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Ameliorative properties of methanol leaf extract of *Momordica charantia* following alloxan-induced cardiotoxicity in rats

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ABSTRACT

Background & Aim: *Momordica charantia*is an extensively distributed plant that is broadly prescribed in African medical system for treatment of various ailments. The plant has a comprehensive range of therapeutic uses. The present study reveals the antioxidative and cardioprotective abilities of methanol leaf extract of *Momordica charantia* (MLEMC) against cardiotoxicity using alloxan-induced animal model.

Experimental: The ameliorative effect of the methanol leaf extract of Momordica charantia (MLEMC) was studied in alloxan-induced cardiac injury in 50 rats divided into five groups (A-E) (n =10) i.e. group A control, group B was toxicant group, group C animals received glibenclamide treatment while groups D and E received extracts at 200 and 400 mg/kg doses, respectively, for 28 days. Histopathological changes, serum cardiac injury markers such as myeloperoxidase (MPO) activity, nitric oxide (NO) contents; oxidative status, blood pressure, electrocardiogram, cardiac P38 and CRP were evaluated.

Results: The extract-treated group showed a decreased level of oxidant markers such as malondialdehyde(MDA) and hydrogen peroxide(H_2O_2) but increased levels of protein thiols, non-protein thiols, glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and superoxide dismutase (SOD) indicating its anti-oxidant potential. Heart sections revealed mild distortion of the cardiac architecture compared to toxicant group while decreased expression of cardiac P38 and CRP in extract-treated groups was observed.

Recommended applications/industries: The plant extract exhibited anti-oxidant and anti-inflammatory effects, thereby displaying cardio-protective property which propose the plant as a good natural source for herbal nutraceuticals.

1. Introduction

Cardiovascular disease has become an important problem globally. The World Health Organisation (WHO) has projected that it will turn into a primary source of death globally by 2020 (Abubaker *et al.*, 2012). Cardiac toxicity is a major complication seen in alloxan-induced diabetes mellitus. Alloxan toxicity involves creation of reactive oxygen species (ROS) that exacerbate cardiac injury; various ROS generated in alloxan-induced diabetes can mutilate membranes, DNA and proteins (e.g. receptors, structural proteins and enzymes), resulting to cardiac failure and cell death (Ojha *et al*., 2011).

The heart contains fewer free radical detoxifying substances than do other metabolic organs making it highly vulnerable to the free radicals generated by alloxan administration in diabetes (Hiroyuki *et al.*, 2001). Alloxan causes severe oxidative stress in the myocardium leading to cardiac necrosis. The free radicals and lipid peroxidation generated by alloxancan cause irreparable myocardial injury in experimental myocardial infarction.

Diabetes increases free radicals generation leading to lipid peroxidation, expression of adhesion molecule, metalloproteinase activation, altered vasomotion and apoptosis resulting to atherosclerosis causing cardiac injury, thus natural antioxidants products possessing free-radical scavenging effects are proposed to be useful in the amelioration of CVDs caused by alloxan-induced diabetes. This implies that plants having antioxidant capacity, may exhibit a shielding role in cardiovascular maladies (Viswanatha *et al.*, 2010). Hence, attention to investigation of plants as prospective sources of novel drugs is on the rise.

Momordica charantia (MC) belongs to family Cucurbitaceae having tropical and subtropical habitat. The plants is also used to treat ailments comprising hyperlipidaemia, microbial infections, digestive maladies and menstrual hitches (Yibchok-Anun *et al.*, 2006). It contains phenols with antioxidant properties (Tshepiso *et al.*, 2016); the bioactive constituents in *M. charantia* have been shown to possess anti-hyperglycaemic capability in diabetic subjects (Wehash *et al.*, 2012). The aim of this study was to explore the antioxidative and cardioprotective abilities of methanol leaf extract of *Momordica charantia* (MLEMC) against cardiotoxicity using alloxan-induced animal model in order to enhance the scientific knowledge of ethnomedicine.

2. Materials and Methods

2.1. Preparation of extract

Fresh whole plant of *Momordica charantia* was collected from the Botanical garden, University of Ibadan. The identification and authentication of the plant were carried out at the Department of Botany, University of Ibadan and the Voucher Specimen Number wasUIH-22563**.** The voucher specimen was maintained at the Herbarium of the Department of Botany, University of Ibadan**.** The leaves were dried at room temperature (27 ± 2^{o}C) and pulverized to a fine powder using an electric blender. The powder (400 g) was soaked and extracted in 90% methanol (1L) using Soxhlet extractor for 3 days until complete extraction. The extracts were filtered using Whatman no 1 filter paper and the filtrate was evaporated to dryness by a rotary evaporator at 190-220 rpm and 40 - 50^{0}C for 24 h under reduced pressure to give amorphous solid mass.

2.2. Experimental animals (Wistar rats)

Male Wistar rats (weight 150–200 g; n = 50) gotten from Animal house facility of Veterinary Medicine Faculty, University of Ibadan, Ibadan were utilised for this investigation. These rats were retained at the Experimental Animal House in rat cages and adapted to the prevailing conditions for 14 days at room temperature (22 ± 2 ºC) in a 12 h bright/12 h dusky cycle with humidity of 55 ± 5% and nourished with rabbit cubes. The animals were permitted access to clean water without restriction and were given compassionate attention with reference to those principles itemized in directives for care and usage of laboratory animals.

2.3. Ethical approval

The procedure for this work was ratified by the Ethics Committee, Animal Care and Usage Research, University of Ibadan (UI-ACUREC/App/2015/044). All research practices in this experiment were accomplished according to the Animal Ethics Team, University of Ibadan and the National Institute of Health Guide for Care and Use of Research animals

(NIH, 1985) and globally acknowledged ethics for laboratory animal usage and care.

2.4. Research design

Cardiac injury was triggered in rats by intraperitoneal administration of newly constituted alloxan (100 mg/kg) solubilised in citrate buffer solution at pH 4.5 to induce diabetes, (Patel *et al.*, 2007). After two days, blood was taken from each rat and glucose concentration determined to confirm diabetes. Animals with fasting blood glucose level >200 mg/dl was said to be diabetic (Thirumalai *et al.*, 2011) and used for the study; fasting BGLs were determined by glucometer (Roche, Mannheim, Germany). After injection of alloxan (48 h), elevated serum glucose level was measured to established hyperglycaemia. Forty rats having blood glucose level above 200 mg/dl were regarded as diabetic and have cardiac injury and used in the study (n = 40) in addition to ten normal rats that were not injected with alloxan.

Rats were arbitrarily distributed into 5 groups (A-E) of ten rats per group as follows:

Group A were normal non diabetic rats non treated control rats and received the vehicle which was 2.5% tween 80 in normal saline (NDNT).

Group B were the diabetic control rats that received no treatments following induction of diabetes (DNT).

Group C were the diabetic rats treated with glibenclamide 4 mg/kg body weight (DTG).

Group D were diabetic rats that received methanol leaf extract of *Momordica charantia* (MLEMC) at dosage of 200 mg/kg body weight (DTMC200).

Group E were diabetic rats that received MLEMC at dosage of 400 mg/kg body weight, DTMC400).

Treatments were done orally with treatment volumes estimated using the mean weight of the rats in each group and the administration lasted 28 days. Collection of serum samples was done before induction and on days 14 and 28 of the experiment, then the blood pressure and electrocardiogram (ECG) measurements were done a day before the sacrifice of the rats, the rats were then compassionately sacrificed 24 hrs following the last treatment, hearts were harvested for antioxidant studies, histological tissue processing and immunohistochemical staining, serum samples were collected for biochemical analysis.

Cardiac injury by alloxan-induced diabetes and cardioprotective activity of the extract was assessed by estimation of serum nitric oxide (NO) level and myeloperoxidase (MPO) activity which are indicators of cardiac damage, analysis of its effect on oxidative stress makers, lipid peroxidation assay, antioxidant system, immunohistochemical staining of the heart and histological assessment of the sections of heart tissue.

2.5. Ameliorative influence posed by methanol leaf extract of M. charantia (MLEMC) on alloxan-induced cardiotoxicity

2.5.1. Serum and tissues preparation for biochemical assays

Collection of blood samples was donebefore induction and on days 14 and 28 after treatment using heparinized capillary tubes into dry plain tubes via the retro-orbital plexus and permitted to coagulate. Blood sample centrifugation was performed at 4×10^3 rev/min for 0.25 h with the serum collected and kept in fridge at -4°C till the period of analysis. The male albino rats (Wistar strain) given different treatments were humanely sacrificed on the 29th day after termination of the experiment via cervical disarticulation with the hearts quickly excised, washed in cold normal saline to eradicate blood, quickly blotted and weighed on digital scale (B303 Mettler-Toledo, Switzerland) and instantly preserved in ice to avoid denaturation of biomolecules.

The heart tissue was homogenized in K phosphate buffer (0.1 M, pH 7.4), with centrifugation of the ensuing homogenate done at 10^4rpmfor 0.25 h to achieve cardiac post mitochondrial fraction (PMF) which was then preserved at –20 °C till the period of biochemical analysis.

2.5.2. Biochemical assays

Generation of hydrogen peroxide (H_2O_2) was evaluated by the procedure of Wolff (1994). Malondialdehyde (MDA) in cardiac PMFwas quantified as TBARS (thiobarbituric acid reactive substance) and was assessed by procedure of Varshney and Kale (1990). Assessment of cardiac protein thiols (PT) and non-protein thiols (NPT) was achieved via the technique of Sedlak and Lindsay (1968). The cardiac glutathione (GSH) composition was evaluated via the procedure of Moron *et al.* (1979). Procedure of Buetler *et al.* (1963) was engaged to appraise theactivity of cardiac glutathione peroxidase (GPX), while the method of Habig *et al.* (1974) was employed to

evaluate glutathione-s-transferase (GST) activity. Cardiac superoxide dismutase (SOD) activity was assessed by technique of Misra and Fridovich (1972) with minor adjustment (Oyagbemi *et al.*, 2015).

Cardiac nitric oxide (NO) content was evaluated using the methd of Olaleye *et al.* (2007) through indirect measurement of nitrite concentration. Serum myeloperoxidase activity was measured according to the technique of Kruidenier *et al*. (2003).

2.5.3. Blood pressure and electrocardiogram

After the treatment period, blood pressure data, comprising diastolic, systolic and mean arterial pressures were evaluated short of anaesthesia, by tail plethysmography via electro sphygmomanometer (Vannimwegen *et al.*, 1973). Electrocardiogram was determined according to the method of Normann *et al.* (1961).

2.5.4. Immunohistochemistry of cardiac P38 and CRP

Immunohistochemistry was done from paraffin sectioned cardiac tissues as described by Oyagbemi *et al*. (2015) with P38 and CRP distinctly employed as primary antibodies of interest.

2.5.5. Histopathological studies

Examination of renal and cardiac histology was performed according to routine histology techniques as described by Drury *et al*. (1976).

2.6. Statistical analysis

Data were stated as Mean ± standard deviation (SD) for ten rats in the five group of rats. Statistical analysis was done via Graph Pad prism version 5.Significance of mean difference was evaluated via one-way ANOVA; 95% confidence interval. Tukey post hoc tests were performed for comparing all groups with control and comparing all pairs of groups (Redmond and Colton, 2001).

3. Results and discussion

3.1.Influence of MLEMC on alterations in cardiac oxidative stress parameters of alloxan induced rats

As shown in Figure 1 and Figure 2, alloxan administration produced significant (P<0.05) increases in H_2O_2 generation and MDA in cardiac tissues, relative to control. However, treatments with MLEMC (200 and 400 mg/kg) and glibenclamide (4 mg/kg) significantly (P<0.05)reduced the levels of these oxidative stress pointers in cardiac tissuesrelative to diabetic control. This implies that alloxan administration caused initiation of oxidative stress in cardiac tissues but the oxidative stress was mitigated by MLEMC and glibenclamide administration.

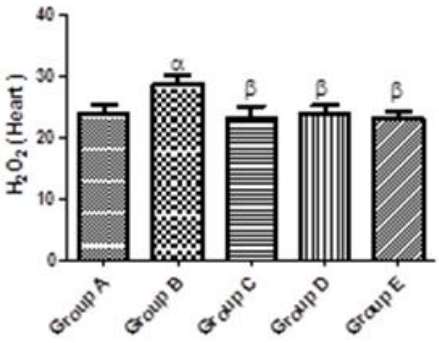

Figure 1. Hydrogen peroxide (H_2O_2, µmole/min/mg protein) level in the heart. α Significant increase when compared with normal control; β Significant reduction when compared with diabetic control (P<0.05). Group A, normal non-diabetic and non-treated control animals; group B, diabetic non-treated control animals; group C, diabetic animals treated with glibenclamide; group D, diabetic animals treated with MLEMC (200 $mgkg^{-1}$); E diabetic animals treated with MLEMC (400 $mgkg^{-1}$).

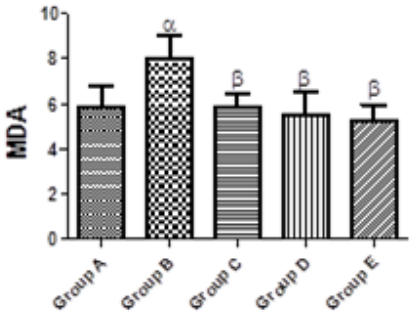

Figure 2. Malondialdehyde (MDA, µmol formed MDA/mg protein) level in the heart.
α Significant increase when compared with normal control; β Significant reduction when compared with diabetic control at P<0.05. Group A, normal non-diabetic and non-treated control animals; group B, diabetic non-treated animals control; group C, diabetic animals treated with glibenclamide; group D, diabetic animals treated with MLEMC (200 $mgkg^{-1}$); group E, diabetic animals treated with MLEMC (400 $mgkg^{-1}$).

3.2. Influence exerted by MLEMC on the antioxidant system of cardiac tissues of alloxan induced rats

The concentrations of non-protein thiol (NPT), protein thiol (PT) and GSH in the heart are shown in Table 1. Rats exposed to alloxan exhibited significant decrease (P<0.05) in cardiac NPT, PT and GSH relative to normal control. Treatments with MLEMC (200 and 400 mg/kg) and glibenclamide (4 mg/kg) significantly (P<0.05) elevated the levels of these non-enzymatic antioxidant system (NPT, PT and GSH) in the heart relative to diabetic control.

Table 1. Influence exerted by methanol leaf extract of *M. charantia* on antioxidant system of the cardiac tissues of alloxan-triggered diabetic rats

Parameters	Group A	Group B	Group C	Group D	Group E
Non-protein thiol	74.57 ± 3.53	70.40^{α} ± 3.36	75.57^{β} ±1.30	75.34^{β} ± 3.46	75.85^{β} ± 3.59
Protein thiol	33.61 ± 3.36	27.56^{α} ± 2.45	28.39 ±2.21	31.16^{β} ± 1.39	32.10^{β} ± 2.21
GSH	93.13 ± 2.25	90.51^{α} ±1.93	91.71 ± 3.51	92.32 ± 3.55	93.04^{β} ± 2.64

Results expressed in Mean ± SD, n=10. $^{\alpha}$Significant decrease relative to normal control at P<0.05.$^{\beta}$Significant increase relative to diabetic control at P<0.05. NPT & PT: Non Protein thiol and protein thiol respectively (μmol /mg protein), GSH: reduced glutathione (μmol /mg protein). Group A NDNT (non-diabetic non treated), Group B DNT (diabetic non treated), Group C (Diabetic treated with glibenclamide), Group D (Diabetic treated with 200 mg/kg MC), Group E (Diabetic treated 400 mg/kg MC).

Results expressed in Mean ± SD, n=10. $^{\alpha}$Significant decrease relative to normal control at P<0.05.$^{\beta}$ Significant increase relative to diabetic control at P<0.05. NPT & PT: Non Protein thiol and protein thiol respectively (μmol /mg protein), GSH: reduced glutathione (μmol /mg protein). Group A NDNT (non-diabetic non treated), Group B DNT (diabetic non treated), Group C (Diabetic treated with glibenclamide), Group D (Diabetic treated with 200 mg/kg MC), Group E (Diabetic treated 400 mg/kg MC).

3.3 Influence of MLEMC on the antioxidant defence system components (GPx, GST and SOD) of the cardiac tissues of alloxan induced diabetic rats

Outcomes indicated that actions of enzymatic antioxidants GPx, GST and SOD decreased significantly in cardiac PMF of diabetic rats compared to normal control (Table 2). The administration of MLEMC and glibenclamide significantly improved the antioxidant defence system compared to the diabetic group.

Table 2. Influence exerted by methanol leaf extract of *M. charantia* on antioxidant system of the cardiac tissues of alloxan-triggered diabetic rats.

	Group A	Group B	Group C	Group D	Group E
GPx	198.16 ± 5.92	186.91^{α} ± 4.55	198.38^{β} ± 1.75	196.13^{β} ± 1.66	196.47^{β} ± 3.91
SOD	11.33 ± 1.43	8.57^{α} ± 1.20	12.22^{β} ± 0.77	11.78^{β} ± 0.94	11.63^{β} ± 0.67
GST	0.43 ± 0.09	0.39^{α} ± 0.08	0.40^{β} ± 0.08	0.43^{β} ± 0.09	0.46^{β} ± 0.08

Results expressed as Mean ± SD, n=10. $^{\alpha}$Significant decrease relative to normal control at P<0.05. $^{\beta}$Significant increase relative to diabetic control at P<0.05.

GPx: glutathione peroxidase, (µmol/mg protein); SOD: superoxide dismutase, U/μg protein; GST: Glutathione-S-transferase (mmol 1-chloro-2,4-dinitrobenzene-GSH complex formed/min/mg protein). Group A, normal non-diabetic and non-treated control animals; Group B, diabetic non-treated animals control; Group C, diabetic animals treated with glibenclamide; Group D, diabetic animals treated with MLEMC 200 $mgkg^{-1}$); Group E (diabetic animals treated with MLEMC 400 $mgkg^{-1}$).

3.4. Influence of MLEMC on the markers of cardiac injury (NO, MPO) in the serum of alloxan induced rats

The results (Table 3) showed significant decline in serum concentration of NO in alloxan-triggered diabetic rats comparatively to control group, while supplementation with MLEMC and glibenclamide significantly elevated the serum concentration of NO relative to diabetic group.

Furthermore, significant increase (P<0.05) was noted in the activity of serum MPO in alloxan-triggered diabetic rats relative to control rats, but administration of MLEMC and glibenclamide significantly reduced the activity of serum MPO.

Table 3. Influence of MLEMC on the markers of cardiac injury (NO, MPO) in the serum of alloxan induced rats

	Group A	Group B	Group C	Group D	Group E
NO	3.62 ± 0.21	2.82^{α} ± 0.39	3.59^{β} ± 0.32	3.53^{β} ± 0.26	3.49^{β} ± 0.43
MPO	40.61 ± 3.40	51.03^{α} ± 5.29	44.73^{β} ± 4.19	43.37^{β} ± 4.19	42.40^{β} ± 2.53

Results expressed as Mean ± SD, n=10. αSignificant decrease relative to normal control at P<0.05. $^{\beta}$Significant increase relative to diabetic control at P<0.05.
NO: Nitric oxide μmol nitrite/mg protein; MPO: myeloperoxidase (µmol/min/mg protein). Group A, normal non-diabetic and non-treated control animals; Group B, diabetic non-treated animals control; Group C, diabetic animals treated with glibenclamide; Group D, diabetic animals treated with MLEMC 200 $mgkg^{-1}$); E (diabetic animals treated with MLEMC 400 $mgkg^{-1}$).

3.5 Influence of MLEMC on electrocardiogram and blood pressure parameters of alloxan induced rats.

Electrocardiographic assessment (Table 4) revealed significant reduction in heart rate of alloxan-triggered diabetic rats relative to control rats, supplementation with MLEMC and glibenclamide ameliorated this effect by producing a significant elevation in heart rate (to the near normal values) relative to diabetic rats. However, the result indicated significant increase in the P duration, PR interval, QRS duration, QT interval, QT corrected (Bazett) interval and R amplitude in alloxan-triggered diabetic rats relative to control rats. Supplementation with MLEMC and glibenclamide ameliorated these electrocardiographic abnormalities by causing significant reduction of these electrocardiographic parameters (to the near normal values) relative to diabetic group.

Alloxan induction triggered significant elevation in systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) relative to control rats, while treatment with MLEMC provoked significant reduction in the values of DBP,SBP and MAP (from hypertensive values to near normal values) relative to diabetic group (Table 5).

Table 4. Influence of MLEMC on electrocardiographic parameters on alloxan induced rats

	Group A	Group B	Group C	Group D	Group E
HR/**min**	248.3±19.43	214.3^{α}±12.66	238.6^{β}±16.52	234.8^{β}±15.84	248.0^{β}±10.56
P wave ***ms***	22.0 ± 3.0	38.67^{α}±8.02	26.2^{β} ± 2.28	26.2^{β} ± 7.63	23.72^{β}± 4.95
PR int ***ms***	38.7 ± 7.64	58.7^{α}±8.58	42.2^{β}± 7.43	47.4^{β} ±6.73	40.3^{β}±3.10
QRS ***ms***	15.7 ± 2.31	25.3^{α}± 1.53	19.4^{β}± 3.05	22.4^{β} ± 4.77	18.25^{β}±1.71
QT int ***ms***	76.0 ± 4.36	108.8^{α}±9.54	74.8^{β}± 5.67	80.8^{β}± 6.48	81.25^{β}±9.73
QTc ***ms***	154 ± 12.53	180.7^{α}±18.0	158.4^{β}±16.49	160.0^{β}±13.09	162.3^{β}±20.76
R amp ***mV***	0.5 ± 0.16	0.8± 0.09^{α}	0.5± 0.08^{β}	0.7^{β}± 0.15	0.5^{β}± 0.31

Values expressed as mean ± SD. $^{\alpha}$indicate significant increasewhen compared with group A, $^{\beta}$indicate significant decreasewhen compared with group B at P<0.05. Group A NDNT (not diabetic not treated), Group B DNT (diabetic non treated), Group C (Diabetic treated with glibenclamide), Group D (Diabetic treated with 200mg/kg MC), Group E (Diabetic treated 400mg/kg MC).

Table 5. Effects of MLEMC on blood pressure parameters of alloxan induced diabetic rats

Groups	Group A	Group B	Group C	Group D	Group E
SBP	140.67 ± 4.32	161.00^{α} ± 5.66	143.89^{β} ± 4.04	138.33^{β} ± 4.16	143.43^{β} ± 3.61
DBP	122.40 ± 5.66	150.40^{α} ± 3.54	118.25^{β} ± 4.57	125.40^{β} ± 4.28	122.44^{β} ± 5.03
MAP	128.40 ± 5.68	152.50^{α} ± 0.71	100.83^{β} ± 2.93	131.40^{β} ± 2.30	130.88^{β} ± 3.36

Results expressed as Mean ± SD, n=10. $^{\alpha}$Significant increase relative to normal control at P<0.05. $^{\beta}$Significant decrease relative to diabetic control at P<0.05.
SBP: systolic blood pressure (mmHg); DBP: diastolic blood pressure (mmHg); MAP: mean arterial pressure (mmHg). Group A, normal non-diabetic and non-treated control animals; Group B, diabetic non-treated animals control; Group C, diabetic animals treated with glibenclamide; Group D, diabetic animals treated with MLEMC (200 $mgkg^{-1}$); Group E, diabetic animals treated with MLEMC (400 $mgkg^{-1}$).

3.6.Influence exerted by MLEMC on antibodiesexpression in cardiac tissues of alloxan induced rat

Immunohistochemistry showed positive immunoreativity with greater expressions of P38 in cardiac tissue of rats induced with alloxan than the control which showed lower expression (Figures 3A and 3B) also, the groups treated with MLEMC and glibenclamide showed lower expression of P38 in the heart similar to control (Figures 3C-3E). This effect seemed to be considerably stronger with MLEMC at 400 mg/kg dosage. CRP immunoreactivity in the heart tissues showed that alloxan-induced diabetic rats demonstrated a strong staining intensity (higher expression) for CRP, relative to control group which showed weak staining intensity (lower expression) for CRP (Figures 4A and 4B). The MLEMC and glibenclamide treated group displayed faint CRP staining (lower expression) similar to control in the heart tissues (Figures 4C-4E).

Figure 3. Immunohistochemical staining for cardiac p38. Group A (control) shows low expressions of p38; Group B (untreated diabetic control) shows higher expressions of p38 than control; Group C (diabetic treated with glibenclamide) shows lower expressions of p38 similar to control; Group D (diabetic treated with MLEMC 200 $mgkg^{-1}$) shows lower expressions of p38 similar to control; Group E (diabetic treated with MLEMC 400 $mgkg^{-1}$) shows lower expressions of p38 similar to control. The slides were counterstained with high definition Haematoxylin and viewed x 400.

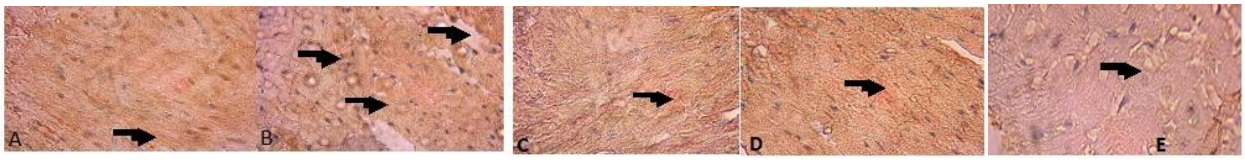

Figure 4. Immunohistochemical staining for cardiac CRP. Group A (control) shows low expressions of CRP, Group B (diabetic untreated) shows higher expressions of CRP than control; Group C (diabetic treated with glibenclamide) shows lower expressions of CRP similar to control; Group D (diabetic treated with 200 $mgkg^{-1}$ MLEMC) shows lower expressions of CRP similar to control; Group E (diabetic treated with 400 $mgkg^{-1}$ MLEMC) shows lower expressions of CRP similar to control. The slides were counterstained with high definition Haematoxylin and viewed x 400.

3.7. Influenceexerted by MLEMC on the myocardial histoarchitecture of alloxan induced diabetic rats

Investigation of cardiac tissues histologically established the biochemical findings of cardiac injury owing to alloxan induction in diabetic rats. Hearts from the alloxan-induced diabetic rats presented widespread haemorrhagic lesions in addition to coronary vessels congestion, myocardial fatty infiltration and myocardial infiltration by inflammatory cells (Figure 5B), while the hearts from the control group presented a typical histological architecture in the heart, devoid of obvious lesions (Figure 5A).

However, significant progress in cardiac architecture was seen in rats treated with MLEMC and glibenclamide, as regenerating myofibrils were seen in MLEMC-treated rats, lesions were scarcely seen in cardiac tissues of the treated group, though areas of mild infiltration of the myocardium were observed with 200 mg/kg dose of MLEMC. The greater dose of 400 mg/kg elicited the supreme maintenance of cardiac architecture, rats treated with glibenclamide showed focal area of mild myocardial fatty infiltration. This implied that MLEMC and glibenclamide, ameliorated the damage in cardiac histoarchitecture considerably since the tissue histological appearance inclined towards that in the control group (Figures 5C-5E).

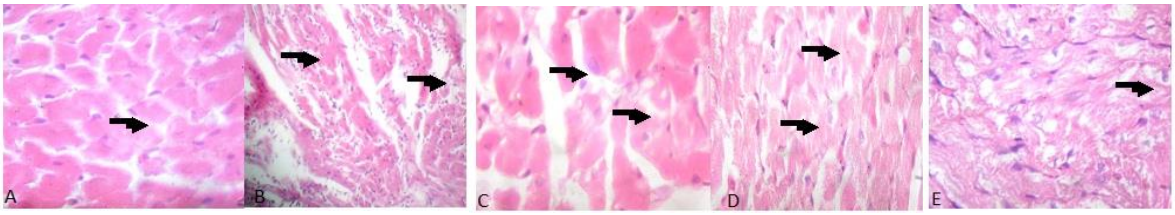

Figure 5. Photomicrograph of heart sections of rat. A (control) showing no visible lesion; B (untreated diabetic) showing haemorrhagic lesions,fatty infiltration of the myocardium, and infiltration of the myocardium by inflammatory cells; C (diabetic treated with glibenclamide) showing focal area of mild fatty infiltration of the myocardium; D (diabetic treated with 200 mgkg-1 MLEMC) showing mild fatty infiltration of the myocardium and regenerating myofibrils; E (diabetic treated with 400 $mgkg^{-1}$ MLEMC) mild disseminated fatty infiltration of the myocardium, mild infiltration of inflammatory cells.

3.8. Cardioprotective activity

In this work, the reaction of cardiac tissues to alloxan-induced toxicity was investigated together with associated blood pressure and ECG changes in Wistar rats. Alloxan caused significant myocardial damage as indicated by cardiac injury markers, ECG abnormalities, oxidative stress markers, immunohistochemistry and histopathological analysis. The significant increase in cardiac injury markers indicates necrosis of myocardium and leakage of plasma membrane (Sabeena Farvin *et al.*, 2004). Cardiotoxicity associated with alloxan-induced diabetes mellitus has been reported (Sharma and Kar, 2014). The release of ROS from oxygen molecules is an incriminating factor eliciting injury to cells, tissues and biomolecules after alloxan administration in diabetes thus contributing to diabetic complications including cardiac injury.

This study revealed that alloxan-induced hyperglycemia was complemented with a significant elevation in H_2O_2generation and lipid peroxidation; elevation in the level of these markers in cardiac tissues is connected to nitrosative and oxidative stress, which is unevenness amidst generation of RNS or ROS and antioxidant defense system. Both H_2O_2 and MDA are deployed as bio-indicators of oxidative stress in cells and tissues (Opara, 2002).Oxidative stress evoked by alloxan causes elevated peroxidation of membrane lipids (Naziroglu *et al.*, 2011).Concurrent supplementation of MLEMC and glibenclamide to the diabetic rats dwindled the H_20_2 and MDA level in the cardiac tissue significantly thus mitigating the oxidative stress triggered by alloxan. The ability of this plant to quench these radicals is an indication of its cardioprotective property.

This study also noted that alloxan administration causes significant drop in the level of antioxidant defence system both non-enzymic antioxidant (PT, NPT and GSH) and enzymic antioxidant (GPX, GST and SOD) in the cardiac PMF. Decreased level of non-enzymic antioxidant and decreased actions of antioxidant enzymes observed in this work suggests elevated ROS production in untreated diabetic rats indicating amplified oxidative stress (Wu *et al.*, 2016); treatment with MLEMC significantly raised ($P<0.05$) the levels of these non-enzymic and enzymic antioxidants in cardiac tissue. Significant increased level of non-enzymic antioxidant and in actions of the antioxidant enzymes in cardiac tissue by MLEMC further demonstrated the antioxidant potentials of *M.charantia*.

Furthermore, significant decline in serum NO was observed in alloxan-induced non treated rats. Hyperglycaemia causes alterations in vascular structure and function by decreasing NO bioavailability and increasing hydrogen peroxide generation in the vascular endothelium. NO is known to protect the vascular systems in various major organs. These shielding actions of nitric oxide are owing to its antioxidant, anti-adhesion, and anti-inflammatory potentials (Phillips *et al.*, 2009). NO is a vasodilator, thus low serum NO level due to alloxan administration causes constriction of blood vessels leading to hypertension. Nitric oxide generated from vascular endothelium aids in the maintenance of continuous vasodilator tone necessary for regulation of blood flow, blood pressure, vasodilation and platelet aggregation (Richard and Joseph, 2010). NO prevents platelet activation, limits endothelial leukocyte adhesion and regulates contraction of myocardium. Additionally, it performs a serious role in the development of known

cardiovascular conditions, comprising hypotension, hypertension, and atherosclerosis. Therefore, endothelial dysfunction and hypertension are triggered by diminished bioavailability of NO (Cristina *et al.*, 2013).

Reduction in bioavailability of NO is positively associated with hypertension together with other cardiovascular complications (Chand *et al.*, 2015), thus enhanced activity of NO elicits a vital function in limitation of vascular resistance and raised blood pressure in hypertensive subjects (Solano and Goldberg, 2006). Administration of MLEMC restored the serum NO to its near normal level thus abrogating the hypertension. Similarly, oxidative stress, inflammation and cardiac impairment were ameliorated by MLEMC typified by diminished serum myeloperoxidase (MPO) level in the rats. MPO is known to cause inflammation and apoptosis (Amjad *et al.*, 2018). Furthermore, the roles of MPO in oxidative stress and inflammation are elicited at cellular level via its enzymatic activity leading to production of reactive molecules with capability to oxidize lipids, antioxidants and proteins of LDL (Zhang *et al.*, 2015). MPO is an indicator of heart failure, cardiac injury, inflammation and oxidative stress (Gjin, 2019); therefore, higher MPO activity is indicative of cardiac impairment.

Myeloperoxidase can cause progression of atherosclerotic lesion, thereby making plaques unstable (Nathaniel *et al.*, 2017).There is elevated level of serum MPO in patients with cardiovascular diseases (Kutter *et al.*, 2000). Since, MLEMC could regularise the aforementioned high MPO level, it suggests that MLEMC possesses anti-inflammatory, antioxidant and cardioprotective potentials.

Therefore, in this experiment, MLEMC ameliorated the deleterious effects of cardiac injury by elevating the *in vivo* antioxidant status, decreasing the oxidative stress markers, inflammation and cardiac damage as well as enhancing NO bioavailability.

The result revealed a significant elevation in blood pressure parameters (SBP, DBP and MAP) following alloxan administration. This is in consonant with previous report that alloxan causes increase in blood pressure (Taiye *et al.*, 2016). Administration of MLEMC significantly reduced these elevated blood pressure parameters to their near normal values

The blood pressure-lowering capability of *M. charantia* may be accrued to its relaxant influence on vascular smooth muscles. The mechanism of action of vasorelaxation agent is stimulation of cyclic AMP which through an energy-consuming Ca^{2+}-binding pathway leading to decreased sarcoplasmic Ca2+ and, hence, relaxation. It may be assumed that MLEMC exert its vaso-relaxant influence via obstruction of extracellular Ca^{2+}influx, restriction of intracellular Ca^{2+} discharge, and opening of K+ pathways (Jonathan and Paul, 2011).

The ameliorative effects of *M. charantia* on cardiac injury was further established by studying its effects on the ECG abnormalities produced by alloxan administration in the diabetic rats.

Electrocardiography is a vital clinical check aimed at diagnosis of heart abnormalities. Assessment of ECG revealed that alloxan treated rats showed various statistically significant abnormalities in ECG parameters relative to control rats. Following alloxan administration, the cardio-toxic effects observed in this experiment entailed significant decrease in heart rate (bradycardia), significant QT interval and QTc prolongations when compared with the control. QTc prolongation is a proven prognostic sign of cardiovascular collapse and poisoning. Likewise, Prolonged QT interval has been associated with hypertension and cardiac arrest (Holditch *et al.*, 2015). However, administration of MLEMC rectified the QT prolongation by diminishing the QTc interval triggered by alloxan administration bringing them to near normal values.

Hence, MLEMC demonstrated a significant anti-hypertensive and cardioprotective effect.

Other abnormalities observed in the ECG due to alloxan administration were significant prolongation of the P duration, PR interval, QRS complex and R amplitude (Ramp),

However, concurrent treatment with MLEMC at 200 and 400 mg/kg doses brought about a noticeable and significant amelioration in these abnormalities and brought the parameters to near normal level. QT interval describes the period from commencement of QRS complex to the close of T wave. QT is the extent of time needed for speedy influx of $Ca^{2+}$and Na+, ensuing in myocardial depolarization andK+ outflow, which eventually causes repolarization (Moskovitz *et al.*, 2013). QT is typically modified to QTc value due to the reliance of frequency of depolarization and repolarization on heart rate. Protracted QT/QTc interval

implies hindered cardiac repolarization and increased risk of ventricular arrhythmias (Witchel and Hancox, 2000).

Immunohistochemistry showed higher expression of p38 and CRP in cardiac tissue of the alloxan-induced rats than control, while administration of MLEMC and glibenclamide showed low expression of these proteins in the cardiac tissue similar to control. P38 is known to be involved in apoptosis, while CRP is involved in inflammation and it determines the extent of cardiac injury. Thus down regulation of these proteins by MLEMC is suggestive of its anti-apoptotic, anti-inflammatory and cardioprotective potentials.

Histopathological findings showed various pathologies including inflammatory cell infiltration, disseminated haemorrhagic lesions in the myocardium, and fatty infiltration of myocardium of rats treated with alloxan, while supplementation with MLEMC and glibenclamide reduce these pathologies in the cardiac tissue.

Overall, this study clearly demonstrates the ameliorative properties of MLEMC against alloxan-induced cardiac damage.

4. Conclusion

This study shows that MLEMC exhibits pronounced capacity in the amelioration of alloxan-induced cardiovascular dysfunction. Alloxan administration causes varying cardiovascular abnormalities including oxidative stress, inflammation, hypertension, ECG abnormalities, blood pressure changes and histopathological abnormalities. Administration of MLEMC mitigated all the abnormalities and restored the parameters to their near normal levels. It can be concluded that MLEMC administration ensued to amelioration of noxious progressions triggered by alloxan. MLEMC possesses a significant medicinal value in the treatment of cardiac injury, thus the plant might be a promising therapeutic line of approach to myocardial dysfunction.

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