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Antidiabetic and anti-oxidant effects of methanol leaf extract of *Momordica charantia* following alloxan-induced hyperglycaemia in rats

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ABSTRACT

Background & Aim: The plant *Momordica charantia* is highly valuable having a wide range of medicinal uses with tropical and subtropical regions distribution. This study was conducted to appraise the medicative properties of methanol leaf extract of *Momordica charantia* on alloxan-induced diabetic rats.

Experimental: The antidiabetic influence of methanol leaf extract of *Momordica charantia* (MEMC) was investigated in alloxan-induced diabetes in rats. 50 rats allocated into five groups (A-E) (n =10) were utilized in this study: group A was normal control, groups B to E were induced with alloxan with diabetes established, while group B was not treated, group C received glibenclamide treatment while groups D and E were administered extracts at 200 and 400 mg/kg doses, respectively, in a study that continued for 28 days. Changes in blood glucose levels, body weight, haematology, serum chemistry, antioxidant system and histopathology changes were evaluated.

Results: MEMC elicited significant drop in blood glucose level from diabetic to near normal level, restoring the body weight, haematological and serum biochemical parameters to the basal non diabetic level; likewise the MEMC-treated group elicited a decreased level of oxidant markers such as malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) but increased concentration of protein thiols (PT), non-protein thiols (NPT), glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and superoxide dismutase (SOD) indicating its anti-oxidant potential. The pancreas section also revealed repair of distorted pancreatic architecture in MEMC-treated group compared to diabetic group.

Recommended applications/industries: The plant exhibited anti-hyperglycaemic, anti-diabetic and anti-oxidant abilities hence could be explored for the treatment of diabetes mellitus.

1. Introduction

The main feature of diabetes mellitus is hyperglycemia initiated by defective insulin discharge, insulin action/sensitivity or both (American Diabetes Association, 2009; Anees *et al.*, 2013). Insulin and oral hypoglycemic agents being major curative options for diabetes have noticeable side effects (Noor *et al.*,2008). Hence, getting novel preventive approaches and therapy for diabetes is necessary. Since natural remedies are somehow safe than these drugs, herbalism has turned out to be a substitute in management of diabetes (Murphy, 2000).

Plants contain many bioactive elements with therapeutic capability making the evaluation of plant products for diabetes treatment a subject of interest with *Momordica charantia* being one of the traditionally useful plants for disease management.

*M. charantia*L. (bitter melon), considered as natural cure for diabetic treatment (Joseph and Jini, 2013) is a widely distributed plant tropically and subtropically. The plant is very rich in phenolic compounds with antioxidant and antimutagenic potentials (Nguyen, 2014; Preeti *et al.*, 2017). On account of these, many clinical studies have reported that bitter melon extracts possess various phytochemicals with anti-hyperglycemic property (Kandangath *et al.*, 2015; Basavaraj *et al.*, 2017).

The current experiment was conducted to appraise the medicative properties of MEMC, (methanol leaf extract of *Momordica charantia,* family Cucurbitaceae) on alloxan-induced diabetic rats and to suggest the probable mechanisms of action because ethnobotanical survey on plants with antidiabetic potential had earlier ben conducted in Ibadan, Nigeria showing that this plant is one of such used in this region. Alloxan-triggered oxidative stress causes mutilation of pancreatic beta cells ensuing in hyperglycemia in rats (Ankur and Shahjah, 2012). Conditions including cardiovascular malady, diabetes mellitus, benign and malignant growth etc. are triggered by oxidative damage, which is an outcome of persistent hyperglycemia. It is therefore important to assume this study in order to assess the antidiabetic action of *M. charantia* and its antioxidant property with respect to intestinal tissue as described in this study.

We hypothesized that the leaves contained active phytochemical components that are capable of synergistically exerting antidiabetic effect in alloxan-induced diabetic animal prototypes.

2. Materials and Methods

2.1. Chemicals

Alloxan monohydrate (2,4,4,6-tetra oxohexahydropyrimidine), methanol and tween-80 were procured from Sigma-Aldrich Chemie Gmbh, (Steinheim, Denmark). Glibenclamide (a reference antidiabetic drug), was purchased from a local medical store and stored according to the given directives (i.e., below 25°C). Reagents used in this experiment including normal saline and distilled water were of analytical grade.

2.2. Experimental animals (Wister rats)

Male adult Wistar strain albino rats (average weight 150–200 g; n = 50) were deployed for this test. The method of Laura *et al.* (2006) was used for animal preparation. The animals were kept in rat cages in Research animal division of Veterinary Medicine Faculty, University of Ibadan and adapted for seven days at room temperature (22 ± 2 ºC) in a condition of 12 h light/12 h dark cycle with humidity of 55 ± 5% and nurtured using commercial rabbit cubes. The animals had continuous accessibility to clean water. All investigation done on the laboratory animals in this study followed the Standard Operation Procedures (SOPs) as approved by the Nationwide Institute of Health Directives for Care and Usage of Laboratory animals (NIH, 1985).

2.3. Extract preparation

Momordica charantia harvested from botanical garden, University of Ibadan, was recognized and authenticated at University of Ibadan Botany department with Voucher Specimen Number given asUIH-22563.

The room temperature of (27 ± 2^{o}C) was used to dry the leaves, which was ground to powdery form (400 g) then soaked and extracted in 90% methanol (1L) for 3 days till thorough extraction. This is followed by filtration and evaporation to dryness at 190 rpm and 45 -50^{0}C for 1 day using lowered pressure to produce the extract. The yield of the extract was 14%.

2.4. Ethical approval

This study protocol was ratified by the Animal Care and Use *Research* Ethics Committee of the University of Ibadan (UI-ACUREC/App/2015/044). All processes were executed as permitted by Animal Ethics team, University of Ibadan, Nigeria.

2.5. Experimental procedure

A solitary injection intraperitoneally (i.p.) of newly constituted alloxan monohydrate (100 mg/kg) (Patel *et al.*, 2007) solubilised in citrate buffer solution at pH 4.5 (Sheweita *et al.*, 2002) was used to induce hyperglycaemia in eighteen-hour starved adult Wistar rats (n = 50) weighing 150–200 g with feeding sustained thereafter. Blood was collected from each rat after two days for glucose level determination to substantiate diabetes. Rats having blood glucose concentration >200 mg/dl were seen as diabetic (Thirumalai *et al.*, 2011) and used in this experiment (n = 40); estimation of fasting BGLs was done by commercially available glucose kit glucometer (Roche, Mannheim, Germany) centred on glucose oxidase technique (Trinder, 1969).

Forty rats with serum glucose concentration beyond 200 mg/dl were known to be diabetic and selected for this study in addition to ten normal rats that were not injected with alloxan. The rats were thereafter grouped into 5 with 10 animals per group with procedure carried out as stated below:

Group A: Normal non-diabetic non-treated control rats administered with 2.5% Tween 80 in normal saline by daily oral gavage (NDNT).

Group B: Diabetic control rats received no treatments after induction of diabetes (DNT) with alloxan.

Group C: Diabetic rats treated with glibenclamide at 4 mg/kg body weight by daily oral gavage (DTG).

Group D: Diabetic rats received MEMC, 200 mg/kg body weight by daily oral gavage (DTMC200).

Group E: Diabetic rats received MEMC, 400 mg/kg body weight by daily oral gavage (DTMC400).

Antidiabetic property of MEMC was evaluated by assessing the influence of its continuous usage on serum glucose concentration and body weight of the rats. Blood glucose concentrations and body weight of the rats were evaluated before the commencement of the experiment (pretreatment) with the measurements taken on 7th, 14th, 21stand 28th days (Aslan *et al.*, 2006) with changes in the serum glucose concentration and body weight measured on these days.

The influence of alloxan induced diabetes, as well as glibenclamide and *Momordica charantia* treatments were further investigated on the haematology, serum lipid profiles; histopathological alterations in the pancreas of normal, diabetic control and diabetic treated rats were also observed.

2.6. Collection of blood and serum sample

Collection of blood samples was done via the retro-orbital vein with capillary tube, which was directed straight to the heparinised bottles lowered well below the capillary tube for adequate outflow of blood for haematological studies; clean non-heparinised bottles were used to collect the other set of blood samples and permitted to coagulate. The serum was separated and centrifuged (4000 rpm for 15 mins) into clean sample bottles and kept in the fridge at -4^{0}C till time of biochemical analysis.

Measurements were taken before the diabetes induction (basal), fourteen and twenty eight days following the start of treatment (Post-treatment).

The male Wistar rats given different treatments were humanely sacrificed on the 29th day after the experiment was terminated by cervical dislocation with the animal dissected and the intestine swiftly removed, cleaned in cold normal saline and instantly preserved in ice to avoid damage of biomolecules. The pancreas was also removed for histological studies. For histological studies of pancreas, the excised pancreas were washed, blotted dry, weighed and a part cut for histological examination. Paraffin section of the pancreas was prepared by fixing the pancreas in 10% neutral buffered formalin.

2.6.1. Homogenization

The intestine was removed and cleaned in normal saline solution, then blotted by filter paper and weighed. It was sliced into fragments and homogenised in ten volumes of the homogenising buffer (0.1 M phosphate buffer, pH 7.4) by Teflon homogenizer. Centrifugation of resulting homogenate was done at 10^4rpm for 0.25 hr using cold centrifuge (4^{0}C) to achieve post mitochondrial fraction (PMF). The supernatant was taken for biochemical analysis.

2.7. Influence of the extract on haematological parameters

Haematocrit tubes were used to collect blood samples by ocular puncture then conveyed to EDTA tubes and positioned in haematology analyzer (Abacus Junior®, Budapest-Hungary) for blood indices estimation in order to evaluate the influence of chronic MEMC administration upon haemoglobin (Hb) and cell counts of the diabetic rats. Data were taken before the diabetes induction (basal), fourteen and twenty eight days following the start of treatment (Post-treatment).

Haemoglobin estimation was done by cyanohaemoglobin technique as described by Jain (1986); determination of erythrocyte and leucocyte count was achieved by the method of Coles (1986), while packed cell volume (PCV) was assessed via the technique of Duncan *et al*., (1994). Differential white blood cell count was estimated by the procedure described by Blumenreich (1990) while Platelets count was determined by the methods described by Wiener (1963).

2.8. Influence of MEMC on lipid profile of diabetic rats and other biochemical parameters

The influence of MEMC was investigated on lipid profile of treated diabetic rats by evaluating the serum levels of cholesterol and triglyceride. Serum sample was used for these biochemical analyses. Commercial test kits obtained from Sigma^{R} Diagnostic, UK were used for all the biochemical parameters measured. Estimation of complete cholesterol and triglyceride concentration of each sample was distinctly done by enzymatic colorimetric technique (Zoppi and Fellini, 1976) with the aid of reagent kits. Lipid levels were determined prior to diabetes induction (Basal), 14th and 28th days post-treatment. Each sample absorbance comprising the reaction combination in the presence or absence of serum was observed at 540nm wavelength in UV spectrophotometer. Total cholesterol or triglyceride was determined by the equation: Total cholesterol (mg/dl) = ODs/ODst × 2 ×10^{2}, ODs = Test sample optical density and ODst = optical density of standard.

Serum HDL was evaluated by the techniques of Anouarand Jacques (2015) while evaluation of serum LDL was done with procedure of Matthias and Nader (2000).

2.9. Antioxidant activity

Antioxidant capability of MEMC was assessed by studying its influence on oxidative stress indicators and on antioxidant enzymes level in intestinal tissues. Generation of hydrogen peroxide was estimated with the procedure of Werner (2003). The method of Varshney and Kale (1990) was employed for lipid peroxidation (Malondialdehyde, MDA level) estimation.

Determination of intestinal non-protein thiol and protein thiol concentrations was achieved using Jakob and Colin's principle (Jakob and Colin, 2014). Assessment of intestinal GSH followed the technique of Trent and Lynette (2012). Measurement of the intestinal GPX activity was credited to procedures of Wayne and Craig (1990). Glutathione-S-transferase (GST) activity was assessed following the techniques of Lila *et al*., (2014) while SOD activity in the intestinal homogenates was assessed using the procedures of Christine and Joseph (2010).

2.10. Histopathological studies

10% buffered formalin was used to fix the pancreatic tissues, then dehydrated in ethanol of increasing concentration (50%-100%) and cleared in xylene. Thereafter, tissues processing with embedding in paraffin wax was done and cut to 5-6 µm thickness using microtome. This was stained using haematoxylin and eosin (H and E) aimed at photomicroscopic interpretations of histologic architecture of pancreas underneath light microscope (Drury *et al*., 1976).

2.11. Statistical analysis

Data were stated as Mean ± standard deviation, analyzed using descriptive statistics. We determined significance of difference in means by one-way analysis of variance (ANOVA; 95% confidence interval), then by Turkey post hoc tests through version 5 of Graph Pad prism statistical package. Values of $\alpha_{0.05}$ were regarded being significant (Betty and Jonathan, 1976).

3. Results and discussion

Oral administration of MEMC provoked a significant decline in blood glucose concentration of diabetic rats (Table 1). The decline exerted by MEMC at 200mg/kg (50%) and 400 mg/kg (51%) doses were comparable to that of typical drug, glibenclamide (53%) on day 28

with highest decline in blood glucose by MEMC being 51%, which was achieved by 400mg/kg on day 28.

The condition of diabetes mellitus (DM) is connected by disorders of carbohydrate, protein and fats breakdown, with subsequent dysfunction of various organs and resultant hyperlipidaemia associated with hyperglycemia (Upendra *et al.*, 2010).

Alloxan, a beta cytotoxin is recognised to evoke diabetes in animals by initiating damage to the pancreatic beta cell leading to reduction in endogenous insulin release and decreased glucose utilization by the tissues (Omamoto *et al.*, 1981). Various metabolic abnormalities including hyperglycemia (Chude *et al.*, 2001), diminished protein content (Gul and Rahman, 2006) and decrease in body weight occur due to insulin deficiency. Prompt glycaemic control is vital for reduction of fatalities of diabetic complications. In the present study, anti-hyperglycemic property of MEMC was assessed and validated owing to the continuous lessening of serum glucose concentration in diabetic rats to normal levels produced by oral supplementation of MEMC daily for 4 weeks indicating its usefulness in overt cases of DM and its complications.

The anti-hyperglycemic effect may be due various mechanisms comprising insulinomimetic activity, glucose uptake enhancement by peripheral tissue, hindrance of endogenous glucose formation or initiation of gluconeogenesis in the liver and muscle, prevention or amelioration of pancreatic injury and suppression diabetes-induced oxidative stress. Experimental confirmation achieved from this study reveals that *M. charantia* possesses antidiabetic and antioxidant properties.

Our results validated that MEMC (200 mg/kg and 400 mg/kg doses) exhibited significant antihyperglycemic activity and antihyperlipidemic properties with alleviation of alloxan induced pancreatic damage comparable to the typical drug, glibenclamide (4 mg/kg) in alloxan-induced diabetic rats. Therefore, the plant could be adopted for the inhibition/therapy of hyperglycemia and hyperlipidemia.

Table 1: Influence of MEMC on serum glucose level of alloxan induced diabetic rats.

Groups	Concentration of blood glucose (mg/dl)					
	Pre diabetic	Diabetic (Pre-Rx)	Post-Rx			
			Day 7	Day 14	Day 21	Day 28
A (NDNT)	74.00±3.36	83.70±2.51	71.30±3.51	88.80±3.00	91.90±3.92	90.60±3.86
B (DNT)	72.60±3.79	287.30±3.62^{a}	292.90±2.83^{a}	294.37±3.53^{a}	289.00±3.71^{a}	293.00±4.52^{a}
C (DTG)	81.50±3.34	292.88±4.93^{a}	248.00±3.73^{ab} (15)	184.75±2.26^{abc} (37)	165.75±3.63^{abcd} (43)	136.25±3.69^{abcde} (53)
D(DTMC 200)	72.20±3.06	282.88±3.80^{a}	261.0±4.39^{ab} (7)	242.13±2.64^{abc} (14)	207.38±4.45^{abcd} (27)	142.00±3.23^{abcde} (50)
E(DTMC 400)	72.90±2.44	285.80±4.45^{a}	217.8±5.03^{ab} (24)	177.70±3.83^{abc} (38)	184.00±3.86^{abcd} (36)	141.20±3.84^{abcde} (51)

Results conveyed by way of mean ± standard deviation (SD), n=10. ^{abcde} indicate significant difference ($\alpha_{0.05}$) when compared with basal, pre-treatment, day7, day14 and day 21 post-treatment respectively. Numbers in parenthesis signify decrease (%) in blood glucose concentration estimated for treatment groups relative to pre-treatment values. Group A NDNT (non diabetic non 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).

3.1. Influence of MEMC on body weight of diabetic rats

Stability was observed in body weight of vehicle control animals (NDNT) while diabetic rats exhibited significant decrease in body weight up till 28 days (Table 2). Alloxan triggered weight reduction, which was upturned by glibenclamide and MEMC. Significant elevation in body weight was perceived in the glibenclamide and extract (200 mg/kg and 400mg/kg) treated groups starting from day 14 for 200 mg/kg dose and day 21 for 400 mg/kg dose of MEMC and this continued on day 28.Diabetes is connected with weight decline (Huang *et al.*, 2000), this study has shown the capability of MEMC to reverse the weight loss caused by diabetes. The reversal of weight loss by MEMC may be as a result of reversal of gluconeogenesis and glycogenolysis, confirming the antidiabetic activity of the MEMC.

3.2. Influence of methanol leaf extract of Momordica charantia on haematological parameters (PCV, Hb level and RBC count) of diabetic rats

From the result as presented in Table 3, reduction in PCV of diabetic rats on day 14 was noted, but subsequently on day 28, there was an elevation in PCV of the glibenclamide and extract treated rats, whereas the decline continued in diabetic non treated group. The haemoglobin levels of all groups were initially reduced on day 14 with the diabetic non treated and glibenclamide groups significantly reduced, subsequently on day 28, there was elevation in HB levels of the MC treated and glibenclamide groups, whereas the reduction continued in diabetic non treated group.

Red cell count of all diabetic rats was decreased on day 14. However, there was a rise in the red cell count on day 28 in glibenclamide- and MEMC-treated groups.

The effect of MEMC administration on PCV, Hb level and RBC when evaluated indicated an increase at day 28 following initial reduction at day 14. The WBC, neutrophils and lymphocytes also increased on day 28 following the initial reduction; this could indicate the immune-stimulatory activity of the extract, thereby supporting its immune-enhancement potential (Shuo *et al.*, 2017). MEMC increased platelet count implying that the plant can contribute an essential function in the reduction of blood loss and repair of vascular injury. The current study showed that MEMC possesses the capability of nullifying toxic changes in the haematological parameters caused by the alloxan administration to the rats and restore the haematological parameters to their near basal values.

Table 2: Influence of MEMC upon body weight of alloxan induced diabetic rats.

Groups	Body weight (g)					
	Pre- diabetic (Basal)	Diabetic (Pre-Rx)	Post-Rx			
			Day 7	Day 14	Day 21	Day 28
A (NDNT)	168.35±3.98	165.40±2.80	163.83±2.82	172.04±3.45	168.36±3.75	178.54 ± 3.79
B (DNT)	142.38±3.78	146.77±3.82	137.55±4.98^{b}	134.13±4.49^{ab}	114.37±4.26^{abcd}	102.00±4.87^{abcde}
C (DTG)	168.38±2.60	143.87±3.85^{a}	149.00±3.52^{ab}	154.90±2.62^{abc}	158.75± 3.63^{abc}	165.25 ±3.97^{bcde}
D(DTMC200)	141.14±2.61	132.89±3.38^{a}	129.78± 2.16^{a}	135.20±2.92^{ac}	134.82± 3.96^{ac}	140.00 ±3.23^{bcde}
E(DTMC400)	150.49±2.36	135.65±3.96^{a}	130.74±3.81^{ab}	132.94±4.97^{a}	139.34± 2.41^{acd}	150.20 ±4.78^{bcde}

Results relayed by way of mean ± standard deviation (SD), n=10. ^{abcde} indicate significant difference ($\alpha_{0.05}$) when compared with basal, pre-treatment, day7, day14 and day 21 post-treatment respectively. Group A NDNT (non diabetic non 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 3: Influence exerted by MEMC on PCV, Hb level and RBC count of alloxan triggered diabetic rats.

Groups/Parameters		A (NDNT)	B (DNT)	C (DTG)	D (DTMC200)	E (DTMC400)
PCV (%)	Basal	44.86±1.86	48.00 ±3.27	44.71± 3.45	45.57±3.78	45.67±3.87
	Day 14	45.75 ±1.35	38.50±1.29^{ab}	41.80±0.84^{b}	42.80±3.03^{c}	43.00±5.85^{c}
	Day 28	45.88 ±3.70	35.33±6.43^{ab}	43.00±6.21^{d}	46.00±3.07^{cd}	44.63±2.77^{c}
Hb (g/dl)	Basal	14.49 ±1.61	16.46 ± 1.22	14.73±1.57	15.29±1.21	15.11±1.39
	Day 14	14.27 ±0.27	13.28 ±0.10^{a}	11.26±1.40^{abc}	14.36±0.98^{c}	14.66±0.21^{c}
	Day 28	14.32 ±1.61	11.20±0.75^{ab}	15.13±1.31^{c}	15.21±0.95^{c}	15.01±0.82^{c}
RBC count (x10^{6}µl)	Basal	7.50 ± 0.29	8.00 ± 0.06	7.42±0.61	7.53±0.73	7.55±0.78
	Day 14	7.56 ± 0.21	6.49 ± 0.46^{ab}	7.19±0.17	6.87±0.43^{d}	7.42±0.35
	Day 28	7.48 ± 0.72	6.24 ± 1.53^{a}	7.44±1.77	7.39±0.27	7.50±0.63

values stated as mean ± SD, n= 10.(^{abcd}) indicate significant difference ($\alpha_{0.05}$) relative to basal, normal control, diabetic control and standard drug respectively. Group A NDNT (non diabetic non 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). PCV: Packed cell volume, Hb: Haemoglobin concentration, RBC count: Red blood cell count.

3.3. Influence posed by MEMC on haematological parameters (WBC, neutrophils, lymphocytes and platelets) of diabetic rats

WBC count of diabetic animals increased on day 14, the increase was sustained in diabetic untreated group, however, reduction in MEMC treated and glibenclamide group ensued on day 28 relative to diabetic non-treated group.

The neutrophil count was elevated in all groups at day 14 but by day 28, administration of MEMC caused significant reduction in neutrophil count when compared with diabetic non-treated group. However, administration of MEMC caused increase in the lymphocyte count compared with diabetic non-treated group; platelet count was slightly reduced but not significantly by the 400 mg/kg extract comparatively to the basal values. Both 200 mg/kg and 400mg/kg MEMC caused increase in platelet count on day 28 (Table 4).

Table 4: Influence posed by MEMC on WBC count, neutrophils, lymphocytes and platelets of diabetic rats.

Groups/ Parameters		A (NDNT)	B (DNT)	C (DTG)	D (DTMC200)	E (DTMC400)
WBC(10^3/µl)	Basal	6.00±1.10	5.80±1.20	5.69±1.45	7.52± 1.36	7.53±1.40
	Day14	6.83±2.41	10.93±2.41^{ab}	7.78±3.73	6.80±2.28	6.49±2.65
	Day 28	7.38±1.05	11.08±1.46^{ab}	7.59±0.14^{c}	7.04±0.47	7.45±0.74^{c}
Neutrophil (10^3cells/ µl)	Basal	2.90±0.01	2.89±0.07	2.86±0.05	2.90±0.04	2.92±0.07
	Day 14	2.91±0.03	4.45±0.03	3.04±0.02^{ab}	2.63±0.04^{abc}	2.85±0.03^{abc}
	Day 28	2.92±0.03	4.41±0.03	2.98±0.03^{abc}	3.03±0.04^{abc}	2.96±0.04^{cd}
Lymphocyte (10^3cells/µl)	Basal	5.58±0.97	6.61±0.59	6.97±0.41	6.82±0.39	6.77±0.40
	Day 14	6.15±0.57	4.62±0.48	4.89±0.49^{a}	4.65±0.56	6.34±0.38
	Day 28	6.08±0.13	4.55±0.15	5.71±0.52	5.97±0.46	6.67±0.33
Platelet (10^3cells/µl)	Basal	189.86±21.3	185.29±16.26	183.71±22.34	181.71±20.38	186.00±21.59
	Day 14	196.00±23.0	114.00±37.6^{ab}	175.20±57.2^{c}	172.88±34.69	175.88±16.3^{c}
	Day 28	190.20±15.9	110.00±20.9^{b}	160.25±21.3^{c}	182.00±25.9^{c}	184.00±22.4^{c}

Values relayed as mean ± SD, n= 10. (^{abcd}) indicate significant difference ($\alpha_{0.05}$) relative to basal, normal control, diabetic control and standard drug respectively. Group A NDNT (non diabetic non 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). WBC: White blood cell count

3.4. Influence posed by Methanol leaf extract of Momordica charantia on serum biochemical parameters (total cholesterol, triglyceride, HDL and LDL) of diabetic rats

Diabetic rats showed significant rise in serum total cholesterol, triglycerides and LDL and significant drop in HDL when related to non-diabetic rats. However, supplementation with MEMC and glibenclamide significantly dropped the serum total cholesterol, triglycerides and LDL levels with the extent of decline greater than that caused by glibenclamide. Significant elevation in serum HDL level was observed after treatment with MEMC and glibenclamide (Table 5).

Oral administration of MEMC caused decline in total cholesterol and triglyceride concentration in diabetic rats as shown in this study. Increased triglycerides, total cholesterol and low density lipoprotein (LDL) and diminished high density lipoprotein (HDL) levels indicates diabetic dyslipidaemia, which constitutes serious cardiovascular menace element suffered by diabetics (Vergès, 2015; Iciar *et al.*, 2018). In diabetes, hypertriglyceridemia and hypercholesterolemia remain the most common lipid abnormalities (Jacobson *et al.*, 2007). Repeated treatment with MEMC for 4weeks significantly lessened the absolute cholesterol, triglycerides and LDL levels but caused elevated HDL levels. The observed antihyperlipidemic properties of MEMC could be as a result of reduced cholesterogenesis and fatty acid production via

blockade of pancreatic cholesterol esterase and pancreatic lipase enzymes respectively (Birari and Bhutani, 2007). The HDL level was significantly increased by the MEMC administration; this implies that the plant may decrease death resulting from diabetic complications by ameliorating diabetes-induced dyslipidaemia.

Table 5: Influence posed by MEMC on serum biochemical parameters (total cholesterol, triglyceride, HDL and LDL) of alloxan-induced diabetic rats.

Groups/ Parameters		A (NDNT)	B (DNT)	C(DTG)	D(DTMC200)	E(DTMC400)
Total cholesterol (mg/dl)	Basal	112.40±3.35	113.86±2.73	116.43±2.17	115.63±2.57	112.47±2.68
	Day 14	112.34±2.57	148.57±3.50$^{\alpha\gamma}$	124.80±4.35$^{\alpha\beta\varepsilon}$	123.83±4.83$^{\alpha\beta\varepsilon}$	132.38±3.25$^{\alpha\gamma\varepsilon\sigma\theta}$
	Day 28	118.86±3.45	151.89±4.18$^{\alpha\gamma}$	120.23±4.29$^{\beta\gamma\varepsilon}$	115.79±3.24$^{\beta\varepsilon}$	113.04±3.67$^{\beta\sigma}$
Triglyceride (mg/dl)	Basal	116.00±4.32	113.45±6.23	115.88±4.67	117.68±3.88	116.47±3.45
	Day 14	115.32±3.35	152.16±4.39$^{\alpha\gamma}$	124.35±4.24$^{\alpha\gamma\varepsilon}$	126.04±3.65$^{\alpha\gamma\varepsilon}$	125.69±3.85$^{\alpha\gamma}{}_{\varepsilon}$
	Day 28	117.78±5.22	154.67±4.15$^{\alpha\gamma}$	121.21±6.29$^{\varepsilon}$	117.89±4.20$^{\beta\varepsilon}$	118.67±4.88$^{\beta\varepsilon}$
HDL(mg/dl)	Basal	29.40±1.64	29.43±1.79	31.43±1.78	29.63±1.62	32.14±1.41
	Day 14	30.44±1.67	22.86±1.25$^{\alpha\gamma}$	30.25±1.60$^{\varepsilon}$	29.95±1.03$^{\varepsilon}$	31.38±1.75$^{\varepsilon}$
	Day 28	30.18±1.91	23.34±1.65$^{\alpha\gamma}$	31.45±1.79$^{\varepsilon}$	29.86±2.45$^{\varepsilon}$	31.87±1.78$^{\varepsilon}$
LDL(mg/dl)	Basal	59.80±0.85	61.74±2.46	61.82±2.88	62.46±1.83	57.04±1.97
	Day 14	58.84±2.56	95.28±4.69$^{\alpha\gamma}$	69.68±3.45$^{\alpha\gamma\varepsilon}$	68.67±4.65$^{\alpha\gamma\varepsilon}$	75.86±3.58$^{\alpha\gamma\varepsilon\sigma\delta}$
	Day 28	58.12±4.22	97.62±4.25$^{\alpha\gamma}$	64.54±3.86$^{\beta\gamma\varepsilon}$	62.35±1.98$^{\beta\varepsilon}$	57.44±4.48$^{\beta\varepsilon\sigma}$

Results expressed in Mean ± SD, n = 10.$^{\alpha\beta\gamma\varepsilon\sigma\delta}$ indicates significant difference ($\alpha_{0.05}$) when compared to basal, day 14, normal control, diabetic control, standard drug and MC 200, respectively. Group A NDNT (non diabetic non 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). HDL: high density lipoproteins; LDL: low density lipoproteins

3.5. Effects of MEMC on intestinal hydrogen peroxide (H_2O_2) generation in alloxan-induced diabetic rats

Significant decline in hydrogen peroxide generation was seen in the intestinal post mitochondrial fractions of MEMC treated diabetic rats relative to diabetic control (Figure 1). The same result was achieved with the reference drug, glibenclamide. This implies MEMC significantly decreased the generation of this oxidative stress marker.

Oxidative stress is an incriminating influence for pathogenesis of diabetes and diabetes related complications (Asmat *et al.*, 2016) characterized by decrease in antioxidant status, proteins glycation, enzymes inactivation and variations in the structural roles of collagen basement membranes. Therefore, oxidative stress reduction is vital in amelioration of hyperglycemia in diabetes and its complications. Morphological and functional variations have been described in the intestine of diabetic rats (Tormo *et al.*, 2002), thus we assessed the extent of oxidative stress triggered by alloxan diabetes in rats intestinal tissues and the ameliorative effects of *Momordica charantia* in the tissues for the first time in this study.

SOD, GPx and GST constitute a defense system that counteracts reactive oxygen species (ROS). SOD is the foremost enzyme that participates in antioxidant defense by depressing the balanced state level of O_2^-. During hyperglycemia, auto-oxidation of glucose occur leading to superoxide and free radicals production resulting in lipid peroxidation in lipoproteins. GPx speeds up hydroperoxides reaction with reduced glutathione to produce glutathione disulphide (GSSG) plus reduction product of hydroperoxide (Pastore *et al.*, 2003). In our study, diminished actions of these enzymes in diabetic rats with the achievement of near normalcy in *M. charantia* treated rats implies that MEMC quenched the oxidative stress provoked by alloxan in the intestinal tissues of the rats. Endogenous

antioxidant defense system is strengthened by natural antioxidants reinstating the optimal balance by mopping off the reactive species. Thus, *M. charantia* is a plant of immense value in this regard.

Glutathione (GSH) performs an immense task in the coordination of body's antioxidant defense system. Reduced concentration contributes to diabetes complications (Fallon *et al.*, 2018). The results disclosed that diabetic rats displayed perceived low levels of non-enzymatic antioxidant (GSH, protein thiol, non protein thiol) relative to the control rats. The levels of these antioxidants were significantly elevated in diabetic rats treated with *M. charantia,* likewise, the plant extract caused an elevation in antioxidant enzymes (SOD, GPx and GST) actions, while diminishing the generation of oxidative stress markers (H_20_2) and lipid peroxidation (MDA) rats intestinal tissues; this implied that the plant possesses antioxidant properties, thus protecting the pancreas from free radicals.

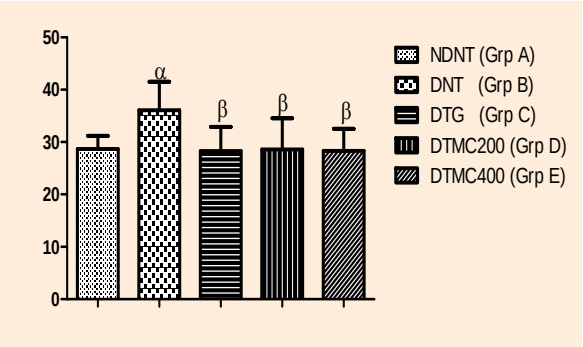

Figure 1: Effects of methanol extract of *M. charantia* on intestinal hydrogen peroxide (H_20_2) level of alloxan-induced diabetic rats. α Significant increase relative to normal control; β Significant reduction when compared with diabetic control at $\alpha_{0.05}$. H_20_2: hydrogen peroxide *µ*mol /ml.

Since *M. charantia* could ameliorate the oxidative stress in intestine of alloxan triggered diabetic rats, it is most likely that the plant possesses gastrointestinal protective ability. Destruction of beta cells in rats occurs within 2-3 days following alloxan administration (Adeghate and Ponery, 2002). Pancreatic beta cells are easily damaged by free radicals due to the reduced level of enzymes that scavenge free radical in pancreatic tissue. The results of this work suggest that MEMC causes regeneration/proliferation of pancreatic beta cells perhaps owing to inhibition of alloxan-induced free radicals generation. Pancreas is known to contain stable (quiescent) beta cells with regenerative potential, after damage caused by alloxan, the surviving cells and preexisting differentiated cells proliferate by replication to replace lost cells (Spinas, 1999).

3.6. Influence of Methanol leaf extract of Momordica charantia on intestinal malondialdehyde (MDA) level of diabetic rats

Supplementation with MEMC elicited a significant drop in malondialdehyde (MDA) level in rats intestinal tissue relative to untreated diabetic rats, the same result was observed for glibenclamide (Figure 2). This implies that MEMC triggered a significant lessening in lipid peroxidation in the intestinal post mitochondrial fraction (PMF).

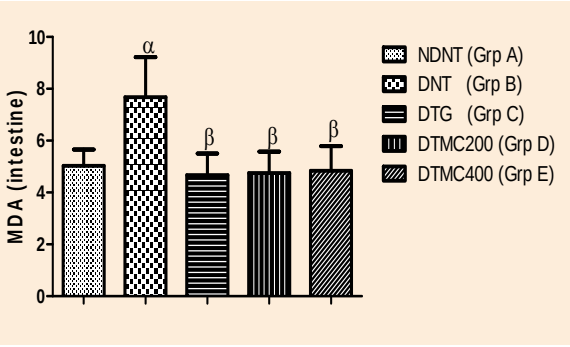

Figure 2: Effects of methanol extract of *M. charantia* on intestinal malondialdehyde (MDA) level of alloxan-induced diabetic rats.α Significant increase relative to normal control;β Significant reduction when compared with diabetic control at $\alpha_{0.05}$; MDA: malondialdehyde, (µmol formed MDA/mg protein).

3.7. Influence of MEMC on antioxidant system of the intestinal tissues diabetic rats

MEMC Administration caused elevated level of non-protein thiol, protein thiol and reduced glutathione (GSH), the same result was observed for glibenclamide (Table 6). 200 mg/kg dose of MEMC triggered significant elevation in GSH level relative to untreated diabetic rats.

Table 6: Influence of MEMC on antioxidant system of the intestinal tissues of alloxan-induced diabetic rats.

Parameters	A (NDNT)	B (DNT)	C (DTG)	D(DTMC200)	E(DTMC400)
Non protein thiol	77.22±6.62	69.94±9.47	74.53±8.67	71.33±9.40	75.13 ± 7.16
Protein thiol	25.05±5.44	18.90±4.83	22.39±2.30	24.23 ± 4.39	26.04±6.64$^{\beta}$
GSH	94.36±4.48	83.94±1.93	92.74±3.87	95.84±3.86$^{\beta}$	92.28 ±3.74

Results expressed in Mean ± SD, n=10. $^{\beta}$ Significant increase compared with diabetic control ($\alpha_{0.05}$). 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 DTG (Diabetic treated with glibenclamide), Group D DTMC 200(Diabetic treated with 200mg/kg MC), Group E DTMC400 (Diabetic treated 400mg/kg MC).

3.8. Influence exerted by MEMC on antioxidant defence system (GPx, GST and SOD) of the intestinal tissues diabetic rats

Significant ($\alpha_{0.05}$) reduction was observed in GPx, GST and SOD activities in the intestinal post-mitochondrial fractions of the diabetic rats relative to control group, but activities of these antioxidant enzymes were significantly ($\alpha_{0.05}$) elevated in MEMC- and glibenclamide treated rats relative to the diabetic non-treated group (Figures 3, 4 and 5).

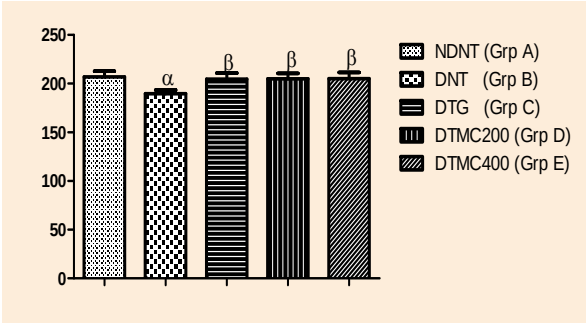

Figure 3: Effects of methanol extract of *M. charantia* on antioxidant defense system (GPx) of the intestinal tissues of alloxan-induced diabetic rats. α Significant reduction when compared with normal control; β Significant increase when compared with diabetic control at $\alpha_{0.05}$. GPx: glutathione peroxidase, (µmol /mg protein)

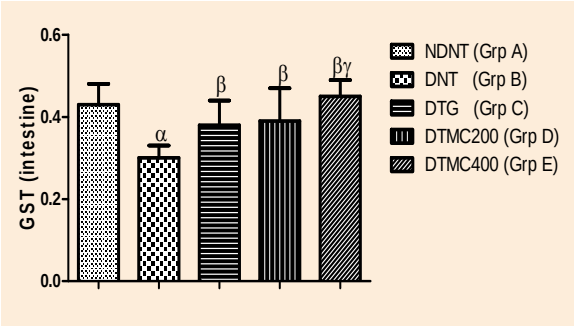

Figure 4: Effects of methanol extract of *M. charantia* on antioxidant defence system (GST) of the intestinal tissues of alloxan-induced diabetic rats.α Significant reduction when compared with normal control; β Significant increase when compared with diabetic control; γ Significant increase when compared with standard drug at $\alpha_{0.05}$. GST: Glutathione-S-transferase (µmol/min/mg protein).

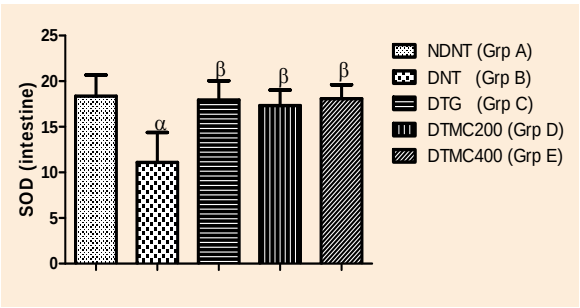

Figure 5: Effects of methanol extract of *M. charantia* on antioxidant defense system (SOD) of the intestinal tissues of alloxan-induced diabetic rats. α Significant reduction when compared with normal control; β Significant increase when compared with diabetic control at $\alpha_{0.05}$. SOD: superoxide dismutase, U/μg protein.

3.9. Influence of methanol leaf extract of Momordica charantia upon pancreatic tissues of diabetic rats

Histological changes produced in the pancreas by alloxan induction and treatment with MEMC and glibenclamide are presented in Figures 6(A-E). Histological analysis of pancreatic sections showed that control group possesses the regular histo-architecture of pancreas. Histopathological evaluation revealed that there was no necrotic lesions in the islet tissues and the alveolar cells in non-diabetic non treated group (Group A, NDNT) (Figure 6A), the plates showed typical exocrine acini with varying volume of zymogen granules (blue arrows); the intralobular and interlobular ducts were typical and some contained pancreatic discharge. There was fatty infiltration of the few islets, which vary in size.

There was islet tissue necrosis with moderately destroyed alveolar cells in the diabetic non treated group (Group B, DNT). The islets were few and far apart with widespread destruction of islets of Langerhans and decreased sizes of islets. Multi focal area of inflammation was observed in the parenchyma (Figure 6B).

The glibenclamide treated group presented mark of minor necrosis of the pancreas about the islet tissues, typical exocrine acini with varying volume of zymogen granules, intralobular and interlobular ducts were typical with some containing pancreatic discharge. There was congestion of blood vessels and fatty infiltration of few islets, which vary in size. Restoration of typical cellular population of the islets was observed (Figure 6C). In MEMC treated rats, in the 200mg/kg dose, the architecture of the pancreas was intact, normal exocrine acini with varying volume of zymogen granules were seen, the intralobular and interlobular ducts were typical and contained pancreatic discharge. Normal cellular population of the islet was partially restored; there was enlargement of β-cells with hyperplasia. There was multi-focal area of moderate inflammation of the parenchyma. The islets seem typical in varying sizes and in good number (green arrows) (Figure 6D). For the 400 mg/kg dose of MEMC, photomicrograph revealed typical exocrine acini with deep-staining zymogen granules, intralobular and interlobular ducts were typical and contained pancreatic discharge, the islets appear normal in diverse sizes few and far apart. There was multi-focal area of moderate inflammation of the parenchyma (Figure 6E).

Histological studies of pancreas revealed that MEMC administration might have repaired the pancreas impaired by alloxan. The extract reinstated the integrity and possibly, roles of injured pancreatic tissues. Therefore, it is possible that *M. charantia* was responsible for the beta cells proliferation and restoration of the normal pancreatic architecture as revealed by histopathological studies, thereby substantiating the cytoprotective action of the plant. Furthermore, we propose that radical scavenging (Mijitaba *et al.*, 2013, Aytaç, 2016) and antioxidant (Tasaduq *et al.*, 2003) properties of MEMC may contribute significantly in shielding the pancreas from the oxidants produced by alloxan.

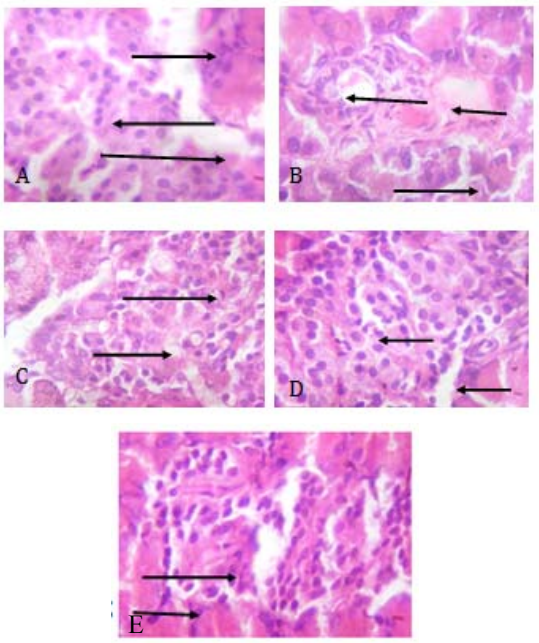

Figure 6: Photomicrograph of Pancreas, A (NDNT) normal exocrine acini. B(DNT) necrosis, multi focal area of inflammation of the parenchyma. C(DTG) normal exocrine acini, congestion of vessels, fatty infiltration of islets. D(DTMC200) normal exocrine acini, moderate inflammation of the parenchyma. E(DTMC400) normal exocrine acini, The islets appear normal in varying sizes few and far in between. (x 400, H and E).

The present study explored and compared the antidiabetic activity of standard drug glibenclamide with the *M. charantia* extract. It was observed that glibenclamide significantly diminished the serum

glucose level relative to diabetic control. Inhibition of free radical generation and potentiation of plasma insulin influence through increased pancreatic release of insulin by the surviving beta cells or its discharge from bound form may account for the significant antidiabetic activity of glibenclamide. Comparatively, glibenclamide, the reference hypoglycemic drug employed in this study did not bring about such influence to the level of MEMC (though the dosage of MEMC was much higher).

This is the first study to assess the influence of MEMC on intestinal tissues after alloxan- induced damage and on biochemical effect after such treatment.

4. Conclusion

It can be concluded that *M. charantia* reduces blood glucose level, reduces oxidative stress and elevates the level of antioxidant defense system in experimental diabetic rats. Thus MEMC possesses anti-hyperglycemic action. MEMC also showed enhancement in indices including body weight, haematology, and regeneration of pancreatic cells as well as improvement in the intestinal antioxidant status; therefore, we may also conclude that MEMC possesses gastrointestinal protective ability.

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