Sitagliptin–Moringa oleifera coadministration did not delay the progression nor ameliorated functional and morphological anomalies in alloxan-induced diabetic nephropathy

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Abstract:
OBJECTIVE: Sitagliptin (ST) and Moringa oleifera (MO) Lam (Moringaceae) are used concomitantly by diabetic patients, with no study ascertaining for potential favorable or otherwise renal implications. We investigated the effect of coadministration of ST and MO leaf extract on functional and morphological biomarkers of alloxan-induced diabetic nephropathy (DN).

MATERIALS AND METHODS: Diabetes was induced with a single dose of 150 mg/kg of alloxan intraperitoneally. Seven groups of eight rats per group were used, with Groups I, II, and VII as normal (NS), diabetic control (DC), and postprandial controls. Groups III, IV, V, and VI were diabetic rats on ST, MO, ST and MO (SM), for 42 days with 2 weeks delayed treatment in a postprandial hyperglycemic group (PPSM), respectively. Serum urea, albumin, electrolyte levels, lipid profile, and kidney tropism were determined in addition to histological examinations.

RESULTS: There was a significant increase (P<0.05) in kidney tropism comparing all drug-treated groups and DC to normal rats. Significant increases in serum urea were observed (P=0.02) in DC, MO-treated, and SM-treated rats compared to normal rats and also in serum triglyceride (P<0.05) in MO-treated and SM-treated rats compared to controls and other drug-treated groups. A mild reduction in severity of pathologic lesions was observed (glomerulosclerosis Grade 1) in SM-treated rats compared to a marked necrosis in DC (Grade 3).

CONCLUSION: The coadministration of ST–MO did not delay the progression of functional anomalies and renal injury nor ameliorated the lesions associated with chronic DN in Wistar rats.

Keywords: Sitagliptin, Moringa oleifera, diabetic nephropathy, renal function biomarkers

Introduction

Diabetic nephropathy (DN) is a progressive development of renal insufficiency caused by angiopathy of capillaries in the kidney glomeruli in the setting of hyperglycemia. DN occurs in about one-third of all people with diabetes and is the leading cause of end-stage renal disease and renal failure in developed and developing countries.[1] DN is associated with increased cardiovascular mortality in diabetic patients and is more prevalent in African Americans, Asians, and Native Americans than Caucasians.[2] The cardinal lesion of DN resides in renal glomeruli and is called diabetic glomerulosclerosis. Persistent albuminuria, increased levels of creatinine, and blood urea nitrogen are...
The ability of oral antidiabetic agents to ameliorate DN is just as important as their capability to control blood glucose. Sitagliptin (ST), a dipeptidyl peptidase-4 (DPP-4) inhibitor, was approved by the Food and Drug Administration (the United States of America) in October 2006 as the first DPP-4 inhibitor for the treatment of type 2 diabetes mellitus (T2DM). Low dose of ST with minimal antihyperglycemic effect has been documented to ameliorate certain morphological and functional markers in an animal model of DN. However, in clinical practice, ST has been associated with acute drug-induced interstitial nephritis. Reducing the risk of DN in addition to intensive glycemic control, and blood pressure regulation, involves the use of low-dose angiotensin-converting enzyme inhibitors (ACEIs) either as renoprotective or as mainstay in therapy. Studies using ST in diabetic rats reveal the augmentation of the renovascular effect of angiotensin, calling for caution and closer examination in hypertensive diabetic patients. In addition, the concurrent use of DPP-4 inhibitors and ACEI in diabetic patients has been associated with an increased risk of angioedema. This evidence limits the use of ACEIs in the management of nephropathy in DPP-4 inhibitor-treated diabetic patients. The use of angiotensin-receptor blockers as alternatives to ACEIs in Nigeria has also been limited due to high cost. In other words, there is a need to look into the safety and suitability of other possible, readily available, and cheaper alternative agents that patients on DPP-4 inhibitors are likely to resort to in preventing and reducing the progression of DN.

Due to these challenges of treatment, poor resource settings, and health system problems in Africa, herbal medications are also often used alongside orthodox medicines by patients in the management of T2DM. There is a high prevalence of herb use in Sub-Saharan Africa, with little or no validation of the folkloric information with respect to ascertaining for efficacy and safety. However, some herbs have shown antihyperglycemic activities when evaluated using available experimental techniques and one example is *Moringa oleifera* (MO). The leaves are either steamed and eaten as a vegetable, dried powder dissolved in water and drank or as a decoction in alcohol. However, studies report conflicting effects on the hepatorenal system; while ameliorative effects were seen with heavy metal-induced renal/hepatic toxicity, adverse effects were mainly associated with chronic use in diabetic and nondiabetic rats. The increase in consumer awareness and acceptance of MO for the management of diabetes invariably increases the possibility of its concomitant use with ST in patients. Drug–herb interactions involving orthodox antidiabetic agents and medicinal plants have been seen in animal studies and clinical practice. Therefore, the concomitant use of MO and ST, in addition to a possible synergistic antidiabetic effect, might have potential favorable or otherwise renal/hepatic implications. Reviews indicate that herbal medicine is not been incorporated into clinical practice because of inadequate information on the adverse effects following the chronic use of herbs and insufficient data on drug–herbal interactions.

The study investigated the effect of concomitant administration of ST and 50% ethanol leaf extract of MO Lam on progression and possible amelioration of DN using renal function biomarkers and morphology (serum electrolytes, urea, albumin and renal tropism, and histology) and lipid profile in alloxan-induced nephropathy in diabetic rats.

**Materials and Methods**

**Materials**
The following materials were used:

- Leaves of MO Lam (Moringaceae) from Zaria, Nigeria.
- ST (Merck Sharp and Dohme [MSD] Pharmaceuticals in Switzerland, Batch no: A000512). Procured directly through the MSD office in Lagos, Nigeria.
- Alloxan monohydrate (Sigma Aldrich, St. Louis, Missouri, USA). Procured through the licensed agent in Nigeria.
- Accu-Chek® glucometer. Procured through the licensed agent in Nigeria from Roche Ltd, India.

**Collection, identification, and extraction of plant material**
The branches of MO Lam (Moringaceae) with leaves and flowers were collected from Graceland, Zaria, Nigeria by Dr. (Mrs.) Comfort Olurishe. The plant was identified and authenticated by a botanist Mr. S. U. Gallah, in the Department of Biological Sciences, Ahmadu Bello University, Zaria, and given a voucher specimen number (571). The leaves are green to dark green, tripinnate and feathery with elliptical leaflets 1-2 cm long and cream-coloured flowers borne in sprays and pleasantly fragrant. The name of the plant was checked with www.theplantlist.org and the common names of the plant include drumstick tree, horseradish tree, and Ben
oil tree. The leaves were then harvested, washed with distilled water, dried under shade until constant weight was obtained, and then pulverized with mortar and pestle. Dried and pulverized leaves were weighed (500 g) and macerated in a percolator with 2 L of 50% ethanol for 72 h under room temperature. Thereafter, the extract was obtained on filtration using a filter paper. The resulting extract was dried using a rotavapor at 50°C–60°C to obtain a brownish greasy residue.

Induction of experimental diabetes
Animal handling and care was according to the recommended guidelines (European community guideline/EEC, 1986). Ethical clearance number obtained from the departmental ethical committee in January 2014 is DAC/IW-OT/067/14. Wistar rats of both sexes were weighed and fasted overnight but allowed free access to water. Their fasting blood glucose levels were determined on day 0 using the glucose oxidase method with the aid of glucose strips using an Accu-Chek® meter. A single dose of alloxan 150 mg/kg (freshly prepared in 0.9% normal saline) was administered intraperitoneally to the rats with the aid of a 1-mL needle and syringe. Rats in Groups VI and VII had only postprandial hyperglycemia at the time of the experiment.

Preparation and administration of the drug and extract
ST is readily soluble in water. The solution was prepared on a daily basis by dissolving 100 mg in 4 mL of distilled water to obtain a stock solution of 25 mg/mL. A dose of 50 mg/kg was administered by oral gavage to each rat. As MO leaf extract is also readily soluble in distilled water, 1.2 g of the leaf extract was weighed and dissolved in 8 mL of distilled water to obtain a stock solution of 150 mg/mL. A dose of 300 mg/kg was administered orally to the rats.

Determination of serum electrolytes
Sodium (Na+), potassium (K+), chloride (Cl+), and bicarbonates (HCO₃⁻) were assayed using the ion-selective electrode method (Potentiometry) with the aid of an Audicom (AC 9900). Three hundred microliters of each test sample was introduced into customized sample fills and fed back into the Audicom. The specific ions to be analyzed were selected, and the Audicom ran the analysis based on the potentiometric principle.

Determination of serum urea and albumin
The principle of determination of serum urea is based on the diacetylmonomoxime method using thiosemicarbazide. The concentration of serum albumin was determined using an autoanalyzer (Rayto Chemray 120) with the aid of Randox kits procured from SEPPIM SAS, in France. Moreover, the principle is based on a colorimetric reaction with the reagent (bromocresol green) in an acidic medium (pH of 4.20).

Determination of lipid profile
The lipids investigated included triglycerides (TGs), total cholesterol (TC), high-density lipoproteins (HDLs), and low-density lipoprotein (LDL). The determination was carried out with an autoanalyzer (Rayto Chemray 120) using Randox kits.

Histological examination
At the end of 42 days, the rats were euthanized using deep anesthesia, and the kidneys were removed, weighed, and fixed in 10% buffered neutral formalin and processed for histopathology studies according to the prescribed method. It involved dehydration, clearing, impregnation, and embedding. The kidneys were dehydrated by graded concentrations of ethanol, impregnated through two changes of molten paraffin wax for 1 h each and embedded in molten paraffin wax to solidify. The blocked embedded tissue was mounted on a wooden block and trimmed. Sections were deparaffinized with xylene and stained with hematoxylin and eosin (H&E) stain. The samples were thereafter observed and photographed, and the severity of glomerular lesions was graded according to the method described by Schäfer et al. i.e.
• Grade 1: Minimal (single glomerulus affected and thickening of mesangium)
• Grade 2: Mild (multiple glomeruli affected and thickening of mesangium)
• Grade 3: Moderate (multiple glomeruli affected and segmental or nodular thickening of the mesangium with synechia of one Bowman’s capsule)
• Grade 4: Marked (majority of glomeruli affected with synechia of Bowman’s capsule or complete sclerosis)
• Grade 5: Severe (general sclerosis of glomeruli in all areas).

Data analysis and presentation of data
All data obtained were entered into the SPSS version 17 statistical package which was used for all statistical analysis. Single-point variables were analyzed using one-way analysis of variance followed by Levene’s test of homogeneity of variance, and Games–Howell or Horchberg post hoc tests were used as appropriate. Results of the study are expressed as mean ± standard deviation. P \leq 0.05 were considered to be statistically significant. Data are presented as tables and charts as well as line graphs as applicable to the collected data. Histological observations are presented as photomicrographs.

Results

Effect of coadministration of sitagliptin and ethanol leaf extract of Moringa oleifera on serum electrolytes in rats
The coadministration of ST and MO did not bring about statistically significant changes in the serum levels of electrolytes compared to DCs and the single agents [Table 1].

Effect of coadministration of sitagliptin and ethanol leaf extract of Moringa oleifera on mean serum levels of urea and albumin in rats
The coadministration of ST and MO, and MO only, increased urea levels compared to DC. Significant increases were observed in diabetic (P = 0.020) and postprandial controls (PPCs) (P = 0.018) compared to normal control [Figure 1a]. No significant effect was seen in albumin levels in the combination group, and a significant increase was observed in normal control (P = 0.034) and ST groups (P = 0.048) compared to DC [Figure 1b].

Effect of coadministration of sitagliptin and ethanol leaf extract of Moringa oleifera on relative kidney weights of rats
A significant increase (P = 0.022) was observed in the combination group (ST and MO), ST-alone-treated rats (P = 0.016), and MO-treated rats (P < 0.001) compared to normal control [Figure 2]. There was no significant decrease in relative kidney weight in the postprandial drug-treated group compared to PPC.

Effect of coadministration of sitagliptin and ethanol leaf extract of Moringa oleifera on lipid profile in rats
There was a statistically significant increase in the level of triglycerides in rats treated with ST and MO compared to rats treated with only ST (P = 0.024) and rats in normal control (P = 0.005) and PPC (P = 0.043) groups. There was no statistically significant difference in the levels of total cholesterol, HDLs, and LDL in the drug-treated groups compared to control. Considerable but nonsignificant decreases were found in TC, TG, and LDL in postprandial-treated group compared to PPC [Table 2].

Effect of coadministration of sitagliptin and ethanol leaf extract of Moringa oleifera on histology of the kidney of rats
Section in normal control shows well-defined Bowman’s space and normal tubules. There was a mild difference in the severity of pathologic lesions seen in sections of kidney of rats treated with ST and MO, as this showed necrosis of most renal tubular epithelial cells with thin Bowman’s space characterized as glomerulosclerosis. Grade 1 compared to DC. Rats in DC had marked

Table 1: Effect of coadministration of sitagliptin and ethanol leaf extract of Moringa oleifera on serum electrolytes in rats

| Group  | Potassium (K⁺) | Sodium (Na⁺) | Chloride (Cl⁻) | Bicarbonate (HCO₃⁻) |
|--------|----------------|--------------|----------------|---------------------|
| NC     | 4.57±0.30      | 137.53±2.57  | 108.86±3.71    | 22.66±2.77          |
| DC     | 5.11±0.30      | 138.62±4.98  | 106.92±4.43    | 23.55±3.59          |
| ST     | 4.76±0.86      | 138.54±6.60  | 109.53±4.60    | 22.53±4.51          |
| MO     | 4.74±0.43      | 130.42±11.70 | 110.53±7.47    | 21.48±5.89          |
| SM     | 5.13±0.28      | 129.35±7.23  | 102.00±6.56    | 20.22±4.65          |
| PPC    | 4.88±0.36      | 135.10±4.27  | 106.58±2.79    | 17.34±9.07          |
| PPSM   | 4.81±0.16      | 138.07±1.49  | 112.20±9.83    | 22.75±4.67          |

ST (50 mg/kg), MO extract (300 mg/kg). Values are mean±SD. No significant difference (P>0.05) between groups (one-way ANOVA). Duration of treatment=42 days. NC=Normal control, DC=Diabetic control, ST=Sitagliptin, MO=Moringa oleifera, SM=ST and MO, PPC=Postprandial control, PPSM=Ameliorative on ST and MO. Ameliorative=Group that received treatment at onset of complication, SD=Standard deviation.
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Figure 2: Effect of coadministration of sitagliptin and ethanol leaf extract of Moringa oleifera on relative kidney weight of rats. Sitagliptin (50 mg/kg), Moringa oleifera extract (300 mg/kg), ameliorative = group that received treatment at onset of complication. Values are mean ± SD. Significant difference A (P = 0.037), B (P = 0.016), C (P < 0.001), D (P = 0.022) compared to normal saline control. One-way ANOVA (Hochberg post hoc test). Duration of treatment = 42 days. Key: NS=Normal control, DC=Diabetic control, ST=Sitagliptin, MO=Moringa oleifera, PPC=Postprandial control, PPSM=Ameliorative on sitagliptin and Moringa oleifera

Table 2: Effect of coadministration of sitagliptin and ethanol leaf extract of Moringa oleifera on lipid profile in rats

| Groups     | Serum lipids (mmol) |
|------------|---------------------|
|            | TC                  | TG                  | HDL                | LDL                |
| NC         | 2.55±0.38           | 0.56±0.23**         | 0.74±0.11          | 1.49±0.24          |
| DC         | 3.23±0.52           | 0.85±0.25*          | 0.74±0.14          | 2.10±0.44          |
| ST         | 3.12±0.67           | 0.73±0.24*          | 0.84±0.12          | 1.82±0.56          |
| MO         | 3.28±0.94           | 1.60±1.02           | 0.69±0.12          | 1.86±0.88          |
| SM         | 3.63±0.19           | 1.35±0.19           | 0.78±0.19          | 2.23±0.45          |
| PPC        | 3.15±0.74           | 0.82±0.17*          | 0.76±0.17          | 2.01±0.63          |
| PPSM       | 2.71±0.08           | 0.77±0.24*          | 0.79±0.15          | 1.63±0.11          |

ST (50 mg/kg), MO extract (300 mg/kg). Values are means±SD. Significant decrease *P<0.05, **P<0.005. For NC (P=0.005), ST (P=0.024), PPC (P=0.043), PPSM (P=0.048), compared with SM. One-way ANOVA. (Games–Howell post hoc test). Duration of treatment=42 days. NC=Normal control, DC=Diabetic control, ST=Sitagliptin, MO=Moringa oleifera, SM=ST and MO, PPC=Postprandial control, PPSM=Ameliorative on ST and MO, TC=Total cholesterol, TG=Triglyceride, HDL=High-density lipoproteins, LDL=Low-density lipoprotein, Ameliorative=Group that received treatment at onset of complication, SD=Standard deviation

Figure 1: Mean serum levels of urea and albumin following coadministration of sitagliptin and ethanol leaf extract of Moringa oleifera in rats. Values are mean ± SEM. Significant difference for (urea mmol/l) A (p=0.020), B (p=0.018) compared to NS. Significant difference for (Albumin g/l) A (p=0.034), B (p=0.048), compared to DC. (one-way ANOVA). Games–Howell post hoc test. Duration of treatment = 42 days. (a) Serum urea mmol/l. (b) serum albumin g/dl

Discussion

Preventing the development and slowing down the progression of DN remains one of the major goals in the management of diabetes mellitus. The functional and structural abnormalities that have been observed in kidneys of diabetic animals resemble that seen in human DN in many respects.[22] These anomalies have been reported within 14 days of alloxan-induced DN.[16]

In this study, diabetic rats showed an increase in serum sodium and potassium levels compared to negative saline control as similarly demonstrated in the previous studies.[23] There was no alteration in the serum levels of electrolytes following coadministration of ST and MO as the values were comparable to DC. Decreases in serum potassium level with high dose of MO have been previously demonstrated, suggesting that the long-term consumption of high dose of MO may affect potassium levels.[24] However, in the present study, electrolyte levels following MO administration remained comparable to DC at the dose of 300 mg/kg. Increased serum levels of potassium and sodium are characteristic of T2DM, and this is a consequence of reduced erythrocyte Na+–K+ ATPase activities implicated in the pathogenesis of nephropathy and neuropathy.[25]
Careful monitoring of serum levels of electrolytes is indicated in diabetes especially where medications that alter Na\(^+\)–K\(^+\) ATPase pump are used as this could have deleterious implications.

Increased serum level of urea is another diagnostic criterion for renal damage. The study demonstrated significantly higher levels of urea in diabetic and postprandial DCs compared to normal control rats. The serum level of urea increased following coadministration of ST and MO compared to normal and DC, indicating the possibility of onset of functional anomalies in the kidneys. The very high levels of urea seen in MO-alone group as similarly reported in diabetic and nondiabetic rats\(^{[13]}\) could be contributory, as no significant difference was recorded with rats that received only ST. This is contrary to reports from previous studies, where very low doses of ST (5 and 10 mg/kg) had produced decreases in serum levels of urea in diabetic rats.\(^{[26]}\) The difference in the present study where ST was used in therapeutic doses is likely related to the extremely high urinary concentrations that result from rapid renal elimination of the drug as seen in rodents.\(^{[6]}\) High serum urea levels are indicative of renal toxicity, and renal toxicity of ST specifically in rodents has been discovered to involve histological changes, which indicates a relatively nonspecific cytotoxicity associated with high renal concentrations.\(^{[27]}\)

Serum albumin levels were significantly lower in the diabetic rats compared to negative control and ST as single-agent-treated rats. Nevertheless, the combination treatment did not increase serum albumin levels in diabetic rats nor in the postprandial hyperglycemic rats as values were comparable to DC. Lower baseline levels of serum albumin have been associated with a rapid decline in kidney function.\(^{[28]}\) The lower level indicates increased clearance by the kidneys, leading to reduced serum levels. Human and animal studies have revealed that serum albumin decreased significantly in patients with reduced creatinine clearance,\(^{[24,29]}\) which is an important biomarker and prognostic indicator of renal function. Seemingly, the concomitant treatment with ST and MO had compromised the positive effect hitherto seen in serum albumin levels in ST-alone-treated rats as similarly seen in serum urea levels.

As regards kidney trophism, the coadministration did not decrease kidney trophism compared to DC and remained significantly higher than normal control. Rats that received only ST followed the same trend, and this has been similarly reported.\(^{[6]}\) However, studies using very
low doses of ST for 28 days in diabetic rats show significant decrease in kidney weights. The initial physiological change in patients with DN is glomerular hyperfiltration, while the initial morphological change is glomerular hypertrophy evidenced as kidney hypertrophism. The finding suggests that the coadministration may not prevent glomerular hypertrophy.

Histopathological findings of the kidneys showed a mild tendency of the coadministration of the drug and extract to reduce the extent of tubulointerstitial damage (renal tubular necrosis) and adherence of glomerulus to Bowman’s capsule (glomerulosclerosis). Nonetheless, mild morphological alterations seen may not necessarily imply an absence of functional anomalies, evident from increased levels of urea seen above. In addition, there was no ameliorative effect on lesions in already damaged kidneys following delayed treatment in the postprandial hyperglycemic group. Administration of ST also showed a mild reduction in severity of tubular necrosis and glomerulosclerosis, and this has been similarly demonstrated for ST. However, other authors recorded marked reductions in tubular and glomerular lesions with very low doses of ST, i.e., 10 mg/kg, which could also be subclinical as an antidiabetic agent. The mild tendency of the coadministration to delay the progression of kidney lesions could be consequent of ST effect as MO has also been associated with kidney toxicity, especially in high doses and on prolonged use. Using the effect on renal lesions, the coadministration of the drug and extract for a period longer than used in this present study may delay the progression of renal injury. Nevertheless, the fact that the coadministration did not ameliorate the lesions in evident DN coupled with functional anomalies already seen calls for legitimate apprehension on chronic use. In addition, this study further confirms the important role of postprandial hyperglycemia in the etiology of DN as seen in the severity of glomerular and tubular lesions with complete sclerosis and synechia of the Bowman’s capsule in the postprandial hyperglycemic control compared to the DC. The coadministration of ST and MO had little or no effect in ameliorating these lesions.

Research has shown the evidence linking hyperlipidemia to renal injury and also that lipids can modulate the progression of chronic renal diseases and may even be primary factors in the pathogenesis of renal tissue injury. Decreased levels of triglycerides were recorded following ST treatment as similarly demonstrated in human studies, where the effects seen were more evident in settings of prandial hyperlipidemia. This could be due to increased Glucagon like polypeptide (GLP)-1 concentration from DPP-4 inhibition, which through some mechanisms limits the release of triglycerides into the circulation after lipid-containing meals. However, there were increased levels of triglycerides following MO administration, which is in contrast to the decreased levels seen in other studies that were conducted in rats having hyperlipidemia and not conventional circulating levels of serum lipids. The evidence from the previous studies reveals that the individual agents demonstrate better antihyperlipidemic effects, especially in settings of high postprandial lipids and not on conventional circulating levels of serum lipids. In this study, the coadministration of ST and MO increased the serum levels of triglycerides compared to levels in normal and DCs and in ST-treated rats. This finding also suggests that the combination treatment may not prevent renal tissue injury as studies have demonstrated that hyperlipidemia in concert with hyperglycemia has been implicated in the development of renal injury in several animal models.

Summary

The coadministration of ST and MO leaf extract did not alter the levels of serum electrolytes and albumin, did not reduce kidney hypertrophism but increased serum urea and triglyceride levels in diabetic rats compared to DCs. Kidney histology showed mild improvements in pathologic lesions with no amelioration of the lesions evident from postprandial hyperglycemia.

Conclusion

ST–MO coadministration did not delay the progression of functional anomalies and renal injury, nor ameliorated significantly the lesions associated with long-standing DN in Wistar rats. Cautionary measures are, therefore, recommended with concurrent use, particularly with long-term administration.

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Conflicts of interest

There are no conflicts of interest.

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