ) was dissolved in 0.1M citrate buffer prior to use and administered intraperitoneally to induce diabetes. After 72 hours of STZ administration, the rats with >250 serum glucose level were selected for the study as mentioned in the table 1.

Evaluation of the cardioprotective activity of MDR extracts on Stress and clozapine induced cardiotoxicity on Diabetic rats [7-9].

Evaluation of anti-diabetic activity: Induction of diabetes in Wistar rat[10, 11], biochemical parameters on the 16th day of the study, blood was collected by carotid bleeding. The blood was allowed clot for 30min at room temperature. The serum was separated by centrifugation at 2500 rpm for 15min. Serum was analysed for measuring the serum CK-MB, LDH , and AST. Other biochemical parameters like Estimation of triglyceride[12], serum total cholesterol[13], Total Protein[14] and enzyme level s using standard diagnostic kits using Auto-analyser.

Evaluation of endogenous antioxidant enzymes: The heart tissues from animals were processed and homogenized in 10% chilled tris hydrochloride buffer (10 mM, pH 7.4) by tissue homogenizer (Remi Motors, Mumbai, India) and centrifuged at 7500 rpm for 15 min at 4°C using Eppendorf 5810 R high speed cooling centrifuge. The clear supernatant was used for the estimation of SOD, CATALASE, GSH, LIPID PEROXIDASE content.

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

RESULTS

Cardioprotective activity of MDR extracts on stress and clozapine-induced cardiotocicity on Diabetic rats.

Morphological parameters:

Bodyweight: Clozapine causes a decrease in body weight,in this study the average body weight in Clozapine group showed significant decrease in body weight compared to normal group as well as AQEMD+ stress and clozapine treated groups as shown in Table 2.Rats treated with HAEMD (200&400mg/kg) have shown significant regaining in body weight compared on 0th day and 16th day.

Wet heart weight: Clozapine also causes an increase in heart weight, in this study, the average of heart weight in the Clozapine group statistically significant compared to the normal group as well as AQEMD+ stress and clozapine treated groups in figure 1. Rats treated with HAEMD (200&400mg/kg) have shown significant difference in heart weight compared to clozapinecontrol group

ECG Changes: Due to the stress and administration of clozapine, there was significantly increased QT and ST ($p<0.05$) intervals in control animals when compared to the normal group. Whereas AQEMD 200mg/kg and 400mg/kg respectively, HAEMD 200mg/kg and 400mg/kg respectively administration significantly decreased QT interval ($p<0.05$) and ST interval, heart rate is non-significant compared to control group. AQEMD 400mg/kg, HAEMD 400mg/kg administration is significant compared to normal $p<0.05$.

Serum parameters (CKMB, LDH and SGOT): Effect of extracts of MDR on stress and clozapine induced cardiotoxicity on rats is shown in Table 4. The 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 of the rats with Vitamin E have lowered the three mentioned serum parameters 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 the mentioned serum parameters when compared to standard vitamin-E treated animals.

Cardioprotective activity of MDR extracts on Stress and clozapine

Induced cardiotoxicity on diabetic rats(Endogenous antioxidant enzymes)

Evaluation of antioxidant markers (SOD, Catalase, GSH and MDA): Effect of extracts of MDR on stress and clozapine induced cardiotoxicity on rats is shown in Table 5. Rats treated with clozapine have shown **SOD**, **Catalase** and **Glutathione (GSH)** levels significantly lower on 16^{th} day (*P<0.05*) when compared to that in normal rats. Rats treated with vitamin-E have shown significantly higher (*P<0.05*) levels of the mentioned antioxidant markers when compared to the clozapine control rats. Both the extracts of MDR have shown dose dependent cardioprotective activity, whereas the extract possess least activity at the lower dose (100mg/kg), 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 increasing the mentioned anti-oxidant marker levels when compared to standard vitamin-E treated animals

Endogenous lipid peroxidase (MDA) levels: The MDA levels were found to be significantly higher (*P<0.05*) in stress and clozapine induced rats, when compared to that of normal rats. Treatment of the rats with Vitamin E have lowered the MDA levels 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 MDA levels when compared to standard vitamin-E treated animals.

DISCUSSION:

Diabetes is a prime risk factor for cardiovascular diseases (CVD). It affects the heart muscle, causing both systolic and diastolic heart failure(15). Influence of diabetes on stress and clozapine inducing cardiotoxicity is the main aim to assess the protective effects of different extracts of *Momordica dioica*Roxb, It

has been well established that active constituents play an important role in the troubles of cardiac disorders by different free radical scavenging activity [16]. The morphological changes like body weight show a significant reduction in the stress and clozapine induced cardiotoxicity and reduction is due to the reduced ingestion of food and its major adverse effects like hyperglycaemia, which causes elevation of certain markers leading to disease condition [10]. The AQEMD & HAEMD showed the protective action in maintaining the rats body weight which was decreased by the clozapine produced cardiotoxicity where as in the control group animals the body weight of rats was decreased upto 9.52% whereas the treatment animals with the different extracts of MDR showed AQEMD (400mg/kg) 1.05%, HAEMD (400mg/kg) showed regaining of the body weight (Table 2).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 ECG findings in stress[17] and clozapine induced cardiotoxicity, the extracts have shown effects even on the ECG of the rats which were treated with clozapine control. The ECG in control group animals wave showed elongation of the ST interval and QT interval 82.4% & 85.01% & respectively when compared to normal (Table5). The extracts AQEMD (400mg/kg) and HAEMD (400mg/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 & HAEMD. The activity was found to be dose dependent and produced a non-significant effect at a 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. In the case of heart weight, the control group has shown upto 58.75% increase where the MDR extracts also have shown a protective effect on the weight of heart. The AQEMD (400mg/kg) produced only 0.4% decrease, HAEMD (400mg/kg) shown 0.2% decrease, in heart weight as shown in (Table 7). 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 MDR shows the significant changes in the biochemical parameters like CK-MB, LDH & SGOT, the rats treated with stress and clozapine caused marked increase in the level of CK-MB, LDH & SGOTindicating the tissue damage in the toxic conditions. In the present study extracts AQEMD & HAEMD of dose 200mg/kg showed significant decrease in CK-MB, LDH & SGOTlevels compared to the control group. Where in the control group the CK-MB, LDH &SGOT levels were increased 34.20%, 54.91% , 27.64% respectively. Whereas animals treated with the different extracts showed CK-MB, LDH & SGOT levels AQEMD (100mg/kg) 57.68%, 80.45%, 49.74% AQEMD (200mg/kg) 85.2%, 83.6%, 78.54% & AQEMD (400mg/kg) 95.2%, 93.6%, 98.54% HAEMD (100mg/kg) 51.14%, 93.25%, 71.49% , HAEMD (200mg/kg) 83.83%, 93.85% 92.03% & HAEMD (400mg/kg) 83.93%, 99.85% 92.03% respectively were decreased as shown in (Table 4). The activity of the extract was very much nearer to the standard drug Vit – E & was found to be significant at higher level of extract tested.

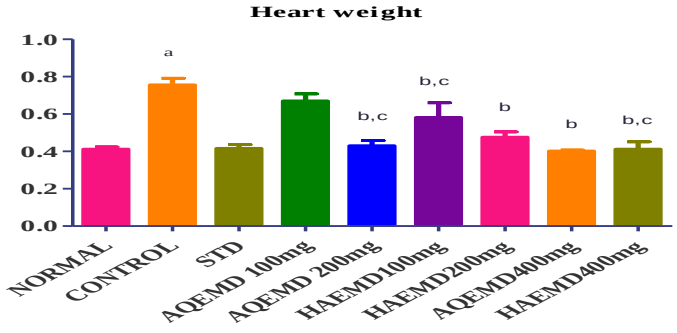


Figure 1: Cardioprotective activity of MDR extracts on Stress and clozapine induced cardiotoxicity on Diabetic rats. (Wet heart weight of rats per 100gm of b.w)

Values are in Mean±SEM, n=6, [a]significant when compared to normal ,[b] Significant when compared to control ,[c] Significant when compared to standard

Table 1: Treatment schedule to evaluate extracts of MDR in Stress and clozapine induced cardiotoxicity on Diabetic rats.

Group	Induction of Cardiotoxicity	Treatment
Normal (1ml/kg)	0.5%NaCMC 1ml/kg body weight, for 15 days(vehicle)	Vehicle p.o.1ml/kg for 15 days(vehicle)
Control (clozapine, 25mg/kg)	Daily 25mg/kg of clozapine in PBS was given for 7 days through i.p. along with stress on the 14th and 15th day.	Received a daily dose of a vehicle (p.o) for 15 days
Vitamin –E (10 mg/kg) and Glibenclamide 1.5mg/kg	----------do----------	Received a daily dose of vit E (10 mg/kg B.W p.o in 0.5% Na CMC) for 15 days
HAEMD 100mg/kg	----------do----------	Received a daily dose of HAEMD (100mg/kg B.W p.o in 0.5% Na CMC) for15 days
HAEMD 200mg/kg	----------do----------	Received a daily dose of HAEMD(200mg/kg B.W p.o in 0.5% Na CMC) for15 days
HAEMD 400mg/kg	----------do----------	Received a daily dose of HAEMD(400mg/kg B.W p.o in 0.5% Na CMC) for15 days
AQMD 100mg/kg	----------do----------	Received a daily dose of AQMD (100mg/kg B.W p.o in 0.5% Na CMC) for15 days
AQMD 200mg/kg	----------do----------	Received a daily dose of AQMD (200mg/kg B.W p.o in 0.5% Na CMC) for15 days
AQMD 400mg/kg	----------do----------	Received a daily dose of AQMD (400mg/kg B.W p.o in 0.5% Na CMC) for15 days

Table 2: Cardioprotective activity of MDR extracts on Stress and clozapine induced cardiotoxicity on Diabetic rats,(Body weight of rats per 100gm of b.w)

Group	Body weight (gm)		▼ % in body weight
	On 0th day	On 16th day	
Normal	197.05±4.61	205.00±4.01	0.00%
Diabetic Control	198.37±6.11	178.30±5.17[a]	9.52%
Standard	185.00±3.12	186.00±3.62	0.00%
AQEMD 100mg	193.30±1.29	176.70±4.57[b]	5.69%
AQEMD 200mg	193.30±.4.47	185.50±3.71[b]	2.2%[b]
AQEMD 400mg	186.70±5.11	178.30±6.79[b]	1.05%
HAEMD 100mg	176.20±8.54	177.50±4.56[b]	0.00%
HAEMD 200mg	186.70±3.57	186.70±3.0[b,c]	0.00%[b]
HAEMD 400mg	196.70±3.57	198.30±3.8[b]	0.00%[b]

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

Table 3: Effect of extracts of MDR on non-serum parameters on Stress and clozapine-induced cardiotoxicity on Diabetic rats.(Non-serum parameters: ECG)

Group	ST interval (mSec)	QT interval (mSec)	Heart rate (Beats/min)
Normal	38.23±1.56	67.88±1.56	349.50±16.17
Control	52.16±2.86[a]	78.86±1.99[a]	298.31±12.47
Standard	37.34±1.03	65.89±1.55	372.52±17.93
AQEMD 100	43.14±1.65[b]	69.66±1.85[b]	380.84±14.64
AQEMD 200	39.96±1.95[b]	67.44±1.63[b]	376.40±18.69[b]
AQEMD 400	37.96±1.95	66.44±1.63[b]	347.44±1.63[b]
HAEMD 100	38.23±1.20[b]	69.40±1.78	366.3±18.96[b, c]
HAEMD 200	37.98±1.43[b]	68.48±1.83[b]	356.42±19.13[b, c]
HAEMD 400	36.96±1.95	66.44±1.63[b]	337.44±1.63[b]

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

Table4: Cardioprotective activity of MDR extracts on Stress and clozapine induced cardiotoxicity on Diabetic rats, (Serum parameters)

Group	CK MB(U/L)	LDH(U/L	SGOT(U/L)	Total Cholestrol (Mg/Dl)	Total Protein (G/Dl)	Triglycerides(Mg/Dl)
Normal	35.88±1.67	137.80±5.30	39.86±2.36	82.16±1.76	6.900±0.17	81.73±0.58
Control	77.51±4.42[a]	295.20±3.77[a]	71.58±1.58[a]	150.70±4.74	2.00±0.30	131.8±1.53
Standard	44.34±4.87	14518±0.14	47.49±4.31	104.90±11.73	5.66±0.52	98.87±4.67
AQEMD 100	64.86±5.88	216.10±8.10[b]	70.07±1.69[b]	143.30±7.74	1.88±0.17	131.8±4.00
AQEMD 200	61.72±6.43[b]	167.70±2.51[b]	52.49±3.47[b]	119.60±8.65[b]	3.50±0.48	106.6±5.62[b]
AQEMD 400	43.86±4.60[b, c]	149.60±2.11[b]	44.71±3.32[b,c]	116.0±9.77[b]	5.45±0.55[b,c]	95.54±7.75[b,c]
HAEMD 100	72.57±3.51[b]	296.80±10.01[b]	55.35±2.17[b]	143.30±7.74	1.88±0.33	130.2±3.69[b]
HAEMD 200	61.14±4.32[b]	239.90±13.87[b]	52.10±5.17[b]	119.60±8.64[b]	3.21±1.14[b]	106.6±5.62[b]
HAEMD 400	46.78±6.15[b, c]	188.10±4.07[b]	38.18±5.75[b,c]	78.030±9.77[b]	5.66±0.48[b]	97.21±7.67[b,c]

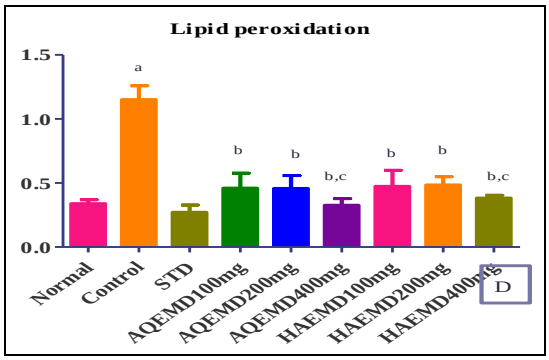
Values are in Mean±SEM, n=6 P<0.05, [a]significant when compared to normal, [b] Significant when compared to control, [c] Significant when compared to standard

Table5. Cardioprotective activity of MDR extracts on Stress and clozapine induced cardiotoxicity on diabetic rats: Endogenous antioxidant enzymes

Group	SOD(U/L)	CATALASE (U/L)	GSH (U/L)	Lipid peroxidation(U mol/g)
Normal	17.62±0.82	7.24±0.69	50.08±1.54	0.34±0.03
Diabetic Control	10.16±0.44[a]	4.42±0.93[a]	14.53±0.12[a]	1.15±0.10[a]
Standard	14.80±1.13	7.368±0.30	32.18±6.57	0.27±0.05
AQEMD 100	11.89±0.88[b]	5.97±0.65	18.55±2.78[b]	0.46±0.11[b]
AQEMD 200	13.63±1.49[b]	5.97±0.64[a]	19.62±2.40[b]	0.45±0.09[b]
AQEMD 400	14.93±1.67[b,c]	6.15±1.17[b]	31.01±6.84[b]	0.32±0.05
HAEMD 100	11.31±0.94[b]	4.37±0.47[b]	24.70±4.22[b]	0.43±0.20[b]
HAEMD 200	13.70±1.35[b]	5.64±0.53[b]	23.10±5.96[b]	0.46±0.19[b]
HAEMD 400	15.74±1.47[b,c]	6.43±0.43[b]	36.28±7.53[b]	0.30±0.08[b,c]

Values represent Mean ± SEM (n = 6) P<0.05, [a]significant as compared to the normal,[b] significant as compared to the control, [c] Significant when compared to standard

Figure 2, Cardioprotective activity of MDR extracts on Stress and clozapineinduced cardiotoxicity on diabetic rats, (Endogenous antioxidant enzymes)Histopathological observation of heart section of clozapine treated rats.



Values represent Mean ± SEM (n = 6) P<0.05, [a]significant as compared to the normal,[b] significant as compared to the control, [c] Significant when compared to standard

Figure 3, Effect of extracts of MDR on heart histology in stress and clozapine induced cardiotoxicity on diabetic rats.

(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 the 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 the clozapine treated group.
(D) Histology of heart tissue treated with AQEMD (400 mg/kg) group returned the injured heart to quite normal when compared to the clozapine treated group.
(E) Histology of heart tissue treated with HAEMD (400 mg/kg) group also showed activity in protecting the heart myocardium as compared to clozapine treated group

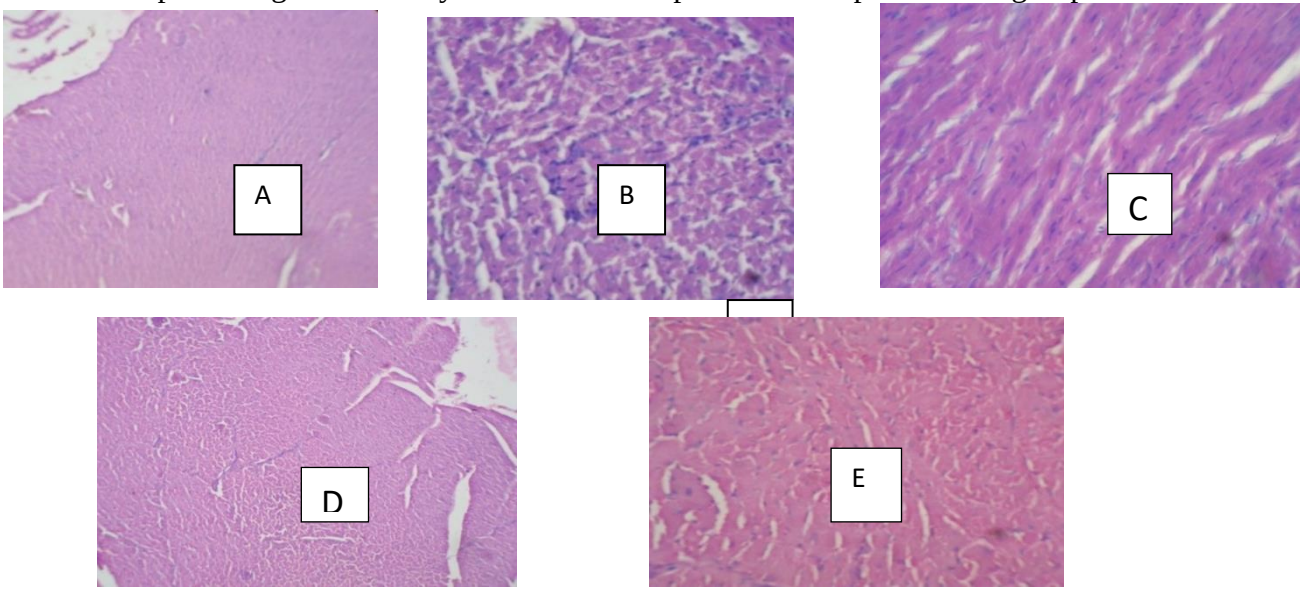


Figure 3: Effect of extracts of MDR on heart histology in stress and clozapine induced cardiotoxicity on diabetic rats

The recorded data were in accordance with this study 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, Interaction of free radicals with damage to DNA, proteins, and lipids[18].*SOD (Superoxide dismutase)*SOD scavenges superoxide radical and prevents further oxygen cantered free radicals' generation.It is suggested that stress and clozapine induced depletion of SOD enzyme is due to loss of copper and zinc, which is essential for SOD activity. The present study extracts AQEMD & HAEMD at a 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 59.24% whereas the animals treated with different extracts AQEMD (100mg/kg) 62.11%, AQEMD (200mg/kg) 78.12% & AQEMD (400mg/kg) 98.12%. HAEMD (100mg/kg) 59.87%, HAEMD (200mg/kg) 75.45% &%, HAEMD (400mg/kg) 85.45% the SOD levels were increased as shown in (Table 5). The activity of the extract was very much nearer to the standard drug Vit – E & was found to be significant at higher level of extract tested. Selenium containing enzyme CAT converts H_2O_2 to water and thereby scavenges hydroperoxides and lipid peroxides, thus protect cells against reactive oxygen species by interrupting the propagation of the lipid peroxidation reaction[19].The present study extracts AQEMD & 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 69.24% whereas the animals treated with different extracts AQEMD (100mg/kg) 72.11%, AQEMD (200mg/kg) 88.12% & AQEMD (400mg/kg) 98.12% HAEMD (100mg/kg) 79.87%, HAEMD (200mg/kg) 85.45% & HAEMD (400mg/kg) 99.87%, the CAT levels were increased as shown in (Table 5). The activity of the extract was very much nearer to the standard drug Vit-E & was found to be significant at higher level of extract tested. The study results reported that the glutathione redox cycle is the most important intracellular antioxidant system which maintains cell integrity and participation in cell metabolism. Stress and clozapine induced glutathione upregulation is due to enhancement of de novo GSH synthesis under conditions of oxidative stress or glutathione depletion.The present study extracts AQEMD & 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% & AQEMD (400mg/kg) 92.12% HAEMD (100mg/kg) 82.07%, HAEMD (200mg/kg) 95.45% & HAEMD (400mg/kg) 99.45% the GSH levels were increased as shown in (Table 5.22 and Graph 5.13).. The activity of the extract was very much nearer to the standard drug Vit – E & was found to be significant at higher level of extract tested. The results of the reported that the degradation product of lipid hydroperoxides is considered as an index of lipid peroxidation. Accumulating evidence suggests that lipid peroxidation due to ROS generation is one of the prime factors in cardiotoxicity. In the present study the control group animals MDA levels were significantly increased up to 69.24% whereas animals treated with different extracts AQEMD (100mg/kg) 59.23 &, AQEMD (200mg/kg) 89.21% & AQEMD (400mg/kg) 99.21% HAEMD (100mg/kg) 57.43%, HAEMD (200mg/kg) 87.54% & HAEMD (400mg/kg) 97.54% decrease in the MDA levels significantly as shown in (Table5and figure2). Supporting the hypothesis that the mechanism of cardiotoxicity is related to the involvement of ROS in clozapine induced lipid peroxidation. The activity of the extract was very much nearer to the standard drug Vit – E & was found to be significant at higher level of extract tested.Histopathological evaluation of the treatment with AQEMD & HAEMD, lower dose (200 mg/kg) showed moderate to weak recovery activity in protecting the heart cells from stress and clozapine induced cardiotoxicity in both normal and diabetic group[20] compared to control group, whereas high dose (400 mg/kg) returned the injured

heart to quite normal. Now, it could be decided that the cardioprotective activity was dose and time dependent. Overall AQEMD & HAEMD had shown very potential cardioprotective activity at a dose of 400 mg/kg.

CONCLUSION:

The results of the study concluded that based on the observation and results obtained it is evident that MDR extractsdemonstrate that aqueous and hydroalcoholic extracts of fruits of MDR possess promising antioxidant and cardioprotective activity when tested *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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Conflict of interest: The authors declare no conflicts of interest.

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