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Effects of Momordica balsamina on markers associated with renal dysfunction in a diet-induced prediabetic rat model

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Abstract

Introduction

Prediabetes is an intermediate state of progression from normoglycemia to overt type 2 diabetes. Literature suggests that managing prediabetes is beneficial in delaying the onset of diabetes. Previous studies have shown that *Momordica balsamina* (MB) possesses anti-hyperglycaemic effects; hence in this study, its effect on renal function in diet-induced pre-diabetes was explored.

Methodology

Male Sprague-Dawley rats (130-180 g) were divided into six groups of six rats per group; Normal control, Prediabetic (PD), Metformin (500 mg/kg, p.o.) treated PD without dietary intervention (DI), MB (250 mg/kg, p.o.) treated PD without DI, Metformin treated PD with DI and MB treated PD with DI. The animals were treated for 12 weeks of the last 32 weeks where fluid intake and urinary output was measured. After 12 weeks, the animals were sacrificed, plasma, urine, and kidney samples were collected for biochemical analysis.

Results

The untreated prediabetic (PD) rats presented with increased fluid intake and urine output; increased creatinine, urea, and uric acid plasma concentrations; sodium retention; potassium loss; increased aldosterone and kidney injury molecule (KIM-1) concentration; and increased urinary podocin mRNA concentration. However, MB administration attenuated the renal markers and oxidative stress and decreased the urinary podocin mRNA expression with or without diet intervention.

Conclusion

MB administration, regardless of diet modification, attenuates renal dysfunction in an experimentally induced prediabetic state. This suggests that MB has beneficial properties in the management of pre-diabetes associated kidney dysfunction.

Introduction

Prediabetes is a state of abnormal glucose homeostasis that is characterised by intermediate hyperglycaemia, elevated glycated haemoglobin and impaired glucose tolerance (1). It is said to be as a result of chronic consumption of a diet that consists of food rich in carbohydrates and saturated fats coupled with a lifestyle of physical inactivity (2). The prevalence of prediabetes is markedly increasing in developed and developing countries especially in Africa, and the International Diabetes Federation (IDF) has predicted that the number of prediabetic (PD) individuals is expected to rise from 280 million to about 398 million in 2030 (3). While abnormal glucose metabolism is often associated with overt T2DM, studies have shown that these abnormalities begin in the PD state with a sedentary lifestyle (4). Although prediabetes is a precursor for type 2 diabetes mellitus (T2DM) however complications such as

macrovascular and microvascular complications including diabetes nephropathy are already manifested (5). Hyperglycaemia -induced oxidative stress is a major cause for the development and progression of diabetic microvascular complications such as diabetic nephropathy (DN) (6). DN accounts for about 40% of new cases (7). A higher proportion of individuals with type 2 diabetes are found to have microalbuminuria and overt nephropathy shortly after the diagnosis of their diabetes (8). DN is characterized by an enlargement of the glomerular mesangium, which is instigated by mesengial cell proliferation and excessive accumulation of an extracellular matrix (9). Hyperglycaemia-mediated oxidative stress plays a key role in the advancement of DN and therefore, glycaemic control remains the focal target in delaying the onset and progression of diabetic nephropathy (10). Some studies have demonstrated that cytokines, chemokines, growth factors, adhesion molecules, nuclear factors as well as immune cells such as monocytes, lymphocytes and macrophages are all involved in DM pathogenesis and of course play an important role in DM complications (11). There are several approaches to the management of diabetic nephropathy however, adequate glycaemic control is the most important (12). Dietary intervention is an essential part of the treatment of diabetes mellitus at all stages (13). A combination of pharmacological and dietary interventions are also recommended for the management of DN progression which aim to improve insulin sensitivity (14). Prolonged use of synthetic oral hypoglycaemic agents is associated with adverse effects and toxicity, and can lose their potency over time (15). Traditionally, various plants have been shown to alleviate diabetes-associated complications. Of interest to our study is *Momordica balsamina* (MB) commonly known as “Intshungu”, a plant widespread in Namibia, Botswana, Swaziland and all provinces of South Africa. In our laboratory, medicinal plants including MB have been shown to be beneficial in managing hyperglycaemia and associated complications (16, 17). Extending further, in this study we sought to investigate this medicinal plant on renal function in a diet-induced pre-diabetes rat model.

Materials And Methods

Crude extract extraction

Momordica balsamina (MB) was identified by a botanist at the University of KwaZulu Natal, Westville. The plant was harvested at the University of KwaZulu Natal in Westville. The leaves were washed three times with water to remove any residual dirt.

Extraction Method

Methanolic extract (ME) was extracted from MB leaves by using a standard protocol that has been validated in our laboratory with minor modifications (18). Briefly, the air-dried MB leaves were sequentially extracted twice at 24 h intervals at room temperature using methanol (45 mL) and deionised water (45 mL) on each occasion. The solvent was removed from the crude extract under reduced pressure at 55 ± 1 ºC using rotatory evaporator to yield dichloromethane solubles (DCMS) and ethyl acetate soluble (EAS).

Animals

Thirty-six (36) male Sprague Dawley rats with body weight 150–180g were used for this study. The rats were obtained from the Biomedical Research Unit (BRU), University of KwaZulu-Natal (UKZN). The animals were kept and maintained in a standard animal facility under controlled environmental conditions at room temperature (22 ± 2°C), humidity (55 ± 5%) and 12h day:12h night cycle. The animals consumed standard rat chow (Meadow Feeds, South Africa) and water ad libitum for 2 weeks to acclimatize before being exposed to the experimental diet (high–fat high–carbohydrate). The components of the high–fat high–carbohydrate (HFHC) diet are carbohydrate (55% Kcal/g), fats (30% Kcal/g) and proteins (15% Kcal/g). All experimental procedures in this study were carried out in absolute compliance with the animal ethics and animal guidelines of the Animal Research Ethics Committee (AREC, ethics no: AREC/062/018M) of the UKZN, Durban, South Africa.

Induction Of Prediabetes

After acclimatization, the animals were divided into 2 main groups: the non-prediabetic control group (n = 6) and the prediabetic group (n = 30). The normal control (NC) animals (negative control) were given normal diet (ND) and water ad libitum for 20 weeks while the prediabetic animals were given HFHC diet and drinking water supplemented with fructose (15%) for 20 weeks to induce prediabetes. At 20th week, prediabetes was confirmed via assessment of fasting blood glucose concentration and oral glucose tolerance test (OGTT) as described by (19).

Experimental Protocol

After prediabetes induction, the non-prediabetic control group (Group 1) continuously fed on ND and received diluted dimethyl sulphoxide, DMSO (2 ml DMSO: 19 ml normal saline) as a vehicle for 12 weeks while the prediabetic animals (n = 30) were further divided into 5 groups (n = 6). All the prediabetic animals continuously fed on either HFHC or ND and were treated with either oral administration of MB (250mg/kg) or metformin (MET, 500mg/kg) every third day for 12 weeks. The prediabetes control group, PD (Group 2) rats were continuously fed on the HFHC diet and received the diluted DMSO (vehicle) for 12 weeks. The ND + MET (Group 3) rats changed the diet to ND and received MET orally, whereas the HFHC + MET (Group 4) rats were continuously fed on the HFHC diet and received MET orally. The ND + MB (Group 5) rats changed the diet to ND and received MB orally while HFHC + MB (Group 6) rats continuously fed on the HFHC diet and were treated with MB. The fluid intake and urine volumes were assessed in all the animals at 20th week and every 4 weeks (24th, 28th and 32nd week). These therefore means the animals were place at metabolic cages on the day fluid intake and urinary output measurement. After the 12 weeks of treatment, the animals were sacrificed, blood samples and the kidneys were collected from all the animals for biochemical analysis.

Blood Collection And Tissue Harvesting

All the animals were placed in a gas anaesthetic chamber (Biomedical Research Unit, UKZN, Durban, South Africa) and anaesthetised with 100 mg/kg of Isofor (Safeline Pharmaceuticals (Pty) Ltd, Roodeport, South Africa). In an unconscious state, blood samples were collected from all the animals via cardiac puncture into different pre-cooled EDTA containers. The blood samples were centrifuged (Eppendorf centrifuge 5403, Germany), 503 g for 15 minutes at 4°C to obtain plasma. Thereafter, the plasma samples were aspirated into plain sample bottles and stored in a Bio Ultra freezer (Snijders Scientific, Tilburg, Holland) at -80°C until ready for biochemical analysis. Also, the kidneys were removed, rinsed with cold normal saline solution, weighed on the weighing balance, snapped frozen in liquid nitrogen and stored at -80°C in a Bio Ultra freezer for biochemical analysis of selected parameters.

Biochemical Analysis

The biochemical analysis of kidney function parameters (such as creatinine, urea, uric acid, albumin and total protein) and electrolytes (Na^+ and K^+) were determined at 32nd week in the plasma and urine samples by using their respective assay kits (Elabscience Biotechnology Co., Ltd., Houston, TX, USA) as instructed by the manufacturer. The kidney injury molecule (KIM-1) and plasma aldosterone concentrations were determined using respective specific ELISA kits as instructed by the manufacturer (Elabscience Biotechnology Co., Ltd., Houston, TX, USA) via the microplate reader, Spectrostar Nano spectrophotometer (BMG Labtech, Ortenburg, LGBW, Germany)

Lipid Peroxidation And Antioxidant Status (Dup: Abstract ?)

The lipid peroxidation was assessed by determination of the concentration of malondialdehyde (MDA) in the kidney homogenized tissue according to the previously established protocol (Mkhwanazi et al. 2014). The antioxidant status of the kidney homogenate was assessed by determination of the concentration of superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant capacity (TOAC) by using their specific assay kits according to the instruction of the manufacturer (Elabscience Biotechnology Co., Ltd., Houston, TX, USA).

Urine Rna Isolation

RNA was isolated from urine (4 ml) by using ZR Urine RNA Isolation KitTM (Zymo Research Corp, Irvine, USA) according to the manufacturer’s protocol. The purity of the RNA was confirmed by the relative absorbance of ratio 260/280 nm via Nanodrop 1000 spectrophotometer (Thermo Scientific, USA). Urine RNA (100 ng) was reverse transcribed to complementary DNA (cDNA) by using iScriptTM cDNA Synthesis Kit (Bio Rad, California, USA) through incubation in a thermal cycler (SimpliAmp Thermal Cycler, Applied biosystems, Life technologies).

Urine Complementary Dna (Cdna) Synthesis

For cDNA synthesis, urine RNA (2 µL) was mixed with 5X iScript reaction (4 µL), iScript reverse transcriptase enzyme (1µL) (Bio Rad, USA) and nuclease-free water to a final volume of 20 µL. The mixture was incubated in the thermal cycler (SimpliAmp Thermal Cycler, Applied biosystems, Life technologies) at 25oC for 5 minutes, 42oC for 30 minutes and finally at 85oC for 5 minutes. Thereafter, the synthesized cDNA was stored at -80°C until use for real-time PCR (Polymerase chain reaction).

Real-time Pcr

The urinary mRNA level of podocin was quantified by real-time PCR lightcycler (Roche LightCycler 96, USA). cDNA template (2 µL), SYBR Green PCR master mix (5 µL) (Bio Rad, USA), podocin forward primer (1 µL), podocin reverse primer (1 µL) and nuclease-free water were mixed to a final volume of 10 µL. Thereafter, the sample mixtures were cycled 40 times at 95oC for 10 seconds, 60oC for 20 seconds and 72oC for 20 seconds in the lightcycler (Roche LightCycler 96, USA). All the samples were run in duplicate, and β-actin mRNA levels were used as a housekeeping gene to normalize the podocin mRNA level. The sequences of the used oligonucleotide primers (Metabion International AG, Planegg, Germany) were as followed: podocin forward $5-TGG\forall GCTGAGGCAC\forall AGA-3$, podocin reverse 5 $-AGAATCTCAG\mathbb{C}G\mathbb{C}ATCCT-3$.

Statistical analysis

The data were presented in mean ± SEM and analysed via a two-way Analysis of Variance (ANOVA) with the Bonferroni test as a post hoc test by using GraphPad Prism 5 software. The results were considered to be statistically significant from $p < 0.05$ and above.

Results

Effects of MB on blood glucose.

Figure 1 below shows fasting blood glucose concentrations in the normal control (NC), prediabetic control (PD), diet intervention and metformin (DI + MET), high fat-high carbohydrate and metformin (HFHC + MET), diet intervention and *Momordica balsamina* (DI + MB) and high fat-high carbohydrate and *Momordica balsamina* (HFHC + MB) groups after 12 weeks of treatment. By comparison to NC, there was a significant increase in fasting blood glucose concentrations in the PD group ($p < 0.05$, Fig. 2). The administration of the medicinal plant, MB in both the HFHC and diet intervention groups resulted in significant reduction in fasting blood glucose concentration by comparison to PD ($p < 0.05$, Fig. 2). A similar observation was shown by the DI + MET treatment group as compared the PD group ($p < 0.05$, Fig. 2).

Fluid Intake And Urine Output

Figure 2 shows fluid and urine output in non-prediabetic control (NC) prediabetic control (PD), MB and high fat high carbohydrate (HFHC + MB) and MB and diet intervention (DI + MB) metformin and high fat high carbohydrate (MET + HFHC), metformin and diet intervention (MET + DI) measured every 4th week for 12 weeks. The fluid intake and urine output of PD rats were significantly increased in comparison to the normal control rats (NC) throughout the treatment period (0–12 weeks) ($p < 0.05$). However, the administration of MB with or diet intervention significantly decreased the fluid intake and urine output in comparison to PD ($p < 0.05$). A similar observation was shown by the DI + MET treatment group as compared the PD group.

Creatinine

Figure 3a, b show urinary and plasma creatinine concentrations in the normal control (NC), prediabetic control (PD), diet intervention and metformin (DI + MET), high-fat/high-carbohydrate diet and metformin (HFHC + MET), diet intervention and MB (MB + DI + MB) and high-fat/high-carbohydrate (HFHC + MB) groups after 12 weeks of treatment. For plasma creatinine, in comparison with the NC group, the PD group showed a significant increase in plasma creatinine concentration ($p < 0.05$) (Fig. 3a). The administration of MB compound in both the diet intervention and high-fat/high-carbohydrate treatments resulted in a significant decrease in plasma creatinine concentration when compared with the PD group ($p < 0.05$). For urinary creatinine, in comparison with the NC group, the PD group showed a significant decrease in urinary albumin concentration ($p < 0.05$) (Fig. 3b). The administration of MB in both the diet intervention and high-fat/high-carbohydrate treatments resulted in a significant increase in urinary creatinine concentration when compared with the PD group ($p < 0.05$). A similar effect was observed with the MET + DI-treated group when compared with the PD group ($p < 0.05$). See Fig. 3a,b.

Electrolytes Concentration (Sodium And Potassium)

Figure 4a,b show plasma and urinary potassium (K^+) in the normal control (NC), prediabetic control (PD), metformin and diet intervention (MET + DI), metformin and high-fat/high-carbohydrate diet (MET + HFHC), MB and diet intervention (Re + DI) and MB and high-fat/high-carbohydrate diet (Re + HFHC) groups after 12 weeks of treatment. In Fig. 4A, there is a significant increase in urinary k^+ concentrations in the PD group when compared to the NC group ($p < 0.05$). In contrast, after 12 weeks of treatment with MB, there was a significant decrease in urinary K^+ concentration when compared with the PD group. In Fig. 4B, there was a significant decrease in plasma K^+ concentration in the PD group when compared to the NC group ($p < 0.05$ Fig. 4B). In contrast, after 12 weeks of treatment with the MB, there was a significant increase in plasma K^+ concentration in the MB-treated groups when compared with the PD group ($p < 0.05$). A similar effect was observed with the MET + DI-treated group when compared with the PD group. See Fig. 4A,

Total Protein

Figure 5 below shows plasma total protein concentrations of PD rats were significantly decreased by comparison to the NC control rats ($p < 0.05$). The administration of metformin with diet intervention as well as BA with or without dietary intervention significantly increased the plasma total protein concentrations when compared to the PD rats. As shown in Fig. 5, total protein concentrations were significantly increased in PD rats when compared to the NC control rats ($p < 0.05$). In comparison to the PD rats, the MB and metformin-treated rats with diet intervention had a significantly decreased urinary total protein while the MB treated rats without diet intervention only had a significantly decreased total protein.

Lipid Peroxidation And Antioxidant Status

Diet-induced pre-diabetes led to a significant increase in renal MDA level whilst decreasing renal SOD and GPx levels as observed in the PD group relative to that of the normal control (NC) control group ($p < 0.05$; **Table 1**). Treatment with MB considerably decreased MDA levels and increased renal concentrations of SOD and GPx when compared to the PD group ($p < 0.05$; **Table 1**). The HFHC + MET group showed significant increased MDA levels when compared with the normal control (NC) group ($p < 0.05$; **Table 1**). However, treatment with metformin significantly decreased SOD and GPx levels when compared with the PD group ($p < 0.05$; **Table 1**).

Impacts of MB on oxidative stress and antioxidants concentrations of pre-diabetic animals for a treatment period of 12 weeks. Values are presented as means ± SEM (n = 6) in each group.

Groups	MDA A (nmol/ g kidney tissue)	GPx (ng/mL)	SOD (ng/mL)
NC	1.89 ± 0.022	84.92 ± 0.023	1833 ± 0.021
PC	1.31 ± 0.079*	66.43 ± 0.032*	1613.23 ± 0.032*
DI + MET	1.33 ± 0.037α	82.70 ± 0.043α	1814.24 ± 0.010α
HFHC + MET	2.01 ± 0.024*	81.65 ± 0.020α	1889.65 ± 0.019α
DI + MB	1.34 ± 0.025α	72.98 ± 0.021α	1807.16 ± 0.048α
HFHC + MB	1.59 ± 0.018α	70.40 ± 0.02α	1621.74 ± 0.010α
* $p < 0.05$ compared to normal control (NC), α $p < 0.05$ compared to pre-diabetic control group (PD)			

KIM-1

Figure 6 shows the KIM-1 concentration in the normal control (NC), prediabetic control (PD), diet intervention and metformin (DI + MET), high-fat/high-carbohydrate diet and metformin (HFHC + MET), MB and diet intervention and MB (DI + MB) and MB and high-fat/high-carbohydrate diet + MB (HFHC +

MB) groups after 12 weeks of treatment. In comparison with the NC group, the PD group showed a significant increase in KIM-1 concentration ($p < 0.05$) (Fig. 6). The administration of MB in both the diet intervention and high-fat/high-carbohydrate treatments resulted in a significant decrease in KIM-1 concentration when compared with the PD group ($p < 0.05$).

Plasma Aldosterone

Figure 7 shows aldosterone concentration in the normal control (NC), prediabetic control (PD), diet intervention and metformin (DI + MET), high-fat/high-carbohydrate diet and metformin (HFHC + MET), diet intervention and MB (DI + MB) and high-fat/high-carbohydrate diet and MB (HFHC + MB) groups after 12 weeks of treatment. In comparison with the NC group, the PD group showed a significant increase in aldosterone concentration ($p < 0.05$) (Fig. 7). The administration of MB in both the diet intervention and high-fat/high-carbohydrate treatments resulted in a significant decrease in aldosterone concentration when compared with the PD group ($p < 0.05$). A similar effect was observed with the DI + MET-treated group when compared with the PD group ($p < 0.05$). See Fig. 7.

Urine Podocin Mrna

Figure 8 shows the levels of the mRNA expression of urinary podocin in the normal control (NC), prediabetic control (PD), diet intervention and metformin (DI + MET), high-fat/high-carbohydrate diet and metformin (HFHC + MET), diet intervention and MB (DI + MB) and high-fat/high-carbohydrate diet and MB (HFHC + MB) groups after 12 weeks of treatment. In comparison with the NC group, the PD group was shown to have high levels of podocin mRNA expression ($p < 0.05$) (Fig. 8). The administration of MB in both the diet intervention and high-fat/high-carbohydrate treatments resulted in significantly reduced levels of podocin mRNA when compared with the PD group ($p < 0.05$). A similar effect was observed with the MET + DI-treated group when compared with the PD group ($p < 0.05$). See Fig. 8

Discussion

Prediabetes is condition characterized by intermediate hyperglycaemia, proteinuria, increased glomerular filtration rate as well as electrolyte imbalance which are all risk factors of chronic kidney disease (CKD) (20). Prediabetes is managed by the combination of pharmacotherapy and lifestyle modifications such as dietary intervention (21). Previous studies have reported on the hypoglycaemic, renoprotective and hepatoprotective properties of MB in Streptozotocin induced diabetic rats (22). However, the effects of this medicinal plant on the risk factors associated with the development of DN have not yet been investigated in prediabetes. Hence, this study sought to investigate the effects of MB on the selected risk factors of DN in both the absence and presence of dietary intervention in a diet-induced prediabetic rat model. In agreement with previous reports, the use of MB crude extract in HFHC induced prediabetic rat model, significantly attenuated blood glucose concentration in this study.

Sustained hyperglycaemia has been shown to activate the intrarenal renin aldosterone system which is thought to increase Na^+ retention at various sites of the kidney such as distal tubule and collecting duct of the nephron (23).In the current study, decreased urinary and increased plasma sodium levels were observed in the prediabetic rats. However, the administration of MB restored both urinary and plasma sodium concentration in prediabetic rats. The significant increase in the excretion of sodium in the MB treated group may suggest the inhibition of increased proximal reabsorption of sodium which is often seen in diabetes, thus increasing the sodium-potassium pump activity (24). The metformin treated rats showed similar effects; however, this was only when used in conjunction with the normal diet. Kidneys maintain the optimum chemical composition of body fluids by removing Na^+, K^+, urea, and creatinine (25). In patients with T2DM, the insulin-mediated uptake of glucose is impaired, but the cellular uptake of potassium remains normal (26). Hyperkalaemia can be caused by an increase in plasma tonicity that results from the redistribution of potassium from the intracellular space to the extracellular space (27). The efflux of potassium from the cell is thought to be due to intracellular dehydration, which results from the osmotically-induced transcellular movement of water (28). Potassium and angiotensin II are the major regulators of aldosterone secretion (29). Interestingly, the prediabetic rats showed decreased urinary potassium levels when compared to the normal control group. On the contrary, the administration of the MB resulted in an increased urinary potassium level in the prediabetic rats. It has been illustrated in many *in-vitro* investigations that renal injuries are related with the accumulation of reactive oxygen species (ROS) under hyperglycemic conditions (30). In prediabetes, intermediate hyperglycaemia can simply inactivate antioxidant enzymes such as SOD and GPx by glycating these proteins and inducing oxidative stress which in turn causes lipid peroxidation (31).

In the enzymatic antioxidant defence system, SOD is one of the important enzymes which scavenge the superoxide radicals by converting them to H_2O_2 and molecular oxygen (32). The observed decrease in SOD activity in diabetic control rats could result from inactivation by H_2O_2 or by glycosylation of the enzyme, which has been reported to occur in diabetes. The decreased activity of SOD and GPx in the prediabetic state may be due to inactivation caused by reactive oxygen species. Additionally, the SKN-1/Nrf are pathway which is responsible for the production of antioxidant has been shown to be down-regulated in diabetes (33). The results herein showed that elevated concentrations of MDA, a marker of lipid peroxidation, were restored after treatment with MB. MB is used traditionally as a medicinal plant, and the leaves, seeds, fruits are consumed as food, which has been reported to contain antioxidants, as it contains triterpenes, flavonoids, saponins and carotenoids (34). Kidney injury is associated with an increase in urinary KIM-1. KIM-1 a transmembrane protein serves as a useful biomarker for renal proximal tubule injury facilitating the early diagnosis of the disease (35). Renal tubular damage, as evidenced by increased levels of KIM-1, is evident even prior to the development of pre-diabetes and overt kidney disease (35). The plasma KIM-1 concentration were found significantly increased in pre-diabetic animals in comparison with non-prediabetic animals, demonstrating the existence of tubular damage at the early stage of DN. The administration of MB resulted in reduced plasma KIM-1 concentration in pre-diabetic treated animals when compared to pre-diabetic animals. Notably, MB coupled with dietary intervention showed a significant decreased in plasma KIM-1 concentration to within the non-prediabetic KIM-1 levels.

The results obtained suggest that the use MB is effective in alleviation renal injury in pre-diabetic animals. This may also be due to its effects as a glucose-lowering abilities as reported in the streptozotocin model for type 1 diabetes, which encompasses kidney dysfunction ameliorative effects by attenuating DN, associated with its ability to improve, lipid metabolism, antioxidative and anti-inflammatory functions.

Hyperglycaemia increases renal angiotensin II (Ang II), which induces oxidative stress, endothelial damage and disease pathology including vasoconstriction, thrombosis, inflammation and vascular remodeling (36). This imbalance can predispose the individual to tissue damage (36). Hyperglycaemia also increases the production of aldosterone, which has an unknown contribution to tissue damage (37). Evidently in this study there was an increase in aldosterone in the prediabetic rats. However, the administration of MB-treated group showed a significant decrease in aldosterone concentration. This suggests that MB normalizes oxidase activity and improves endothelial function (38). Podocytes, which are one of the main cell types that constitute the glomerular filtration barrier, are injured in diabetes, resulting in proteinuria and DN (39). The proteinuria is also associated with tubular atrophy, as studies have shown that the presence of albumin in kidney tubules induces inflammations, subsequently cell death (40). Podocin connects nephrin and CD2AP via its C-terminus to form a “zipper-type” filtration barrier and to maintain the normal structures and functions of the glomerular filtration membrane (41). Nephrin is the earliest discovered protein that is specifically located in the podocyte slit membrane area (41). Previous studies have shown that the expression of nephrin and podocin was higher in diabetic rats than that in normal controls, which is shown by an increase in podocin in prediabetic rats. However, the administration of MB significantly lowered podocin. This may be attributed to the significant improvement in renal antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) that was observed in MB-treated groups. Also considering that podocytes are insulin-sensitive cells, it can also be argued that their survival could be associated with increase in insulin sensitivity as result of MB administration. However, further studies are required gain clear insights.

Conclusion

High Fat High Carbohydrate induced kidney dysfunction and injury as displayed by abnormal plasma and urinary electrolyte, increased urinary KIM-1 and podocin. The oral treatment of MB to prediabetic rats improved glycaemia with concomitant attenuation of renal injury and dysfunction reinstated renal. Taken together, MB administration demonstrate benefits in attenuating kidney function aberrations associated with prediabetes.

Declarations

Author Contributions

Conceptualisation, B., P.S.N. and N, A.K.; methodology, B., A.S., A.M.A. software, B.; validation, B., P.S.N. and N.H.S.; formal analysis, B. and P.S.; investigation, B; resources, A.K., P.N. and.; data curation, B; writing

—original draft preparation, B; writing—review and editing, P.S.N, N.H.S. and A.K; visualisation, B; supervision, P.S.N. and A.K.; project administration A.K.; P.S.N; funding acquisition, P.S.N. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The animal study protocol was approved by the Institutional Animal Research Ethics Committee of the University of KwaZulu-Natal UKZN AREC reference number: AREC/062/018M).

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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Figures

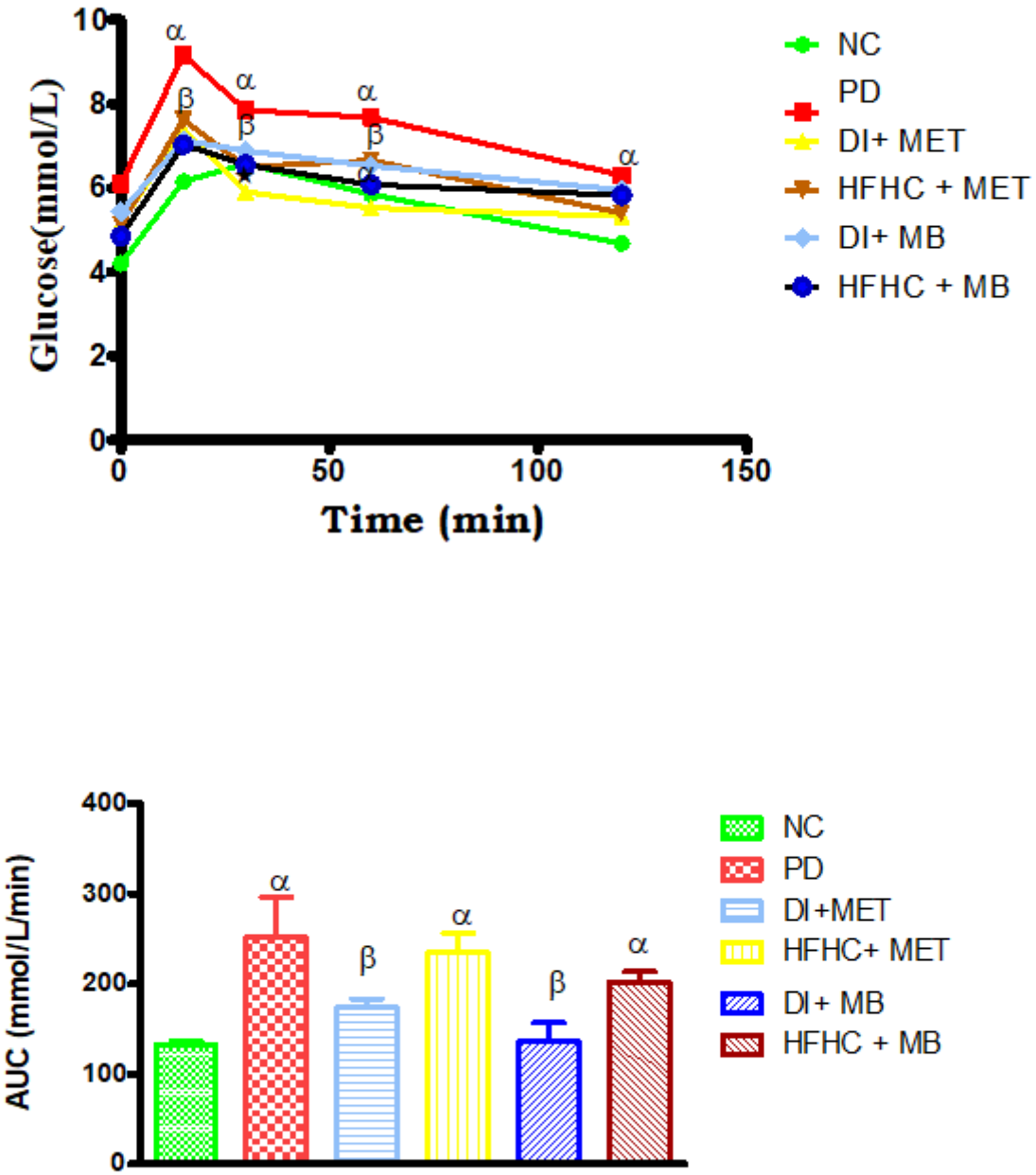


Figure 1

The effects of MB and AUC on blood glucose. Values are presented as means ± SEM (n = 6) in each group. α $p < 0.05$ compared to normal control (NC), β $p < 0.05$ compared to pre-diabetic control (PD).

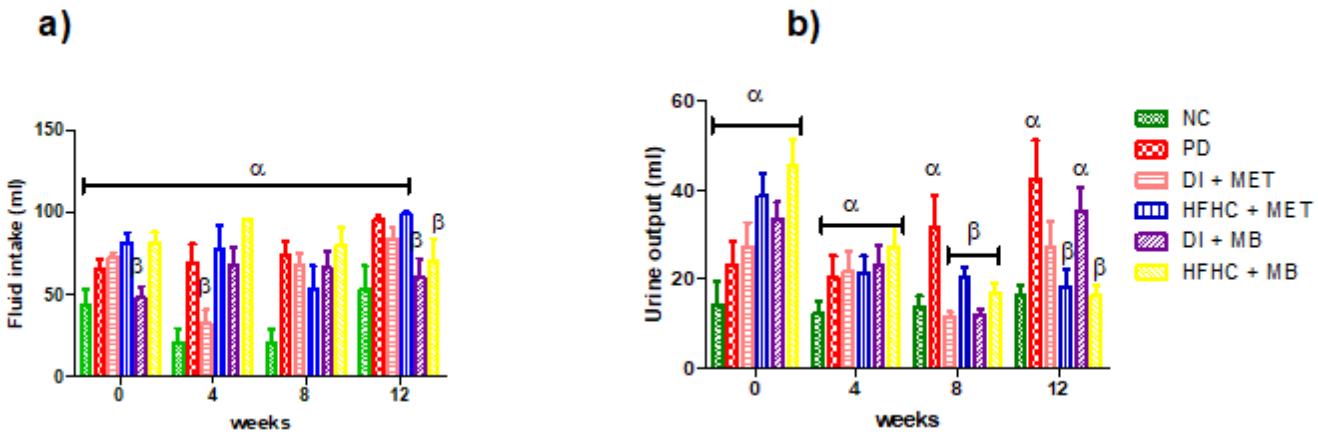


Figure 2

Effects of MB on fluid intake (a) and urine output (b) in non-prediabetic and prediabetic rats with or without diet intervention. α p<0.05 (vs. NC), β p<0.05 (vs. PD).

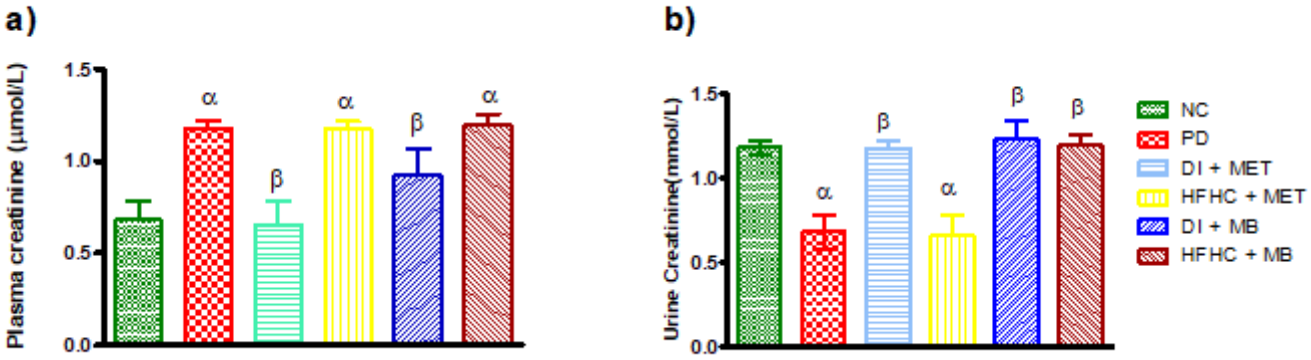


Figure 3

Effects of MB on plasma creatinine (a), urine creatinine (b) in non-prediabetic and prediabetic rats with or without diet intervention. α p<0.05 (vs. NC), β p<0.05 (vs. PD)

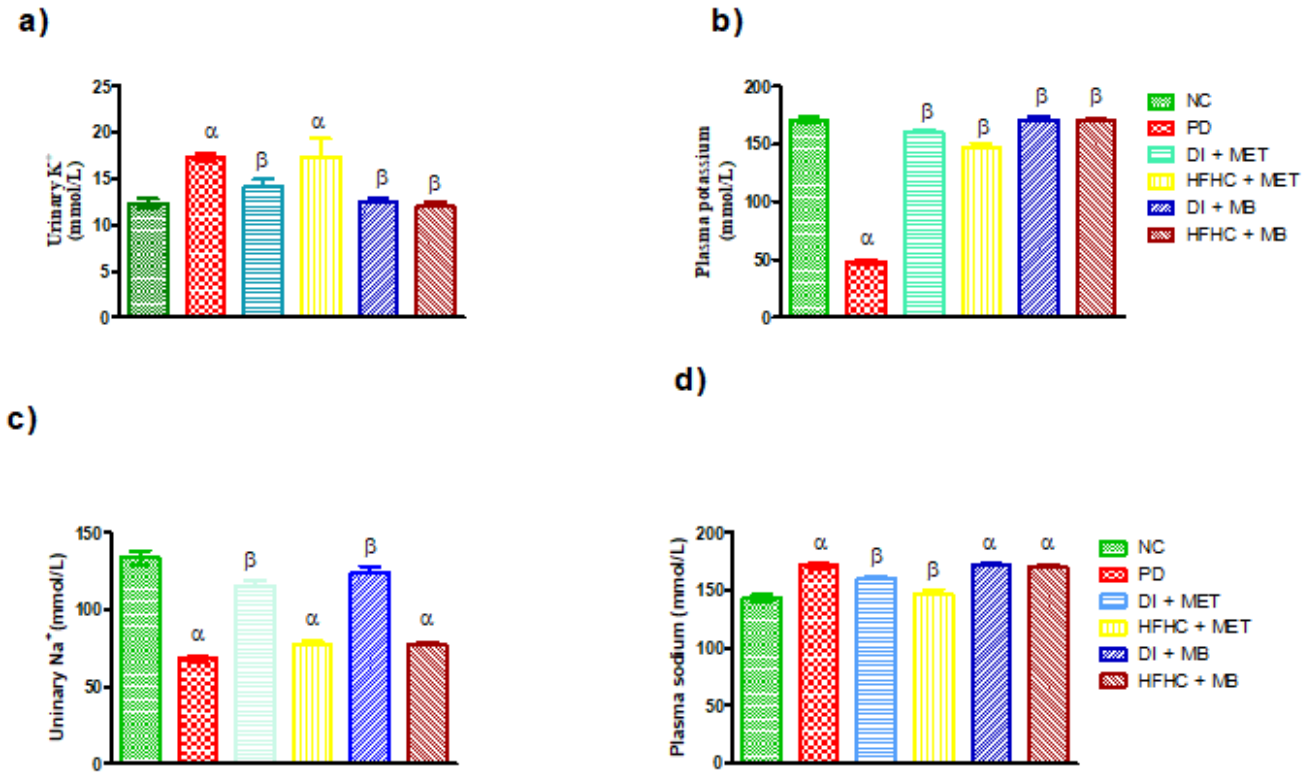


Figure 4

Effects of MB on urinary Potassium(a), plasma Potassium(b), urinary Sodium(c) and plasma Sodium (d) in non-prediabetic and prediabetic rats with or without diet intervention. α $p<0.05$ (vs. NC), β $p<0.05$ (vs PD).

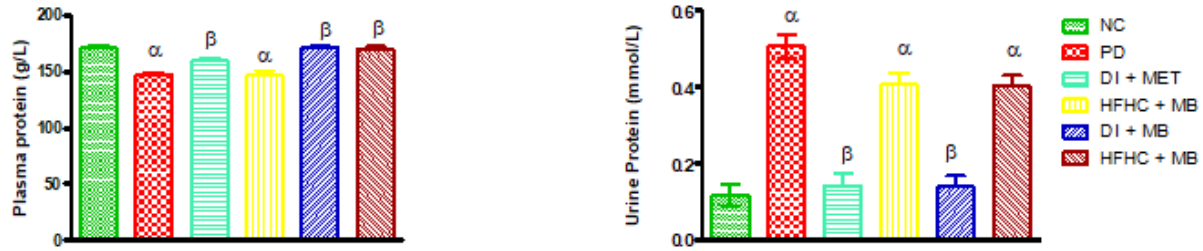


Figure 5

Effects of MB on a) plasma total protein (b) and urine total protein in normal control and prediabetic rats with or without diet intervention. α p<0.005 (vs. NC), β p<0.05 (vs. PD).

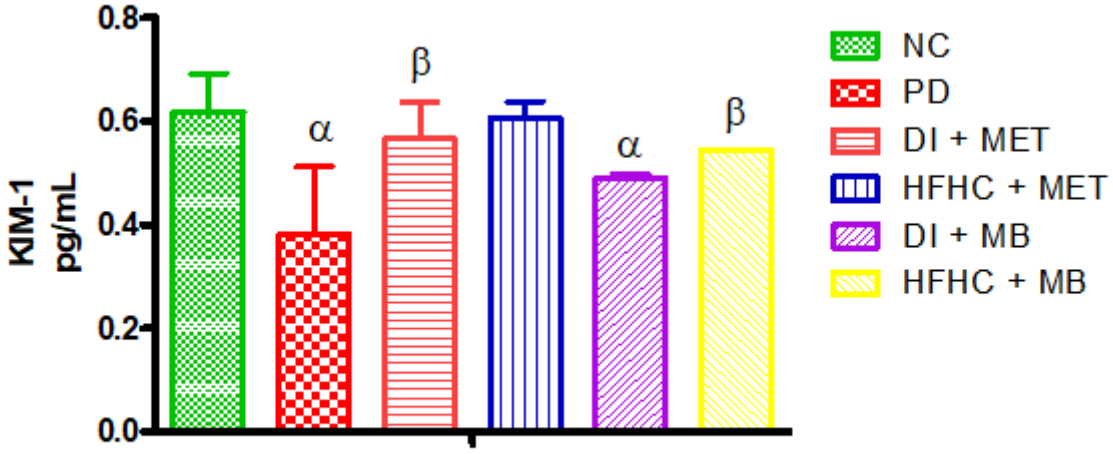


Figure 6

The effects of MB on KIM-1 concentration of pre-diabetic animals during the treatment period. Values are presented as means ± SEM (n = 6) in each group. α p < 0.05 compared to the non-pre-diabetic control (NC), β p < 0.05 compared to the pre-diabetic control (PD).

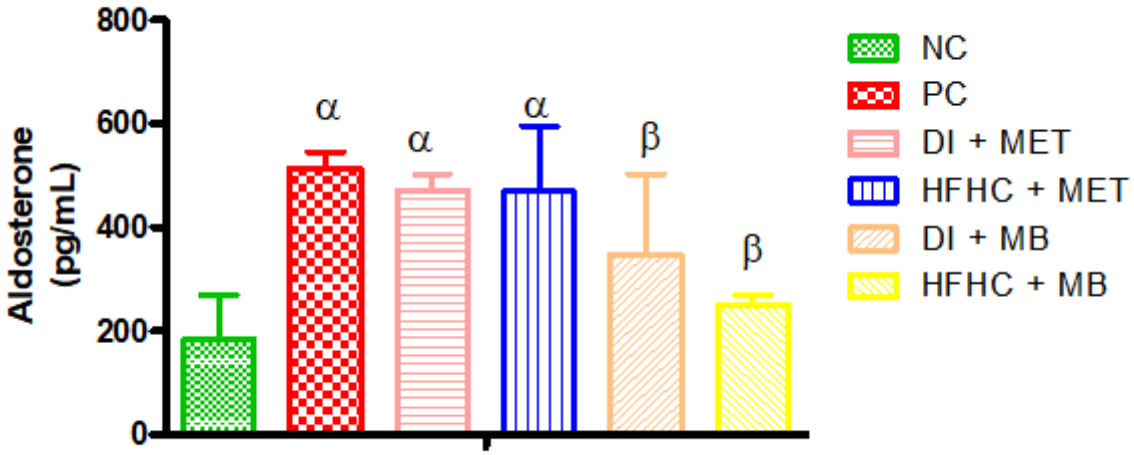


Figure 7

The effects of MB on aldosterone concentration of pre-diabetic animals during the treatment period. Values are presented as means ± SEM (n = 6) in each group. α p < 0.05 compared to non-prediabetic (NC), β p < 0.05 compared to pre-diabetic control group (PD).

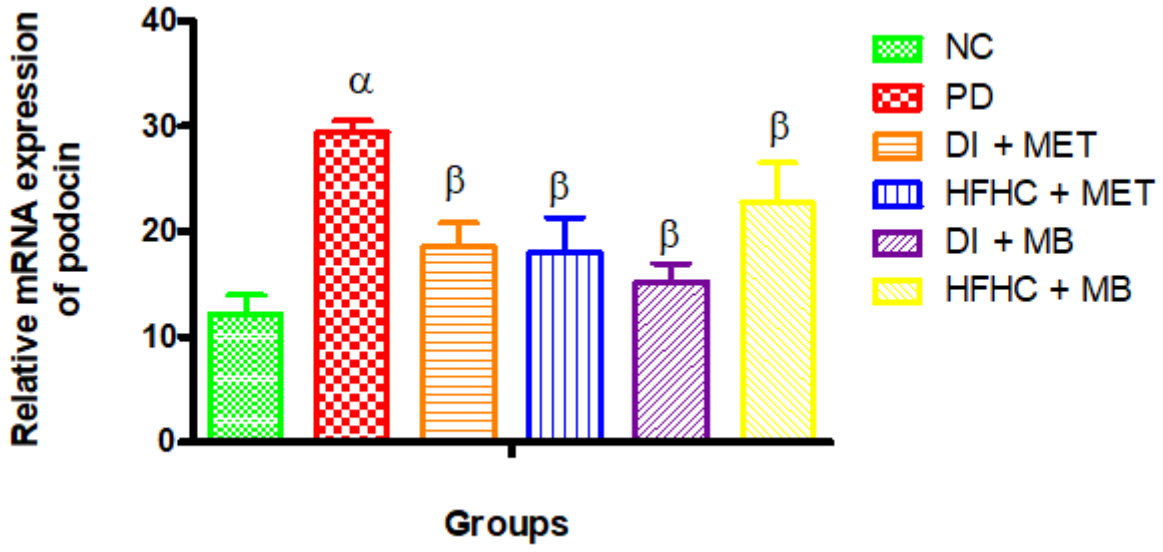


Figure 8

The effects of MB on relative mRNA expression of urinary podocin levels of pre-diabetic animals after the treatment period. Values are presented as means ± SEM (n = 6) in each group. α $p < 0.05$ compared to the non-prediabetic control (NC), β $p < 0.05$ compared to the pre-diabetic control (PD).