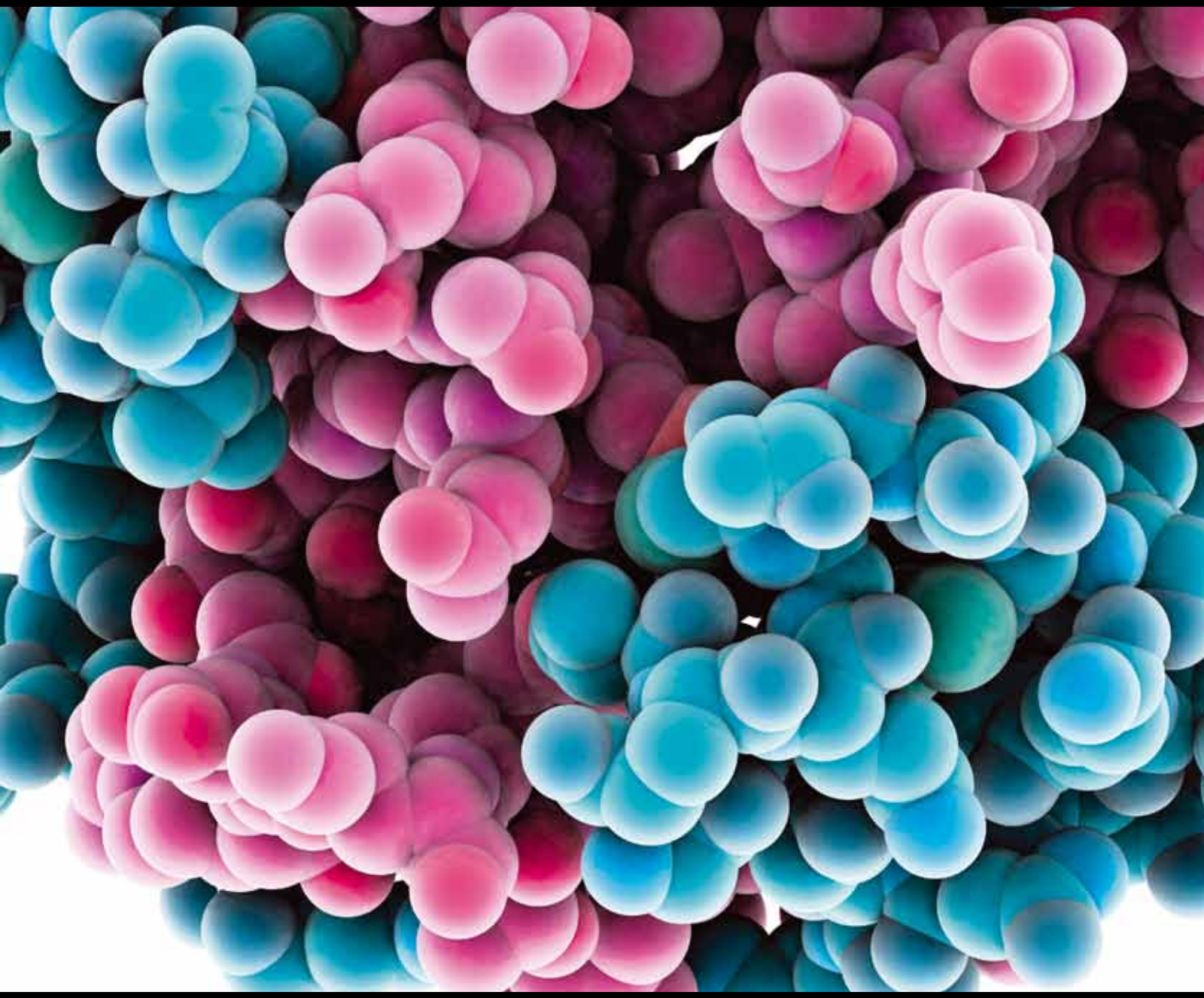
Journal of Diabetes Research

Traditional Medicine in Management of Type 2 Diabetes Mellitus

Guest Editors: Syed Ibrahim Rizvi, Elena Matteucci, and Pinar Atukeren



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Editorial

Traditional Medicine in Management of Type 2 Diabetes Mellitus

Syed Ibrahim Rizvi,[1] Elena Matteucci,[2] and Pinar Atukeren[3]

[1] *Department of Biochemistry, University of Allahabad, Allahabad 211002, India*
[2] *Department of Clinical and Experimental Medicine, Pisa University, 56126 Pisa, Italy*
[3] *Department of Biochemistry, Cerrahpasa Medical School, Istanbul University, 34303 Istanbul, Turkey*

Correspondence should be addressed to Syed Ibrahim Rizvi; sirizvi@gmail.com

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The incidence of type 2 diabetes mellitus has now reached epidemic proportions. Although the disease manifests in the form of hyperglycemia, the cause could be varied ranging from disturbance in insulin secretion, insulin action, insulin resistance, glucose production and glucose uptake, interplay between different hormones, and various kind of stress. Due to such varied etiology, the management of diabetic condition poses a great medical challenge. No single agent has so far been unequivocally accepted as the antidiabetic drug.

Alternative systems of medicine based on traditional wisdom have thrived through ages and are still practiced by a large population for the management of diabetes. A large number of plants have proved their efficacy in management of diabetes especially hyperglycemia. In many cases, scientific studies have validated the antidiabetic nature of plant-based medicines, and the bioactive principle has been isolated and characterized. It is important that more research is done to understand the mechanism(s) involved in the antidiabetic action of large number of plant-based medicines used as traditional therapy for the management of diabetic condition.

The present special volume has brought together some interesting papers reporting the findings of the use of traditional medicines for the treatment of diabetes mellitus.

S. I. Rizvi and N. Mishra provide a good review of the antidiabetic potential and the bioactive compounds present in *Ficus religiosa*, *Pterocarpus marsupium*, *Gymnema sylvestre*, *Allium sativum, Eugenia jambolana, Momordica charantia*, and *Trigonella foenum-graecum*. All these plants are widely used in the Indian subcontinent for the management of diabetic condition.

O. Eleazu et al. explore the chemical composition of cocoyam and unripe plantain flours and their potential in the dietary prevention of diabetic complications.

D. Bailbè and colleagues evaluate the effects of Subetta (containing release-active dilutions of antibodies to beta-subunit of insulin receptor and antibodies to endothelial nitric oxide synthase) in Goto Kakizaki diabetic rats and demonstrate that 28-day administration improves glucose control to an extent similar to that of Rosiglitazone.

H. Y. Jin et al. have tested the efficacy of DA-9801, a mixture of extracts from *Dioscorea japonica* and *Dioscorea nipponica* in the treatment of diabetic peripheral neuropathy in experimental diabetes. They have also presented a comparison of the effect of DA-9801 with lipoic acid.

Statins are very widely used during dyslipidemia. Since type 2 diabetes is frequently associated with dyslipidemia, it is important to investigate the effect of statins on glucose homeostasis in diabetes. Wang et al. report that simvastatin may cause hyperglycemia and have an adverse effect on glucose homeostasis in diabetic rats.

Syed Ibrahim Rizvi
Elena Matteucci
Pinar Atukeren

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Research Article

Ameliorative Potentials of Cocoyam (*Colocasia esculenta* L.) and Unripe Plantain (*Musa paradisiaca* L.) on the Relative Tissue Weights of Streptozotocin-Induced Diabetic Rats

C. O. Eleazu,[1] M. Iroaganachi,[2] and K. C. Eleazu[3]

[1] *Department of Biochemistry, National Root Crops Research Institute, P.O. Box 380, Umuahia, Abia State, Nigeria*
[2] *Department of Food Science and Technology, Abia State Polytechnic, Aba, Nigeria*
[3] *Department of Biochemistry, Michael Okpara University of Agriculture, P.O. Box 380, Umuahia, Abia State, Nigeria*

Correspondence should be addressed to C. O. Eleazu; eleazon@yahoo.com

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Aim. To investigate the ameliorating potentials of cocoyam (*Colocasia esculenta* L.) and unripe plantain (*Musa paradisiaca* L.) incorporated feeds on the renal and liver growths of diabetic rats, induced with 55 and 65 mg/kg body weight of Streptozotocin. *Method*. The blood glucose level of the rats was measured with a glucometer, the protein and glucose and specific gravity (SPGR) in the urine samples of the rats were measured using urine assay strips and urinometer respectively. The chemical composition and antioxidant screening of the test feeds were carried out using standard techniques. *Results*. Administration of the test feeds for 21 days to the diabetic rats of groups 4 and 5, resulted in 58.75% and 38.13% decreases in hyperglycemia and amelioration of their elevated urinary protein, glucose, SPGR, and relative kidney weights. The diabetic rats administered cocoyam incorporated feeds, had 2.71% and 19.52% increases in weight and growth rates, the diabetic rats administered unripe plantain incorporated feeds had 5.12% and 29.52% decreases in weight and growth rates while the diabetic control rats had 28.69%, 29.46%, 248.9% and 250.14% decreases in weights and growth rates. The cocoyam incorporated feeds contained higher antioxidants, minerals and phytochemicals except alkaloids than unripe plantain feed. *Conclusion*. Cocoyam and unripe plantain could be useful in the management of diabetic nephropathy.

1. Introduction

Diabetes is one of the most challenging diseases of the 21st century that affects essential biochemical pathways of the body (carbohydrate, protein, and lipid metabolism) and whose prevalence is rising globally, including the rural Nigerian populations [1, 2]. Due to the inability of the modern therapy to control all the pathophysiological aspects of the disorder as well as the enormous cost it poses on the economy of the developing nations of the world, alternative strategies are urgently needed [3]. The use of medicinal plants in the traditional management of diabetes mellitus could play an important role in the lives of rural people, particularly in remote parts of developing countries which are poorly served with health facilities.

During diabetes, the liver has been reported to decrease in weight due to enhanced catabolic processes such as glycogenolysis, lipolysis, and proteolysis, which is the outcome of lack of insulin in the liver cells while the kidney has been reported to increase in weight due to glucose overutilization and subsequent enhancement in glycogen synthesis [4], lipogenesis, and protein synthesis. These changes could lead to serious microvascular renal complications, which involve a series of metabolic changes in the pathogenesis of diabetic nephropathy. Moreover, despite much research work, the diabetic kidney epidemic keeps increasing, and over 40%

of diabetic patients worldwide have been reported to develop severe diabetic nephropathy [5]. Patients with diabetic kidney failure undergo either painful dialysis or kidney transplant [6] which is costly and harmful.

The diets/medicinal plants that are commonly used in the management of diabetes in Nigeria include acha (*Digitaria exilis*), breadfruit (*Treculia africana*), and beans (*Phaseolus vulgaris*) [7]. However, diabetic patients have often complained of the monotony of staying on a particular diet (personal communication), and this has therefore increased the research into other plants with similar antidiabetic potentials as the ones being used.

Plantain (*M. paradisiaca*) belongs to the "Musaceae" family and it is cultivated in many tropical and subtropical countries of the world. Plantain is a source of starchy staple for millions of people in Nigeria. Unripe plantain contains low quantities of minerals and sugars. Although unripe plantain has been scientifically documented as a hypoglycemic plant [7], there is paucity of information in the literature on its use in the management of diabetic complications.

Cocoyam (*Colocasia esculenta L.*) is a herbaceous perennial plant belonging to the "Araceae" family. In most African countries, cocoyam is mainly cultivated by small-scale farmers [8]. Like many plants of the Araceae family, cocoyam grows from the fleshy corm (tuber) that can be boiled, baked, or mashed into a meal and used as staple food or snack. The corms supply easily digestible starch and are known to contain substantial amounts of protein, vitamin C, thiamine, riboflavin, and niacin and significant amounts of dietary fiber [9]. The flour of cocoyam can be used for the preparation of soups, biscuits, bread, beverages, and puddings. Cocoyam has also been reported in folklore medicine in the management of diabetes mellitus. However, there is no scientific documentation on its role in the management of diabetic complications.

Since the use of medicinal plants in the traditional management of diabetes mellitus could serve as a good alternative for the management of this disease and its complications, we decided to commence a preliminary investigation with the following objectives:

(1) investigating the ameliorating potentials of unripe plantain and cocoyam on the renal and liver growths of diabetic rats induced with two different concentrations of streptozotocin (55 and 70 mg/kg body weight);

(2) determining the chemical composition of cocoyam and unripe plantain flours.

2. Materials and Methods

2.1. Plant Materials. The cocoyam variety (*Colocasia esculenta L.*) known locally in Nigeria as *Edeofe* was freshly obtained at harvest from National Root Crops Research Institute, Umudike, Nigeria, while the false horn unripe plantain variety (*M. paradisiaca*) was bought from Umuahia Main Market, Abia State, Nigeria. They were authenticated in the Department of Botany, Michael Okpara University of Agriculture, Umudike, Nigeria.

2.2. Chemicals. Streptozotocin (STZ), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, and standard quercetin were products of Sigma-Aldrich Chemical Company, UK. All other chemicals that were used in the experiments were bought from HosLab, Umuahia, Abia State, Nigeria, and were of analytical grade.

2.3. Processing of the Plant Materials. The samples were properly washed, peeled, and oven dried at 50°C for 48 hours until constant weight was obtained before being pelletized and incorporated into the rat feeds.

2.4. Proximate Analysis. The moisture, crude protein, lipid, crude fibre, and ash contents of the cocoyam and unripe plantain incorporated feeds were carried out using the methods of the Association of Analytical Chemists [10]. Triplicate samples were incinerated in a muffle furnace (Thermodyn Type 1400 Furnace, Dubuque, IA, USA) at 600°C until a constant weight was obtained. The total carbohydrate content of the samples was obtained by difference (100 − (%moisture + %ash + %lipid + %crude protein)) [10]. The energy value of the test feeds was calculated from the Atwater Formula of 4, 9, and 4 by multiplying the total carbohydrate content by 4, percentage lipid by 9, and percentage protein by 4, respectively, and taking the sum of the products.

2.5. Phytochemical Analysis. The gravimetric method of Harbone [11] was used in the determination of the percentage alkaloid contents of the cocoyam and unripe plantain incorporated feeds while the AOAC methods (1990) were used in the determination of the flavonoid, saponin, and tannin composition of the test feeds.

2.6. Mineral Analysis. The atomic absorption spectrophotometer (Analyst 200, Perkin Elmer, Waltham, MA, USA) was used in the analysis of Fe, Zn, Mg, and Ca; the flame photometric method was used for the analysis of K while the molybdate method [12] was used for the analysis of phosphorous contents of the cocoyam and unripe plantain incorporated feeds.

2.7. Rapid Thin Layer Chromatography (TLC) Free Radical Scavenging Screening. The TLC screening of the antioxidant activity of the methanolic extracts of the cocoyam and unripe incorporated feeds was carried out using the DPPH method as proposed by Mensor et al. [13] with minor modifications. With the aid of a capillary tube, stock solutions (100 mg/mL instead of 1 mg/mL) of the extracts were spotted on a silica gel Thin Layer Chromatographic (TLC) Plate and developed with a solvent system of ethanol : methanol (90 : 10). After development, the chromatograms were dried and sprayed with a 0.3 mM solution of the stable DPPH free radical. The plates were visualized for the presence of yellow spots, and the degree of activity was determined qualitatively from observation of the yellow colour intensity. Yellow spot formed (within 30 minutes of spraying) against a purple background was taken as a positive result. Quercetin was used as the positive control for this assay.

2.8. Animal Experiments

2.8.1. Selection of Animals. Forty male albino rats of the Wistar strain (146.76–228.74 g) obtained from the animal house of the Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria, were used for the study. The rats were kept in metabolic cages in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria. The rats were acclimatized for two weeks to their diets and water prior to the commencement of the experiment and were maintained under a constant 12 h light and dark cycle and at room temperature. The experimental procedures were approved by the Ethical Committee of Michael Okpara University of Agriculture, Umudike, Nigeria. The National Institutes of Health Principles of Laboratory Animal Care [14] were observed.

2.9. Induction of Diabetes. Freshly prepared solution of streptozotocin (0.1 g dissolved in 5 ml of freshly prepared sodium citrate buffer 0.1 M, pH 4.5) was injected intraperitoneally to the rats at a dosage of 65 mg/kg body weight at fasting state [15]. Blood was collected from the tail vein, and the blood glucose concentration was analyzed prior to the commencement of the dietary feeding using a blood glucose meter (Double G Glucometer, USA) and subsequently, twice in a week, throughout the experiment. The STZ-treated rats with fasting blood glucose levels > 200 mg/dL after seven (7) days of induction of STZ were considered to be diabetic. The severity of diabetes was checked in the 24-hour urine samples of the STZ-treated rats using Urine Glucose Detection Strips (Clinistix, Bayer Health Care, USA) and Urine Reagent Strips for urinalysis (qualitative and quantitative) tests for glucose, protein, ketone, and bilirubin (CONDOR-TECHO URS-10, Condor-Teco Medical Technology Co., Ltd., China). The specific gravity of the urine samples was determined with a urinometer. The rats were also observed for physical activity such as excessive thirst (polydypsia) and excessive hunger (polyphagia).

2.10. Experimental Procedure. The experimental rats with stable diabetic condition were then divided into 4 subgroups (groups 2 to 5) with six rats per group while the nondiabetic rats formed the first group as follows:

group 1: normal rats administered standard rat pellets (nondiabetic control);

group 2: diabetic control rats administered 55 mg/kg body weight STZ;

group 3: diabetic control rats administered 70 mg/kg body weight STZ;

group 4: diabetic rats administered cocoyam incorporated feed;

group 5: diabetic rats administered unripe plantain incorporated feed.

Their diets and water were both administered *ad libitum* for 21 days, after which the rats were anesthetized with chloroform and their liver and kidney collected and weighed. The body weights and feed intakes of the rats were recorded on a daily basis, using an electronic weighing balance (Model Scout Pro, Ohaus Corporation, USA), and were calculated as

$$\begin{aligned}
&\text{Percentage change in weight} \\
&\quad = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100, \\
&\text{Feed intake} = \text{Feed administered} - \text{Residue}, \\
&\text{Percentage change in fasting blood glucose (FBG)} \\
&\quad = \frac{\text{Initial FBG} - \text{Final FBG}}{\text{Initial FBG}} \times 100, \\
&\text{Percentage growth rate} \\
&\quad = \frac{\text{Final weight} - \text{Initial weight}}{\text{Experimental duration}} \times 100, \\
&\text{Relative liver weight } (\text{g}/100\,\text{g}) \\
&\quad = \frac{\text{Total liver weight}}{\text{Final body weight}} \times 100, \\
&\text{Relative kidney weight } (\text{g}/100\,\text{g}) \\
&\quad = \frac{\text{Total kidney weight}}{\text{Final body weight}} \times 100.
\end{aligned} \tag{1}$$

2.11. Statistical Analysis. Data was subjected to analysis using the Statistical Package for Social Sciences (SPSS), version 15.0. Results were presented as the means ± standard deviations of triplicate experiments. One-way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $P < 0.05$ using the Duncan Multiple Range Test.

3. Results

The administration of STZ at dosages of 55 and 70 mg/kg body weight to the rats of groups 2 to 5 produced stable diabetic condition within 7 days in most of the experimental rats. Administration of the cocoyam incorporated feed to the diabetic rats of group 4 resulted in a 58.75% decrease in the resulting hyperglycemia while the administration of the unripe plantain incorporated feed to the diabetic rats of group 5 resulted in a 38.13% decrease in the resulting hyperglycemia compared with the diabetic controls and the non-diabetic rats (Table 1).

The diabetic rats of groups 2 to 5 had varying levels of glucose and protein in their urine by the 1st and 2nd weeks of the experimentation (Table 2) which indicated the severity of their diabetic condition. However, by the last week of the experimentation, administration of the test diets (cocoyam and unripe plantain) to the diabetic rats of groups 4 and 5 resulted in their excretion of trace/low amounts of glucose and proteins in their urine.

Table 1: Fasting blood glucose of diabetic and nondiabetic rats (mg/dL).

	Week 0	Week 1	Week 2	Week 3	PC (%)
Group 1	70.67 ± 10.60	87.00 ± 7.55	92.00 ± 8.00	93.67 ± 8.50	−7.67 (increase)
Group 2	93.00 ± 1.52[b]	232.67 ± 12.36[b]	251.00 ± 12.37[b]	280.00 ± 5.29[b]	−20.34 (increase)
Group 3	107.00 ± 9.17[c]	242.00 ± 20.88[b]	229.40 ± 10.03[b]	269.50 ± 13.87[b]	−11.36 (increase)
Group 4	57.00 ± 6.54[a]	373.00 ± 126.06[b]	176.00 ± 37.56[b]	153.88 ± 30.09[b]	58.75 (decrease)
Group 5	55.00 ± 12.25[b]	210.00 ± 9.80[b]	111.00 ± 11.43[a]	129.92 ± 52.80[ab]	38.13 (decrease)

Values are given as mean ± SD. $n = 6$; [a]$P < 0.05$ versus diabetic control; [b]$P < 0.05$ in comparison with normal control within the groups (column); PC: percentage change in fasting blood glucose.

The specific gravity of the urine of the diabetic rats in groups 2 to 5 was elevated, ranging from 1.06 to 1.07 by the 1st and 2nd weeks of the experimentation (Table 2). However, by the last week of the experimentation, administration of the test diets to the diabetic rats of groups 4 and 5 resulted in the amelioration of the elevated specific gravities of their urine.

The body weights of the diabetic control rats of groups 2 and 3 as well as the diabetic rats administered unripe plantain incorporated feed decreased by 28.69, 29.46, and 5.12%, respectively. On the contrary, the body weights of the diabetic rats administered cocoyam incorporated feed increased by 2.71% compared with the non-diabetic rats administered standard rat pellets whose body weights increased by 6.21% (Table 3).

The percentage growth rates of the diabetic control rats of groups 2 and 3 as well as the diabetic rats administered unripe plantain incorporated feed decreased by 248.9, 250.14, and 29.52%, respectively. On the contrary, the percentage growth rates of the diabetic rats administered cocoyam incorporated feed increased by 19.52% compared with the non-diabetic rats administered standard rat pellets whose percentage growth rates increased by 60.14% (Table 3).

The liver weights of the diabetic rats of groups 2 and 3 showed a significant decrease ($P < 0.05$) compared with the nondiabetic rats. In addition, there was no significant difference ($P > 0.05$) in the liver weights of the diabetic rats administered cocoyam feed and the liver weights of the two groups of diabetic control rats. However, the liver weights of the diabetic rats administered unripe plantain feed were significantly lower ($P < 0.05$) than the liver weights of the two groups of diabetic control rats (Table 3).

There were no observed significant differences ($P > 0.05$) in the kidney weights of the nondiabetic, diabetic control, and diabetic rats administered cocoyam and unripe plantain feeds, respectively (Table 3).

The relative liver weights of the diabetic control rats administered STZ at a dosage of 55 mg/kg body weight and the diabetic rats administered unripe plantain incorporated diets were not significantly different from each other ($P > 0.05$) while the relative liver weights of the diabetic rats administered cocoyam incorporated feed differed significantly from the relative liver weights of the two groups of diabetic control rats ($P < 0.05$) (Table 3).

The relative kidney weights of the diabetic control rats were significantly higher than those of the non-diabetic rats and diabetic rats treated with cocoyam and unripe plantain feeds ($P < 0.05$). In addition, there was no significant difference in the relative kidney weight of the diabetic rats administered cocoyam incorporated feeds and the nondiabetic rats ($P > 0.05$) (Table 3).

The feed intake of both the experimental and nondiabetic rats increased by the last week of the experimentation (Table 5).

The feed composition that was given to group 4 diabetic rats comprised 77% cocoyam flour, 9% soya bean flour, 4% vitamin mixture, 2% salt, 4% banana flavour, and 4% groundnut oil while the feed composition that was given to group 5 diabetic rats comprised 77% unripe plantain flour, 9% soya bean flour, 4% vitamin mixture, 2% salt, 4% banana flavour, and 4% groundnut oil.

The proximate composition of the cocoyam incorporated feed indicated that it contained, on average, 3.64% moisture, 10.67% ash, 1.51% crude fibre, 3.42% lipids, 8.44% crude protein, 73.83% carbohydrate, and 359.86 Kcal/100 g of energy while that of the unripe plantain incorporated feed contained, on average, 3.41% moisture, 8.93% ash, 8.52% lipid, 9.76 protein, 69.39% carbohydrate, and 393.24 Kcal/100 g of energy (Table 6).

The Thin Layer Chromatographic screening of the methanolic/ethanolic extracts of the unripe plantain and cocoyam incorporated feeds indicated that they possessed considerable antioxidant activities, though the antioxidant activity of unripe plantain was lower than that of cocoyam as well as standard quercetin (Table 7).

The mineral analysis of the cocoyam incorporated feeds showed that it contained, on average, 38.41 mg/100 g Mg, 113.78 mg/100 g Ca, 35.38 mg/100 g K, 195.81 mg/100 g P, 1.84 mg/100 g Fe, and 0.8 mg/100 g Zn while the plantain incorporated feed contained, on average, 23.64 mg/100 g Mg, 95.76 mg/100 g Ca, 31.48 mg/100 g K, 172.80 mg/100 g P, 1.59 mg/100 g Fe, and 0.62 mg/100 g Zn (Figure 1).

The phytochemical analysis of the cocoyam incorporated feed indicated that it contained, on average, 2.65% flavonoid, 1.01% alkaloid, 0.70% saponin, and 1.06% tannin while the unripe plantain incorporated feed contained, on the average, 2.09% flavonoid, 1.84% alkaloid, 0.57% saponin, and 0.89% tannin (Figure 2).

4. Discussion

The STZ rat model of diabetes is one of the most commonly used models of human disease [16] because it mimics many of the acute and chronic complications of human diabetes, and the model has the advantage of being highly reproducible.

Table 2: Biochemical parameters in the urine of diabetic and non-diabetic rats.

	Week 0	Week 1	Week 2	Week 3
Group 1	Glucose: −ve Protein: Nil SPGR: 1.015–1.02	−ve Trace 1.02	−ve Trace 1.02–1.025	−ve Trace 1.02–1.025
Group 2	Glucose: −ve Protein: Nil SPGR: 1.02–1.025	Trace to 2+ 100 mg/dL 1.06–1.07	+ to 2+ 100–300 mg/dL 1.03–1.04	2+ 100–300 mg/dL 1.025–1.03
Group 3	Glucose: Nil Protein: Trace SRGR: 1.02–1.03	Trace to + 30–100 mg/dL 1.05–1.07	Trace to 2+ 30–100 mg/dL 1.04–1.07	2+ to 3+ 10–300 mg/dL 1.05–1.07
Group 4	Glucose: Nil Protein: Trace SRGR: 1.02–1.025	Trace to + 30–100 mg/dL 1.06–1.07	−ve to 2+ 30–100 mg/dL 1.04–1.07	−ve to trace Nil to 30 mg/dL 1.03–1.04
Group 5	Glucose: Nil Protein: Trace SRGR: 1.02–1.025	Trace to + 30–100 mg/dL 1.06–1.07	−ve to 2+ 30–100 mg/dL 1.04–1.07	−ve to trace Nil to 30 mg/dL 1.02–1.05

−ve: negative or absent; +: positive or present.

Table 3: Body weights of non-diabetic and diabetic rats (g).

	Week 0	Week 1	Week 2	Week 3	PG (%)
Group 1	208.37 ± 20.74	203.47 ± 19.15	205.30 ± 20.19	216.10 ± 21.86	60.14 (increase)
Group 2	189.90 ± 36.02	182.20 ± 5.57	162.91 ± 8.40	129.93 ± 5.38	−248.90 (decrease)
Group 3	192.60 ± 23.00	178.33 ± 18.60	142.63 ± 7.51	125.80 ± 4.12	−250.14 (decrease)
Group 4	164.45 ± 20.29	151.45 ± 16.33	147.40 ± 18.38	155.55 ± 14.78	19.52 (increase)
Group 5	151.00 ± 4.24	121.10 ± 2.97	113.80 ± 2.55	114.90 ± 2.69	−29.52 (decrease)

Each value in the table is the average of triplicate experiments ± std. PG: percentage growth rate.

Table 4: Organ weights and relative organ weights of diabetic and non-diabetic rats.

	Liver weight (g)	Kidney weight (g)	Relative liver weight (g/100 g)	Relative kidney weight (g/100 g)
Group 1 (control)	5.85 ± 0.30	1.33 ± 0.33	2.72 ± 0.16	0.61 ± 0.11
Group 2	4.29 ± 0.23[b]	1.34 ± 0.12	3.30 ± 0.02[b]	1.03 ± 0.03[b]
Group 3	4.64 ± 0.12[b]	1.31 ± 0.16	3.69 ± 0.05[a]	1.04 ± 0.09[b]
Group 4	4.55 ± 0.24[b]	1.00 ± 0.16	2.94 ± 0.17[a]	0.64 ± 0.03[c]
Group 5	3.70 ± 0.00[c]	1.00 ± 0.00	3.23 ± 0.09[b]	0.87 ± 0.02[a]

Values are presented as means ± SD. $n = 6$; [a]$P < 0.05$ versus diabetic control; [b]$P < 0.05$ in comparison with normal control; [c]$P > 0.05$ in comparison with normal control.

Table 5: Feed intake of rats (g/week).

	Week 0	Week 1	Week 2	Week 3
Group 1	112.50 ± 2.59	108.13 ± 6.91	117.73 ± 9.45	118.33 ± 7.36
Group 2	100.97 ± 1.82	116.67 ± 2.24	108.03 ± 0.87	110.60 ± 4.12
Group 3	108.30 ± 0.31	101.27 ± 2.00	109.20 ± 1.43	112.24 ± 2.25
Group 4	100.30 ± 0.99	91.45 ± 6.29	108.75 ± 0.07	121.20 ± 4.10
Group 5	78.40 ± 3.39	85.20 ± 1.70	78.50 ± 2.12	91.40 ± 3.39

Each value in the table is the average of triplicate experiments ± std. $n = 6$ rats per group.

Table 6: Proximate composition of cocoyam and unripe plantain incorporated feeds (%).

Parameter	MC	Ash	CF	Lipid	Crude protein	Carbohydrate	Energy value (Kcal/100 g)
Cocoyam	3.64 ± 0.11	10.67 ± 0.04	1.51 ± 0.22	3.42 ± 0.04	8.44 ± 0.03	73.83 ± 0.04	359.86 ± 0.44
Plantain	3.41 ± 0.81[a]	8.93 ± 0.00[a]	1.45 ± 0.10[a]	8.52 ± 0.00[a]	9.76 ± 0.00[a]	69.39 ± 0.00[a]	393.24 ± 0.06[a]

[a]$P < 0.05$ versus cocoyam feed; MC: moisture content; CF: crude fibre.

Table 7: Free radical scavenging activities of the methanolic/ethanolic extracts of cocoyam and unripe plantain incorporated feeds using rapid DPPH TLC screening.

Plant	Antioxidant activity	Intensity of spots
Cocoyam	Moderate	+++
Unripe plantain	Moderate	++
Quercetin	Strong	+++

The degree of activity, determined qualitatively from the observation of the yellow colour intensity: moderate (++), strong (+++).

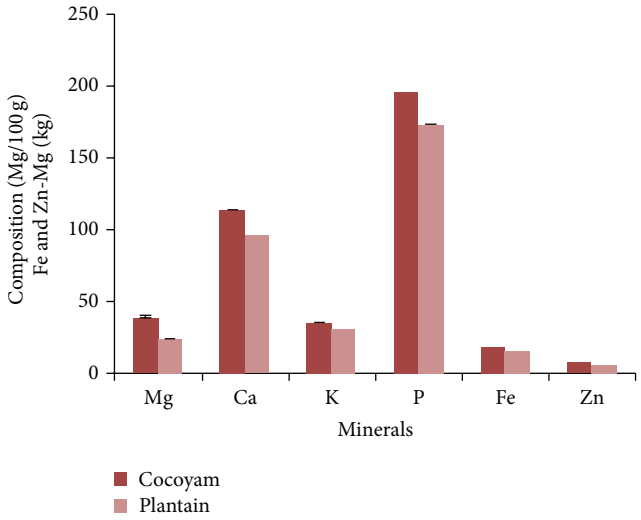

Figure 1: Mineral composition of cocoyam and unripe plantain incorporated feeds.

Findings from this study indicated that the incorporation of 77% cocoyam and unripe plantain into the feeds of the diabetic rats led to 58.75 and 38.13% decreases in their hyperglycemia by the last week of the experimentation, thus confirming the ability of cocoyam and unripe plantain to ameliorate hyperglycemia.

Urinalysis is conducted in almost all disease cases because of its enormous prognostic and diagnostic significance [17].

The excretion of large amounts of glucose in the urine (glucosuria) of the STZ administered rats indicates that their renal threshold of glucose was exceeded since glucosuria occurs when the filtered glucose exceeds the Tm for glucose reabsorption.

The glomerular membrane permits only very small amount of plasma proteins [18]. In 24 hr urine, 1–14 mg/dL of protein may be excreted by the normal kidney [19] while values greater than 30 mg/dL may be indicative of significant proteinuria. Diabetic nephropathy therefore occurs when proteins deposit in the glomerulus [20, 21]. Thus, the occurrence of varying levels of protein in the urine (proteinuria) samples of the diabetic rats of groups 2 to 5 by the 1st and 2nd week of the experiment suggests possibilities of glomerular complication. In addition, the low/trace amounts of detectable proteins in the urine samples of the diabetic rats administered cocoyam and unripe plantain incorporated feeds, by the last week of the experiment, suggest the ability of cocoyam and unripe plantain to ameliorate glomerular

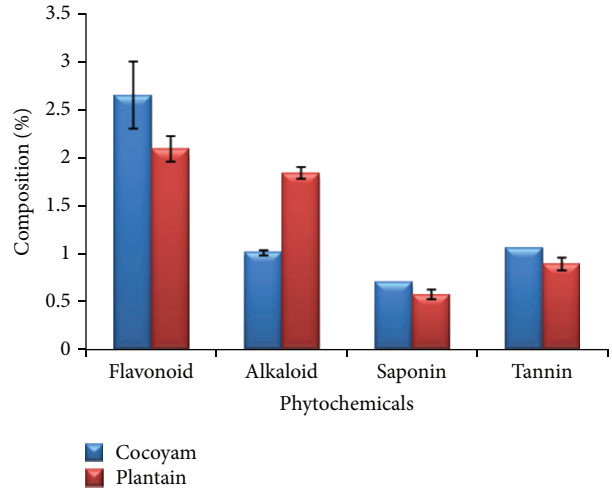

Figure 2: Phytochemical composition of cocoyam and unripe plantain incorporated feeds.

complication in diabetics, and this is a significant finding in this study.

Specific gravity (SPGR) is a urinalysis parameter that aids in the evaluation of kidney function and diagnosis of renal diseases. The kidneys of both humans and other mammals aid in the clearance of various water-soluble molecules via excretion in urine while the concentration of the excreted molecules determines the urine's specific gravity. Random urine may vary in specific gravity from 1.003 to 1.04, and 24-hour urine from normal patients may vary from 1.003 to 1.04 while 24-hour urine from normal patients may vary from 0.016 to 1.025 [22, 23]. However, the specific gravity of rats varies from 1.022 to 1.05 [24]. The elevated levels of SPGR in the urine samples of the diabetic rats of groups 2 and 3 by the 1st week of experimentation, compared with the non-diabetic rats, as observed in this study could be attributed to the elevated levels of glucose as well as protein in their urine, and this may be indicative of other substances that may have permeated the membrane of the glomerular filtrate and were dissolved in the urine. This also suggests, in addition, severe renal complications for the rats of these groups. However, the reduction in the elevated urinary SPGR values of the diabetic rats administered cocoyam and unripe plantain incorporated feeds indicates the ability of cocoyam and unripe plantain to ameliorate glomerular complication in diabetics.

The loss of weight and the decrease in growth rates in the STZ-treated rats despite their increased feed intake, are attributed to the fact that STZ-induced diabetes is characterized by severe loss in body weight, and this reduction is due to loss or degeneration of structural proteins, as the structural proteins are known to be a major contributor to body weight.

Although STZ is a diabetogenic agent, intraperitoneal injections of it in experimental rats have been reported to induce kidney, pancreatic, liver, and uterine tumors in laboratory animals [25].

Diabetic glomerular hypertrophy constitutes an early event in the progression of glomerular pathology which occurs in the absence of mesangial expansion [26].

The increase in the liver weight in proportion to the body weights of the diabetic control rats of groups 2 and 3, compared with the control, as observed in this study is attributed to increased triglyceride accumulation leading to enlarged liver as a result of increased influx of fatty acids into the liver induced by hypoinsulinemia and the low capacity of excretion of lipoprotein secretion from liver resulting from a deficiency of apolipoprotein B synthesis. The findings of this study are in agreement with those of previous researchers [15, 27]. However, the decrease in liver weights in proportion to body weights of the diabetic rats administered cocoyam and unripe plantain feeds indicates the ability of cocoyam and unripe plantain to ameliorate diabetic liver hypertrophy, and this is another significant finding in the present study.

In addition, the increased weight of the kidney in proportion to the body weights of the STZ diabetic control rats of groups 2 and 3, as observed in this study is indicative of diabetic glomerular hypertrophy. However, the decreased weight of the kidney in proportion to the body weight of the diabetic rats administered unripe plantain incorporated feeds indicates the potentials of unripe plantain in ameliorating diabetic kidney hypertrophy while the decreased weight of the kidney in proportion to the body weights of STZ diabetic rats administered cocoyam incorporated feeds which did not differ significantly from the nondiabetic rats suggests the kidney ameliorative potentials of cocoyam in diabetics by maintaining or regenerating the renal cell histoarchitecture, and this is another significant finding in the present study.

The results of the TLC antioxidant screening of the cocoyam and unripe plantain incorporated feeds indicate their antioxidant activities.

The higher quantities of flavonoids, saponin, tannin, Ca, Mg, Fe, Zn, K, P, and crude fibre but lower quantities of alkaloids in the cocoyam incorporated feed compared with the unripe plantain feed are another significant finding in the present study.

Flavonoids, alkaloids, tannins, and flavonoids, as polyphenolic compounds, have been associated with hypoglycemic activity [28]. The inhibition of the glycolytic activity of brush border enzymes by polyphenolic compounds seems to be one of the factors that stimulates hypoglycemic action in some medicinal plants [7]. In addition, flavonoids, as antioxidants, may prevent the progressive impairment of pancreatic beta cell function due to oxidative stress, thereby reducing the occurrence of diabetes. Flavonoids like myricetin, a polyhydroxylated flavonol, stimulate lipogenesis and glucose transport in the adipocytes, hence lowering blood sugar [28, 29]. The alkaloid l-ephedrine promotes the regeneration of islets of the pancreas, following destruction of the beta cells, hence restoring the secretion of insulin and thus corrects hyperglycemia [28]. Tannins inhibit the activities of digestive enzymes such as trypsin and amylase. The tannin epigallocatechin-3-gallate has been reported to exhibit antidiabetic activity demonstrated [29].

Iron influences glucose metabolism and insulin action as well as interferes with insulin inhibition of glucose production by the liver [30].

Magnesium is a cofactor of the glycolytic enzyme hexokinase and pyruvate kinase. It also modulates glucose transport across cell membranes [31, 32]. Zinc plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells [33]. Zinc also influences glyceraldehyde-3-phosphate dehydrogenase in the glycolytic pathway [34].

Dietary fibre decreases the absorption of cholesterol from the gut in addition to delaying the digestion and conversion of starch to simple sugars, an important factor in the management of diabetes. Dietary fibre also functions in the protection against cardiovascular disease, colorectal cancer, and obesity [35]. Thus, we may not be wrong to assume that the presence of higher quantities of flavonoids, saponin, tannin, Ca, Mg, Fe, Zn, K, P, and crude fibre as well as antioxidant activity in cocoyam than unripe plantain flour could have contributed to the higher amelioration of hyperglycemia and renal growth that we observed in this study.

5. Conclusion

The study showed that the use of cocoyam and unripe plantain flours in the dietary management of diabetes mellitus could be a breakthrough in the search for plants that could prevent the development of diabetic nephropathy. Finally, cocoyam flour contains higher quantities of flavonoids, saponin, tannin, Ca, Mg, Fe, Zn, K, P, and crude fibre as well as antioxidant activity but lower quantities of alkaloids than unripe plantain flour.

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Review Article

Traditional Indian Medicines Used for the Management of Diabetes Mellitus

Syed Ibrahim Rizvi[1] and Neetu Mishra[2]

[1] *Department of Biochemistry, University of Allahabad, Allahabad 211002, India*
[2] *Centre of Food Technology, University of Allahabad, Allahabad 211002, India*

Correspondence should be addressed to Syed Ibrahim Rizvi; sirizvi@gmail.com

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Plants have always been a source of drugs for humans since time immemorial. The Indian traditional system of medicine is replete with the use of plants for the management of diabetic conditions. According to the World Health Organization, up to 90% of population in developing countries use plants and its products as traditional medicine for primary health care. There are about 800 plants which have been reported to show antidiabetic potential. The present review is aimed at providing in-depth information about the antidiabetic potential and bioactive compounds present in *Ficus religiosa, Pterocarpus marsupium, Gymnema sylvestre, Allium sativum, Eugenia jambolana, Momordica charantia*, and *Trigonella foenum-graecum*. The review provides a starting point for future studies aimed at isolation, purification, and characterization of bioactive antidiabetic compounds present in these plants.

1. Introduction

Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medical care policy issues [1]. According to International Diabetes Federation (IDF), the number of individuals with diabetes in 2011 crossed 366 million, with an estimated 4.6 million deaths each year [2]. The Indian subcontinent has emerged as the capital of this diabetes epidemic. The reported prevalence of diabetes in adults between the ages of 20 and 79 is as follows: India 8.31%, Bangladesh 9.85%, Nepal 3.03%, Sri Lanka 7.77%, and Pakistan 6.72% [3].

Indians show a significantly higher age-related prevalence of diabetes when compared with several other populations [4]. For a given BMI, Asian Indians display a higher insulin level which is an indicator of peripheral insulin resistance. The insulin resistance in Indians is thought to be due to their higher body fat percentage [5, 6]. Excess body fat, typical abdominal deposition pattern, low muscle mass, and racial predisposition may explain the prevalence of hyperinsulinemia and increased development of type 2 diabetes in Asian Indians.

Diabetes is characterized by metabolic dysregulation primarily of carbohydrate metabolism, manifested by hyperglycemia resulting from defects in insulin secretion, impaired insulin action, or both [7]. Uncontrolled diabetes leads to a plethora of complications affecting the vascular system, eyes, nerves, and kidneys leading to peripheral vascular disease, nephropathy, neuropathy, retinopathy, morbidity, and/or mortality.

According to the World Health Organization (WHO), up to 90% of the population in developing countries uses plants and its products as traditional medicine for primary health care [8]. The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India [9]. There are about 800 plants which have been reported to show antidiabetic potential [10]. A wide collection of plant-derived active principles representing numerous bioactive compounds have established their role for possible use in the treatment of diabetes [10].

The most common and effective antidiabetic medicinal plants of Indian origin are Babul (*Acacia arabica),* bael *(Aegle marmelose),* church steeples *(Agrimonia eupatoria),* onion *(Allium cepa),* garlic *(Allium sativum),* ghrita kumara

(Aloe vera), neem *(Azadirachta indica),* ash gourd *(Benincasa hispida),* Beetroot *(Beta vulgaris),* fever nut *(Caesalpinia bonducella),* bitter apple *(Citrullus colocynthis),* ivy gourd *(Coccinia indica),* eucalyptus *(Eucalyptus globules),* banyan tree *(Ficus benghalenesis)*, gurmar *(Gymnema sylvestre),* gurhal *(Hibiscus rosa-sinesis)*, sweet potato *(Ipomoea batatas),* purging Nut *(Jatropha curcas)*, mango *(Mangifera indica),* karela *(Momordica charantia),* mulberry *(Morus alba),* kiwach *(Mucuna pruriens),* tulsi *(Ocimum sanctum),* bisasar *(Pterocarpus marsupium),* anar *(Punica granatum),* jamun *(Syzygium cumini),* giloy *(Tinospora cordifolia),* and methi *(Trigonella foenum-graecum).* All these plants are a rich source of phytochemicals.

The present review presents the antidiabetic efficacy of some important plants used in traditional system of medicine in India for the management of type 2 diabetes mellitus.

2. Indian Medicinal Plants with Antidiabetic Potential

2.1. Ficus religiosa. Ficus religiosa, commonly known as peepal in India, belongs to family Moraceae. *Ficus religiosa* has been reported to be used in the traditional system of Ayurveda for the treatment of diabetes [11]. *F. religiosa* has been shown to possess a wide spectrum of *in vitro* and *in vivo* pharmacological activities: antidiabetic, hypolipidemic, anticonvulsant, anti-inflammatory, analgesic, antimicrobial, antiviral, antioxidant, antitumor, antiulcer, antianxiety, anthelmintic, antiasthmatic, immunomodulatory, estrogenic, endothelin receptor antagonist, apoptosis inducer, cognitive enhancer, and antihypertensive [12].

Decoction prepared from the bark is used in treatment of diabetes [13]. The plant is believed to contain several bioactive principles including tannins, saponins, polyphenolic compounds, flavonoids, and sterols. Sitosterol-d-glucoside present in the bark of *Ficus religiosa* is believed to elicit hypoglycemic activity in rabbits [14]. The bioactive components present in *Ficus* are leucocyandin 3-O-beta-d-galactosyl cellobioside, leucopelargonidin-3-O-alpha-L rhamnoside [15, 16]. The phytoconstituents present in *Ficus* can impart a significant antidiabetic effect. It has been reported to contain phytosterols, flavonoids, tannins, and furanocoumarin derivatives, namely, bergapten and bergaptol [17].

The leaves of *Ficus religiosa* have also been studied for antihyperglycemic activity [18]. Oral incorporation of aqueous extract of *Ficus religiosa* for 21 days caused a significant lowering in blood glucose levels, and an elevated level of insulin has been observed. The skeletal muscle is an important site for insulin-stimulated glucose uptake. Decrease in muscle and hepatic glycogen in diabetes was observed to be corrected by peepal extract [19, 20].

Secondary complications of diabetes that is hypercholesteremia and hypertriglyceridemia were found to decrease through significantly reduced serum triglycerides and total cholesterol levels in STZ-diabetic rats [21]. Administration of aqueous extract of bark at the dose of 500 mg/kg has been reported to ameliorate blood glucose level, hepatic enzymes, and lipid parameters in streptozotocin-induced diabetic rats [22].

Oxidative stress is one of the major etiologies in the pathogenesis and complications of type 2 diabetes. *F. religiosa* has been reported to modulate the enzymes of antioxidant defence system to combat oxidative stress. Restoration of glutathione and inhibition of malondialdehyde content has shown the antioxidative property of *Ficus religiosa* [23].

2.2. Eugenia jambolana. Eugenia jambolana (black plum or jamun) belongs to the family Myrtaceae. The most commonly used plant parts are seeds, leaves, fruits, and bark. *Eugenia jambolana* is an evergreen tropical tree of 8 to 15 m height, with smooth, glossy turpentine-smelling leaves. The bark is scaly gray, and the trunk is forked. There are fragrant white flowers in branched clusters at stem tips and purplish-black oval edible berries. The berries contain only one seed. The taste is generally acidic to fairly sweet but astringent. This tree is known to have grown in Indian subcontinent and in other regions of South Asia such as Nepal, Burma, Sri Lanka, Indonesia, Pakistan, and Bangladesh from ancient time.

Jamun has been reported to be used in numerous complementary and alternative medicine systems of India and, before the discovery of insulin, was a frontline antidiabetic medication even in Europe. The brew prepared by jamun seeds in boiling water has been used in the various traditional systems of medicine in India [24].

Eugenia jambolana is one of the widely used medicinal plants in the treatment of diabetes and several other diseases. The plant is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaempferol, myricetin, and hydrolysable tannins (1-0-galloyl castalagin and casuarinin). The seeds also contain alkaloid jambosine and glycoside jamboline, which slows down the diastatic conversion of starch into sugar [25].

The whole plant of *Eugenia jambolana* is reported to show antioxidative defence due to numerous phytochemical constituents present in it. The bark of jamun is rich in several bioactive compounds including quercetin, betulinic acid, B-sitosterol, eugenin, ellagic and gallic acid [26], bergenin [27], tannins [28], and flavonoids. Fruits contain glucose, fructose, raffinose [29], malic acid [30], and anthocyanins [31]; leaves are rich in acylated flavonol glycosides [32], quercetin, myricetin, and tannins [33] all of which have hypoglycemic ability.

The blood glucose-lowering effect of *Eugenia jambolana* may be due to increased secretion of insulin from the pancreas or by inhibition of insulin degradation [34]. *Eugenia jambolana* is also reported to have lipid-lowering effect evidenced by reduction of blood cholesterol, triglycerides, and free fatty acids [35]. This effect has been reported to be due to the presence of flavonoids, saponins, and glycosides in the extract which is reported to decrease the activity of enzyme 3-HMG Co-A reductase in liver [36]. *Eugenia jambolana* seed extract is reported to reduce blood pressure probably due to the ellagic acid present in it [33].

Addition of ethanolic extract of seeds and seed powder of *Eugenia jambolana* in alloxan-induced diabetic rats showed

significant reduction in blood sugar level and enhancement in the histopathology of pancreatic islets [37]. Decrease in glycosuria and blood urea levels has also been reported. Similar kind of results has also been reported in numerous studies done on dogs and rabbits [38, 39].

Eugenia jambolana fruit juice is diuretic and has been reported to provide a soothing effect on human digestive system [40]. The gastroprotective effect has also been reported in jamun seeds. Elevation of antioxidant status and mucosal defensive properties might be the possible mechanisms behind gastroprotective properties present in jamun. Presence of flavanoids in the seeds provides the gastric ulcer protective activity to jamun [40]. Jamun shows antiviral activity against goat pox and the highly pathogenic avian influenza (H5N1) virus [41, 42].

The efficacy of *Eugenia jambolana* has also been tested in preclinical and clinical studies [43, 44] for hypolipidemic [45], anti-inflammatory, [46], neuropsychopharmacological [47], antiulcer, [48], antibacterial [49], anti-HIV [50], antidiarrhoeal [49], and antihypertensive activities [47].

2.3. Momordica charantia. *Momordica charantia* (bitter gourd or karela) belongs to the family Cucurbitaceae. Fruit as a whole and fruit's seeds are the parts most frequently used for therapeutic benefits. *Momordica charantia* is a popular fruit used for the treatment of diabetes, cardiovascular diseases, and related conditions amongst the indigenous population of Asia, South America, and East Africa. It is often used as a vegetable in diet. Bitter gourd contains bioactive substances with antidiabetic potential such as vicine, charantin, and triterpenoids along with some antioxidants [51]. Several preclinical studies have documented the antidiabetic and hypoglycaemic effects of *Momordica charantia* through various hypothesised mechanisms [52].

Several studies have demonstrated antibacterial, antiviral, anticancer, and antidiabetic activities, in *Momordica charantia* [53, 54]; however, the antidiabetic activity has been widely reviewed. In several animal studies, bitter gourd has been reported to ameliorate the metabolic syndrome, where diabetes is one of the risk factors [55–57]. In a study conducted on Taiwanese adults, a significant reduction in waist circumference, improvement in diabetes, and symptoms of metabolic syndrome has been observed [58].

The hypoglycemic and lipid-lowering properties of bitter melon have been observed [59]. Studies have shown that *Momordica charantia* can repair damaged β-cells thereby stimulating insulin levels [60] and also improve sensitivity/signalling of insulin [57]. Bitter gourd is also reported to inhibit absorption of glucose by inhibiting glucosidase and suppressing the activity of disaccharidases in the intestine [61].

Ethanolic extract of *Momordica charantia* is reported to show antihyperglycemic effect in normal and streptozotocin diabetic rats which might be due to inhibition of glucose-6-phosphatase and also stimulation of the activity of hepatic glucose-6-phosphate dehydrogenase [62]. Studies have reported that triterpenoids may be the hypoglycemic components present in karela which could be responsible for activation of AMP-activated protein kinase [63]. The blood glucose-lowering activity of karela has been reported in several animal models [64].

Bitter melon is also effective in loosening adiposity. It is reported to decrease the weight of epididymal and retroperitoneal white adipose tissues [54]. Bitter melon is found effective in augmenting skeletal muscle strength, an effect which could be due to higher mRNA expression for the glucose transporter 4 [55]. Extracts/fractions of *Antidesma madagascariense* and *Momordica charantia* were found to significantly inhibit the activity of α-glucosidase, a key carbohydrate hydrolyzing enzyme. However, glycogen-loaded mice showed significant depressive effect on increasing the level of postprandial blood glucose after ingestion of *Momordica charantia* [65]. Presence of saponins to some extent might justify the inhibitory activities on α-amylase and α-glucosidase. Saponins are also supposed to stimulate insulin secretion [66].

2.4. Ocimum sanctum. *Ocimum sanctum* L. (holy basil or tulsi) belongs to the family Lamiaceae. Every part of the plant is used as a therapeutic agent against several diseases. *Ocimum* (holy basil) is reported to grow worldwide. Nutritional and chemical composition of holy basil makes it a plant with immense potential. Eugenol, the active constituent present in *O. sanctum* L., has been found to be responsible for its therapeutic potential [67]. Major bioactive constituents present in the leaves and stems of holy basil include flavonoids, saponins, tannins, triterpenoids, rosmarinic acid, apigenin, isothymusin, isothymonin, cirsimaritin, orientin, and vicenin. Tulsi leaves oil contains eugenol, ursolic acid, carvacrol, linalool, limatrol, and caryophyllene along with eugenol. Seeds oil is known to have fatty acids and sitosterol while seed mucilage contains some sugars. Anthocyanins are present in green leaves. Furthermore, tulsi is also rich in vitamins, minerals, chlorophyll, and many other phytonutrients.

Antidiabetic properties of tulsi were appreciated in Ayurveda [68]. A significant reduction in blood glucose, glycosylated hemoglobin, and urea along with a simultaneous increase in glycogen, hemoglobin, and protein in streptozotocin-induced diabetic rats has been observed when rats were supplemented with ethanolic extract of *O. sanctum* [69]. Leaf extract of *O. sanctum* L has been reported to stimulate the physiological pathways of insulin secretion [70]. *O. sanctum* L. showed serum glucose-lowering effect when the extract was given to normal rats for 30 days [71]. *O. sanctum* L. is reported to reduce the serum level of cortisol and glucose in male mice showing its antiperoxidative effect [72].

Studies have reported that oral administration of alcoholic extract of leaves of *O. sanctum* L. significantly reduced blood sugar level in normal, glucose-fed hyperglycemic, and streptozotocin-induced diabetic rats. Improvement in the action of exogenous insulin in normal rats has also been recorded [73]. Mixed extract of *P. marsupium* and *O. sanctum* has been recorded to not only rectify dyslipidemia but also restore the endogenous antioxidant levels in alloxan-induced diabetic rats [74].

Chloroform extracts of aerial parts of tulsi have been able to ameliorate the derangements in lipid metabolism caused due to diabetes mellitus in alloxan-induced diabetic rats. The extract significantly decreased elevated level of serum glucose and also reversed the cholesterol, triglyceride, and LDL values [75].

The hydroalcoholic extract of *O. sanctum* L. given to stress-induced male Wister rats is reported to significantly prevent the chronic resistant stress induced rise in plasma cAMP level, myocardial superoxide dismutase, and catalase activities [76]. Ursolic acid isolated from *O. sanctum* L. has been reported to protect heart cells from Adriamycin-induced lipid peroxidation [77]. *O. sanctum* L is also used to control blood cholesterol. A marked decrease in serum cholesterol, triacylglycerol, and LDL + VLDL cholesterol as compared to untreated cholesterol-fed group was observed in cholesterol-fed rabbits when supplemented with *O. sanctum* L. seed oil for four weeks [78]. A similar kind of study performed on normal albino rabbits showed lowered levels of serum total cholesterol, triglyceride, phospholipids, and LDL-cholesterol and a significant boost in the HDL-cholesterol and total fecal sterol contents with incorporation of fresh leaves of tulsi [79].

Along with antidiabetic and cardioprotective effects, *O. sanctum* L. has also been suggested to acquire antifungal [80], antimicrobial [81], analgesic [82], anthelmintic [83], anti-stress [9], antifertility [84], anti-inflammatory [85], antioxidant [78, 86], gastroprotective [87], immunomodulatory [88], antithyroidic [89], anticancer [90], and radioprotective effects [91, 92]. Tulsi is reported to provide a protection for central nervous system [93] and against sexually transmitted diseases [94].

2.5. Pterocarpus marsupium. *Pterocarpus marsupium* (indian kino tree, bijasar) belongs to the family Fabaceae. Plant parts used most commonly are heart wood, leaves, flowers, bark, and gum. *Pterocarpus marsupium* grows very well in India, Nepal, and Sri Lanka. As per Ayurveda, it is one of the most versatile medicinal plants with a wide spectrum of biological activities. Every part of the tree has been acknowledged for its therapeutic potential. This tree grows up to 30 metres in height. Compositional studies on bijasar have shown this plant to be a good source of polyphenols. *P. marsupium* contains terpenoids and phenolic compounds: β-sitosterol, lupenol, aurone glycosides, epicatechins, and iso-flavonoids [95, 96].

P. marsupium is known for its antidiabetic activity [97]. Besides eliciting a strong antidiabetic property, *Pterocarpus marsupium* is reported to be effective against several diseases. It is reported to be antiobesity, antihyperlipidemic [98], anti-inflammatory, anthelmentic [99, 100], antioxidative, antitumorigenic and antiulcerative [71, 101].

Pterocarpus marsupium is reported to have not only hypoglycemic property but also β-cell protective and regenerative properties [102], effects which have been attributed to the flavonoid content in the plant. Complete restoration of normal insulin secretion and regeneration of beta cells have been reported in various experimental models of diabetes [103, 104]. A methanolic extract of *Pterocarpus marsupium* when supplemented for 7 and 14 days to STZ-diabetic rats showed normalization of streptozotocin-distressed serum glucose by correcting glycosylated hemoglobin (HbA1c), serum protein, insulin, alkaline and acid phosphatase, and albumin levels [105].

The blood sugar-lowering activity has been endorsed to be due to the presence of tannates in the extract of the plant. Antihyperlipidemic activity is contributed probably to the marsupin, pterosupin, and liquiritigenin present in the plant [106]. (−) Epicatechin has been shown to have insulinogenic property by enhancing insulin release and conversion of proinsulin to insulin. (−) Epicatechin has also been shown to possess insulin-like activity [107, 108]. Epicatechin has also been shown to strengthen the insulin signalling by activating key proteins of that pathway and regulating glucose production through AKT and AMPK modulation in HepG2 cells [109].

2.6. Trigonella foenum-graecum. *Trigonella foenum-graecum* (fenugreek, methi) belongs to the family Fabaceae. Seeds and leaves are the most frequently used parts of the plant. *Trigonella foenum-graecum* L. (fenugreek) is cultivated throughout India and in some other parts of the world as a semiarid crop [80]. It is used both as a vegetable and as a spice in India. Fenugreek is well known for its pungent aromatic properties, and it is a flavoring agent in food [110]. Studies on different experimental models have proved that fenugreek has strong antidiabetic properties [111, 112]. Human studies have also confirmed the glucose and lipid-lowering ability of fenugreek [113].

Several studies have demonstrated that fenugreek seed extract, mucilage of seeds, and leaves can decrease blood glucose and cholesterol levels in humans and experimental diabetic animals [114, 115]. The therapeutic potential of fenugreek is primarily due to the presence of saponins [116], 4-hydroxyisoleucine [117], and trigonelline, an alkaloid [118] and a high-fiber content [119].

The antihyperglycemic effect has been correlated with decline in somatostatin and high plasma glucagon levels [120]. Fenugreek seed powder has been shown to normalize the activity of creatinine kinase in liver, skeletal muscles, and heart of diabetic rats [121]. The antihyperglycemic effect of fenugreek has been hypothesized to be due to the amino acid 4-hydroxyisoleucine which acts by the enhancement of insulin sensitivity and glucose uptake in peripheral tissues [122]. The steroids present in methi have been reported to reduce blood glucose level when supplemented to diabetic rats [123]. A considerable increment of the area of insulin-immunoreactive β cells has been observed [124].

A study on intestinal and renal disaccharidases activity in STZ-induced diabetic rats proved the beneficial effects of fenugreek seed mucilage by enhancing the reduction in maltase activity during diabetes [125]. The optimistic influence of fenugreek supplementation on intestinal and renal disaccharidases has been reported [126]. A marked reduction in renal toxicity has been observed when fenugreek oil is incorporated in the diet of alloxanized rats [125].

2.7. Gymnema sylvestre. Gymnema sylvestre (gurmar) belongs to the family Asclepiadaceae. It is a herb native to the tropical forests of India and Sri Lanka. *G. sylvestre* is a large climber, with roots at nodes. It is a potent antidiabetic plant used in ayurvedic preparations. Several studies have proved its antidiabetic potential in animal models [125]; when combined with acarbose it is reported to reduce intestinal transport of maltose in rats [127]. Absorption of free oleic acid in rats has also been reduced [128].

Aqueous extract of *G. sylvestre* has been reported to cause reversible increases in intracellular calcium and insulin secretion in mouse and human β cells with type 2 diabetes [129]. Regeneration of the cells in the pancreas might raise the insulin levels [130]. *G. sylvestre* can also help prevent adrenal hormones from stimulating the liver to produce glucose in mice, thereby reducing blood sugar levels [131]. A group of triterpene saponins, known as gymnemic acids and gymnemasaponins are found to be present in *G. sylvestre* which are responsible for the reported pharmacological properties.

Oral administration of *Gymnema* is reported to be effective against chronic inflammation [132], obesity [133, 134], and pancreatic β cell dysfunction [135]. *G. sylvestre* suspension shows tremendous diabetic potential against alloxan-induced diabetic albino male rats [136]. The hypoglycemic effect of ethanolic extract of *G. sylvestre* is reported to be due to enhanced effect of insulin which comes into play by increasing either the pancreatic secretion of insulin from β cells or its release from the bound form [130, 137, 138]. A significant correlation between the good glycemic control and phospholipid levels has been observed [139]. Oral administration of *G. sylvestre* to rats has been reported to result in increased utilization of glucose and/or by decreasing mobilization of fat [136]. A significant reduction in body weight, plasma proteins, and total hemoglobin levels has also been observed [136].

2.8. Allium sativum. Allium sativum (garlic) commonly called lahsun belongs to the family Amaryllidaceae. Leaves and bulb are the parts frequently used. As per Ayurveda it is a miraculous plant used against a variety of problems including insect bites, intestinal worms, headache, and tumors [140]. Garlic is also used in folk medicine for the management of cardiac diseases, cancer, parasitic, fungal diseases, and diabetes [141, 142]. The principle bioactive components present in garlic are allicin, allixin, ajoene, and other organosulphur compounds.

Biological and therapeutic functions of garlic are basically due to the organosulphur compounds they possess [143]. These chemical components are thought to exhibit numerous biological effects including lowering of cholesterol and glucose, cancer prevention, and antimicrobial properties [144]. Studies have proved that the consumption of garlic significantly decreased fasting blood sugar levels [145]. Diallyl trisulfide has been proved to improve glycemic control in STZ-induced diabetic rats. [146] Incorporation of garlic juice resulted in better utilization of glucose in glucose tolerance tests performed in rabbits, while allicin at a dose of 250 mg/kg was 60% as effective as tolbutamide in alloxan-induced diabetic rabbits [147].

Garlic may act as an antidiabetic agent by increasing either the pancreatic secretion of insulin from the β cells or the release of bound insulin [148]. Allicin is supposed to enhance serum insulin by combining with cysteine and sparing it from SH group reactions [147]. The beneficial effects of N-acetylcysteine, an organosulfur from allium plants, on serum lipids and glucose are related to its antioxidant property. N-Acetylcysteine is reported to reduce the oxidative stress by improving the endogenous antioxidant defences [149].

Allicin, a sulfur-containing compound, is responsible for the pungent flavour and significant hypoglycemic activity in garlic. This effect is supposed to be due to enhanced hepatic metabolism, release of insulin, and/or insulin-sparing effect [150, 151]. S-allyl cystein sulfoxide the precursor of allicin is reported to control lipid peroxidation and hyperglycemia in rats [152].

Cardiovascular complications of diabetes are reported to be prevented by the consumption of garlic [153]. Saponins are reported to reduce serum cholesterol levels [154]. Garlic juice has been found to exert antioxidant and antihyperglycemic effects in alloxan-induced diabetic rats [155].

Phytochemicals present in garlic also show antioxidative property evidenced by scavenging of reactive oxygen species [156] and increasing cellular antioxidant enzymes: superoxide dismutase, catalase, and glutathione peroxidase [157]. Garlic alone and with ginger and turmeric when tested against oxidative stress in streptozotocin (STZ)-nicotinamide diabetic rats showed 80–97% increment in the signs of hyperglycaemia and dyslipidaemia, 26–37% increase in the production of insulin and enrichment in the antioxidant defence system along with a 60–97% decrease in lipid peroxidation [158]. Administration of raw garlic homogenate was found to normalise both hepatic TBARS and GSH levels and also improve insulin sensitivity and oxidative stress in fructose-fed rats [159]. Numerous studies report that aged garlic extract inhibit the generation of glycation-derived free radicals and AGEs *in vitro*. S-Allyl cysteine, one of the bioactive ingredients of aged garlic, is a known antioxidant that possesses the capacity to inhibit AGEs synthesis [160].

3. Conclusion

As per Ayurveda, there exists a huge collection of plants with antidiabetic potential. Only few of them have been scientifically proven and a lot more have yet to be explored and proved. *Ficus religiosa, Gymnema sylvestre, Allium sativum, Trigonella foenum graecum, Pterocarpus marsupium, Ocimum sanctum, Momordica charantia, Eugenia jambolana,* and *Ficus religiosa* have shown varying degrees of hypoglycemic activity. These plants have also been reported to contribute in control of complications of diabetes. Future studies may target isolation, purification, and characterization of bioactive compounds present in these plants. The outcome of such studies may provide a starting point for development of potential antidiabetic drugs. This review may be helpful in the management of diabetes.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Research Article

The Novel Oral Drug Subetta Exerts an Antidiabetic Effect in the Diabetic Goto-Kakizaki Rat: Comparison with Rosiglitazone

Danielle Bailbé,[1] Erwann Philippe,[1] Evgeniy Gorbunov,[2] Sergey Tarasov,[2] Oleg Epstein,[2] and Bernard Portha[1]

[1] *Laboratoire B2PE (Biologie et Pathologie du Pancréas Endocrine), Unité BFA (Biologie Fonctionnelle et Adaptive), Equipe 1, Université Paris-Diderot et CNRS EAC 4413, Bâtiment Buffon, 5éme étage, Piéce 552A, 4, rue Lagroua Weill Hallé, Case 7126, 75205 Paris Cedex 13, France*
[2] *OOO "NPF "MATERIA MEDICA HOLDING", 3rd Samotyochny Per., 9, Moscow 127473, Russia*

Correspondence should be addressed to Evgeniy Gorbunov; gorbunovea@materiamedica.ru

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The aim of the present study was to evaluate the potential antidiabetic effects of two-component drug Subetta and its components (release-active dilutions of antibodies to β-subunit insulin receptor (RAD of Abs to β-InsR) and to endothelial nitric oxide synthase (RAD of Abs to eNOS)) in Goto-Kakizaki (Paris colony) (GK/Par) diabetic rats. Subetta was administered orally for 28 days once daily (5 mL/kg) and compared to its two components (2.5 mL/kg), Rosiglitazone (5 mg/kg), and vehicle (5 mL water/kg). At day 28, fasting plasma glucose levels were significantly decreased only in Subetta and Rosiglitazone groups as compared to vehicle ($P < 0.01$): 147 ± 4 mg/dL and 145 ± 4 mg/dL and 165 ± 4 mg/dL, respectively. The data of glucose tolerance test showed that Subetta and RAD of Abs to β-InsR (similar to Rosiglitazone) prevented significantly ($P < 0.01$) the age-related spontaneous deterioration of glucose tolerance as seen in the control group. Subetta and RAD of Abs to β-InsR did not significantly modify the glucose-induced insulin secretion. Chronic administration of Subetta and RAD of Abs to β-InsR improves glucose control, to an extent similar to that of Rosiglitazone. We hypothesize that Subetta and RAD of Abs to β-InsR mostly act via an insulin-sensitizing effect upon target tissues.

1. Introduction

According to the WHO (2012), more than 347 million people worldwide suffer from diabetes mellitus, and among these 90% have type 2 diabetes. That is why type 2 diabetes is ranked high in prophylactic, therapeutic, and rehabilitation programs worldwide. Despite the high efficacy of per oral antidiabetic drugs in treatment of type 2 diabetes, there are a number of limitations associated with their side effects (hypoglycemia, heart failure, body weight gain, lactic acidosis, low tolerability of some drugs, necessity of multiple-dose administration, etc.). The search for new targets, as well as development of innovative approaches for effective and safe action on these targets remains a topical issue. The use of the release-activity phenomenon, which consists in the modifying action exerted by specifically processed ultradilutions on the starting substance [1, 2], could lay the foundation for one of those innovative methods. The drugs of this class containing the so-called release-active dilutions of antibodies [3] demonstrated a fundamentally new pro-antigen (cotargeted with antigen) targeted activity, based on the ability of release-active dilutions of antibodies to modify the nature of antigen-target (molecule-target) interaction via the mechanism of conformational modification. The efficacy and safety of the drugs were intensively studied and proved in different experimental models and in clinical studies as well [1, 4–18].

Subetta contains release-active dilutions of antibodies to β-subunit insulin receptor and antibodies to endothelial NO synthase. In experimental model of streptozotocin-induced diabetes Subetta showed pronounced antihyperglycemic activity, which is comparable to that of the reference drug Rosiglitazone: Subetta decreases high plasma levels of glucose, urine levels of ketone bodies and improves glucose

uptake in peripheral tissues [7]. Toxicological studies proved a high safety of the drug. The aim of the present preclinical study was to evaluate the potential antidiabetic effects of Subetta and its components in GK/Par diabetic nonobese rats.

2. Materials and Methods

Male diabetic (GK) Goto-Kakizaki rats were obtained from the Paris colony (GK/Par), maintained at the University Paris-Diderot animal core, in accordance with accepted standards of animal care as established in the French National Center for Scientific Research guidelines. The characteristics of the adult GK/Par rats have been described previously [19]. Adult male animals (10-week old) were used for this study.

The animals were kept in animal room under artificial light from 8 am to 8 pm. All animals were fed *ad libitum* with a commercial pelleted chow (diet 113, SAVE, Villemoisson-sur-Orge, France) and had free access to tap water.

Animals were assigned randomly to six different groups ($n = 12$ in each group). There was no statistically significant difference among groups with respect to body weight and blood glucose on Day 0 (d0).

The first group (PGK1 group) was given release-active dilutions (ultrahigh dilutions) of antibodies to β-subunit insulin receptor (RAD of Abs to β-InsR) (OOO "NPF "MATERIA MEDICA HOLDING", Moscow, Russia) (2.5 mL/kg body weight) once a day, as a solution in distilled water (2.5 mL/kg body weight), so that the total volume administered 5 mL/kg body weight. The second group (PGK2 group) was given release-active dilutions (ultrahigh dilutions) of antibodies to endothelial NO synthase (RAD of Abs to eNOS) (active pharmaceutical ingredient of drug "Impaza," OOO "NPF "MATERIA MEDICA HOLDING", Moscow, Russia) (2.5 mL/kg body weight) once a day, as a solution in distilled water (2.5 mL/kg body weight). The third group (PGK3 group) was given Subetta (5 mL/kg body weight) once a day, as a water solution (2.5 mL/kg body weight of RAD of Abs to β-InsR + 2.5 mL/kg body weight of RAD of Abs to eNOS). The dose of Subetta was the same as that used in the previous study in rats with streptozotocin-induced diabetes (5 mL/kg body weight), where drug showed significant antihyperglycemic efficacy [7]. So, Subetta contains RAD of Abs to β-InsR and RAD of Abs to eNOS. Ultrahigh dilutions of antibodies were obtained using routine methods described in the European Pharmacopoeia (6th Edition, 2007) as previously detailed [5] Antigen affinity purified rabbit polyclonal antibodies to β-subunit of insulin receptor or to endothelial nitric oxide synthase were produced from rabbit antiserum in accordance with the requirements imposed on animal immunosera for human use as described in the European Pharmacopeia (6th Edition, 2007). All dilutions were prepared in glass vials. Rabbit polyclonal antibodies to β-subunit insulin receptor or antibodies to endothelial NO synthase were mixed with a solvent (ethanol-water solution) and shaken for 1 min. to produce C1 dilution. All subsequent dilutions consisted of one part of the previous dilution to 99 parts of solvent (ethanol-water solution for intermediate dilutions and distilled water for preparation of the final dilution), with succession between each dilution. So, RAD of Abs to β-InsR, RAD of Abs to eNOS, and Subetta contain release-active dilutions of respective initial substances (or its combination in case of Subetta), which were diluted up to receiving mixture of final dilutions C12 + C30 + C200. Solutions were prepared in sterile conditions, avoiding direct intense light, and were stored at room temperature. RAD of Abs to β-InsR, RAD of Abs to eNOS, and Subetta were provided by OOO "NPF "MATERIA MEDICA HOLDING" as a ready-to-use solution in distilled water.

The fourth group (H_2O) received vehicle only (distilled water; 5 mL/kg body weight) and was used as the control diabetic group for RAD of Abs to β-InsR, RAD of Abs to eNOS, and Subetta. The fifth group (Rosi) received Rosiglitazone (ref 223207-34-1, Interchim, France) (5 mg/kg body weight, in 5 mL/kg body weight) as a solution in 1% carboxy-methyl-cellulose (CMC) (ref 5678, Interchim, France). The sixth group (CMC) receiving carboxy-methyl-cellulose only (1% CMC, 5 mL/kg body weight) was used as the control diabetic group for Rosi. The drugs and the vehicles were orally administered (using a gastric tube) in one daily dose (at 9.00) for four weeks (d1 to d28). Animal body weight, food intake, water intake, and basal (at 9.00, i.e., in the nonfed state) plasma glucose and insulin levels were checked twice a week throughout the protocol. On d0 and d28, whole blood HbA1c was measured in each group. On d1 and d28, basal plasma glucagon, GLP-1, leptin, and adiponectin levels were measured in each group.

Oral glucose tolerance tests (OGTT, 2 g glucose/kg body weight) were sequentially performed on each rat from each group: two days before the first administration of any drug (d0), on the first day of treatment (d1) (at 12.00 i.e., 3 hours after the drug administration), and on the last day of the treatment period (d28) (at 12.00 i.e., 3 hours after the drug administration). Each OGTT was performed at 12.00 in nonanaesthetized rats fasted from 9.00 (postabsorptive state). Blood samples were collected sequentially from the tail vein before (0) and 5, 10, 15, 30, 60, and 120 min after glucose intake. They were then centrifuged and the plasma was separated. Plasma glucose concentration was immediately determined on a 10 μL aliquot and the remainder was kept at −20°C until insulin radioimmunoassay.

Plasma glucose was determined with a glucose analyser (Beckman). Immunoreactive insulin in the plasma was estimated with an ultrasensitive ELISA for rat (ref 80-INSRTU-E01 insulin kit from ALPCO/Eurobio). GLP-1 (ref YK160 GLP1 EIA kit from Yanaihara Institute/AbCys), glucagon (ref YK090 glucagon EIA kit from AbCys), leptin (ref 22-LEPMS-E01 leptin EIA kit from ALPCO/Eurobio), and adiponectin (ref 22-ADPRT-E01 adiponectin ELISA kit from ALPCO/Eurobio) were assayed from the same aliquot (50 microliters) of plasma. HbA1c (ref 280-008EX Micromat II from Bio-Rad) was measured from 10 microliters of whole blood.

Insulin and glucose responses during the glucose tolerance test were calculated as incremental plasma insulin values integrated over the 120 min period following the glucose

Table 1: Effect of test articles and appropriate controls on Goto Kakizaki/Par male rats basal (non-fed state) plasma glucose level (mg/dL; M ± SEM) during treatment period (28 days) ($n = 12$ in each group).

Groups	Plasma glucose level (mg/dL)							
	Day 1 (d1)	Day 4 (d4)	Day 8 (d8)	Day 12 (d12)	Day 16 (d16)	Day 20 (d20)	Day 24 (d24)	Day 28 (d28)
RAD of Abs to β-InsR	168 ± 8	167 ± 8	166 ± 8	165 ± 7	164 ± 6	153 ± 5**	156 ± 4*	153 ± 4**
RAD of Abs to eNOS	163 ± 10	159 ± 9	159 ± 8	179 ± 9*	169 ± 10	159 ± 10	167 ± 9	167 ± 11
Subetta	167 ± 3	165 ± 4	158 ± 5	163 ± 5	160 ± 7	152 ± 5*	158 ± 5	147 ± 4$^{***\#\#}$
Rosi	169 ± 3$^{\#\#}$	154 ± 5	156 ± 5	153 ± 4	148 ± 3	150 ± 4	151 ± 5	145 ± 4
H2O	176 ± 6	176 ± 10	174 ± 12	169 ± 10	157 ± 6*	162 ± 10	165 ± 5*	165 ± 4*
CMC	186 ± 9	172 ± 8	167 ± 13	157 ± 8**	148 ± 5**	144 ± 4**	155 ± 4***	149 ± 5**

$^{*}P < 0.05$ (versus d1)
$^{**}P < 0.01$ (versus d1)
$^{***}P < 0.001$ (versus d1)
$^{\#\#}P < 0.01$ (versus control (H_2O or CMC, resp.)).

Table 2: Whole blood HbA1c (%; M ± SEM), basal (non-fed state) plasma insulin (ng/mL; M ± SEM), and GLP-1 (ng/mL; M ± SEM) in Goto Kakizaki/Par male rats ($n = 12$ in each group).

Groups	Whole blood HbA1c level (%)		Plasma insulin level (ng/mL)		Plasma GLP-1 level (ng/mL)	
	Day 0 (d0)	Day 28 (d28)	Day 1 (d1)	Day 28 (d28)	Day 1 (d1)	Day 28 (d28)
RAD of Abs to β-InsR	8.38 ± 0.27$^{ND=4}$	8.76 ± 0.26	2.7 ± 0.4	2.7 ± 0.4	7.70 ± 0.74$^{\#}$	7.24 ± 0.49$^{\#}$
RAD of Abs to eNOS	8.90 ± 0.21	9.85 ± 0.44*	2.8 ± 0.4	2.7 ± 0.4	6.10 ± 0.77	5.42 ± 0.66
Subetta	9.32 ± 0.50$^{ND=3}$	8.87 ± 0.13	3.0 ± 0.3	2.2 ± 0.2**	5.70 ± 0.44	6.56 ± 0.61
Rosi	8.78 ± 0.24	9.18 ± 0.29	1.8 ± 0.4$^{\#\#}$	1.2 ± 0.1$^{*\#}$	5.96 ± 0.52	5.51 ± 0.58
H2O	9.15 ± 0.17	8.77 ± 0.12	3.2 ± 0.4	2.4 ± 0.3***	5.30 ± 0.49	5.30 ± 0.50
CMC	9.30 ± 0.21$^{ND=6}$	9.20 ± 0.39$^{ND=2}$	4.0 ± 0.5	2.0 ± 0.3**	5.55 ± 0.47	4.58 ± 0.44

ND = 2, 3, 4 or 6: number of rats, for which whole blood HbA1c level (%) was not determined because of failure of the assays or insufficient number (volume) of samples
$^{*}P < 0.05$ (versus d0 or d1, resp.)
$^{**}P < 0.01$ (versus d1)
$^{***}P < 0.001$ (versus d1)
$^{\#}P < 0.05$ (versus control (H_2O or CMC, resp.))
$^{\#\#}P < 0.01$ (versus control (H_2O or CMC, resp.)).

injection (AUC insulin; ng/mL/120 min) and corresponding incremental integrated blood glucose values (AUC glucose; g/L/120 min) (sum of value at t_n − value t_0, for $t_n = 5$, 10, 15, 30, 60, and 120 min).

Statistical analysis was performed with following software-R version: 2.13.1, RCOM server version: 2.1. All the results are presented as means ± S.E.M. and statistical significance of differences between means values was evaluated by Mann-Whitney and Wilcoxon tests for unpaired and paired data, respectively.

3. Results and Discussion

All rats entered the study survived until the end of the study. Weight gain and water intake in RAD of Abs to β-InsR, RAD of Abs to eNOS, Subetta, and CMC groups were similar to those of the control H_2O group during the four-week period (data not shown). RAD of Abs to β-InsR, RAD of Abs to eNOS, and CMC had no significant effect on basal food intake (versus the H_2O control group) while in Subetta group food intakes on d1 and d28 were slightly but significantly ($P < 0.05$) lower as compared to the H_2O control group: 69 ± 1 g/kg/day versus 74 ± 1 g/kg/day and 66 ± 1 g/kg/day versus 71 ± 1 g/kg/day, respectively. In the Rosi group, weight gain of the GK rats was similar to that of CMC rats, whereas water and food intakes on d28 were significantly lower ($P < 0.01$) as compared to the CMC control group: 88 ± 2 mL/kg/day versus 62 ± 1 g/kg/day and 102 ± 3 mL/kg/day versus 71 ± 2 g/kg/day, respectively.

Chronic treatment with Subetta and RAD of Abs to β-InsR, but not with RAD of Abs to eNOS, prevented diabetes progression and significantly decreased plasma glucose as compared with baseline values (153 ± 4 dg/mL versus 168 ± 8 dg/mL ($P < 0.01$) and 147 ± 4 dg/mL versus 167 ± 3 dg/mL ($P < 0.001$), resp.) and with H_2O control group as well in case of Subetta (147 ± 4 dg/mL versus 165 ± 4 dg/mL ($P < 0.01$)) (Table 1). Quite unexpectedly, CMC, used as a control for Rosi, exerted a slight but significant ameliorating effect on plasma glucose. This observation could reflect a delayed gastric emptying/intestinal absorption due to its high fibers content. Probably, it is the reason why Rosi exerts significant antihyperglycemic effect only on d1 as compare to CMC group. However, its effect still remained significant on d28 as compared to H_2O group ($P < 0.01$).

Baseline and final values of HbA1c, insulin, GLP-1, adiponectin, leptin, and glucagon are shown in Table 2 and Table 3. CMC had no significant effect on the above-mentioned parameters as compared to the H_2O control

Table 3: Basal (non-fed sate) plasma adiponectin (ng/mL; M ± SEM), leptin (ng/mL; M ± SEM), and glucagon (ng/mL; M ± SEM) in Goto Kakizaki/Par male rats ($n = 12$ in each group).

Groups	Plasma adiponectin level (ng/mL)		Plasma leptin level (ng/mL)		Plasma glucagon level (ng/mL)	
	Day 1 (d1)	Day 28 (d28)	Day 1 (d1)	Day 28 (d28)	Day 1 (d1)	Day 28 (d28)
RAD of Abs to β-InsR	9539.2 ± 529.2	9751.4 ± 273.5	1669.2 ± 142.9	1664.1 ± 106.5	196.5 ± 25.0	230.3 ± 21.4
RAD of Abs to eNOS	10362.6 ± 433.9	10773.3 ± 612.5	1437.9 ± 86.5	1673.7 ± 106.2$^{**\#\#}$	242.1 ± 29.0	184.2 ± 19.2
Subetta	10635.9 ± 416.5	11133.9 ± 567.4	1924.9 ± 328.4	1869.8 ± 236.4	233.0 ± 24.3	165.3 ± 25.5
Rosi	13816.2 ± 507.1$^{\#\#\#}$	14486.9 ± 436.4$^{\#\#\#}$	1293.0 ± 85.3$^{\#\#}$	1334.1 ± 61.6	152.0 ± 11.9	211.4 ± 28.7
H2O	10197.3 ± 392.9	9797.8 ± 482.0	2266.1 ± 210.1	2125.6 ± 254.5	199.9 ± 20.0	202.9 ± 21.0
CMC	9686.2 ± 415.5	9496.3 ± 356.0	2354.0 ± 413.9	2034.7 ± 307.0	205.4 ± 29.6	232.8 ± 49.3

$^{**}P < 0.01$ (versus d1)
$^{\#\#}P < 0.01$ (versus control (H_2O or CMC, resp.))
$^{\#\#\#}P < 0.001$ (versus control (H_2O or CMC, resp.)).

group. Animals in Rosi group as compared to CMC control group displayed considerably higher level of adiponectin ($P < 0.001$) on d1 and d28 (increased by 43% and 53%, resp.) and lower level of leptin on d1 (decreased by 45% on d28 ($P < 0.01$)). Treatment with RAD of Abs to β-InsR resulted in increasing of plasma GLP-1 by 39% on d1 and by 37% on d28 as compared to H_2O control group ($P < 0.05$). RAD of Abs to eNOS significantly decreased plasma leptin by 17% on d28 only ($P < 0.01$ versus H_2O control group).

OGTT showed that glucose intolerance spontaneously deteriorated with aging (at least within the time-window 10–14 wks.) in the male GK/Par rats in both control groups (H_2O and CMC) (Figure 1). Animals in RAD of Abs to β-InsR, Subetta, and Rosi groups exhibited significantly lower postoral glucose loading glucose levels than those in the controls: AUC glucose variations during the 28-days period were lower by 41% ($P < 0.001$), 59% ($P < 0.05$), and 41% ($P < 0.05$) as compared to respective controls (Figure 3). This establishes that both RAD of Abs to β-InsR and Subetta exert positive long-term effect upon glucose homeostasis in GK/Par rat model of T2D, which is comparable with Rosi effect. Herewith Subetta effect exceeds RAD of Abs to β-InsR effect.

The followup of glucose-induced insulin secretion (GSIS) showed that only treatment with Rosi resulted in lowering of insulin secretion in response to the oral glucose by the end of the four-week period as compared to baseline value ($P < 0.05$) (Figures 2 and 3).

Type 2 diabetes is a complex, heterogenous, and polygenic disease. The primary defects in insulin secretion and the development of insulin resistance contribute to the etiology of type 2 diabetes. Impaired postprandial insulin secretion because of functional defects and the loss of pancreatic β-cells leads to hyperglycemia and further decline in insulin sensitivity [20, 21]. Therefore, individuals with type 2 diabetes experience both reduced insulin secretion and insulin action. Thus, an animal model that mimics the pathogenesis and clinical features of human type 2 diabetes should have both of these traits. Among the animal models currently available, the Goto-Kakizaki rats exhibit inherited polygenic hyperglycemia, with low postprandial insulin secretion and insulin resistance, and they are widely used in experimental studies [22, 23].

In the present study, we evaluated the potential antidiabetic effects of two-component drug Subetta and its components RAD of Abs to β-InsR and RAD of Abs to eNOS in GK/Par diabetic rats. Herein, we showed that chronic oral administration of Subetta and RAD of Abs to β-InsR significantly attenuated fasting hyperglycemia and improved glucose homeostasis in GK/Par rats. The mechanism of Subetta action is not clear enough and seems to be complex because of the variety of the results received for its components and for the drug.

When we administered RAD of Abs to β-InsR for 4 weeks, it did not improve the HbA1c, insulin, adiponectin, leptin, and glucagon levels, but it did significantly increase GLP-1 levels at both stages of the treatment (on d1 as well as on d28). In spite of the significant increase of GLP-1 levels in RAD of Abs to β-InsR group, which is known to enhance satiety and causes inhibition of food intake and body weight gain in animal models and type 2 diabetes patients [24], RAD of Abs to β-InsR had no significant effect on basal food and water intake and weight of the GK rats. Subetta did not affect GLP-1; however, food intake was slightly but significantly lower in this group as compared to H_2O control group.

Treatment with RAD of Abs to β-InsR decreased plasma glucose starting from d20 to the end of the study. Nevertheless, these plasma glucose levels were still significantly higher than in H_2O controls on d28 ($P = 0.05$). However, the glucose intolerance increased by 24% only in RAD of Abs to β-InsR group, a value significantly lower than that in the H_2O control group. This effect could not be explained via increased GLP-1 level, which directly contributes to the improvement of glucose tolerance by inhibition of glucose production from liver and increasing of glycogen synthase activity [24]. GLP-1 remained unchanged in Subetta group, but the drug exerted a significant positive effect on the glucose intolerance in response to glucose load after chronic (28 days) administration too. More precisely, our data clearly demonstrate that Subetta effect exceeds RAD of Abs to β-InsR effect. It is important to notice that the improvement registered in Subetta and RAD of Abs to β-InsR groups was

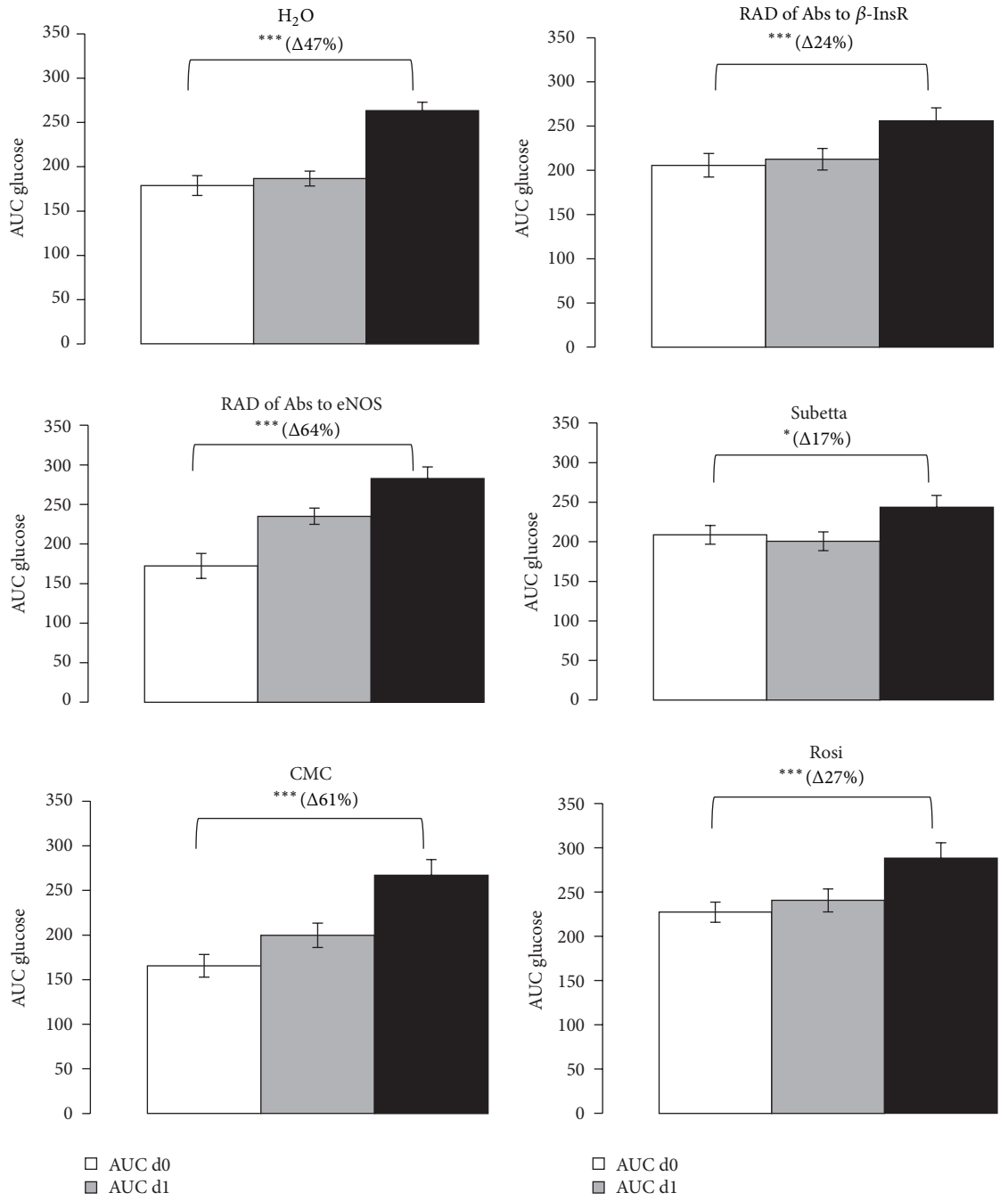

Figure 1: Glucose tolerance to glucose (2 g/kg per os) in adult diabetic male GK/Par rats before (d0), after acute treatment (d1), and after chronic treatment (d28) with RAD of Abs to β-InsR, RAD of Abs to eNOS, Subetta, H_2O (distilled water), Rosiglitazone (Rosi), or carboxymethyl-cellulose (CMC). On d1 and d28, drugs or vehicle were administrated at 9.00. OGTT were performed at 12.00 in nonanaesthetized rats fasted from 9.00 (postabsorptive state). Glucose responses during the glucose tolerance test were calculated as incremental plasma glucose values integrated over the 120 min period following the glucose injection (AUC glucose; g/L/120 min). Each point represents the mean ± S.E.M. of 12 observations/group. $^{***}P < 0.001$ versus the related d0-value within each group. $^{**}P < 0.01$ versus the related d0-value within each group. $^{*}P < 0.05$ versus the related d0-value within each group.

similar to that obtained in the Rosiglitazone-treated group under the same conditions.

RAD of Abs to β-InsR and Subetta have shown only tendency to decrease leptin level at early (d1) stage of the treatment (as compared to H_2O rats) ($P = 0.06$ and $P = 0.05$, resp.) meanwhile RAD of Abs to eNOS decreased it at both stages of the treatment, but significantly at later stage only. It is generally accepted that leptin has a potent inhibitory effect on insulin secretion from pancreatic β-cells *in vitro* and *in vivo* and has additional effect on reducing pre-proinsulin gene

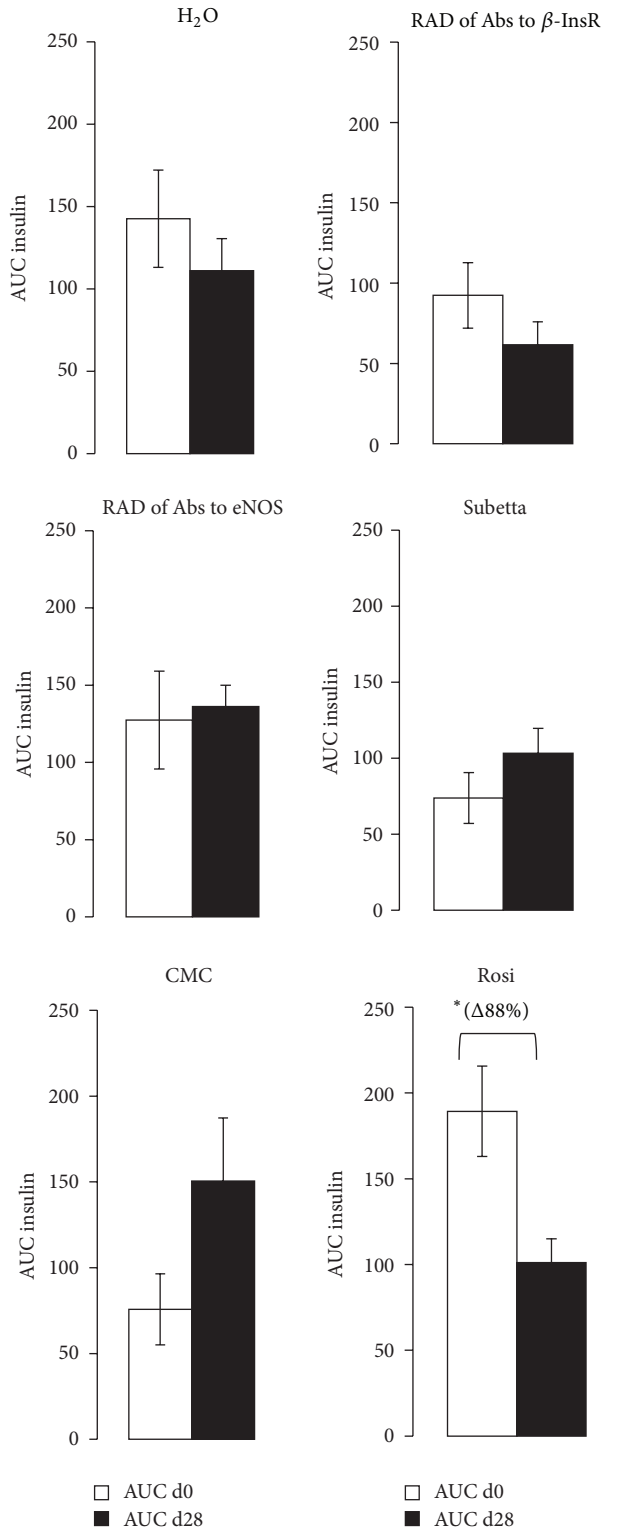

Figure 2: Insulin secretion in response to glucose (2 g/kg per os) in adult diabetic male GK/Par rats before (d0) and after chronic treatment (d28) with RAD of Abs to β-InsR, RAD of Abs to eNOS, Subetta, H_2O (distilled water), Rosiglitazone (Rosi), or carboxy-methylcellulose (CMC). Insulin responses during the glucose tolerance test were calculated as incremental plasma insulin values integrated over the 120 min period following the glucose injection (AUC insulin; g/L/120 min). Each point represents the mean ± S.E.M. of 12 observations/group. $^{*}P < 0.05$ versus d0-ROSI-treated GK/Par group.

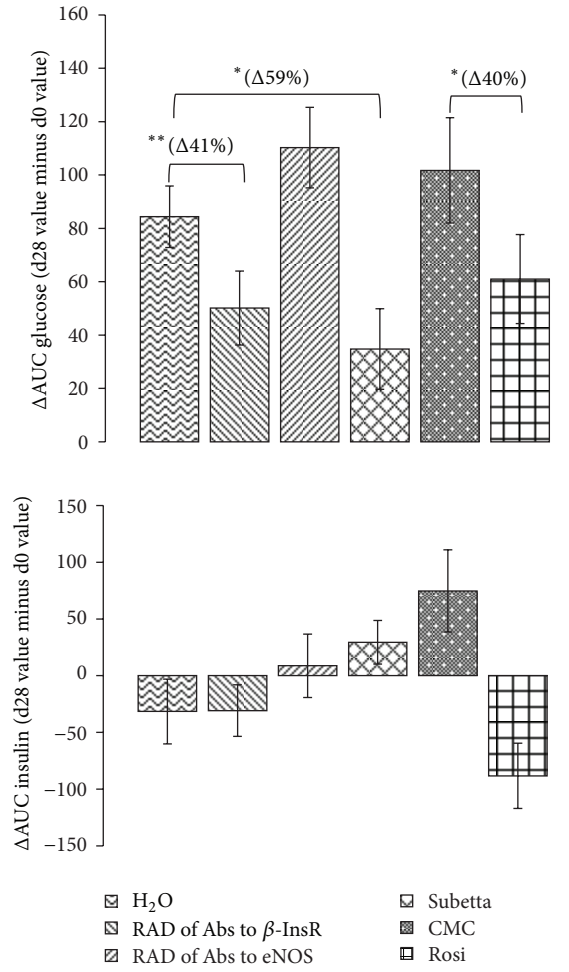

Figure 3: Time-related variations of AUC glucose and AUC insulin values in each group between d0 and d28. Such a calculation (d28 value minus d0 value) enables intergroup comparison. $^{**}P < 0.01$ versus d0-H_2O-treated GK/Par group. $^{*}P < 0.05$ versus d0-CMC-treated GK/Par group.

expression [25], but there were no effects on plasma insulin and insulin secretion levels in GSIS after neither RAD of Abs to eNOS, nor RAD of Abs to β-InsR, nor Subetta treatment.

RAD of Abs to eNOS had not any significant effect on basal (nonfed state) plasma glucose level all along the treatment, despite of an isolated glucose elevation on d12, and glucose intolerance was as high as in H_2O control group. Except for leptin, RAD of Abs to eNOS had no effect on any other basal plasma parameters related to diabetes status. Since RAD of Abs to eNOS is ineffective on GK diabetes, it can be inferred that the antidiabetic activity of Subetta does not rely on its "anti-eNOS" component, but could be related to Subetta "anti-β-InsR" component. On the other hand, an additive effect of RAD of Abs to eNOS might be caused by the improvement of oxidative stress and inflammation involved in diabetic pathology. RAD of Abs to eNOS is an active pharmaceutical ingredient of drug "Impaza" (OOO "NPF "MATERIA MEDICA HOLDING", Moscow, Russia). It has

been reported that it enhances the activity of endogenous endothelial NO synthase. Impaza revealed to be effective as monotherapy for erectile deficiency in the human and it also increases the efficacy of PDE5 inhibitors on combined treatment [4, 12, 13, 26]. The drug also showed endothelial protecting properties [1]. It seems that combination of RAD of Abs to β-InsR and RAD of Abs to eNOS resulting in combination of antidiabetic properties and endothelial protecting properties, respectively, creates the necessary and powerful prerequisites and reasonable background for Subetta application not only for diabetes management, but also for prevention of its complications.

Our findings suggest that Subetta and RAD of Abs to β-InsR action are mostly at the level of insulin action on the target tissues. Taking into account that Subetta (and RAD of Abs to β-InsR as it component) belong to the class of novel drugs and shares its common properties [3], such mechanism might be carried out by modulating effect of Subetta on the β-subunit of the insulin receptor regulating the insulin receptor's kinase activity and consequently activating receptor-associated signaling pathways [27]. Partly direct action of Subetta on insulin receptor has been recently confirmed *in vitro*, where it was shown that Subetta significantly stimulates adiponectin production by mature human adipocytes in the absence of insulin [5], which is known to enhance adiponectin regulation and secretion selectively in adipocytes [28]. In principle, ability of direct activation of insulin receptor and receptor-associated signal pathways in the absence of insulin was shown for L7 (Merck) [29]. Moreover in current *in vivo* study, Subetta showed tendency to increase plasma adiponectin ($P = 0.07$).

Influence on adiponectin production could be an additional mechanism of Subetta action. The most significant role of adiponectin may be that of sensitizing the liver and muscles to the action of insulin in both humans and rodents. Adiponectin appears to increase insulin sensitivity by improving glucose and lipid metabolism [30]; adiponectin improves glucose metabolism apart from insulin signalling [31]; adiponectin regulates the expression of several pro- and anti-inflammatory cytokines. Its main anti-inflammatory function might be related to its capacity to suppress the synthesis of tumor necrosis factor alpha and interferon gamma and to induce the production of anti-inflammatory cytokines such as interleukin-10 and interleukin-1 receptor antagonist [32]; adiponectin has effects on β-cell function and survival, which are well known as key factors in the development of type 2 diabetes along with insulin resistance [25]. Finally, the results of the present study serve as a basis for further experiments which should be performed in order to test our hypothesis for the Subetta mechanism of action.

Rosiglitazone, like other thiazolidinediones, reduces blood glucose levels by sensitizing insulin activity in target tissues, mainly by inhibiting lipolysis in adipose tissue and subsequent reduction of glucose production in the liver and enhancing insulin-mediated glucose disposal in skeletal muscle [33]. Our data in the Rosiglitazone-treated GK rats are consistent with this view, although we did not investigate directly glucose metabolism fluxes. They also match with previous reports in GK rats indicating that chronic troglitazone treatment improves their glucose tolerance through decreased hepatic glucose production and has a limited effect on peripheral insulin sensitivity [34]. Our data in the Rosiglitazone-treated GK rats also showed that plasma leptin and adiponectin levels are, respectively, decreased and increased. Leptin, the product of the ob gene, is a hormone secreted by adipocytes, and increased body fat content is closely correlated with the circulating plasma leptin levels [35, 36]. Leptin has been posited as a humoral signal from adipose tissue that acts on the central nervous system to reduce excess food intake and increase energy expenditure in a negative feedback manner [37, 38]. In the present study, acute exposure of Rosiglitazone significantly reduced plasma leptin levels. This reduction in leptin was associated with improvements in fasting hyperglycemia and glucose tolerance on the long term. In addition, we found that chronic Rosiglitazone tended to reduce food intake, which cannot be mediated by the decrease plasma leptin levels. Such pattern seems paradoxical since chronic Rosiglitazone is reported to increased plasma leptin levels due to the Rosiglitazone-induced increase in adiposity [39] through the activation of the adipocyte transcription factor peroxisome, proliferator-activated receptor-γ which stimulates adipocyte differentiation [40]. The paradoxical GK-response to Rosiglitazone could, at least partly, reflect a defective adipose tissue growth/differentiation in the GK model, as we previously suggested [41, 42]. In this study, chronic Rosiglitazone treatment significantly increased plasma adiponectin levels in the GK/Par rats. Finally, one may retain that both circulating leptin and adiponectin changes after Rosiglitazone in the GK/Par rats may contribute to the improvements of their hyperglycemia and insulin resistance.

4. Conclusion

Taken together, we conclude that Subetta and release-active dilutions of antibodies to β-subunit insulin receptor treatments are effective to significantly improve glucose homeostasis in GK/Par diabetic rats. Herewith Subetta effect exceeds, effect of, mentioned component. Moreover, since Subetta and release-active dilutions of antibodies to β-subunit insulin receptor behave as antihyperglycemic agents, it would be worthwhile to evaluate, after more prolonged administration, their effects on the residual pancreatic β-cell population, the low grade inflammation status of some tissues (pancreas, adipose tissue, liver), the circulating lipid status, and diabetic complications (kidney, heart, brain).

As such, Subetta and release-active dilutions of antibodies to β-subunit insulin receptor may be considered as new candidate antidiabetic drugs in the diabetic patients. Further studies are obviously needed to address more detailed information regarding the mechanisms of action for Subetta in treating diabetes.

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Research Article

Therapeutic Potential of *Dioscorea* Extract (DA-9801) in Comparison with Alpha Lipoic Acid on the Peripheral Nerves in Experimental Diabetes

Heung Yong Jin,[1] Sun Hee Kim,[1] Hea Min Yu,[2] Hong Sun Baek,[1] and Tae Sun Park[1]

[1] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Research Institute of Clinical Medicine of Chonbuk National University, Biomedical Research Institute of Chonbuk National University Hospital, Chonbuk National University Medical School, 634-18, Keum-Am Dong, Jeonju 561-712, Republic of Korea*
[2] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Research Institute of Clinical Medicine, Eulji University Hospital-Eulji University, Daejeon, Republic of Korea*

Correspondence should be addressed to Tae Sun Park; mdjinhy@hanmail.net

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DA-9801, a mixture of extracts from *Dioscorea japonica* Thunb. and *Dioscorea nipponica* Makino, was reported to have neurotrophic activity. Therefore, we investigated the therapeutic potential of DA-9801, in comparison with alpha lipoic acid (ALA), for peripheral nerves preservation in experimental diabetes. Experimental animals were divided into 4 groups, and each group was designated according to the type of treatment administered as follows: normal, DM, DM+DA-9801, and DM+ALA. After 16 weeks, response thresholds to tactile and thermal stimuli were higher in DM+DA-9801 group than in nontreated DM group. This degree of increase in DM+DA-9801 group indicates more therapeutic potency of DA-9801 than ALA. Western blot analysis showed more significant increase in NGF and decrease in TNF-α and IL-6 in DM+DA-9801 group than in DM or DM+ALA groups ($P < 0.05$). IENF density was reduced less significantly in the DM+DA-9801 group than in other DM groups (7.61 ± 0.32, 4.2 ± 0.26, and 6.5 ± 0.30 in DM+DA-9801, DM, and DM+ALA, resp., $P < 0.05$). Mean myelinated axonal area in the sciatic nerves was significantly greater in DM+DA-9801 group than in other DM groups (69.2 ± 5.76, 54.0 ± 6.32, and 63.1 ± 5.41 in DM+DA-9801, DM, and DM+ALA, resp., $P < 0.05$). Results of this study demonstrated potential therapeutic applications of DA-9801 for the treatment of diabetic peripheral neuropathy.

1. Introduction

Half of diabetic patients have chronic complication involving the peripheral nervous system, which results in diabetic peripheral neuropathy (DPN). Besides morbidity and mortality, DPN has diverse symptoms, which leads to the deterioration of the quality of life of diabetic patients [1, 2]. DPN can be managed via pathogenic correction and symptomatic control [3]. Alpha lipoic acid (ALA), gamma linoleic acid, neurotrophic agent, and aldose reductase inhibitor are well known as pathogenic treatments based on multifactorial DPN mediators [3, 4]. Among these, ALA is widely used for the clinical pathogenic treatment of DPN. On the contrary, the therapeutic effectiveness of neurotrophic agents, such as nerve growth factor (NGF), for DPN treatment is not yet confirmed because clinical trials could not yield positive results, although neuronal effects were claimed by several experimental studies [5, 6]. However, defective neurotrophic factors binding to specific receptors have been suggested as one cause of DPN, and preventing this mechanism has been regarded as a possible therapy for DPN.

DA-9801 is a mixture of extracts from *Dioscorea japonica* known as SanYak and *Dioscorea nipponica* known as Buchema [7]. Results of previous studies on DA-9801 showed increased NGF level and improvement of nociceptive pain, although the exact role of NGF and the degree of its neurotrophic effect compared to other agents were not described [8]. In this context, DA-9801, an enhancer of NGF, can then be postulated to play a role in the pathogenic treatment

of DPN similar to other neurotrophic factors. Therefore, in this study, we investigated the neuroprotective potential of DA-9801 on the peripheral nerves of streptozotocin- (STZ-) induced diabetic rats, in comparison with ALA.

2. Materials and Methods

2.1. Animals, Materials, and Experimental Design. Six to eight-week-old male Sprague-Dawley (SD) rats weighing 160 to 180 g were purchased from Damool Science (Daejeon, Chungnam, Korea). Animals were housed in an optimal condition with a 12 hr light and dark cycle. The room temperature was maintained at 23 ± 1°C and humidity at 53 ± 2%. The animals had a free access to food and water. All experiments and protocols were performed after the approval by the Institutional Rat Care and Use Committee of the Chonbuk National University Medical School (CBU 2011-0055). Streptozotocin (STZ) (Sigma Chemical, St. Louis, MO, USA) dissolved in 0.1 mol/L sodium citrate buffer (pH 4.5) was injected intraperitoneally to experimental rats (60 mg/kg body weight) to induce diabetes. Forty-eight hours after STZ injection, rats showing blood glucose levels higher than 350 mg/dL were verified to have diabetes. After subjecting rats into an overnight fasting, blood samples were drawn from the tail vein, and blood glucose levels were measured using Precision Xtra Plus (Abbott Laboratories, MediSence Products, Bedford, MA, USA). In the same manner, age-matched control rats received an equal volume of the vehicle-sodium citrate buffer (pH 4.5). DA-9801 was supplied from Dong-A Pharmaceutical (Yongin, South Korea), while alpha lipoic acid (ALA) was supplied from Bukwang Pharmaceutical (Seoul, South Korea). After 4 weeks of STZ and sodium citrate buffer injection, normal and diabetic rats were randomly selected and divided into 4 groups ($n = 10$ per group) according to the treatment agents: normal (normal control group), DM (nontreated diabetic group), DM+DA-9801 (DA-9801-treated diabetic group), and DM+ALA (ALA-treated diabetic group). DA-9801 was administered orally at a dose of 100 mg/kg/day, while 0.5% ALA, which was in powdered form, was mixed with daily food at a dose of 50 mg/kg/day. On the 8th and 16th weeks of treatment, behavioral assessment and morphometric comparison were performed. On the 17th week, biochemical parameters including NGF, TNF-α, and IL-6 levels in sciatic nerve and spinal cord were measured.

2.2. Behavioral Assessment by Tactile Response and Thermal Response. To measure the allodynia, tactile stimulation was performed using flexible von Frey filament (Stoelting Co., Illinois, USA) to evaluate the withdrawal threshold of hind paw. After adaptation to the testing condition for at least 20 min, rats were placed individually in a plastic cage with 1 cm sized perforated mesh. Von Frey filament, with calibrated bending forces (in g), were applied perpendicularly to the plantar surface of the hind paw to deliver tactile stimuli of varying intensity. This test was conducted according to the procedure of Chaplan et al. [9]. The stimulation was performed five times with 5-sec and interval, and immediate withdrawal in at least one time in the five-time application was determined to be a positive response. To measure thermal response, rats were placed on a hot plate (Ugo Basile, PA, USA) with temperature at 55 ± 1°C. The latency to the first sign of paw licking response to avoid the heat was taken as a threshold for heat sense.

2.3. NGF, TNF-α, and IL-6 Determinations in Sciatic Nerve and Spinal Cord. On the 17th week, sciatic nerves and spinal cord were rapidly removed after killing all rats under deep anesthesia and were prepared for western blot analysis. Sciatic nerve and spinal cord were broken down mechanically with 100–200 μL of Triton lysis buffer. After centrifugation at 13,200 rpm for 15 min at 4°C, the supernatant was transferred to a fresh tube (1.5 mL capacity), and the protein concentration was quantified following Bradford method [10]. Samples were loaded into 10% SDS polyacrylamide gel, and blocking of nonspecific binding was achieved by placing the membrane in a dilute protein solution of nonfat dry milk (5% skim milk) with 1% Tween-20 in PBS for 3 hr at room temperature. The blots were incubated with primary antibodies such as NGF, TNF-α, and IL-6 (1 : 1,000; Abcam, UK) overnight at 4°C. The membranes were washed in 1 TBST by shaking 3 times for 10 min. Incubation with HRP-conjugated secondary antibody donkey anti-goat IgG, HRP conjugate (1 : 2,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was performed on a rocker for 1 hr at 4°C. Protein bands were detected using ECL-plus kit (Amersham Pharmacia Biotech, Buckinghamshire, England). Quantitative image analysis was conducted using LAS 3000 Fuji film and film densitometry was performed using MultiGauge version 3.0 (Fuji film).

2.4. Morphometric Assessment. On the 0th and 8th weeks, 3 × 3 mm tissues were taken from the dorsum of the foot via skin biopsy for immunohistochemical analyses of intraepidermal nerve fiber (IENF). On the 17th week after killing all rats under deep anesthesia, last cutaneous tissue samples from the feet and segments of the sciatic nerve were obtained from each rat. Sciatic nerve tissue samples were immersed in a fixative (2.5% glutaraldehyde in phosphate-buffered saline (PBS)) and incubated overnight at 4°C. These samples were then embedded in JB-4 solution (Polysciences, Inc., Germany), and 1.5 μm transverse sections were stained with toluidine blue. The procedures used for immunohistochemical analysis were the same as those described previously [11]. Skin tissue specimens were fixed with periodate-lysine-paraformaldehyde (PLP) (2% paraformaldehyde, 0.075 M lysine, 0.05 M phosphate buffer pH 7.4, 0.01 M sodium m-periodate) solution for 24 hr. After thorough rinsing in PBS containing 20% glycerol-0.1 M phosphate buffer for 48 hr at 4°C, the tissue specimens were cryoprotected with Tissue-Tec (OCT compound) (Miles, Elkhart, IN, USA). Sections of 40 μm in thickness, cut perpendicular to the dermis, were prepared with a sliding cryostat (Leica CM 1510, Leica Microsystems AG, Wetzlar, Germany) and were immersed in PBS for 15 min at room temperature. Samples were then transferred into microtubes containing Dako Protein Block Serum Free (Dako, Carpinteria, CA, USA) as a blocking buffer supplemented with 3% goat serum. After 30 min of blocking on a shaker table at room temperature, specimen sections were washed with PBS twice for 10 min and then incubated

overnight with primary antibody, rabbit anti-protein-gene-product 9.5 (PGP 9.5) (Biogenesis, Poole, UK) at a dilution of 1 : 100 at 4°C. The antibodies were diluted in antibody diluent (Dako, Carpinteria, CA, USA) supplemented with 1% goat serum. After complete washing, the specimens were incubated with the secondary antibody, goat anti-rabbit IgG-FITC (1 : 200, Vector, UK), for 1 hr at room temperature in a dark room. After washing with PBS, sections were placed on slides and mounted with a fluorescent mounting media (Dako, Carpinteria, CA, USA).

Photomicrographs of the myelinated fiber and IENF were captured using a digital camera (Axiocam HRC, Carl Zeiss, Goettingen, Germany) with a final magnification of 400 and 100 times, respectively. The myelinated fiber or axonal area in the sciatic nerve, represented by the outer or inner border of the myelin sheath, was measured with the aid of analySIS image software (Soft Imaging Systems GmbH, Munster, Germany), and the mean myelinated fiber area was determined. In addition, the thickness of myelin sheath and the diameter of axonal fiber were measured.

PGP 9.5-immunoreactive nerve fibers in the epidermis of each section were counted as described previously [12]. In cutaneous nerves, each nerve fiber with branching points inside the epidermis was counted as one fiber. The number of intraepidermal nerve fibers (IENFs) per length (fibers/mm) was considered as the amount of innervation. In order to avoid any possible bias during preparation and calculation, two independent investigators were blinded to the experimental groups. Moreover, the slides were mixed with a set of normal slides before examination.

2.5. Statistical Analysis. All data were expressed as means ± SD. One-way ANOVA with Duncan's post hoc test was used to compare experimental groups. The confidence interval for testing the differences was 95%, and analyses results with $P < 0.05$ were considered as statistically significant. Statistical analyses were performed using SPSS 12.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Effect of DA-9801 on the Body Weight and Blood Glucose Level. Two weeks after STZ injection, increased blood glucose levels and decreased body weight were observed in diabetic groups compared with normal rats. Treatment with DA-9801 for 16 weeks did not affect on the body weight and blood glucose levels in DM+DA-9801 group. There were no significant changes in body weight and blood glucose levels in the DM+ALA group (Figures 1(a) and 1(b)).

3.2. Effect of DA-9801 on the Tactile Response and Thermal Response. On the 8th week, the paw withdrawal threshold, when stimulated with von Frey filament, was reduced by 59% in the diabetic group compared with normal group. However, this reduction was significantly prevented in DM+DA-9801 and DM+ALA groups compared with nontreated diabetic group (Figure 2(a)). The degree of preventing threshold reduction was observed more potently in DM+DA-9801 than in DM+ALA group. On the 16th week, this pattern of response to von Frey filaments was reversed in the DM+DA-9801 or DM+ALA groups, and more blunted response was observed in the nontreated diabetic group. However, DM+DA-9801 group exhibited significantly preserved sensitivity compared with other diabetic groups ($P < 0.05$) (Figure 2(a)). On the 8th week, the latent time to withdrawal of rat paw on the hot plate was significantly reduced (by 34%) in diabetic group compared with normal group. DA-9801 treatment prevented this hypersensitive response in diabetic group, and the latent time to withdrawal of rat paw was longer in DM+DA-9810 group than in DM+ALA group, although

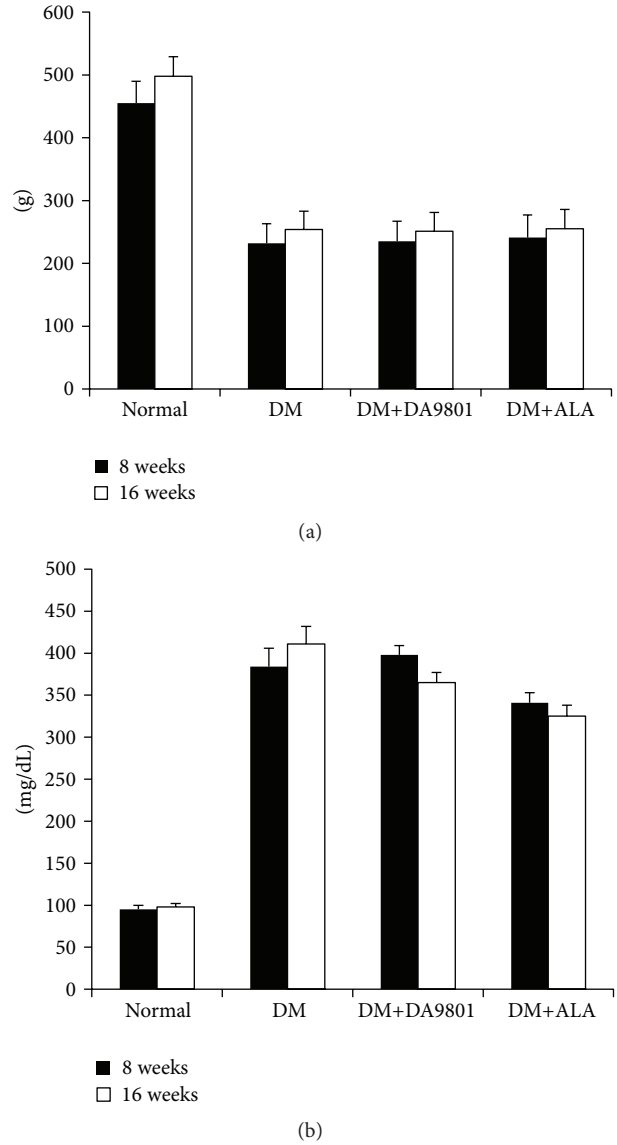

Figure 1: Body weight (a) and blood glucose levels (b) of the experimental groups. Body weight was increased gradually in normal glucose rats. In the diabetic groups, however, it was not increased irrespective of DA-9801 or ALA treatment. DA-9801 and ALA had no effects on blood glucose levels. Data are presented as means ± SD ($N = 10$ in each group). ALA: Alpha lipoic acid and DM: diabetes.

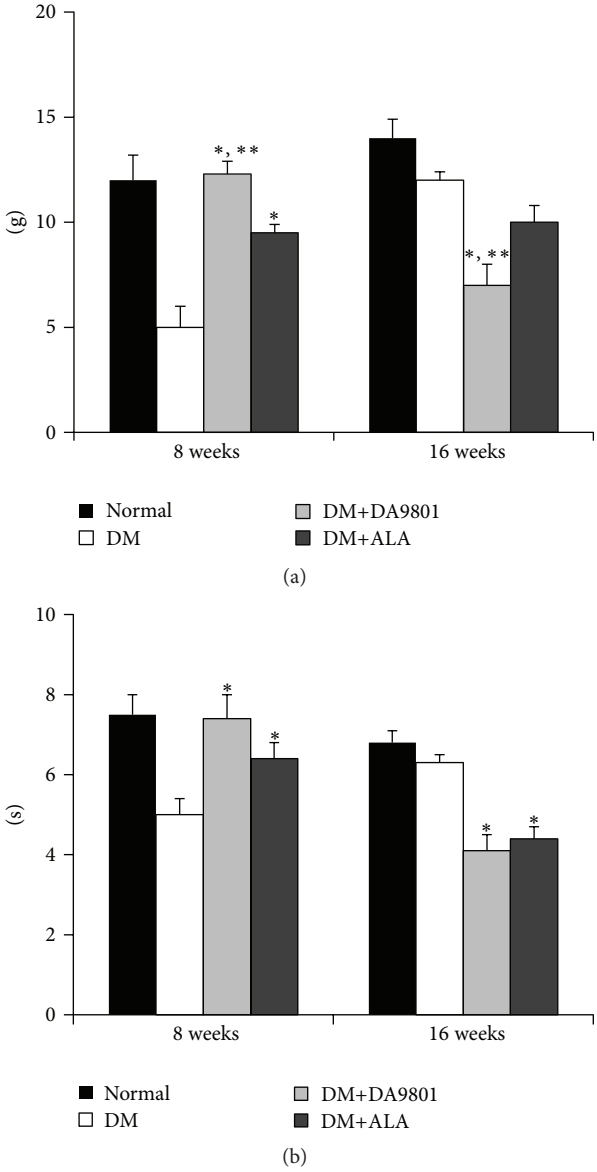

Figure 2: Von Frey filament (a) and hot plate (b) response of the experimental groups. Threshold of tactile response was lower in the nontreated diabetic group as compared with the DM+DA-9801 or DM+ALA groups on the 8th week. On the 16th week, however, this trend was reversed, and the thermal response was also changed in a similar pattern to the tactile response. Data are presented as means ± SD. $^{*}P < 0.05$ versus Normal and $^{**}P < 0.05$ versus DM ($N = 10$ in each group). ALA: Alpha lipoic acid and DM: diabetes.

the difference was not significant. However, this pattern was reversed in the diabetic groups after 16 weeks, DM+DA-9801 and DM+ALA groups showed more preserved sensitivity to hot stimulus compared with the nontreated diabetic group (Figure 2(b)).

3.3. Effect of DA-9801 on the NGF, TNF-α, and IL-6 Levels in Sciatic Nerve and Spinal Cord. NGF level in sciatic nerve and spinal cord was markedly decreased (about 50%) in the non-treated diabetic group compared with those in normal ones. However DM+DA-9801 group recovered NGF level to a similar degree with the normal group. This degree of NGF recovery was more potent in DM+DA-9801 than in DM+ALA group, although ALA is not an NGF inducer (Figure 3(a)). TNF-α and IL-6 levels increased in diabetic condition groups compared with the normal ones. These cytokines in each diabetic group were reduced after DA-9801 treatment, and the degree of reduction was similar in DM+DA-9801 and DM+ALA groups (Figures 3(b) and 3(c)).

3.4. Morphometric and Quantitative Comparisons of Peripheral Nerve among Groups. The comparison of cutaneous peripheral nerve quantity showed markedly reduced IENF density in non-treated diabetic group by about 42–50% compared with normal group. Shortened and degenerated patterns of small peripheral nerve fibers were observed in diabetic groups (Figure 4(a)). However, this morphological change and the reduction of IENF density were blunted in DM+DA-9801 and DM+ALA groups. Furthermore, DA-9801 showed more potent therapeutic potential than ALA, although this difference did not reach statistical significance (Figures 4(a) and 4(b)). The examination of the sciatic nerves also showed similar trend of results on IENF density. Mean area of myelinated axonal fiber was reduced in non-treated diabetic groups compared with the normal ones. This reduction was prevented in the DM+DA-9801 group, which shows more potent therapeutic potential of DA-9801 than ALA. The diameter of myelin sheath and the axonal fiber of DM+DA-9801 or DM+ALA groups increased significantly compared with the non-treated diabetic group (Figure 5).

4. Discussion

Peripheral neuropathy is a common disease in diabetic patients, and an estimate of more than half of all diabetic patients suffer from diverse neuropathic symptoms [13]. Diabetic peripheral neuropathy (DPN) occurs as a result of multiple etiologies and leads to neuronal damage. Affected lower leg (e.g., diabetic foot) may lead to ulcer or amputation, which lowers the quality of life of the patients [14]. The basic pathogenic mechanism of DPN can be divided into metabolic and vascular etiologies. A number of mechanisms suggested to be a pathogenesis of DPN have been investigated, which include polyol pathway, advanced glycation end product, and activation of the proteins kinase, poly ADP-ribose polymerase, and aldose reductase [15, 16]. These can result in increased oxidative stress and attenuation of antioxidative defense mechanism. Therefore, hyperglycemia, a metabolic disruption causing increased oxidative stress in the peripheral nerves, is important to the pathogenesis of DPN [17]. In patients with defective antioxidant response, oxidative stress from hyperglycemia can cause vascular impairment leading to endoneurial hypoxia [18]. In addition to these etiologies, diminished nerve growth factor also causes problems in neuronal survival, growth, and differentiation, although the extent of its contribution to the pathogenesis of DPN is unclear [5, 19]. Previous studies suggested that NGF plays a significant role in the pathogenesis of DPN, and NGF or NGF mimetics administration may recover the neuronal

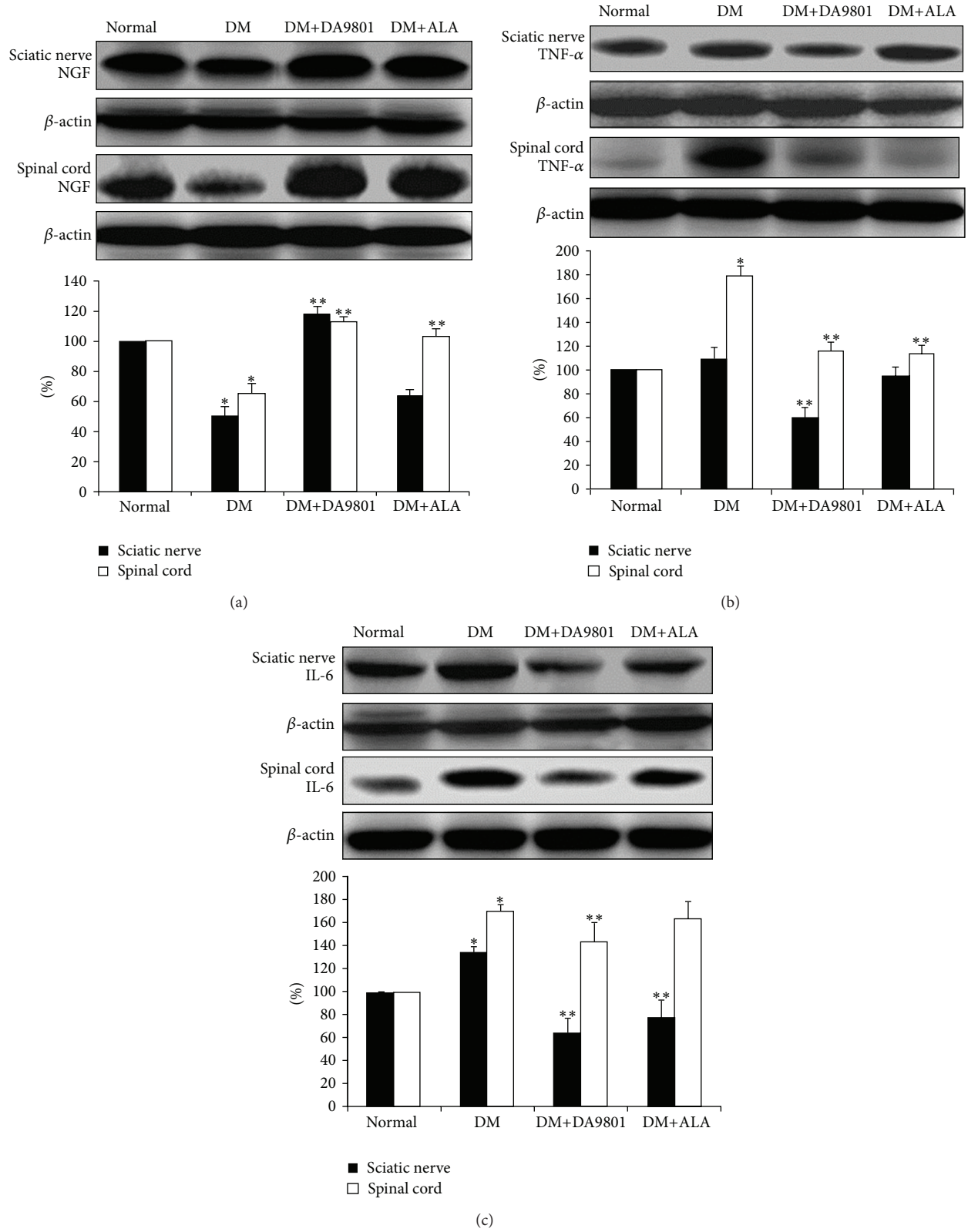

Figure 3: NGF, TNF-α, and IL-6 levels in the sciatic nerve and spinal cord of the experimental groups. Data are presented as means ± SD. $^{*}P < 0.05$ versus Normal and $^{**}P < 0.05$ versus DM ($N = 10$ in each group). ALA: Alpha lipoic acid and DM: diabetes.

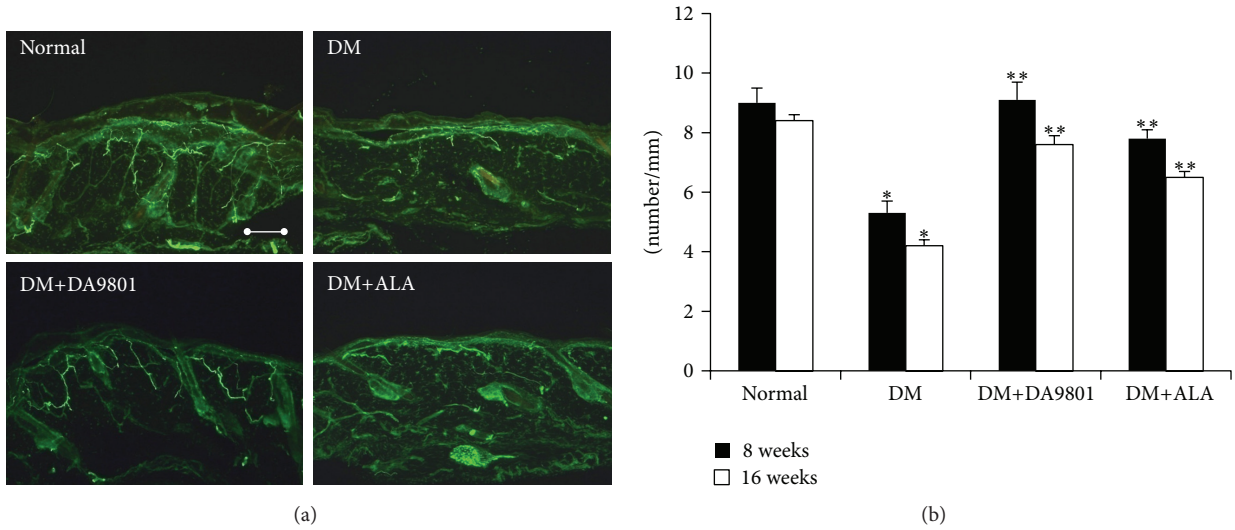

FIGURE 4: Immunohistochemistry of cutaneous small nerve fibers of the foot dorsum (a) and the IENF density (b) of the experimental groups. PGP 9.5-stained small nerve fibers are more preserved and less shortened, and IENF density was higher in the DM+DA-9801 group than its non-DA-9801-treated DM groups. Data are presented as means ± SD. $^{*}P < 0.05$ versus Normal and $^{**}P < 0.05$ versus DM ($N = 10$ in each group). ALA: Alpha lipoic acid and DM: diabetes. Bar indicates the 100 μm.

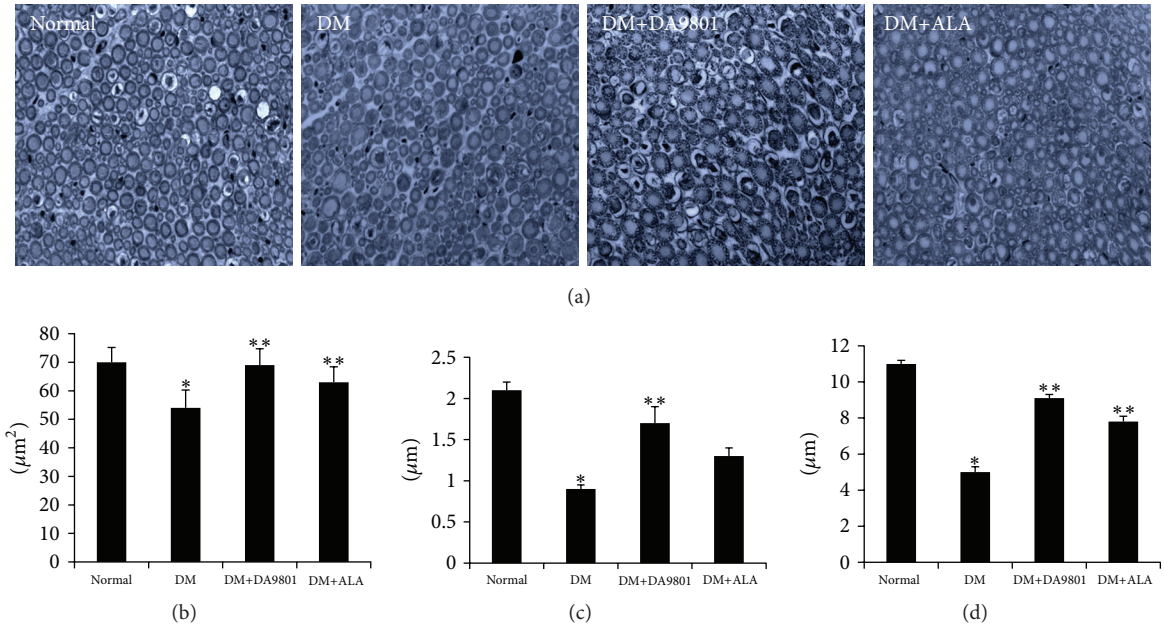

FIGURE 5: Immunohistochemistry of the sciatic nerve (a) and the area of myelinated nerve fiber (b), the diameter of myelin sheath (c), and the diameter of axon (d) of the experimental groups. Myelinated axonal nerve fibers were less degenerated in the DM+DA-9801 and DM+ALA groups. In addition, the size of nerve fibers with or without myelin sheath was increased in the DM+DA-9801 and DM+ALA groups as compared with the DA-9801-treated DM groups. Data are presented as means ± SD. $^{*}P < 0.05$ versus Normal and $^{**}P < 0.05$ versus DM ($N = 10$ in each group). ALA: Alpha lipoic acid and DM: diabetes.

damage caused by DPN, although no successful clinical trials to show the efficacy of NGF were reported [5, 20, 21]. Thus, neuroprotective effect of various neurotrophic or NGF mimetic agents on diabetes had been tested [21, 22].

Hence, in the present study, we investigated the potential of a botanical agent (DA-9801) in increasing NGF levels for the treatment of DPN. A comparative study with ALA, which is a well-known agent for the treatment of DPN, was also conducted.

Various species of Dioscorea has been used as a traditional medicine for diverse diseases including metabolic disorders, inflammatory diseases, pain control, and neuropathic diseases [23–25]. DA-9801 is a mixture of extracts from Dioscorea japonica Thunb (DJ) and Dioscorea nipponica

Makino (DN). Previous studies showed neurotrophic activity of DA-9801 *in vitro* and in experimental animals [7, 8]. However, there are few data to show the degree and the scientific mechanism of the neuroprotective effect of Dioscorea species on diabetic animals. In this study, we assessed the functional parameters, related mediators, and morphometric and quantitative characteristics of DPN in STZ-induced diabetic rats after 16 weeks of DA-9801 treatment. In this study, DA-9801 did not show significant effect on the body weight and blood glucose levels of experimental rats. However, deteriorating sensitivities caused by hyperglycemia were blunted through DA-9801 treatment. The degree of this response was more potent in DM+DA-9801 group than in the DM+ALA group. Histologically, DPN is characterized by axonal degeneration, demyelination, and neuronal atrophy associated with neuronal regeneration failure [26]. These neuronal damage from hyperglycemia leads to diverse neuronal symptom and lower leg problems in diabetic patients. Clinically and experimentally, the assessment of neuropathic symptoms is not reliable as an absolute indicator for the diagnosis or the assessment of the severity of peripheral nerve damage in diabetic patients. Furthermore, DPN can be manifested by positive or negative sensory symptoms, with or without sensory motor deficit. Mixed symptoms of DPN are also possible depending on the nerve damage, which is related to glycemic control and diabetes duration. Therefore, subjective and objective assessment should be incorporated in the diagnosis and assessment of the degree of DPN. Therefore, our data showing response threshold to mechanical and thermal stimuli need to be interpreted in association with the quantity of peripheral nerve. On the 8th week, more sensitive reaction to Von Frey filament and hot plate was observed in the diabetic group than in normal group. However, this sensitivity was reduced through DA-9801 and ALA treatments. This trend was more pronounced in DM+DA-9801 group than in DM+ALA group, although this difference was insignificant. Results also showed decreased IENF density in diabetic rats, which suggests that more hypersensitive reaction may result from degeneration of small nerve fiber in non-treated diabetic group. On the 16th week, the sensitive response observed on the 8th week was reduced in diabetic group compared with the normal ones. However, treatment with DA-9801 and ALA has blunted this insensitivity, in agreement with the results on IENF. These results suggest that DPN progression may be prevented by DA-9801 or ALA treatment, which may also reverse the pattern of mechanical allodynia and thermal response in the 16th week compared with the results in the 8th week in diabetic rats. However, the comparison and interpretation of functional parameters at the later stages of diabetes are difficult because of possible mixed symptoms and sensitivity. Furthermore, body weight difference between normal and diabetic groups may result in different mechanical allodynia and thermal response thresholds. Thus, functional parameters including tactile and thermal responses between normal and diabetic groups in this study need to be compared in consideration with body weight differences, although ANCOVA was used to exclude such bias. These types of behavioral data also require careful interpretation as sick animals, such as those with long-term diabetes, can become unresponsive to sensory stimuli due to generalized cachexia rather than something related to neuronal degeneration or dysfunction.

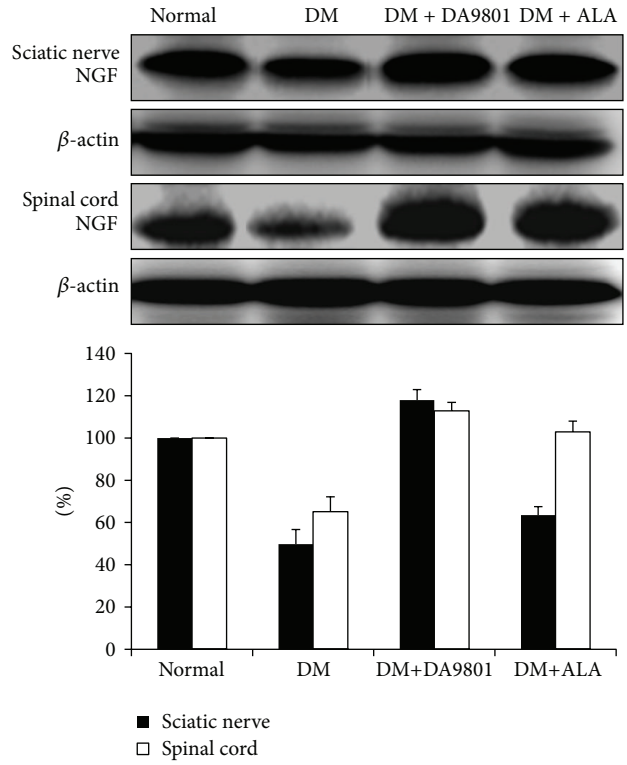

Figure 6

In the body weight and glucose levels, DA-9801 did not show the lowering effect in diabetic group. Of course, DA-9801 is not lowering agent for glucose or body weight, and our animal model was late state type 2 diabetic or type 1 diabetic model using STZ 60 mg/kg. Therefore, we thought that only one oral agent is difficult to lower the blood glucose in this condition, even though DA-9801 may have the glucose lowering effect. Accordingly, we thought that body weight was difficult to be affected without glucose control in this animal model because this agent could not affect on the food intake.

Anti-inflammation, antioxidant, or neuronal regeneration are fundamental mechanisms for nerve protection, although more detailed diverse pathways including several mediators are involved in DPN pathogenesis [27, 28]. These combined pathogenic corrections targeting neurotrophic and anti-inflammation can give more potent benefit than single pathogenic modulation of oxidative stress. The detailed mechanism of NGF in neuronal protection is known to phosphorylate Trk-A and induce PI3K pathways, although direct NGF administration did not reach the nervous tissue [21, 29–31]. Hence, neurotrophin-enhancing agents are more interesting in this respect. To investigate the neuroprotection mechanism of DA-9801 in diabetic rats, we examined the NGF concentration in the sciatic nerve and spinal cord (Figures 6, 7, and 8). However, complete prevention of DPN by single factor correction is difficult due to the complexity

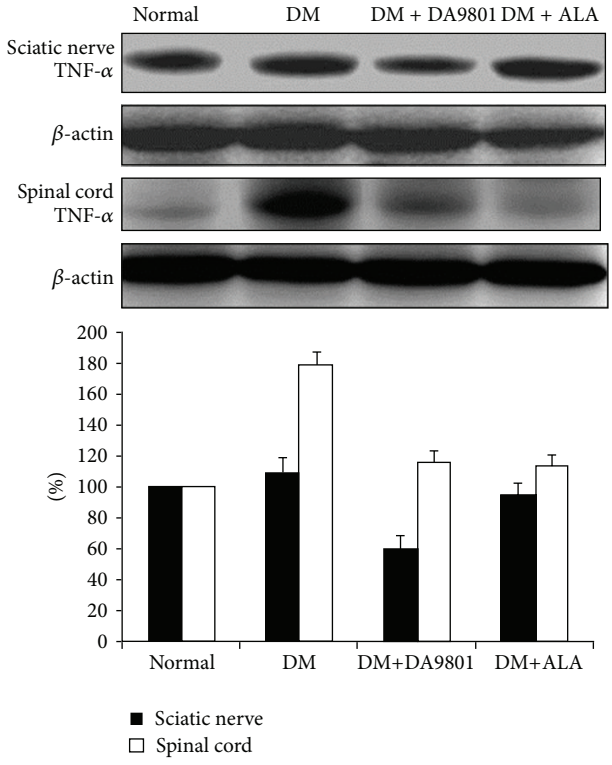

Figure 7

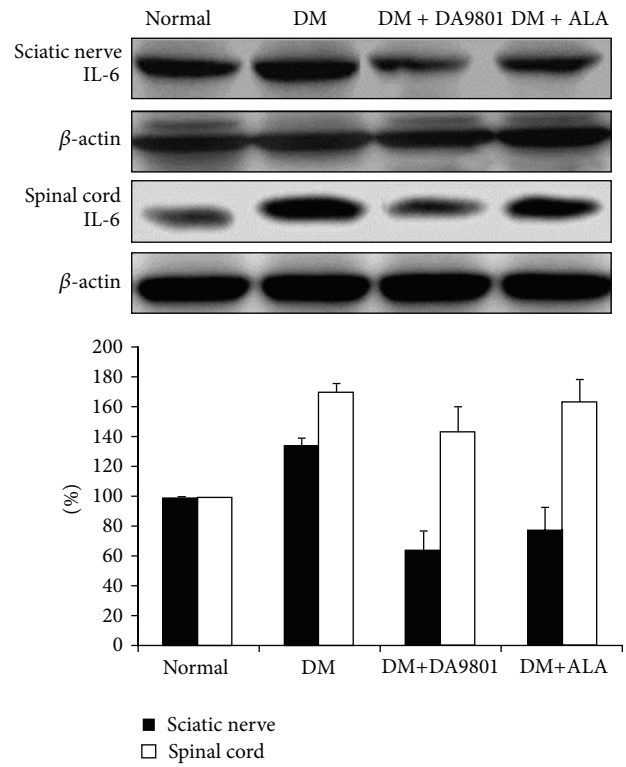

Figure 8

and diversity of etiologies in the pathogenesis. Hence, we measured other mediators, such as TNF-α and IL-6, which are well known to be involved in DPN pathogenesis. In the present study, the increases of TNF-α and IL-6 in diabetic group were reduced after DA-9801 treatment. Therefore, neuronal effect mediated by increased NGF level and anti-inflammation mediated via TNF-α and IL-6 may have played a role in the peripheral nerve protection in DM+DA-9801 group. However, the degree of the contribution of NGF and cytokines on DPN treatment has not been confirmed.

To support our claim of therapeutic potential of DA-9801 for peripheral nerve protection, the structural change of cutaneous small nerve fibers and sciatic nerves was also compared among experimental groups. We found that the quantity of small intraepidermal nerve fibers was more preserved, and the pattern was less degenerated in DM+DA-9801 and DM+ALA groups than in non-treated diabetic group. Moreover, the degree of neuronal protection of DA-9801 was found to be more potent than ALA. Based on IENF density measurements, DM+DA-9801 or DM+ALA groups exhibited less degenerated morphology of myelin sheath and axon compared with the non-treated diabetic group. DA-9801 also showed more potent therapeutic potential than ALA in the present study. Taking all results as a whole, this study showed that DA-9801 can act as a biochemical and morphological protective agent on the peripheral nerves in diabetic patients. However, the potential of DA-9801 to induce other neurotrophic factors or improve other mediators involved in pathogenesis of DPN remains to be determined. Aside from NGF, brain-derived neurotrophic factor, neurotrophin- (NT-) 3, IGF-I, IGF-II, and glial cell-derived neurotrophic factors have also been examined as therapeutic agents [5, 32].

However, this study have several limitations. First, although the NGF agonistic activity and anti-inflammatory effect of DA-9801 was proven to contribute to the prevention of peripheral nerve damage, more detailed mechanism and identification of the active components of Dioscorea species are warranted for the effective use of DA-9801 in DPN management. Second, dose-response effect of DA-9801 on diverse parameters in DPN assessment should be investigated. Third, NGF pathway, in the pathophysiologic treatment of DPN, is considered to lead nerve regeneration as well as neuronal survival. However, an examination of their differences was not performed in the present study. Further investigation on the effects of DA-9801 for the treatment of DPN should be conducted.

In summary, the results of this study suggest the potential of DA-9801 for the prevention of degeneration or induction of regeneration of peripheral nerves in diabetic rats. Furthermore, this therapeutic potential is greater in DA-9801 than in ALA. The effects of DA-9801 are believed to include enhancement of neurotrophic activity and anti-inflammatory response, which are commonly involved in the pathogenesis of DPN. The improvement of NGF and decreased levels of TNF-α and IL-6 may reduce the deterioration of the peripheral nerves by chronic hyperglycemia. However, DA-9801 has not completely prevented the hyperglycemia-induced metabolic disturbance in the peripheral nerves. Therefore, multiple steps involved in the pathogenesis of DPN need

further assessment to attain more effective pathogenic correction or complete prevention of DPN progression.

Abbreviations

ALA: Alpha lipoic acid
DM: Diabetes mellitus
DPN: Diabetic peripheral neuropathy
NGF: Nerve growth factor
IENF: Intraepidermal nerve fiber
IL: Interleukin
TNF: Tumor necrosis factor.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Research Article

The Effect of Simvastatin on Glucose Homeostasis in Streptozotocin Induced Type 2 Diabetic Rats

Lulu Wang,[1,2] Guanglan Duan,[2] Yong Lu,[2] Shuguang Pang,[1,2] Xianping Huang,[2] Qiang Jiang,[2] and Ningning Dang[2]

[1] *School of Medicine, Shandong University, Jinan, Shandong 250013, China*
[2] *Department of Medicine, Jinan Central Hospital Shandong University, Jinan, Shandong 250013, China*

Correspondence should be addressed to Shuguang Pang; shuguangpang@163.com

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Objective. To investigate the effect of simvastatin on glucose homeostasis in streptozotocin induced type 2 diabetic rats. *Methods*. Forty male Wistar rats were randomly divided into four groups. Normal control rats were fed with standard diet, others were fed with high-fat diet. Diabetic rats were induced by a single intraperitoneal injection of STZ. The simvastatin intervention rats were fed with simvastatin during the experiment process, and the simvastatin treatment rats were fed with simvastatin after diabetes rats were induced. We measured body weight, fasting plasma glucose, cholesterol, high-density lipoprotein cholesterol, and triglyceride after an overnight fast. *Results*. The FPG was higher in diabetic rats when compared to normal control ones; the simvastatin intervention rats had a higher FPG compared to the diabetic rats and were more easily be induced to diabetes at the end of 4 weeks, FPG level of simvastatin treatment rats was increased compared with diabetic model rats after 12 weeks. *Conclusion*. These data indicate that simvastatin intervention rats may cause hyperglycemia by impairing the function of islet β cells and have an adverse effect on glucose homeostasis, especially on FPG level.

1. Introduction

Type 2 diabetes mellitus is ordinarily associated with dyslipidemia, which presents a synergistic risk factor for cardiovascular disease [1]. Statins, the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are inhibitors of cholesterol synthesis. Statin therapy is effective for reduction of cardiovascular events [2] and is commonly recognized as being safe and well tolerated [3]. Furthermore, statin therapy has a close relationship with different doses of statin in cardiovascular patients. Intensive-dose statin therapy provides a significant benefit for preventing cardiovascular events compared with moderate-dose statin therapy [4].

More recently, some studies [5] have reported that statin therapy may affect glucose homoeostasis in type 2 diabetes patients. However, the potential of statin use to affect glucose metabolism has been controversial [6]. Some studies provided that statins therapy have been linked to increased risk of developing diabetes mellitus [7, 8], some studies reported that statin therapy had no effect on insulin sensitivity [9–11], and others indicated a beneficial effect on glucose metabolism [12, 13]. The mechanisms by which statins may affect glycemic control have been under discussion [14], but not clear.

The target of the present study is to investigate the effect of simvastatin on FPG in STZ-induced diabetic rats. This research may provide an alternative to the problem of statin therapy. Toward this goal, we observed the different effect between simvastatin intervention rats and simvastatin treatment rats, especially on FPG levels of various periods.

2. Methods

2.1. Materials. Streptozotocin (Art. Number SO-130) was purchased from Sigma. Insulin ELISA test kits (Art. Number 10-1250-01) were purchased from Mercodia. Glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) test kits were detected using automatic biochemistry analyzer. Simvastatin (Sino-American Shanghai Squibb Pharmaceuticals Ltd) was obtained from Squibb.

Table 1: Administration of simvastatin in fluid and food intake and body weight of rats in the different experimental groups.

Groups	Fluid intake (mL/day)	Food intake (g/day)	Weight gain (g/day)
Normal control	24.00 ± 4.00	19.3 ± 0.2	5.20 ± 0.02
Diabetic	116.00 ± 5.00*	28.50 ± 0.40*	3.10 ± 0.03*
Simvastatin intervention	40.00 ± 2.00*#	24.50 ± 0.30*	3.80 ± 0.05*#
Simvastatin treatment	56.00 ± 3.00*#	25.40 ± 0.20*	3.50 ± 0.03*

Data are the mean ± SD for ten animals in each group.
*$P < 0.05$ compared with the control group.
#$P < 0.05$ compared with the untreated diabetic group.

Glucometer and glucose testing strips were productions of Roche (Switzerland). The standard diet was obtained from Animal Experimental Central, Shandong University (Jinan city, Shandong province); and the high-fat diet purchased from Beijing Ke' ao xieli company (Beijing city). The high-fat diet was purchased from standard diet (SD) consists of 6% fat, 64% carbohydrate and 23% protein, and high-fat diet (HFD) consists of 25% fat, 48% carbohydrate, and 20% protein.

2.2. Experimental Animals. Forty male Wistar rats, weighing 180–200 g, were obtained from Animal Experimental Central, Shandong University (Jinan city, Shandong province, China). Guides for the care and use of laboratory animals were approved by the Local Ethics Committee at the Medical College of Shandong University. Rats were housed in wire-floored cages under a 12 h light-dark cycle for at least 7 days prior to treatment and were fed with standard laboratory chow and tap water ad libitum. The room temperature was kept at 22 ± 2°C. All stressful conditions were avoided. Rats were fasted overnight prior to the study and housed in mesh-bottomed cages to minimize coprophagia. Except for the last hour, water was supplied ad libitum.

2.3. Experimental Induction of Diabetes. Food was withdrawn from 12 to 14 hours before the experiment, and diabetes was induced by a single intraperitoneal injection of a prepared solution of STZ (35 mg/kg) in 0.1 mol/L citrate buffer (pH 4.5). The dosing volume was 1 mL/kg. Successful induction of diabetes was confirmed by measuring the FPG concentration in rats 24 h after injection of STZ. The fasting blood glucose level above 11.1 mmol/L was considered diabetic and included in the present study.

2.4. Experimental Design. Wistar rats were fed with standard diet for one week, and then the rats were randomly divided into four groups, with ten rats in each group as follows: group A: normal control rats, Group B: diabetic model rats, Group C: simvastatin intervention rats, and Group D: simvastatin treatment rats. The normal control rats ($n = 10$) were fed with standard diet, receiving vehicle solution (citrate buffer, 1 mL/kg/day); Group B, Group C, and Group D rats ($n = 24$) were injected with STZ (35 mg/kg bodyweight, i.p.) after high-fat diet for 4 weeks. Successful induction of diabetes was confirmed by measuring the FPG concentration in rats 24 h after injection of STZ. The fasting blood glucose level above 11.1 mmol/L was considered diabetic rats. The simvastatin intervention rats received simvastatin (10 mg/kg/day) from the whole experiment and continued for 12 weeks, and simvastatin treatment rats were treated with simvastatin after successful induction of diabetes and continued for 8 weeks.

The following parameters were assayed in each of the study groups during variance period: daily fluid and food consumption, weekly body weight, and blood glucose concentration. Food consumption was determined by subtracting leftovers from the diet provided to rats at 2-day intervals.

The levels of fasting blood glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) were assayed and compared with each group.

2.5. Statistical Analysis. The statistical analyses were conducted using SPSS18.0 software. Data are expressed as the mean value ± standard deviation. All groups of animals were studied in parallel. Comparisons among multiple groups were achieved via one-way ANOVA. Student's t-test was applied to compare probabilities of data between two groups. Values were considered significant when $P < 0.05$.

3. Results

3.1. Fluid and Food Intake and Bodyweight in Different Experimental Groups. Table 1 displays significant differences in fluid and food intakes and bodyweight change between normal control and diabetic model rats. Compared with normal control rats, there is a significantly increased fluid and food intakes and decreased bodyweight in diabetic rats. Intervention with simvastatin rats tended to increase the bodyweight to that seen in diabetic rats, and the effect was less pronounced in the group of rats treated with simvastatin.

3.2. Blood Glucose and Plasma Lipid Profile in Control and Diabetic Rats Are Given in Table 2. Table 2 shows that, in diabetic rats, there was a significant increase ($P < 0.05$) in TC and TG levels, and significant decrease in HDL-C, and the tendency of TC, TG, and HDL-C levels was even worse at the end of the experiment. Compared to untreated diabetic rats, the levels of TC and TG significantly decreased ($P < 0.05$) and the levels of HDL-C increased ($P < 0.05$) in simvastatin intervention rats. Compared to untreated diabetic rats the TC, TG, and HDL-C levels had no significant change in simvastatin treatment rats at 4 weeks, while there was a significant increase ($P < 0.05$) in TC and TG levels, and significant decrease in HDL-C at 12 weeks.

3.3. The Different Effect between Simvastatin Intervention and Treatment Rats on FPG Is Given in Figure 1. As summarized in Table 2 and Figure 1, at the end of 4 weeks and 12 weeks, the fasting blood glucose levels were significantly higher in diabetic animals when compared with control rat values, which is indicating that there was a successful induction of diabetic model rats. Compared with diabetic rats, at the end

Table 2: Effect of intervention and treatment with simvastatin on fasting plasma glucose, serum total cholesterol, HDL-C, and triglycerides.

Groups	FPG (mmol/L)	Total cholesterol (mmol/L)	HDL-C (mmol/L)	Triglycerides (mmol/L)
Normal control				
Baseline	4.5 ± 0.8	2.0 ± 0.2	0.85 ± 0.02	1.3 ± 0.1
After 4 weeks	4.6 ± 0.6	2.1 ± 0.1	0.87 ± 0.01	1.2 ± 0.2
After 12 weeks	4.4 ± 0.3	2.2 ± 0.3	0.92 ± 0.03	1.1 ± 0.2
Diabetic				
Baseline	4.8 ± 0.6	1.9 ± 0.1	0.75 ± 0.01	1.2 ± 0.1
After 4 weeks	12.4 ± 1.2*	3.7 ± 0.8*	0.59 ± 0.02*	2.6 ± 0.3*
After 12 weeks	13.2 ± 0.9*	4.0 ± 0.5*	0.35 ± 0.01*	3.4 ± 0.2*
Simvastatin intervention				
Baseline	4.6 ± 0.1	2.1 ± 0.2	0.83 ± 0.02	1.4 ± 0.1
After 4 weeks	17.5 ± 0.9*#	2.2 ± 0.3#	1.34 ± 0.04*#	1.2 ± 0.3*
After 12 weeks	15.1 ± 0.2*#	2.0 ± 0.1#	1.56 ± 0.02*#	1.3 ± 0.2*
Simvastatin treatment				
Baseline	4.2 ± 0.5	2.0 ± 0.1	0.87 ± 0.03	1.3 ± 0.1
After 4 weeks	13.4 ± 1.5*	3.2 ± 0.1*#	0.62 ± 0.02#	2.2 ± 0.2#
After 12 weeks	16.2 ± 1.0*#	2.1 ± 0.3*#	1.49 ± 0.05#	1.5 ± 0.3#

Data are the mean ± SD for ten animals in each group.
*$P < 0.05$ compared with the control group.
#$P < 0.05$ compared with the untreated diabetic group.

of 4 weeks, treatment of diabetic rats with simvastatin slightly increased FPG ($P > 0.05$) simvastatin intervention rats were significantly increased FPG ($P < 0.05$) and easily induced diabetic rats. At the end of 12 weeks, simvastatin intervention rats were still higher at the level of FPG, while the FPG levels were slightly decreased compared with the end of 4 weeks. At the end of 12 weeks, the simvastatin treatment rats had a significantly higher FPG levels compared with the diabetic rats ($P < 0.05$).

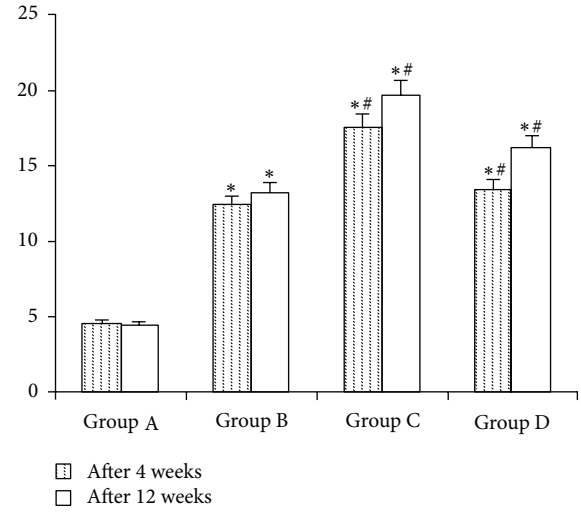

Figure 1: The different effect on FPG in each group of various periods. A: normal control group; B: diabetes model group; C: simvastatin intervention group; D: Simvastatin treatment group.*$P < 0.05$ compared with the control group.#$P < 0.05$ compared with the untreated diabetic group.

4. Discussion

Statins can enhance the expression of low-density lipoprotein (LDL) receptors in the liver and consequently lower the levels of blood LDL cholesterol through inhibiting cholesterol synthesis in the liver [15]. Stains, including both water-soluble and lipid-soluble statins, have been shown to protect against the injury in cardiovascular and renal diseases [16, 17]. Besides their well-established role in lowering cholesterol levels, recent research has demonstrated that statins might have relevant effect on glucose metabolism in animal models and in humans. However, several studies on the effect of statin on glucose metabolism are controversial. Some [18–20] have shown that simvastatin had a benefit effect on insulin resistance; some [21, 22] have reported no benefit on insulin action, and others [23–25] have identified a deterioration in glucose homoeostasis. These mechanisms may be caused by the different property of statins. Water-soluble statins, such as rosuvastatain, are hepatocyte specific and are not probably taken up by pancreatic cells and adipocytes. Lipid-soluble statins, such as simvastatin, enter extrahepatic cells easily and may suppress isoprenoid protein synthesis, consequently attenuating the action of insulin levels. The present study is to investigate the effect of simvastatin on glucose metabolism from the prediabetic rats models. Toward this goal, we observed the different effect between simvastatin intervention rats and simvastatin treatment rats, especially on FPG levels of various periods.

This study shows that the rats with simvastatin intervention at a dose of 10 mg/kg have increased the levels of FPG, and consequently the diabetic model rats were easily induced. Similarly, we observed that FPG was slightly increased in diabetic rats after eight weeks with simvastatin treatment. In addition to the classic function of lowering the cholesterol levels, our study demonstrated that statin could also impact the glucose homeostasis on STZ-induced diabetic rats. Despite the levels of TC and TG decreased and total cholesterol/HDL ratio increased compared with control group, both intervention and treatment with simvastatin rats did not improve the glucose homeostasis, especially on FPG. These results suggest that the rats with simvastatin intervention were more easily induced, the diabetic model rats and the diabetic rats, those treated with simvastatin could also increase the levels of FPG. Therefore simvastatin could not decrease the FPG levels and improve the insulin sensitivity. What is more, simvastatin therapy is associated with a slightly increased risk of diabetes.

In this paper, we demonstrated the different effect on FPG between intervention and treatment with simvastatin rats. The FPG increased greater in simvastatin intervention rats than simvastatin treatment rats. Importantly, the simvastatin intervention rats were more easily induced to be diabetic rats and had a higher FPG compared with simvastatin treatment group. Besides, the results indicated that simvastatin intervention rats were more inclined to have an adverse effect on glucose homeostasis, especially on FPG, compared with simvastatin treatment rats. The biological significance of elevated FPG identified in simvastatin intervention group remains unknown, but some studies [26] would suggest that the abnormality in FPG may translate into the clinical syndrome of diabetes with a rise in glycosylated hemoglobin (HbA1c). In addition, it has provided that there is a graded relationship between FPG and the extent of coronary artery disease severity [27]. Current estimates indicate that simvastatin intervention rats, with prediabetic status, may easily develop diabetic rats induced by STZ. It is noteworthy that, in our current study, the mean FPG value in simvastatin treatment rats increased which is in the impaired fasting glucose range. The worsening of FPG in simvastatin intervention was even more pronounced.

The precise mechanisms by which statins may exert any influence on glucose metabolism are unclear. Statins have the potential to alter glycemic control by decreasing various metabolites such as isoprenoid, farnesyl pyrophosphate, geranylgeranyl pyrophosphate, and ubiquinone (CoQ10), all of which are dependent on mevalonic acid production. Isoprenoid in particular enhances glucose uptake via GLUT-4 in adipocytes [28]. Reduction in CoQ10 may result in delayed ATP production in pancreatic beta cells and thereby impair insulin release [29]. The mechanisms may have the relationship with the property of statins. Simvastatin has been shown to inhibit glucose-induced increase in intracellular calcium in pancreatic beta cells leading to the inhibition of insulin secretion in a dose-dependent manner [30]. Thus, this study shows that simvastatin intervention that effectively increased FPG may promote the development of type 2 diabetes mellitus. The research may provide an alternative to the problem of statin therapy.

The limitations of the research showed that our diabetic rats were followed up for 8 weeks in this experimental study this is an obviously short period considering the natural history of diabetes mellitus. We do not know whether this association would continue, worse, or get better through a long period of followup. The biological importance of elevated FPG in simvastatin intervention and treatment rats identified in this study remains unknown, but data from the JUPITER trial [26] would suggest that the elevated FPG may translate into the clinical syndrome of diabetes with a rise in HbA1C. Besides, it has been demonstrated that there is a graded relationship between FPG and the levels of coronary artery disease severity [31]. The present estimates indicate that most individuals with prediabetic status may eventually develop clinical diabetes mellitus.

5. Conclusion

Our observational study demonstrated that statin therapy is associated with a rise of FPG levels over a mean period of 12 weeks in simvastatin intervention rats. Considering the prevalence of statin usage in current clinical practice, this significant observation merits further investigation. Clinicians should realize this potential adverse association of statin use on FPG, and careful monitoring is advised.

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