

Original Research Article

Antiglycation and Hypolipidemic Effects of Polyphenols from *Zingiber officinale* Roscoe (Zingiberaceae) in Streptozotocin-Induced Diabetic Rats

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Abstract

Purpose: To evaluate the antiglycation and hypolipidemic potential of polyphenols from *Zingiber officinale* in streptozotocin-induced diabetic rats.

Methods: Diabetes was induced in male Wistar rats by single intraperitoneal injection of 50 mg/kg body weight (bw) of streptozotocin. This was followed by oral administration of 500 mg/kg each of free and bound polyphenol extracts of *Z. officinale* to the rats daily for 42 days. Distilled water and glibenclamide (5 mg/kg) were used as normal and positive controls, respectively.

Results: Significant increases ($p < 0.05$) in blood glucose level (369.26 mg/dL), serum advanced glycation end-products (AGEs) (6.80 $\mu\text{g/mL}$), lipid profile and atherogenic indices, with decrease in high density lipoprotein cholesterol (HDL-C) (15.55 mg/dL) were observed in diabetic rats compared to control. Free polyphenol extracts of *Z. officinale* significantly reduced ($p < 0.05$) blood glucose (147.96 mg/dL), serum AGEs (1.98 $\mu\text{g/mL}$), lipid profile and atherogenic indices while it significantly increased HDL-C (23.28 mg/dL). However, bound polyphenol extract did not cause any significant change in the lipid profile of the diabetic rats except for LDL-C.

Conclusion: This study indicates that free and bound polyphenols from *Z. officinale* can ameliorate diabetes as well as its complications, and its effect is comparable to that of the standard drug, glibenclamide.

Keywords: *Zingiber officinale*, Diabetes, Lipid profile, Atherogenic index, Polyphenol, Glycation, Streptozotocin

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia and dyslipidemia which results from defects in both insulin secretion and/or insulin action [1]. The disease is associated with reduced quality of life and increased risk factors for mortality and morbidity. Long-term hyperglycemia is an important factor in the development and

progression of micro- and macro-vascular complications, which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases [2].

Zingiber officinale Roscoe (family: Zingiberaceae) is commonly known as ginger (English), gingembre (French), Afu (Hausa) and Ata-ile (Yoruba). It is used widely for indigestion, stomach-ache, malaria and fevers [3]. Its

combination with lime juice and rock salt increases appetite and stimulates the secretion of gastric juice [4]. The rhizomes of *Z. officinale* are important ingredients of many traditional medicines worldwide. The fresh (or dried) rhizomes of *Z. officinale* are also used as tonics to treat indigestion, dyspepsia, flatulence and nausea. It is also used to treat a variety of human ailments such as rheumatism, asthma, stroke, constipation and diabetes [5].

Polyphenolic compounds are ubiquitous in all plant and are, therefore, an integral part of the human diet [6]. Until recently, most of the nutritional interest in polyphenolic compounds was in the deleterious effects caused by the ability of certain polyphenols to bind and precipitate macromolecules, such as dietary protein, carbohydrate, and digestive enzymes, thereby reducing food digestibility. Recent interest, however, in phenolics has increased greatly because of the antioxidant and free radical-scavenging abilities associated with some phenolics and their potential effects on human health [7].

Though, several studies had been conducted on the possible antidiabetic potential of *Z. officinale* [8,9], none has been done on the antiglycation potential of its polyphenols in diabetic rats. It is based on this premise that the antiglycation and hypolipidemic potential of polyphenols from *Z. officinale* was performed in streptozotocin-induced diabetic rats.

EXPERIMENTAL

Plant material

Zingiber officinale was purchased from the Central Spices Market in Mile 12 area, Ketu, Lagos, Nigeria in May 2012. Identification and authentication of the sample was done by Dr. A. B. Kadirri of the Department of Botany, University of Lagos, Akoka, Lagos and voucher specimen (LUH 4730) was deposited in the university's herbarium.

Chemicals

Streptozotocin was a product of Alexis Biochemical, San Diego CA 92101, USA. The assay kits for glucose and lipid profile were products of Randox Laboratories, Antrim BT41, United Kingdom while advanced glycation end-products (AGEs) ELISA kit was obtained from Cusabio Biotech. Co. Ltd, Hubei Province 430206, China

Animals

Albino rats were obtained from the Animal House of the Department of Biochemistry, Lagos State University, Ojo, Lagos. All the animals were maintained under laboratory conditions of temperature (22 ± 2 °C), humidity (45 ± 5 %) and 12 h day: 12 h night cycle under air-conditioner. They were also allowed access to food (standard pellet diet) and water *ad libitum*. All procedures were performed in compliance with the Guide for the Care and Use of Laboratory Animals [10], as approved by the Research ethical committee of Lagos State University (ref no. RC/BCH/0720).

Preparation of free polyphenol

A known mass (3 kg) of the *Zingiber officinale* was crushed in 80 % acetone (1 : 2 w/v) using a Waring blender (Waring Commercial, Torrington, CT) for 5 min [11]. The sample was homogenized using a Polytron homogenizer (Glen Mills Inc., Clifton, NJ) for 3 min. The homogenates were filtered under vacuum using Buchner funnel and Whatman no. 2 filter paper. The filtrate was concentrated using a rotary evaporator under vacuum and later freeze-dried in a lyophilizer (Ilshin Lab. Co. Ltd, Seoul, Korea). The extract (245.62 g) was stored frozen at - 20 °C until the commencement of the experiment.

Preparation of bound polyphenol

Residue from the free polyphenol extraction was drained and hydrolyzed with 2 L of 4 M NaOH for 1 h with constant shaking [11]. The mixture was acidified with concentrated hydrochloric acid to pH 2, extracted six times with ethylacetate, pooled and concentrated using rotary evaporator and subsequently freeze-dried. The extract (97.35 g) was stored frozen at - 20 °C until the commencement of the experiment.

Induction of experimental diabetes

Diabetes mellitus (Type 2) was induced by single intraperitoneal injection of freshly prepared STZ (50 mg/kg bw) in 0.1 M citrate buffer (pH 4.5). Diabetes was confirmed in these STZ-treated rats over a period of 7 days. In all experiments, rats were fasted for 18 h prior to STZ injection. After 7 days, the blood was collected from the tail and the blood glucose level of each rat determined. Rats with a fasting blood glucose range of 250 – 300 mg/dL were considered diabetic and included in the study.

Experimental design

A total of 40 rats (8 normal; 32 STZ-diabetic rats) were used for this experiment. The rats were randomised into five groups of eight animals each. Group 1 consisted of rats administered with vehicle alone (distilled water) while group 2 comprises STZ-induced diabetic rats only; Groups 3 and 4 consisted of STZ-induced diabetic rats administered 500 mg/kg bw free and bound polyphenol extracts of *Z. officinale* respectively. Group 5 comprises STZ-induced diabetic rats administered glibenclamide (5 mg/kg bw). The extract was suspended in the vehicle (distilled water) and was administered everyday orally using an orogastric tube. Following 42 days of administration [12], animals were decapitated, their blood was collected and serum separated immediately. This was done by centrifuging the blood samples at $1282 \times g$ for 5 min with Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, Essex, England).

Biochemical measurements

Glucose was determined by the glucose oxidase method. Glycated haemoglobin (HbA1c) was determined using Micromat II HbA1c analyzer (Bio-Rad, Deeside, UK) using Glycosal® test cartridge. Serum advanced glycation end products was determined using rat advanced glycation end products (AGEs) ELISA kit (Cusabio Biotech Co., China). Total cholesterol (TC), HDL-cholesterol and triacylglycerol (TG) were measured using colorimetric methods while LDL-cholesterol was calculated by Friedwald's formula [13].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, San Diego MA, USA). The data were

analysed by one way analysis of variance (ANOVA) followed by Bonferroni test. All the results were expressed as mean \pm SEM ($n = 8$) and were considered statistically significant when $p < 0.05$.

RESULTS

Effect on fasting blood glucose

Table 1 shows the result of fasting blood glucose (FBG) level of the animals during the experiment. The diabetic control animals had increasing fasting blood glucose throughout the period of the experiment rising from 262.8 mg/dL on the 1st day to 369.26 mg/dL at the end of the experiment. The administration of free and bound polyphenol extracts of *Z. officinale* to diabetic rats significantly decreased ($p < 0.05$) FBG compared to the diabetic control rats by the 42nd day. However, the administration of glibenclamide to the diabetic animals produced the best result by significantly reducing ($p < 0.05$) FBG drastically by the end of the experiment.

Effect on HbA1c

The effect of the administration of polyphenol extracts of *Z. officinale* on the HbA1c of the streptozotocin induced diabetic rats is shown in Figure 1. The HbA1c level of diabetic control rats (10.33 %) was significantly higher ($p < 0.05$) than the normal control (4.52 %). The administration of free and bound polyphenols from *Zingiber officinale* significantly reduced ($p < 0.05$) HbA1c of the diabetic rats. However, it was only glibenclamide treated diabetic animals that exhibited values that were similar to the control group.

Table 1: Effect of administration of polyphenols from *Z. officinale* on the fasting blood glucose (mg/dL) of normal and streptozotocin-diabetic rats

Group	Glucose (mg/dL)		
	Initial	Final	% change
Normal Control	75.78 \pm 2.70 ^a	80.26 \pm 4.32 ^a	4.55 \pm 0.16 ^a
Diabetic Control	262.08 \pm 8.46 ^b	369.26 \pm 11.34 ^b	44.92 \pm 3.02 ^b
Diabetic + Free polyphenol	285.92 \pm 6.72 ^b	147.96 \pm 9.34 ^c	-51.65 \pm 4.63 ^c
Diabetic + Bound polyphenol	270.40 \pm 9.06 ^b	226.08 \pm 7.15 ^d	-15.46 \pm 1.75 ^d
Diabetic + Glibenclamide	296.64 \pm 8.94 ^b	111.96 \pm 6.04 ^c	-60.17 \pm 3.78 ^c

Values are mean \pm SEM ($n = 8$); values down the vertical columns carrying different superscripts are significantly different from each other ($p < 0.05$)

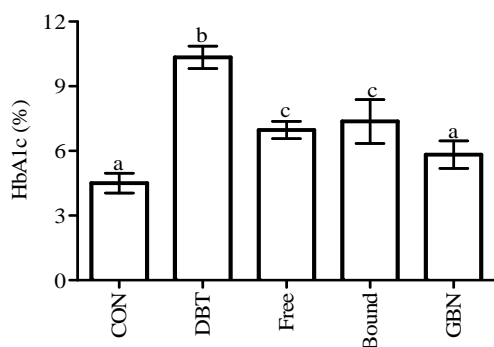


Figure 1: Effect of oral administration of polyphenols from *Z. officinale* on the HbA1c level in normal and streptozotocin-induced diabetic rats. Values are mean \pm SEM ($n = 8$); bars carrying different superscripts are significantly different ($p < 0.05$) from each other. CON: Normal Control, DBT: Diabetic Control, Free: Diabetic rats treated with free polyphenol extract, Bound: Diabetic rats treated with bound polyphenol extract, GBN: Diabetic rats treated with glibenclamide

Effect on AGEs

Advanced glycation endproducts (AGEs) were significantly higher ($p < 0.05$) in the diabetic untreated rats when compared with normal control rats (Figure 2). Administration of free and bound polyphenol extracts of *Z. officinale* to diabetic rats reduced the AGEs significantly ($p < 0.05$) when compared to the diabetic control rats and their values are comparable to the normal control as well as glibenclamide treated diabetic animals.

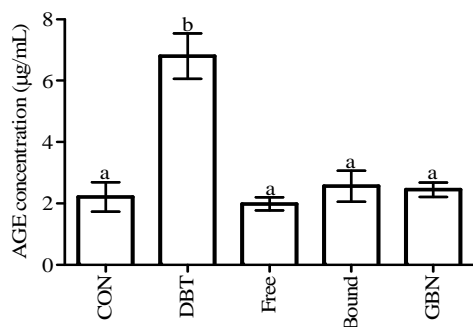


Figure 2: Effect of oral administration of polyphenols from *Z. officinale* on the formation of advanced glycation endproducts (AGEs) in normal and streptozotocin-induced diabetic rats. Values are mean \pm SEM ($n = 8$); bars carrying different superscripts are significantly different ($p < 0.05$) from each other. CON: Normal Control, DBT: Diabetic Control, Free: Diabetic rats treated with free polyphenol extract, Bound:

Diabetic rats treated with bound polyphenol extract, GBN: Diabetic rats treated with glibenclamide

Effect on lipid profile

Table 2 depicts the effect of polyphenolic extracts of *Z. officinale* on the serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triacylglycerol (TG) concentration of STZ-induced diabetic rats. The serum TG, TC and LDL-C levels were significantly higher ($p < 0.05$) in the diabetic control rats compared to the normal control rats while HDL-C was significantly reduced ($p < 0.05$). Treatment with free polyphenols from *Z. officinale* significantly reduced ($p < 0.05$) the level of TC, LDL-C and TG but increased HDL-C and this result is comparable to the values obtained for glibenclamide-treated diabetic rats. Bound polyphenol extract only significantly reduced ($p < 0.05$) LDL-C compared to the diabetic control group.

Effect on atherogenic indices

Diabetic control rats displayed the highest level in all atherogenic indices calculated and are significantly different ($p < 0.05$) from the normal control group except for log (TG/HDL-C) (Table 3). The administration of free and bound polyphenols from *Z. officinale* to diabetic rats significantly decreased ($p < 0.05$) these indices and their values are comparable to standard drug glibenclamide.

DISCUSSION

Streptozotocin-induced diabetes has been described as a useful experimental model to study antidiabetic activity of several agents [14]. The mechanism by which streptozotocin brings about diabetes includes selective destruction of pancreatic β -cells which make cells less active, leading to poor sensitivity of insulin for glucose uptake by tissues [15]. The increased levels of serum glucose in STZ-induced diabetic rats were lowered by the administration of both free and bound polyphenols from *Z. officinale*, though glibenclamide produced better effect. The reduced glucose levels suggests that *Z. officinale* might be exerting insulin-like effect on peripheral tissues by promoting glucose uptake, inhibiting hepatic gluconeogenesis or by absorption of glucose into the muscle and adipose tissues through the stimulation of a regeneration process of the remaining β -cells [16].

Table 2: Effect of oral administration of polyphenols from *Z. officinale* on the lipid profile (mg/dL) of normal and streptozotocin- diabetic rats

Group	Lipid (mg/dL)			
	TC	HDL-C	LDL-C	TG
Normal Control	57.22 ± 7.17 ^a	28.42 ± 2.64 ^a	18.84 ± 3.17 ^a	52.60 ± 4.63 ^a
Diabetic Control	114.64 ± 19.59 ^b	15.55 ± 2.55 ^b	62.54 ± 7.31 ^b	95.68 ± 8.05 ^b
Diabetic + Free	72.28 ± 3.84 ^a	23.28 ± 1.84 ^a	30.38 ± 4.99 ^c	66.46 ± 8.00 ^a
Diabetic+Bound	93.16 ± 7.06 ^b	19.20 ± 4.41 ^b	35.40 ± 3.54 ^c	82.30 ± 5.67 ^b
Diabetic + GBN	63.92 ± 5.28 ^a	24.78 ± 3.76 ^a	19.01 ± 2.88 ^a	55.86 ± 5.95 ^a

Values are mean ± SEM (n = 8); values down the vertical columns carrying different superscripts are significantly different (p < 0.05) from each other; TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TG: triacylglycerol

Table 3: Effect of administration of polyphenols from *Z. officinale* on the atherogenic indices of normal and streptozotocin-induced diabetic rats

Group	Atherogenicity			
	TC/HDL-C	LDL-C/HDL-C	TG/LDL-C	Log(TG/HDLC)
Normal Control	2.17 ± 0.48 ^a	0.67 ± 0.09 ^a	1.97 ± 0.35 ^a	0.27 ± 0.08 ^a
Diabetic Control	10.89 ± 2.00 ^b	7.48 ± 1.78 ^b	10.59 ± 2.09 ^b	0.99 ± 0.08 ^a
Diabetic + Free	3.19 ± 0.32 ^a	1.30 ± 0.19 ^a	2.95 ± 0.47 ^a	0.45 ± 0.07 ^a
Diabetic+Bound	4.85 ± 0.05 ^c	1.83 ± 0.01 ^a	4.47 ± 0.64 ^c	0.63 ± 0.06 ^a
Diabetic + GBN	2.78 ± 0.37 ^a	0.90 ± 0.26 ^a	2.32 ± 0.12 ^a	0.36 ± 0.02 ^a

Values are mean ± SEM (n = 8); values down the vertical columns carrying different superscripts are significantly different (p < 0.05) from each other; TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TG: triacylglycerol

Glycated haemoglobin, often measured as HbA1c has been widely used as an index of glycaemic control in most parts of the world [17]. HbA1c was found to increase in patients with diabetes mellitus due to glycosylation of haemoglobin and the amount of increase was directly proportional to the fasting blood glucose levels. In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including hemoglobin [18]. This is consistent with the result of this study in which the HbA1c was very high in diabetic control rats. However, administration of free polyphenol extracts of *Z. officinale* suppressed this index. This decrease in HbA1c levels in diabetic rats treated with free polyphenol extracts of *Z. officinale* could be due to the decreased glucose levels (good glycemic control).

Hyperglycemia is known to have a range of biological effects which include the production and accumulation of advanced glycation end-products (AGEs). Advanced glycation endproducts (AGEs) have been implicated in diabetic complications because they alter enzymic activity, decrease ligand binding, modify protein half-life and alter immunogenicity [19]. The four-fold increase in the formation of AGEs

in the streptozotocin-induced diabetic control rats was normalised in diabetic rats treated with both free and bound polyphenol extracts of *Z. officinale* and are comparable to the normal control rats and glibenclamide treated diabetic rats. The reduction in the formation of AGEs in diabetic rats treated with polyphenols from *Z. officinale* may be due to the antioxidant potential of these extract and/or their hypoglycaemic potential. Since hyperglycemia is the main precursor to all diabetic-related pathologies, ability of the extracts to lower the glucose level may also reduce the formation of AGE and subsequently diabetic complications.

Diabetes is associated with alterations in the plasma lipid and lipoprotein profile, with an increased risk of coronary heart disease [20]. In this study, elevated levels of serum lipids such as cholesterol and triacylglycerols were noticed in diabetic rats. Under normal circumstances, insulin activates lipoprotein lipase and hydrolyzes triacylglycerols. It also increases uptake of fatty acids into adipose tissue as well as inhibits lipolysis. In cases of insulin deficiency, lipolysis is not inhibited but rather proceeds at a higher rate which finally leads to hyperlipidemia. In this study, diabetic control rats had elevation in the

level of LDL-C (Table 2). Insulin deficiency or insulin resistance may also be responsible for this because insulin has an inhibitory action on β -hydroxyl- β -methyl glutaryl CoA reductase (HMG-CoA reductase), a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue [21]. This results in increased production of cholesterol-rich LDL particles [22]. However, administration of both free and bound polyphenol extracts of *Z. officinale* counteracted this effect in diabetic rats and is comparable to glibenclamide (Table 2).

HDL-C is an anti-atherogenic lipoprotein which transports cholesterol from peripheral tissues into the liver and thereby acts as a protective factor against coronary heart disease [21]. The concentration of HDL-C, which increased after *Z. officinale* free polyphenol extracts administration, might be due to the increase in the activity of lecithin cholesterol acyl transferase (LCAT), which may contribute to the regulation of blood lipids [23]. In this study, administration of free polyphenol extracts of *Z. officinale* lowers serum lipids, decreases LDL-C and also increases the serum HDL-C level in diabetic rats. The antilipidaemic effect of *Z. officinale* may be due to decreased cholesterol synthesis and fatty acid synthesis. Significant lowering of total cholesterol, triacylglycerols, LDL-cholesterol and rise in HDL-cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions [23]. This was achieved by administration of free polyphenol extract of *Z. officinale* to the diabetic rats.

In individuals with diabetes, cardiovascular risk is increased by a cluster of risk factors such as abdominal obesity, impaired fasting glucose, increased blood pressure, low HDL-C, increase in both TGs and LDL-C particles [1]. Although, insulin resistance is crucial to the pathogenesis of this disease, the associated atherogenic lipoprotein phenotype considerably enhances the risk. Hence there was the need to evaluate atherogenic indices from the lipid profile obtained during the experiment. Several lipoprotein-related indices have been postulated to evaluate the risk of cardiovascular diseases in diabetes. These include TC/HDL-C, LDL-C/HDL-C, TG/HDL-C and log (TG/HDL-C) molar ratios and are adjudged to be good predictive value for future cardiovascular events [24]. In this study, diabetic control animals had the highest values in all the atherogenic indices evaluated (Table 3). However, treatment with both free and bound polyphenol extracts of *Z. officinale* reduces the

atherogenic indices of these rats and the values were comparable to that of glibenclamide. The high values of atherogenic indices experienced by diabetic rats may be due to the decreased HDL-C values and hypertriacylglycerolemia which are all due to derangement in lipid metabolism [25].

The difference in the activities of both free and bound polyphenol extracts of *Z. officinale* may not be unconnected to the difference in their structures and compositions. Free polyphenols occur as phenolic acids and flavonoids. They are freely available and more readily absorbed, and thus, exert beneficial bioactivities in early digestion [11]. Bound polyphenolic compounds, on the other hand, are present as a component of plant cell walls. They are present as monomeric, dimeric, or oligomeric compounds, which are esterified to the cell wall. Bound phytochemicals, mainly as β -glycosides, could not be digested by human enzymes, and could survive stomach and small intestine digestion to reach the colon and be digested by bacteria flora releasing phytochemicals with health benefits [11].

CONCLUSION

The results of the present study clearly indicate that free polyphenols from *Z. officinale* rhizome possess the antiglycation and hypolipidemic active principle(s) which probably acts by improving the alterations in the carbohydrate and lipid metabolism of the animals used.

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