

RESEARCH ARTICLE

Effect of coadministration of glibenclamide and methanolic stem extract of *Anisopus mannii* N.E.Br. (*Apocynaceae*) on glucose homeostasis and lipid profile in streptozotocin/nicotinamide-induced diabetic rats

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ABSTRACT

Background: Although the antihyperglycemic activity of stem extracts of *Anisopus mannii* (AM; an antidiabetic ethnomedicinal plant utilized in Northwestern Nigeria) has been reported, the effects of coadministration of the extracts with glibenclamide, a commonly used antihyperglycemic agent, are not known. **Aims and Objectives:** The objectives were to determine the effect of simultaneous administration of AM methanolic stem extract with glibenclamide using streptozotocin/nicotinamide-induced diabetic rats. **Materials and Methods:** After permission from the departmental ethics committee, male Wistar rats were assigned to five groups: Vehicle-treated normal control; vehicle-treated diabetic control; diabetic rats treated with either glibenclamide (0.6 mg/kg) or methanolic stem extract of AM (200 mg/kg), singly or in combination. Fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) were performed at days 14 and 28 of treatment, while lipid profile and the contents of hepatic and skeletal muscle glycogen were determined at the end of the study (day 29). **Results:** When combined, the AM extract significantly reduced ($P < 0.05$) the effect of glibenclamide in lowering the total area under the OGTT curve as well as the FBG level and in increasing the contents of hepatic and skeletal muscle glycogen as well as the blood level of high-density lipoprotein cholesterol. The two treatments also significantly reduced each other's effect in lowering triglycerides, low-density lipoprotein, and total cholesterol. **Conclusion:** The methanolic stem extract of AM (200 mg/kg) combined with glibenclamide (0.6 mg/kg) reduced each other's beneficial effects on glucose homeostasis and lipid profile in streptozotocin/nicotinamide-induced diabetic rats.

KEY WORDS: *Anisopus mannii*; Glibenclamide; Streptozotocin; Rats; Combination


INTRODUCTION

Diabetes mellitus, one of the most common endocrine disorders, is characterized by an absolute or relative lack of insulin resulting in abnormal glucose and lipid metabolism. The global prevalence of the disorder was estimated at 8.8%

in 2017 among people aged 20–79 years and is expected to increase to 9.9% by 2045.^[1] In Nigeria, about 4.7 million people aged 20–79 years suffered from the disease in 2015, translating to 5.7% age-adjusted prevalence.^[2]

The use of herbal medicine to treat various disease conditions, including diabetes mellitus, is on the increase worldwide, due to its accessibility, affordability, and cultural acceptability.^[3]

A Nigerian survey revealed that about one-third of people with diabetes mellitus who are on conventional antidiabetic treatment concurrently use herbal remedies, and most of them failed to inform their physicians about the practice.^[4] Such practice poses an attendant risk of herb-drug interaction,

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which may increase the risk of therapeutic failure as well as adverse effects.^[5]

One such herbal agent is *Anisopus mannii* (AM) (*Apocynaceae*), a medicinal plant traditionally used to treat diabetes mellitus.^[6] Furthermore, traditional medicine practitioners in Sokoto often prescribe the plant in patients with diabetes mellitus who are on treatment to improve well-being and to treat other comorbid disease conditions such as erectile dysfunction (personal communication).

One of the most commonly used antidiabetic agents in Northwestern Nigeria is glibenclamide,^[7] a sulfonylurea agent that acts by increasing the release of insulin from the beta-cells of the islets of Langerhans.

Although several studies have documented the antidiabetic and antihyperlipidemic activities of extracts from the leaf and the stem of AM,^[6,8-10] the effect of coadministration of glibenclamide with extracts from AM is not known.

The current study is aimed at investigating the effect of the simultaneous administration of the methanolic stem extract of AM and glibenclamide on glucose homeostasis and lipid profile of streptozotocin/nicotinamide-induced diabetic rats.

MATERIALS AND METHODS

Drugs and Chemicals

Streptozotocin, nicotinamide, glyburide (glibenclamide), and glucose standard were procured from Sigma-Aldrich Co., USA. Glucose oxidase reagent and assay kits for high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), as well as triglycerides (TGs) were obtained from Randox Laboratories Ltd. (Crumlin, UK). Accu-Chek Active Glucometer (Roche Diagnostics, Germany) was purchased from a reputable store. All chemicals utilized were of analytical grade.

Plant Material

The whole plant of AM was purchased from the forests of Achida, Sokoto state, Nigeria, in October 2016 and the name of the plant was confirmed from the plant list database (<http://www.theplantlist.org>). The plant sample was identified and authenticated at botany unit, Department of Biological Sciences, Faculty of Science, Usmanu Danfodiyo University, Sokoto. A voucher specimen (UDUH/ANS/0141) was deposited in the herbarium of the department.

Preparation of the Plant Extract

The whole plant of AM was dried under shade to constant weight followed by separation of the stems from the leaves. The dried stems were reduced to a powder with a pestle

and mortar, and the powder (50 g) was macerated at room temperature using 250 ml of 99% methanol with intermittent shaking for 48 h. After filtration (through Whatman filter paper 1), which was repeated twice, the extract was reduced to a semisolid mass under reduced pressure at 35°C using a rotary vacuum evaporator. The final drying of the extract was achieved at 40°C in an aerated oven. The dried extract was stored at -4°C until being used.

Phytochemical Analysis

The qualitative phytochemical analysis of the methanolic extract of AM was carried out for the presence of phytosterols, alkaloids, flavonoids, saponins, tannins, cardiac glycosides, and anthraquinones using the methods of Harbone,^[11] Sofowora,^[12] and Trease and Evans,^[13] while the quantitative analysis of the phytoconstituents detected qualitatively was made according to the methods of Ferguson,^[14] Harbone,^[11] Kirk and Ronald,^[15] Bohm and Koupai-Abyazani,^[16] El-Olemy *et al.*,^[17] Obadoni and Ochuko,^[18] and Okeke and Elekwa.^[19]

Animals

Healthy male Wistar rats (weighing 100–120 g) obtained from the Department of Pharmacology, University of Jos, Nigeria, were used in this study. The animals were acclimatized for 2 weeks in an air-conditioned animal room. They were fed *ad libitum* with standard commercial chow (Vital Feeds, Jos, Nigeria) and allowed free access to water. The maintenance and handling of the animals were in accordance with the international guidelines.^[20] The study was performed following permission from the Animal Ethics Committee, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

Induction of Diabetes Mellitus

Male rats were injected intraperitoneally with 65 mg of freshly prepared streptozotocin in 0.1 M citrate buffer at pH 4.5, 15 min following injection of a solution of nicotinamide in normal saline (150 mg/kg in 10 ml/kg). The same procedure was performed daily for 2 days. One week after the last streptozotocin/nicotinamide injection, fasting blood glucose (FBG) level was determined in the rats after an 8 h fast, employing a standardized Accu-Chek® Active Glucometer. Moderately diabetic rats with FBG of 198–252 mg/dl were selected for the current study.^[21] Percentage successful induction was 85% (34 of 40 rats).

Experimental Design

The influence of the extract-glibenclamide coadministration on antihyperglycemic activity of the plant extract was investigated in streptozotocin/nicotinamide-induced diabetic rats. Each animal was orally administered with the same

volume of aqueous solutions of treatment or vehicle relative to body weight (10 ml/kg) by gavage.

Forty rats were randomly divided (using decision analyst software) into five equal groups (one normal and four diabetic groups) comprising eight rats each:

- Group 1: Normal vehicle-treated control rats (randomly selected before the induction of diabetes) were given distilled water alone
- Group 2: Diabetic vehicle-treated control rats were given distilled water alone
- Group 3: Diabetic rats were given a methanolic stem extract of AM (200 mg/kg b.w./day) dissolved in distilled water
- Group 4: Diabetic rats were given aqueous solution of the standard antidiabetic drug glibenclamide (0.6 mg/kg b.w./day)
- Group 5: Diabetic rats were given a combination of glibenclamide (0.6 mg/kg) and the methanolic extract of AM (200 mg/kg) dissolved in distilled water.

Assessment of Biochemical Parameters from Blood, Skeletal Muscle, and Liver Samples

Oral glucose tolerance test (OGTT) was conducted on 8 h fasted rats at days 0, 14, and 28. The five groups of rats (a normal group and four diabetic groups comprising eight animals each) were administered orally with their respective treatments. Thirty minutes later, glucose (2 g/kg b.w. kg.) was administered orally to each rat by gavage. Blood samples were collected from the tail vein by needle puncture, and blood glucose levels determined employing a standardized Accu-Chek® Active Glucometer at 30 min (just before the administration of the respective treatments), 0 min (just before the administration of oral glucose), and 30 min, 60 min, 90 min, and 120 min after glucose loading. Glucose tolerance was determined using the total area under the OGTT curve, which was calculated from the baseline (0 min) and divided by time period.^[22]

Body weights of the rats were measured and recorded at baseline and then every 2 weeks. The animals were sacrificed on day 29. Blood samples were collected from the animals, following light chloroform anesthesia, for the estimation of LDL-C, HDL-C, TC, and TG using commercially available kits (Randox, UK) following the manufacturer's instructions. The rats were then dissected, and the liver and femur skeletal muscle samples were collected for the determination of their glycogen contents according to the method of Kemp and Van Heijningen.^[23] The glucose equivalents of the glycogen concentration were determined using glucose oxidase method (Glucose oxidase reagent, Randox Laboratories Ltd., Crumlin, UK).

Statistical Analysis

Data analysis was performed using GraphPad Prism and expressed as mean \pm standard error. One-way analysis of

variance (ANOVA) was worked out, followed by Tukey Kramer multiple comparison test. $P < 0.05$ was regarded as statistically significant.

RESULTS

Phytochemical Analysis

Qualitative phytochemical analysis of the dried methanolic stem extract of AM (yield: 17.66% w/w) revealed that phytosterols, alkaloids, flavonoids, saponins, and tannins were present, but the cardiac glycosides and anthraquinones were absent in the extract. Quantitative analysis revealed the presence of 17.50% tannins, 8.00% alkaloids, 4.00% saponins, 3.00% flavonoids, and 0.01% steroids.

Effect of Coadministration of AM Extract with Glibenclamide on OGTT, Tissue Glycogen, Body Weight, and Lipid Profile in Streptozotocin/Nicotinamide-induced Diabetic Rats

The fasting levels of blood glucose, TC, LDL-C, and TGs were found to be significantly increased, while the level of HDL-C, as well as hepatic and skeletal muscle contents of glycogen, was observed to be significantly decreased in vehicle-administered diabetic group compared with vehicle-administered normal control group.

At baseline, treatment with glibenclamide (at 0.6 mg/kg) or the methanolic extract of AM (200 mg/kg) singly, but not in combination, resulted in a significantly larger reduction in the total area under the curve (tAUC) of the OGTT when compared with the vehicle-administered diabetic rats ($P < 0.05$; Table 1). A significantly greater reduction in the tAUC of OGTT occurs in rats treated with glibenclamide as monotherapy than in rats administered with the glibenclamide-extract combination ($P < 0.05$; Table 1).

At day 14 of treatment, the diabetic groups administered with either AM extract or glibenclamide alone or in combination exhibited a significant reduction in the tAUC of the OGTT as well as in FBG when compared to their vehicle-administered counterparts ($P < 0.05$; Table 1). The reduction in the tAUC of OGTT, but not that of the FBG, was significantly larger in diabetic rats treated with glibenclamide alone than in the group treated with the glibenclamide-extract combination ($P < 0.05$; Table 1).

At 4 weeks post-treatment, there was a significant decrease in the tAUC of OGTT and in the level of FBG in diabetic rats treated with glibenclamide or the AM extract singly or in combination compared with the vehicle-administered controls ($P < 0.05$; Table 1). Such effects were significantly greater in the diabetic group treated with glibenclamide as monotherapy than in their counterparts administered with the glibenclamide-extract combination ($P < 0.05$; Table 1).

Treatment with glibenclamide or the extract singly or simultaneously resulted in a significant elevation in the contents of hepatic and skeletal muscle glycogen as compared to vehicle ($P < 0.05$; Figure 1). The contents of hepatic and muscle glycogen in the group administered with glibenclamide-extract combination were lower than that in the glibenclamide-treated rats ($P < 0.05$; Figure 1) but not the extract-treated group.

At baseline, the body weight of the rats was not statistically different between the groups ($P > 0.05$; Table 2). However, following 2 weeks of treatment, a significantly lower body weight was recorded in the vehicle-administered diabetic group than in the normal group. This pattern continued until the end of the experiment ($P < 0.05$; Table 2). By week 4, the body weight of the three groups of diabetic rats administered with either the extract or glibenclamide, singly or in combination, was significantly lower than that of the vehicle-treated normal control group but higher than that of the vehicle-administered diabetic control ($P < 0.05$; Table 2).

Treatment with the AM extract or glibenclamide, singly or simultaneously, resulted in a significantly higher level of plasma HDL-C and lower levels of plasma TGs as well as TC and LDL-C than vehicle treatment ($P < 0.05$; Figure 2a-d). The extent of the fall in plasma TG, TC, and LDL-C in the group treated with glibenclamide-extract combination was significantly lower than in the group treated with glibenclamide alone ($P < 0.05$; Figure 2a-c). By contrast,

the extent of elevation in plasma HDL-C in the group administered with glibenclamide-extract combination was only significantly lower than in the glibenclamide-treated group but not in the extract-treated rats (Figure 2d).

DISCUSSION

This study revealed that intraperitoneal injection of streptozotocin and nicotinamide resulted in an increase in the blood level of glucose, coupled with a decrease in the contents of hepatic and skeletal muscle glycogen, changes in lipid profile and weight loss. Streptozotocin/nicotinamide intraperitoneal injection is a method widely used to induce

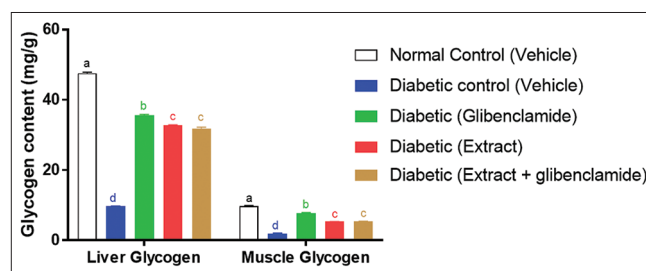


Figure 1: Effect of the methanolic stem extract of *Anisopus manni* alone or simultaneously with glibenclamide on the contents of hepatic and skeletal muscle glycogen of diabetic rats at the end of the study (day 29). Values are the means \pm standard error ($n = 8$). Treatment means with different lower case letters are significantly different at $P < 0.05$

Table 1: Effect of methanolic stem extract of AM alone and in combination with glibenclamide on FBG and OGTT in diabetic rats

Group	FBG level (mg/dl)			Total area under OGTT curve (mg/dl x min)		
	0 week	2 weeks	4 weeks	0 week	2 weeks	4 weeks
Normal control	78.38 \pm 2.74 ^b	74.00 \pm 2.10 ^c	74.13 \pm 1.43 ^d	15,834.00 \pm 960.90 ^d	14,919.00 \pm 329.10 ^d	14,689.00 \pm 279.00 ^d
Negative control	216.13 \pm 5.98 ^a	237.60 \pm 6.75 ^a	258.60 \pm 6.77 ^a	55,044.00 \pm 1528.00 ^a	56,374.00 \pm 910.60 ^a	58,849.00 \pm 919.20 ^a
Glibenclamide (0.6 mg/kg)	209.38 \pm 6.94 ^a	188.40 \pm 7.39 ^b	150.40 \pm 6.97 ^c	42,926.00 \pm 1002.00 ^c	37,119.00 \pm 1019.00 ^c	31,149.00 \pm 1458.00 ^c
Extract (200 mg/kg)	207.50 \pm 4.95 ^a	197.10 \pm 4.90 ^b	179.00 \pm 5.10 ^b	50,229.00 \pm 582.40 ^b	46,427.00 \pm 746.80 ^b	41,083.00 \pm 619.00 ^b
Extract (200 mg/kg) + Glibenclamide (0.6 mg/kg)	206.75 \pm 4.67 ^a	211.00 \pm 7.49 ^b	195.40 \pm 3.39 ^b	51,934.00 \pm 448.10 ^{ab}	49,230.00 \pm 939.10 ^b	44,366.00 \pm 926.60 ^b

Values are the means \pm standard error ($n=8$). Treatment means with different lower case superscript letters in the same column are significantly different at $P<0.05$. OGTT: Oral glucose tolerance test, FBG: Fasting blood glucose

Table 2: Effect of methanolic stem extract of AM alone and in combination with glibenclamide on body weight of diabetic rats

Group	Body weight (g)		
	Baseline (before induction of diabetes)	2 weeks	4 weeks
Normal control	108.25 \pm 1.70 ^a	126.25 \pm 2.36 ^a	143.38 \pm 1.78 ^a
Negative control	109.88 \pm 2.25 ^a	93.13 \pm 1.71 ^c	73.38 \pm 1.89 ^d
Positive control (0.6 mg/kg)	113.38 \pm 2.34 ^a	124.00 \pm 2.28 ^a	134.88 \pm 2.36 ^b
Extract (200 mg/kg)	109.88 \pm 2.47 ^a	114.13 \pm 2.59 ^b	119.25 \pm 2.57 ^c
Extract (200 mg/kg) + Glibenclamide (0.6 mg/kg)	108.25 \pm 1.42 ^a	110.00 \pm 2.01 ^b	115.75 \pm 1.58 ^c

Values are the means \pm standard error ($n = 8$). Treatment means with different lower case superscripts in the same column are significantly different at $P < 0.05$

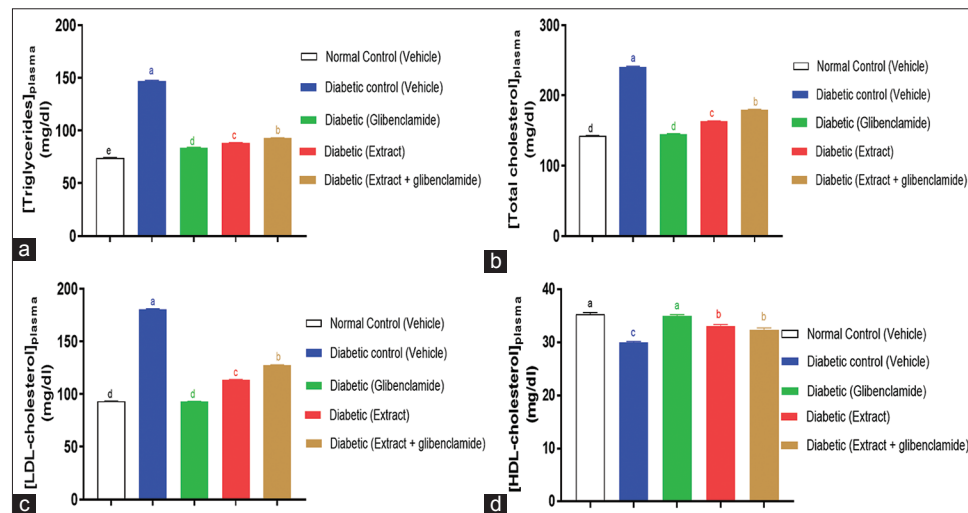


Figure 2: Effect of the methanolic stem extract of *Anisopus mannii* alone or simultaneously with glibenclamide on lipid profile in diabetic rats. (a) Plasma triglycerides at the end of the experiment (day 29 post-treatment). (b) Plasma total cholesterol at the end of the study (day 29). (c) Plasma low-density lipoprotein cholesterol concentration at the end of the experiment (day 29 post-treatment). (d) Plasma high-density lipoprotein cholesterol at the end of the experiment (day 29 post-treatment). Values are the means \pm standard error ($n = 8$). Treatment means with different lower case letters are significantly different at $P < 0.05$

Type 2 diabetes mellitus in rats.^[24] Streptozotocin causes its cytotoxic effect on the beta-cells of the pancreas by inducing DNA alkylation, leading to a fall in the concentration of nicotinamide adenine dinucleotide in the cells^[25] and subsequent generation of oxygen-free radicals.^[26] Administration of nicotinamide tends to cushion the cytotoxic effect of streptozotocin on the beta-cells, resulting in only minor damage to the organ, with moderate and stable hyperglycemia.^[27] In this study, the qualitative and quantitative phytochemical analysis of the methanolic stem extract of AM revealed the presence of certain phytoconstituents, namely, tannins, alkaloids, saponins, and flavonoids, in relatively high yield, while the yield of steroids was found to be low. The extract at 200 mg/kg/day significantly improved all the altered variables as compared to the diabetic control group. The present study also revealed that both the positive control glibenclamide and the extract produced a significant reduction in plasma glucose, a significant improvement in lipid profile (a decrease in TC, LDL-C, and TGs as well as an increase in HDL-C), a significant elevation in the contents of hepatic and skeletal muscle glycogen, and a significant increase in body weight. In addition, the findings of the current study showed that when coadministered (at least at the doses used in the study), the AM extract significantly reduced ($P < 0.05$) the effect of glibenclamide in improving oral glucose tolerance (as evidenced by the reduction in the tAUC of the OGTT), in lowering the level of FBG glibenclamide, and in increasing the contents of hepatic and skeletal muscle glycogen and the blood level of HDL-C. The two treatments also significantly reduced each other's effect in lowering TGs, TC, and LDL-C.

The increase in the blood level of glucose coupled with a decrease in the contents of hepatic and skeletal muscle glycogen, changes in lipid profile and weight loss observed

following streptozotocin/nicotinamide injection accord with the previously documented findings of studies on this animal model.^[24,28] In addition, the observed glucose-lowering effect of glibenclamide is in agreement with the previous research, in which glibenclamide was shown to be effective in animals with moderate diabetes, but not in those with severe diabetes.^[29] Glibenclamide acts by stimulating the release of insulin from beta-cells. The effects of the methanol stem extract of AM on blood glucose and lipid profile are also consistent with the findings of other studies, which showed antihyperglycemic and antihyperlipidemic activities of extracts from AM in diabetic rats.^[6,8-10,30] Some of the phytochemicals found in the extract (tannins, saponins, alkaloids, and flavonoids) have been documented to possess antidiabetic and antihyperlipidemic properties,^[31-37] and any of them, singly or simultaneously, could, therefore, be responsible for the antidiabetic and antihyperlipidemic activities observed in the extract. The observed tendency of both glibenclamide and the extract to reduce each other's beneficial effects on glucose homeostasis supports the previous studies that revealed the potential antagonistic effect of the simultaneous administration of herbal products with conventional antidiabetic agents on blood glucose.^[38,39]

The present study revealed that glibenclamide significantly reduced plasma glucose and improved oral glucose tolerance, a finding which is in agreement with the previous research, in which glibenclamide was shown to be effective in animals with moderate diabetes, but not in those with severe diabetes.^[29] Glibenclamide acts by stimulating the release of insulin from beta-cells. The current study also corroborates the findings of other studies, which showed antihyperglycemic and antihyperlipidemic activities of extracts from AM in diabetic

rats.^[6,8-10,30] The dose of 200 mg/kg for the extract was chosen based on our preliminary findings.

Qualitative and quantitative phytochemical analysis of the methanolic stem extract of AM revealed the presence of certain bioactive phytoconstituents, namely, tannins, alkaloids, saponins, and flavonoids, in relatively high yield. Each of these classes of phytochemicals has been documented to possess antidiabetic and antihyperlipidemic properties,^[31-37] and any of them, singly or simultaneously, could, therefore, be responsible for the antidiabetic and antihyperlipidemic activities observed in the extract.

Strength and Limitations of this Study

To the best of our knowledge, the present study is the first to demonstrate the effect of the methanolic stem extract of AM in elevating the content of the hepatic and skeletal muscle glycogen in diabetic rats – an effect which may be due to either increased glycogenesis or decreased glycogenolysis.^[40]

Furthermore, the current study is the first to reveal that when coadministered (at least at the doses used in the study), the AM extract significantly reduced ($P < 0.05$) the effect of glibenclamide in improving oral glucose tolerance (as evidenced by the reduction in the tAUC of the OGTT), in lowering the level FBG glibenclamide, and in increasing the contents of hepatic and skeletal muscle glycogen and the blood level of HDL-C. The two treatments also significantly reduced each other's effect in lowering TGs, TC, and LDL-C.

Such results support the previous studies that revealed the potential antagonistic effect of the simultaneous administration of herbal products with conventional antidiabetic agents on blood glucose.^[38,39] Although the mechanism of the observed effects is not yet known, the two treatments may act by interfering with the pharmacokinetics and/or the pharmacodynamics of each other. Tannins, the class of phytochemicals found to have the highest yield in the extract, have been documented to form a non-absorbable complex with some drugs,^[41] thus leading to a reduction in the absorption of both the drug and the tannin-containing extract.

There are several limitations of our study. First, we were unable to perform a mass spectrometric analysis to achieve a more precise characterization of the extract components. However, we were able to identify several biologically active compounds that are also found in extracts from other plants. One of the limitations of this study is that in this rat model of diabetes mellitus, we did not determine the possible dose-dependent effect of the extract singly or in combination with glibenclamide, which requires the use of incremental doses; we only investigated the effect at a single dose of 200 mg/kg/day. The dose was chosen based on a preliminary study showing it to be the more effective than the higher dose of 600 mg/kg and, since it is lower, presumably less toxic.

CONCLUSION

This study revealed that concurrent administration of the methanolic stem extract of AM (200 mg/kg) with glibenclamide (0.6 mg/kg) has a potential antagonistic effect on glucose homeostasis and lipid profile in streptozotocin/nicotinamide-induced diabetic rats. Further studies are required to understand the mechanism(s) behind these findings.

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