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Murraya paniculata (L.) (Orange Jasmine): Potential Nutraceuticals with Ameliorative Effect in Alloxan-Induced Diabetic Rats

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Orange jasmine, *Murraya paniculata* (Rutaceae), is a plant from India widely used in folk medicine as antinociceptive, antiinflammatory, and antioxidant. Although oral hypoglycemic agents and insulin are the mainstays of treatment of diabetes mellitus (DM), there is a significant demand for new natural products to reduce the development of diabetic complications. Alloxan-induced diabetic rats were treated for 60 days with a hydroalcoholic extract of *M. paniculata* (MPE), at doses of 100, 200, and 400 mg/kg. MPE decreased glycemia and also cholesterol and triglyceride levels, starting 1 week after treatments, as compared with the same group before treatments. Glucose values were reduced toward normality after 1 week of treatment. MPE hypoglycemic effects were potentiated by glibenclamide and metformin. MPE also decreased fructosamine and glycated hemoglobin values. MPE reduced diabetes-induced morphological alterations of the kidney, pancreas, and liver. MPE acts similarly to glibenclamide and metformin, and its glucose-lowering action is partly a consequence of ATP-sensitive K^+ channel inhibition. MPE may be a potential therapeutic alternative for the treatment of diabetes and its complications. Copyright © 2017 John Wiley & Sons, Ltd.

Keywords: Murraya paniculata; diazoxid; alloxan; hyperglycemia; mechanism of actions; diabetes.

Abbreviations: DZD, Diazoxide; GLI, Glibenclamide; HPLC-DAD, High-performance liquid chromatography with diode array detector; KATP, ATP-sensitive potassium channels; LOD, Limit of detection; LOQ, Limit of quantification; MET, Metformin; MPE, *Murraya paniculata* L. extract; TC, Total cholesterol; TG, Triglycerides; WHO, World Health Organization

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder resulting from insulin deficiency, characterized by hyperglycemia, altered metabolism of carbohydrates, protein, and lipids, and an increased risk of vascular complication. Mortality due to diabetes is more than 80% in the low-income and middle-income countries (Mathers and Loncar, 2006). WHO predicts that diabetes will be the seventh most important cause of mortality in 2030 (Richards et al., 2016), and in 2035, near to 592 million people will die due to diabetes (Tao et al., 2015).

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The complications of diabetes greatly influence the quality of life in patients suffering from diabetes. According to the recent estimates of the International Diabetes Federation (2015), 382 million patients suffer from it globally (Zabetian et al., 2013) and will rise to 5.4% of the population in 2025 (King et al., 1998). Poorly managed diabetes can lead to a large number of complications including retinopathy, neuropathy, nephropathy, cardiovascular diseases, and male impotency (Epstein et al., 1994; Melendez-Ramirez et al., 2010).

The levels of glucose, insulin, fructosamine, and HbA1c are important *in vitro* markers for the diagnosis of metabolic disorders like diabetes. Fructosamine is increased in patients with diabetes mellitus due to the formation of unstable and reversible aldimines that are later rearranged to irreversible and stable ketoamines in the presence of persistent hyperglycemia and has been used as an auxiliary parameter for glycemic control and is related to clinical outcomes and mortality (Armbruster, 1987). The level of glycated hemoglobin

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(HbA1c) is another indicator for long-term glycemic average of serum (Lapolla et al., 2005). Both parameters are complementary and present wide clinical importance in the monitoring of diabetes (Ayyıldız et al., 2015).

Natural antioxidants from plants will alleviate these damages and may be an effective, safe, and economical alternative therapy for diabetes management and organ protection. The use of plants in the management of diabetes is well documented, which is primarily due to antihyperglycemic and/or oxygen radical scavenging of their various phytoconstituents through various mechanisms (Chan et al., 2012). Plant extracts rich in polyphenols, flavonoids, stilbenes, lignans, and other phytochemicals have been used as neutraceuticals and in the management of metabolic disorders, as diabetes mellitus, and its complications (Bahadoran et al., 2013). These products have been used as complementary or alternative medicines, but efforts have been made to identify antidiabetic molecules from natural sources (Fuentes et al., 2016).

Murraya paniculata (L.) Jack belongs to the family Rutaceae. It is a small, tropical, evergreen shrub, bearing small, white, scented flowers. *M. paniculata* is often grown in southern China (Hainan, Guangdong, Fujian, and Guangxi), India, Thailand, Malaysia, and Australia. Traditional knowledge on medicinal use of *M. paniculate* was related by parts of the plant. However, medicinal use of leaves in decoction or infusion is the most common use, but other parts of the plant have been used traditionally as the bark and the roots. In China, *M. paniculata* was used to regulate fertility (Black, 2015), antinociceptive effect (Sharker et al., 2009), as potential therapeutic applications for treatment of toothache (Rahmatullah, 2014) and analgesic activity (Fazal-ur-Rehman et al., 2014) and antimicrobial activity (Menezes et al., 2015). These activities correlated positively with the presence of secondary compound classes. It would be better to group the list of compound class, for example, flavones (myrecetin, gardenin, umhengerin), coumarins (murralongin, isomurralonginol, coumurrayin, toddalenone, auraptene, toddasin), and a bisindole alkaloid named yuehchukene (Wu, 1988; Zhang et al., 2011; Zou et al., 2014). Several flavones called gardenin (i.e. gardenins A, C, and E) have been identified in *M. paniculata* (Kinoshita and Firman, 1996).

M. paniculata (L.) Jack was introduced in Brazil for ornamental purposes for many years being commonly known as orange jasmine. A chemical study of this plant collected in São Paulo, Brazil, showed that it had a different chemical profile, when compared with studies using plant material collected in Asian countries. It is reported to have antinociceptive and antiinflammatory (Narkhede et al., 2012; Podder et al., 2011; Sharker et al., 2009), antidiarrheal (Rahman et al., 2010), and hypoglycemic effects (Gautam et al., 2012a, 2012b).

Hence, the present study was carried out to elucidate the mechanism of blood glucose-lowering action of hydroalcoholic extract from leaves of *M. paniculate* and also performed to evaluate the effect on blood biochemical parameters after 2 months of treatment.

MATERIALS AND METHODS

Botanical material. The leaves of *M. paniculate* (L.) Jack were collected in Crato city in July 2013 in Ceara state, Brazil. The botanical material was identified by Dr Arlene Maria Pessoa da Silva, and a voucher specimen of the material was deposited in the Herbarium Caririense Dárdano de Andrade Lima of the Regional University of Cariri under the number 4301.

Preparation of hydroalcoholic extract. The leaves of *M. paniculata* were submerged in water-ethanol (1:1 *v*/*v*) for 72 h. The extract was then filtered and concentrated using a vacuum rotary evaporator unit (Fisaton) and water bath (214M2 Model Quimis to 60°C). Finally, the crude extract was lyophilized and used for experimental procedures.

Phytochemical analysis. Chemical, apparatus, and general procedures: All chemicals were of analytical grade. Acetonitrile, phosphoric acid, gallic acid, ellagic acid, chlorogenic acid, and caffeic acid were purchased from Merck (Darmstadt, Germany). Catechin, epicatechin, rutin, quercetin, quercitrin, kaempferol, and luteolin were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High-performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector, and LC solution 1.22 SP1 software.

Quantification of compounds by HPLC-DAD: *M. paniculata* hydroethanolic extract at a concentration of 12 mg/mL was injected by means of a model SIL-20A Shimadzu Auto Sampler. Separations were carried out using Phenomenex C_{18} column (4.6 mm × 250 mm × 5 μm particle size). The mobile phase was water with 1% phosphoric acid (*v*/*v*) (solvent A) and HPLC-grade acetonitrile (solvent B) at a flow rate of 0.7 mL/min and injection volume 40 μL. The composition gradient was 5% solvent B reaching 15% at 10 min, 30% solvent B at 25 min, 65% solvent B at 40 min, and 98% solvent B at 45 min. At 50 min, the gradient reached the initial conditions again, following the method described by Menezes et al. (2015) with slight modifications. The sample and mobile phase were filtered through a 0.45-μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standard references were prepared in the HPLC mobile phase at a concentration range of 0.030–0.500 mg/mL. Quantifications were carried out by integration of the peaks using the external standard method, at 254 nm for gallic acid; 280 nm for catechin and epicatechin; 327 for caffeic acid, chlorogenic acid, and ellagic acid; and 356 nm rutin, quercitrin, quercetin, kaempferol, and luteolin. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). All chromatography operations were carried out at ambient temperature and in triplicate. The limits of detection (LODs) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves.

LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve (Boligon et al., 2015).

Drugs. The alloxan and diazoxide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Glibenclamide and metformin were acquired from Biosynthetic Laboratory (São Paulo, SP, Brazil).

Animals. Male Wistar rats (180–250 g) from the Animal House of the Faculty of Medicine of Juazeiro do Norte were maintained under standard environmental conditions (22 ± 1°C, humidity 60 ± 5%, 12-h light/12-h dark cycle) with free access to a commercial diet and water *ad libitum*. The experiments were performed according to the Guide for the Care and Use of Laboratory Animals from the US Department of Health and Human Services and approved by the Institutional Ethics Committee for Animal Experimentation of the Regional University of Cariri, Brazil.

Experimental protocol. The animals were divided into groups of 10–12 rats each. For the induction of diabetes, the animals fasted for at least 12 h and afterwards received alloxan (45 mg/kg) through the penile vein. After 48 h, the diabetic state of the groups was assessed by measurements of serum glucose levels, and the animals presenting glucose levels lower than 250 mg/dL were rejected. The animals were divided into six groups of 10 animals which were treated by gavage with *M. paniculata* extract 100 mg/kg (MPE 100), *M. paniculata* extract 200 mg/kg (MPE 200), *M. paniculata* extract 400 mg/kg (MPE 400), glibenclamide 5 mg/kg (GLI 5), metformin 50 mg/kg (MET 50), and water.

The animals were treated orally for 2 months, starting 48 h after the alloxan-induced diabetes. Blood samples were collected prior to treatment (48 h after alloxan, B = before treatment) and 1 week, 2 weeks, 1 month, and 2 months after treatments (A = after treatment) for the determination of glucose, triglycerides, and total cholesterol. The fructosamine analysis was carried out after 1 month of treatment, while the glycated hemoglobin was performed after 2 months of treatment; at the end of this period, the liver, kidney, and pancreas were removed for performing histopathological analysis.

Determination of biochemical parameters in the rat serum. Blood from the retro-orbital plexus was collected and centrifuged at 3000 rpm for 10 min, and glucose levels were determined by the glucose oxidase–peroxidase enzymatic method (Labtest®, Brazil). Concentrations of serum total cholesterol and triglycerides were measured by standard enzymatic colorimetric methods. Fructosamine and glycated hemoglobin levels were also determined by standard procedures, according to the manufacturer's instructions (Labtest®, Brazil).

Potentiation of the effect of M. paniculata extract. The animals were divided into six groups of six animals, as follows: *M. paniculata* extract (MPE) (10 mg/kg), glibenclamide (GLI) (2 mg/kg), and metformin (MET) (5 mg/kg), a control group (water), and two groups treated with the drugs in combination (MPE 10 mg/kg + GLI 2 mg/kg and MPE 10 mg/kg + MET 5 mg/kg); all animals were treated for 1 week. One hour after the last administration, the animals were weighed; a blood drop arising from the tail of the animal was withdrawn and analyzed with regard to biochemical parameter of glucose in a glucose meter Accu-CHEK® Active type.

Evaluation of M. paniculata extract hypoglycemic activity on diazoxide-induced hyperglycemia in rats. The assays were performed as described by Leahy et al. (1994) and Zhao et al. (2005). Groups of six rats were divided into three groups (normal control (NC), *M. paniculata* extract 100 mg/kg (MPE 100), and glibenclamide 5 mg/kg (GLI 5); after 1 h treated, diazoxide (DZD) 125 mg/kg was administered intraperitoneally. Tail blood was obtained and analyzed regarding biochemical parameter of glucose in a glucose monitor ACCU-type CHEK® Active in time zero (before application of DZD), 60, 120, and 180 min after application.

Histological analyses of untreated and M. paniculata extract-treated diabetic rats. The liver, kidney, and pancreas were excised from (untreated diabetic and diabetic treated for 60 days with MPE, GLI, and MET) rat groups, and after the animal decapitation were fixed in 10% buffered formalin. Paraffin blocks were made for the preparation of 5-μ sections cut with a microtome, and of routine microscopic slices. HE staining performed in all slices were examined afterwards.

Statistical analysis. Statistical analysis was performed using Prism™ v6.0 software (GraphPad Software®, San-Diego, CA, USA), and the results were presented as mean ± SEM and analyzed by ANOVA followed by the Tukey test as a *post hoc* test. When necessary, the non-parametric Kruskal–Wallis test was used.

RESULTS

HPLC fingerprinting of *M. paniculata* hydroethanolic revealed the presence of the gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak

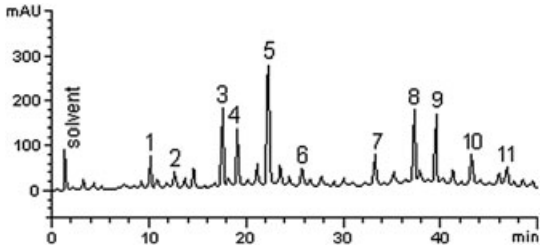

Figure 1. Representative high-performance liquid chromatography profile of *Murraya paniculata*. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), quercetin (peak 9), kaempferol (peak 10), and luteolin (peak 11).

Table 1. Composition of *Murraya paniculata*

Compounds	*M. paniculata* (mg/g)	LOD (μg/mL)	LOQ (μg/mL)
Gallic acid	5.18 ± 0.03 a	0.023	0.076
Catechin	2.05 ± 0.01 b	0.011	0.036
Chlorogenic acid	9.93 ± 0.01 c	0.018	0.059
Caffeic acid	8.06 ± 0.04 d	0.009	0.030
Ellagic acid	14.11 ± 0.02 e	0.020	0.066
Epicatechin	1.98 ± 0.01 b	0.015	0.049
Rutin	5.07 ± 0.03 a	0.007	0.023
Quercitrin	9.83 ± 0.01 c	0.026	0.085
Quercetin	9.46 ± 0.01 c	0.013	0.043
Kaempferol	5.09 ± 0.02 a	0.008	0.026
Luteolin	1.97 ± 0.03 b	0.019	0.063

Results are expressed as mean ± standard deviation (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$.
LOD, limit of detection; LOQ, limit of quantification.

4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), quercetin (peak 9), kaempferol (peak 10), and luteolin (peak 11) (Fig. 1 and Table 1).

The 1-week treatment of diabetic rats with MPE (100, 200, and 400 mg/kg) decreased blood glucose levels by, respectively, 66, 57, and 63% blood glucose, as related to the same group before treatments (Table 2). While the treatment of diabetic rats with GLI 5 and MET 50 brought glycemia to normal levels, hyperglycemia was maintained in untreated diabetic controls (Fig. 1/Table 1). A similar profile was observed in TG and cholesterol levels (Tables 3 and 4). Furthermore, MPE (100, 200, and 400 mg/kg) maintained glucose reduction from 57 to 67% after 1 week and longer treatments, as compared with each group before treatments or to untreated diabetic rats, respectively. After 2 months of treatment, there was a reduction in TG levels in all treated groups, MPE 100 mg/kg reduction of 64%, MPE 200 mg/kg reduction of 58%, and MPE 400 mg/kg reduction of 61%. The previous results show up similar to those found in classic hypoglycemic drugs, MET 50 mg/kg reduction of 65% and GLI 5 mg/kg reduction of 58%. Similar reductions were found for serum cholesterol with reduction of 50, 66, 24, 51, and 45% compared with CD (control), respectively.

After 1 month, the rats treated with MPE showed the following values: serum fructosamine 100 mg/kg, MPE 200 mg/kg (198.70 ± 15.11 μM/L, $^{***}p < 0.001$), and MPE 400 mg/kg (173.50 ± 32.48 μM/L); these values are statistically significant in relation the diabetic control (420.13 ± 34.78 μM/L), and they are similar to GLI 5 mg/kg treated group (229.50 ± 22.35 μM/L, $^{**}p < 0.01$) and to group treated with MET 50 mg/kg (172.90 ± 18.40 μM/L, $^{***}p < 0.001$). Normal untreated control showed a fructosamine value (163.0 ± 12.49 μM/L) (Fig. 2).

After 2 months, the rats treated with MPE showed the following values: serum glycated hemoglobin (HBA1c) 100 mg/kg (6.35 ± 0.40%), MPE 200 mg/kg (5.37 ± 0.30%), and MPE 400 mg/kg (5.71 ± 0.44%); these values are statistically significant in relation to the diabetic control (12.15 ± 0.60%), and they are similar to GLI 5 mg/kg treated group (6.04 ± 0.68%) to group treated with MET 50 mg/kg (6.08 ± 0.55%). Normal untreated control showed a value of glycated hemoglobin (HBA1c) in 2.13 ± 0.68% (Fig. 3).

From Fig. 4, to study the mechanisms of MPE hypoglycemic action, glucose was measured in diabetic animals treated with lower doses of MPE (10 mg/kg) or GLI (2 mg/kg) or MET (5 mg/kg), alone or associated, after 1-week treatments. We showed that while MPE 10 mg/kg did not show a significant reduction of blood glucose levels, its association with GLI decreased these values by 19.77% (21.29 ± 1.00 to 17.08 ± 0.92, $^{*}p < 0.05$) as related to the same group before treatments or to untreated diabetic groups. In this same experiment, it was seen that the association of MPE with MET also decreases the glycemia levels by 22.69% (21.68 ± 1.22 to 16.76 ± 1.29, $^{*}p < 0.05$).

The results showed that hyperglycemia caused by DZD in normal rat was reversed by MPE extract 100 mg/kg in time periods and presented the following values in millimole per liter: 7.24 ± 0.12 in 1 h, 6.62 ± 0.39 in 2 h, and 6.67 ± 0.14 in 3 h. The group treated with GLI showed normoglycemic standard in all analyzed times (1 h: 4.11 ± 0.15, 2 h: 3.53 ± 0.18, and 3 h: 4.47 ± 0.25) (Fig. 5).

Figure 6 shows representative photomicrographs of histological analyses of pancreas, liver, and kidney from untreated and MPE 100, 200, and 400-treated diabetic rats, GLI 5-treated diabetic rats, and MET 50-treated

Table 2. Effect of *Murraya paniculata* on plasma glucose levels in rats with alloxan-induced diabetes

	Blood glucose level in mmol/L at different days after the treatment			
Name of groups	7 days	15 days	30 days	60 days
MPE 100	7.11 ± 0.49***	7.19 ± 0.77***	11.00 ± 0.98***	8.68 ± 0.43***
MPE 200	9.03 ± 1.49**	5.73 ± 0.27***	7.40 ± 0.50***	7.61 ± 0.64***
MPE 400	7.81 ± 0.88***	5.16 ± 0.06***	8.18 ± 0.86***	8.84 ± 1.02***
GLI 5	8.36 ± 1.09***	5.20 ± 0.09***	7.58 ± 0.69***	5.88 ± 0.23***
MET 50	9.71 ± 0.68***	7.38 ± 1.40***	9.28 ± 1.60**	6.99 ± 0.33***
CD	21.05 ± 0.54	18.61 ± 1.34	18.18 ± 1.90	18.14 ± 1.26

Values represent mean ± SEM for blood glucose levels ($n = 6$).
MPE 100, *Murraya paniculata* extract 100 mg/kg; MPE 200, *M. paniculata* extract 200 mg/kg; MPE 400, *M. paniculata* extract 400 mg/kg; GLI 5, glibenclamide 5 mg/kg; MET 50, metformin 50 mg/kg; CD, diabetic control.
$^{*}p < 0.05$.
$^{**}p < 0.01$.
$^{***}p < 0.001$ (significant after Tukey test).

Table 3. Effect of *Murraya paniculata* on plasma triglyceride levels in rats with alloxan-induced diabetes

	Blood triglyceride level in mmol/L at different days after the treatment			
Name of groups	7 days	15 days	30 days	60 days
MPE 100	1.68 ± 0.22***	1.97 ± 0.08***	1.38 ± 0.07***	1.41 ± 0.05***
MPE 200	1.33 ± 0.10**	1.57 ± 0.22	1.33 ± 0.23*	1.63 ± 0.18*
MPE 400	1.46 ± 0.08**	1.54 ± 0.17**	1.21 ± 0.11**	1.53 ± 0.14**
GLI 5	1.84 ± 0.08**	1.31 ± 0.18***	0.79 ± 0.07***	1.62 ± 0.15**
MET 50	0.87 ± 0.05***	1.28 ± 0.23***	0.83 ± 0.09***	1.35 ± 0.08***
CD	3.09 ± 0.18	3.07 ± 0.02	3.20 ± 0.26	3.88 ± 0.44

Values represent mean ± SEM for blood glucose levels ($n = 6$).
MPE 100, *Murraya paniculata* extract 100 mg/kg; MPE 200, *M. paniculata* extract 200 mg/kg; MPE 400, *M. paniculata* extract 400 mg/kg; GLI 5, glibenclamide 5 mg/kg; MET 50, metformin 50 mg/kg; CD, diabetic control.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$ (significant after Tukey test).

Table 4. Effect of *Murraya paniculata* on plasma cholesterol levels in rats with alloxan-induced diabetes

	Blood cholesterol level in mmol/L at different days after the treatment			
Name of groups	7 days	15 days	30 days	60 days
MPE 100	1.98 ± 0.17***	1.81 ± 0.05***	2.15 ± 0.21***	1.36 ± 0.03***
MPE 200	2.81 ± 0.19**	1.42 ± 0.14***	1.94 ± 0.19***	0.93 ± 0.12***
MPE 400	2.58 ± 0.38	1.59 ± 0.12***	2.73 ± 0.21*	2.07 ± 0.56*
GLI 5	1.77 ± 0.20**	1.83 ± 0.12**	1.60 ± 0.10***	1.32 ± 0.19***
MET 50	1.34 ± 0.10***	1.19 ± 0.08***	1.45 ± 0.09**	1.49 ± 0.08**
CD	3.96 ± 0.25	2.54 ± 0.07	2.70 ± 0.08	2.71 ± 0.08

Values represent mean ± SEM for blood glucose levels ($n = 6$).
MPE 100, *Murraya paniculata* extract 100 mg/kg; MPE 200, *M. paniculata* extract 200 mg/kg; MPE 400, *Murraya paniculata* extract 400 mg/kg; GLI 5, glibenclamide 5 mg/kg; MET 50, metformin 50 mg/kg; CD, diabetic control.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$ (significant after Tukey test).

diabetic rats. Normal organs were included for comparisons. The present light microscopic study of diabetic rats revealed pathological changes of both exocrine and endocrine part of the pancreas represented by vacuolation and marked decrease of β cells. Areas with steatosis (lipid degeneration), dilation, and hyperemia of the centrilobular vein in some regions and the presence of inflammatory cells in the vessels were observed in the liver. Partial or total reduction of hair tufts (suggesting atrophy structure) was also observed in the kidneys of diabetic rats. After treatment with MPE, the pancreas appeared similar to the control, but with reduced volume. The hepatic tissue was normal; there was low hyperemia of the centrilobular vein when compared with diabetic control and the absence of inflammatory cells in the vessels. The presence of many glomeruli was observed in the kidney, but some with reduction of glomerular volume. Similar results were observed for the treated diabetic rats with GLI and MET.

DISCUSSION

Diabetes mellitus is a complex metabolic disorder that is thought to be caused by imbalance between free radical formation and the body's ability to produce sufficient amounts of natural antioxidant enzymes (e.g. superoxide dismutase, catalase, and glutathione peroxidase). Several studies have shown a correlation between oxidative stress and damage of cellular organelles and enzymes, lipid peroxidation, and development of insulin resistance (Karunakaran and Park, 2013; Marrazzo et al., 2014). Some herbal medicines have shown beneficial effect in prevention and management of diabetes and its associated complications (Nasri and Rafieian-Kopaei, 2014). Clinically important compounds, such as MET and GLI, when combined with compounds from derivatives of herbal source may increase their therapeutic efficacy (Monago et al., 2016).

M. paniculata (orange jasmine) is a plant from India widely used in folk medicine as antinociceptive (Narkhede et al., 2012; Podder et al., 2011; Wu et al., 2010), antiinflammatory (Narkhede et al., 2012; Wu et al., 2010), and antioxidant (Gautam et al., 2012a; Rohman and Riyanto, 2005). Among its main constituents are flavonoids, indole alkaloids, coumarin, isoflavones, essential oils, polysaccharides, and fatty acids. Although oral hypoglycemic agents and insulin are the mainstays of treatment of diabetes mellitus (DM), there is a significant demand for new drugs of natural origin that can significantly reduce the development of diabetic complications.

In the previous study, we showed the qualitative phytochemical analysis that revealed the presence of

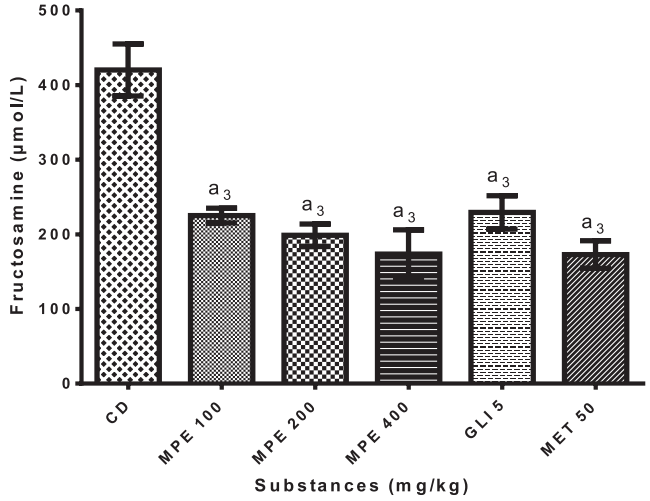

Figure 2. Fructosamine levels after 1 month of treatment of diabetic rats with MPE, glibenclamide, and metformin. $a_3 p < 0.001$ (MPE 100 vs CD; MPE 200 vs CD; MPE 400 vs CD; GLI 5 vs CD; MET 50 vs CD), after analysis of variance (ANOVA) followed by Tukey test.

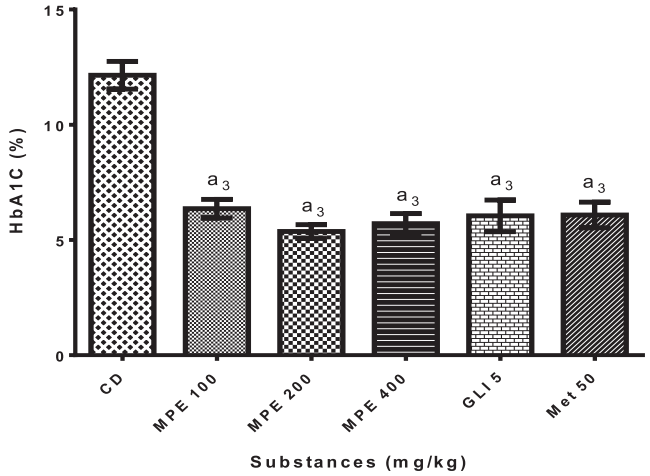

Figure 3. HbA1c evaluation after 2-month treatment of diabetic rats with MPE, glibenclamide, and metformin. $a_3 p < 0.001$ (MPE 100 vs CD; MPE 200 vs CD; MPE 400 vs CD; GLI 5 vs CD; MET 50 vs CD), after analysis of variance (ANOVA) followed by Tukey test.

flavones, flavonoids, tannins, and coumarins. The presence was confirmed rather than revealed. Only data of tannin and phenol derivatives are presented in this manuscript. However, other studies confirm the presence of coumarins, flavones, and flavonols in extract from leaves (Aziz et al., 2010; Zhang et al., 2012, 2010). The flavonols that are listed in Table 1 are common to many plant species, and thus not expected to have any specific role in the management of diabetes. Although this manuscript did not identify the presence of coumarins, flavones or flavonols are examples of classes of compounds that can influence the pathogenesis of diabetes in different action models as antioxidant effect (Sundaram et al., 2014; Testa et al., 2016) and inhibitory activities of α-glucosidase and α-amylase (Zhao et al., 2015), respectively.

We demonstrated in this study that the hydroalcoholic MPE significantly reduces glucose, TGs, and cholesterol levels in alloxan-induced diabetic rats, after its daily administration by gavage for 1 week. Prolonged treatments bring blood glucose concentrations to normality, while cholesterol and TGs decreased about 50 to 70%, respectively. Our data corroborate results for the related species *Murraya koenigii* that caused a decrease of fasting blood glucose in diabetes-induced model (Grover et al., 2003; Yankuzo et al., 2011).

The results of the present study also show that the combination of MPE with GLI or MET, at lower doses, when given for 1 week in alloxan-induced diabetic wistar rats caused more significant reduction in blood glucose level than when they are administered singly that showed additional antihyperglycemic effect which may be due to increased insulin secretion and increased glucose threshold.

Metformin reduces glucose levels primarily by decreasing hepatic glucose production and by increasing insulin action in muscle and fat. The mechanism by which MET reduces hepatic glucose production is contentious, but the preponderance of data indicates an effect on reducing gluconeogenesis. Metformin also may decrease plasma glucose by reducing the absorption of glucose from the intestine (Hardman and Limberd, 2001). MPE, at least in part, shares with MET this mechanism of action. GLI works by inhibiting the ATP-sensitive potassium channels (KATP), in pancreatic beta cells. This inhibition causes cell membrane depolarization and opening of the voltage-dependent calcium channel, which results in increased intracellular calcium concentration in the beta cell and subsequent stimulation of insulin release (Luzi and Pozza, 1997). MPE, at least in part, shares with GLI this mechanism of action.

Diazoxide is used to inhibit inappropriate insulin secretion, causing hypoglycemia (Marks and Samols, 1968). Its major mode of action is the opening of KATP channels in the beta cell membrane, with repolarization, closure of voltage-dependent Ca^{2+} channels, and lowering of $[Ca^{2+}]$ic (Gilon and Henquin, 1992; Trube et al., 1986). However, DZD has been proposed to decrease the efficacy of Ca^{2+} on exocytosis (Flatt et al., 1993; Renstrom and Rorsman, 1996). In order to investigate whether MPE would block DZD hyperglycemia, as GLI does, the effects of both drugs were studied on non-diabetic rats administered with DZD.

Our results show that GLI blocked the increase in glucose levels after DZD; this effect was also observed after MPE, when the animals treated with MPE + DZD behaved similarly to those treated with GLI + DZD. Such results suggest that MPE inhibits the DZD-induced hyperglycemia in a similar way to GLI, that is, by acting as a KATP channel blocker.

Polymethoxylated flavones were also found in the leaves of *M. paniculata* (Wu, 1988; Zhang et al., 2011). This class of compounds presents important metabolic stability and link in membrane transport present in the intestine/liver that showed significant influence in improving oral bioavailability (Walle, 2007). Notably, the polymethoxy flavones and derivatives have attracted some interest because they are less rapidly removed from our systemic circulation and present antidiabetic actions. Myricetin is known to stimulate Ca^{2+} chain mechanism into the pancreatic beta cells, which can characterize the MPE by increased influx of calcium increasing the secretion of insulin from Langerhans islet cells and finally reducing the level of glucose in the blood (Wu, 1988).

Gautam et al. (2012a, b) evaluating the hypoglycemic potential and antioxidant activity of MPE showed an

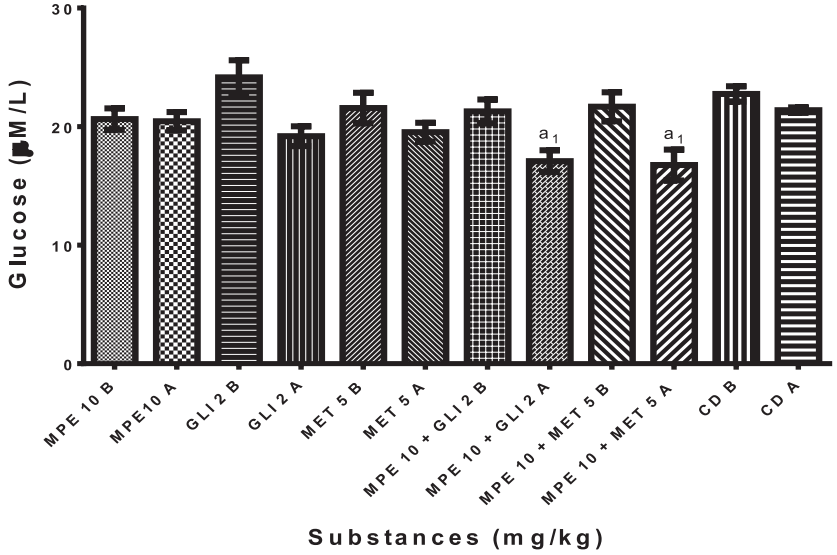

Figure 4. Study potentiation hypoglycemic effect by time–response curve of the oral treatment with *Murraya paniculata* extract on glycemia in Wistar rat serum. The animals were treated with vehicle water (CD), glibenclamide at a dose of 2 mg/kg (GLI 2), metformin at the dose of 5 mg/kg (MET 5), *M. paniculata* extract in the dose 10 mg/kg (MPE 10), *M. paniculata* extract at a dose 10 mg/kg + glibenclamide 2 mg/kg (MPE 10 + GLI 2), and *M. paniculata* extract at a dose 10 mg/kg + metformin 5 mg/kg (MPE 10 + MET 5) ($n = 6$). The means of the treated groups (after treatment) were compared with group B (before treatment), and they were considered significantly different ($a_1p < 0.05$) by two-way ANOVA followed by Tukey test).

Figure 5. Evaluation of hypoglycemic activity of MEP extract on glycemic curve with diazoxide (DZD) in non-diabetic rats ($n = 6$). ($a_2p < 0.01$, $a_3p < 0.001$, $a_4p < 0.0001$ for 1, 2, and 3 h (GLI 5 + DZD)^c vs (CN + DZD)^a; (GLI 5 + DZD)^c vs (MPE 100 + DZD)^b, after analysis of variance (ANOVA) followed by Tukey test.

increased number of islets and the increased percentage of beta cells in the diabetic rats that received the extracts; in the same study, they reported that a possible mechanism for the antihyperglycemic activity was by increasing pancreatic insulin secretion by the beta cells of Langerhans islets (Gautam et al., 2012a, 2012b).

Our histological studies also showed that changes in diabetic organs were partly reversed after MPE treatment for 2 months. The treatment with MPE, MET, and GLI showed a pancreas with diminished damage when compared with the untreated diabetic rats. Similar results were also observed in diabetic liver and kidney.

It has been reported that high levels of glucose also produce chemical alterations in other physiologically abundant proteins such as collagen, hemoglobin, and albumin (Schwartz, 1995). Glycation is a non-enzymatic reaction between sugars and the free amino groups of materials in a hyperglycemic situation such as DM. The glycation of materials induces a wide range of chemical, cellular, and tissue effects and leads to nephropathy, retinopathy, and neuropathy development. Sabbatini et al. (1992) showed that the early glycation products induce glomerular hyperfiltration even in normal rats (Sabbatini et al., 1992). The glycation process is reversible, but over time, it becomes irreversible and early glycation products develop into advanced glycation end products (AGEs). AGE influences charge, solubility, and conformation of extra cellular matrix (ECM). Therefore, the early diagnosis and treatment of hyperglycemia prevents AGE production. And the interest in compounds that could potentially reduce the biological alterations associated with hyperglycemia is intense.

Thus, we showed that the administration of MPE, for 1 and 2 months, to diabetic rats significantly decreased fructosamine and HbA1c levels, as related to untreated diabetic controls. Both fructosamine and glycated

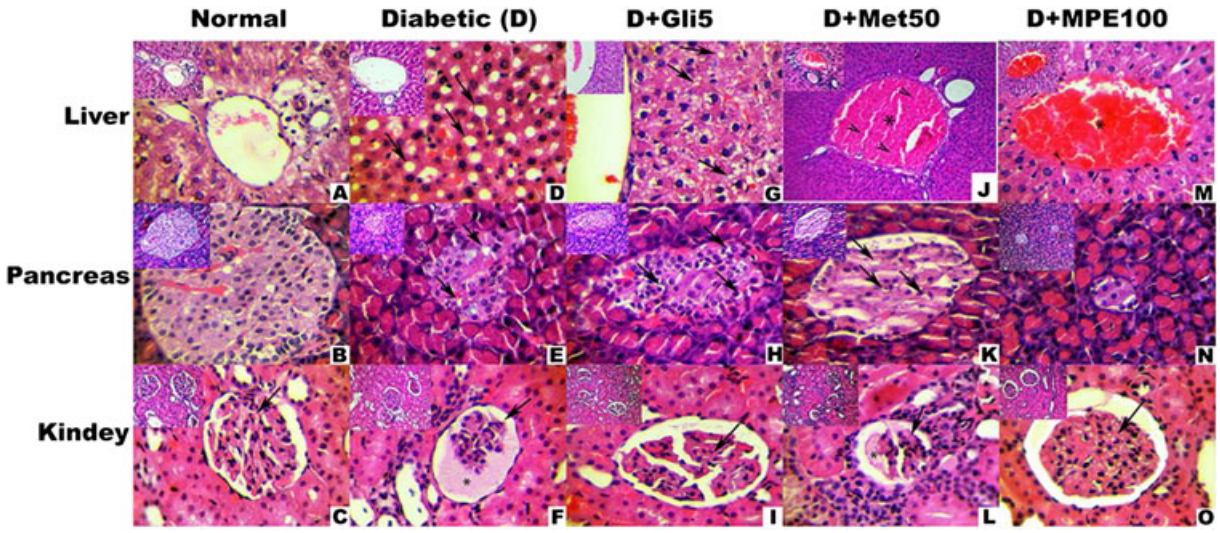

Figure 6. Representative photomicrographs from HE staining of rat liver (upper panel), pancreas (middle panel), and kidney (lower panel) in normal, untreated diabetic, and diabetic rats treated with MPE (100 mg/kg), GLI (5 mg/kg), or MET (50 mg/kg), for 8 weeks (×400 magnification). In the liver, the large arrows indicate the areas of hepatic steatosis (**G** and **D**). The asterisks show the centrilobular vein with the presence of hyperemia, and the small arrows indicate the presence of inflammatory cells (**J**). In the cuts of the pancreatic tissue, the arrows show a cytoplasmic vacuolization (**E** and **H**). In the cuts of the kidney, the large arrows indicate the capillary tufts. The asterisks show the areas of hyalinization, and the small arrows identify cells of the inflammatory infiltrate (**L**). [Colour figure can be viewed at wileyonlinelibrary.com]

hemoglobin are associated with microvascular conditions in diabetic patients and increased risk for mortality and morbidity in hemodialysis patients (Selvin et al., 2010; Shafi et al., 2012).

Previous studies reported that 10% stable reduction in HbA1c determines a 35% risk reduction for retinopathy, a 25–44% risk reduction for nephropathy, and a 30% risk reduction for neuropathy (Calisti and Tognetti, 2005). The decrease in fructosamine and glycated hemoglobin values by *M. paniculata* indicates an important glycemic control in the medium-term and long-term treatment, indicating the beneficial role of MPE in reducing diabetic complications like glycosylation in experimental diabetic rats.

The results of HbA1c and fructosamine-added histopathological evidence cited here indicate that the *M. paniculata* can inhibit the development of microvascular and macrovascular injuries resulting from the DM, confirming recent findings of Zou et al. (2014) that showed a protective effect of extracted total flavonoids in the leaves of *M. paniculata* (L.) Jack on diabetic nephropathy in the rat, where parameters such as albumin, creatinine, urea, and interleukin-6 were significantly reduced by the treatment (Zou et al., 2014). The same study also showed that the flavanoid of *M. paniculata* significantly blocked the decrease of superoxide dismutase and glutathione peroxidase and the increase of malondialdehyde levels in diabetic rats. On the other hand, studies with isolated coumarin of *Murraya* genus, respectively, showed hypoglycemic activity and inhibitory effect on the activity of the enzyme aldose reductase (Gacche and Dhole, 2011; Gupta et al., 2014) and on platelet aggregation (Chia et al., 2008), which are considered to be important causes of diabetic complications.

Another important aspect attributed to *M. paniculata* is its efficacy on other pathologies of inflammatory basis, because according to Narkhede et al. (2012), the extract of *M. paniculata* leaves exhibited significant antiinflammatory action in acute and chronic models of inflammation (Narkhede et al., 2012). These results suggest that MPE can be a protective agent against chronic complications of diabetes where a mix of inflammation, glycation of proteins, and oxidative stress are involved.

CONCLUSION

The results of the present investigation clearly indicate that the leaf extract of *M. paniculata* has a glucose-lowering effect on alloxan-induced diabetic rats. The extract was effective in managing the complications associated with DM, such as hypercholesterolemia and hypertriglyceridemia and also in the reduction of the tissue lesions associated with diabetic state, this effect at least partly explained by antioxidant actions. The hypoglycemic effect of MPE is similar to those of GLI and MET, and this effect, at least partly, is related to the inhibition of KATP. It is recommended that further studies to identify and characterize the active components of this part of the plant be undertaken with a view to determining the molecular bases for the hypoglycemic action. Furthermore, controlled clinical trials are required to confirm its hypoglycemic actions in human subjects.

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Conflict of Interest

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

I.R.A.M., F.A.O.G., and H.D.M.C. conceived the experimental design; T.R.A., P.G.P., and A.C.M. performed extraction of extract and characterization; C.D. A.M., E.S.S., M.R.C., T.R.G., and B.A.F.S. performed the experimental assay; T.F.S. conceived the pathological assay; G.S.B.V., I.R.A.M., and F.A.O.G. drafted the manuscript and provided facilities for the work. All the authors read and approved the final version of the manuscript.

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