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Full Length Research Paper

Catechins as antidiabetic compounds of *Bridelia ferruginea* Benth root bark extract

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The present study was carried out to evaluate the antidiabetic activity of catechins isolated from *Bridelia ferruginea* in previous studies. Epigallocatechin (EGC) and Epigallocatechin gallate (EGCG) isolated from *B. ferruginea* were administrated to streptozotocin-induced diabetic mice to evaluate their anti-hyperglycemic and anti-hyperlipidemic effects. Then, biochemical parameters were assayed in different groups of streptozotocin-induced diabetic mice. The level of fasting blood glucose levels, triglycerides (TG) and total cholesterol (TC) in streptozotocin-induced diabetic mice were significantly decreased after daily oral administration of EGC and EGCG at doses of 10 mg/kg/day, for 21 days. Glucose intolerance was significantly reduced in streptozotocin induced diabetic mice treated with catechins. These results suggest that catechins constituents from *B. ferruginea*, revealed significant anti-hyperglycemic and antihyperlipidemic activity in type 2 diabetes.

Key words: *Bridelia ferruginea*, epigallocatechin, streptozotocin, diabetes, medicinal plant.

INTRODUCTION

Diabetes mellitus (DM) is one of the most severe metabolic disorders characterized by hyperglycemia as a result of a relative or an absolute lack of insulin secretion, or/and insulin action on its target tissue (Leila al., 2007). There are other symptoms, including hyperlipidemia, which can lead to the development of microvascular complication of diabetes ***Sun***th (Taskinen, 2003).

There are mainly two types of diabetes, type 1 and type 2. Type 1 diabetes is known as insulin-dependent-diabetes-mellitus (IDDM), and results from a cellular mediated autoimmune destruction of the β cells of the pancreas leading to absolute insulin deficiency (Gavin et al., 1997). Type I diabetes commonly occurs in child-hood and adolescence, but can occur at any age. This form of the disease may account for 5 to 10% of all cases of diabetes (Stumvoll et al., 2005). Type 2 diabetes, which is responsible for more than 90% of all diabetes patients and previously referred to as non-insulin dependent diabetes mellitus (NIDDM), or adult-onset diabetes, is a term used for individuals who have insulin resistance and usually have relative insulin deficiency (Gavin et al., 1997). The risk of developing this form of diabetes increases with age, obesity and lack of physical activity. Obesity and type 2 diabetes are closely correlated.

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Most of the conventional synthetic chemical antidiabetic drugs have low rates of response; they have also severe adverse effects. Accordingly, it is necessary to introduce more effective hypoglycemic agents with lower adverse effects (Sun et al., 2008).

In our previous studies, the effects of *B. ferruginea* hydroethanolic extract were proven on some parameters of metabolic syndrome in type 2 diabetes (Bakoma et al., 2011); there was lack of apparent toxicity, acute or sub-chronic, at doses greater than those that induce an effect in animal disease models (Bakoma et al., 2013). The ethyl acetate (EtOAc) soluble fraction of the hydroethanolic extract from the roots of *B. ferruginea* were found to be the most active fraction on diabetes and catechins were isolated (Bakoma et al., 2014 ; 2015). To the best of our knowledge, the active ingredients with antidiabetic activity and their probable mode of action have not been investigated so far. The present study was designed to identify the active compounds of *B. ferruginea* using streptozotocin induced diabetic mice.

MATERIALS AND METHODS

Plant material

The roots of *B. ferruginea* were collected in August 2012 from Tsévié area, 35 km North East of Lomé (Togo). Botanical authentication was confirmed at the Department of Botany, University of Lomé, where a voucher specimen of *B. ferruginea* was deposited at the herbarium (No. 83, 2010).

Animals

Male Swiss mice (BW 30 to 35 g) purchased from Elevage Janvier (France) were maintained under standard conditions with a 12 h light/dark cycle and had free access to standard laboratory diet and water. Prior to initiation of dosing, all rats and mice were acclimated for 7 days. After, mice were randomized to different groups on the basis of their body weights. Principles of laboratory animal care as described in the European Community guidelines were followed (Official Journal of European Union L197 vol. 50, July 2007). This study was approved by the ethical committee on animal experimentation of the University of Bordeaux.

Extraction and fractionation

The air-dried and powdered root bark of *B. ferruginea* (1230 g) were sliced and macerated in 9000 ml ethanol-water (80:20) three times for 72 h at room temperature. The extract was then evaporated under vacuum (40°C). The residue (172 g) was dissolved in distilled water and partitioned three times with hexane (3X400 ML), dichloromethane, DCM (3X400 ML), ethyl acetate, EtOAc (3X400 ML).

Ethyl acetate fractions were used to isolate epigallocatechin and epigallocatechin gallate in previous studies (Bakoma et al., 2015).

Diabetes induction and treatment

Forty Swiss male mice (30 to 35 g) were randomly divided into 6 groups of 8 animals. Diabetes was induced in animals of group 2, 3, 4, 5 and 6 with a single streptozotocin (STZ) intraperitoneal injection, at 135 mg/kg weight, in 0.1 M citrate buffer, pH 4.5. Group 1 received the same volume of STZ vehicle (citrate buffer). A week after STZ delivery, mice with blood glucose above 200 mg/dl were included in the study, and 24 h later the animals were treated as follows: group 1 (normal control, NC) and group 2 (diabetic control, DC) received isotonic saline solution; group 3 (EA) received the ethyl acetate fraction (50 mg/kg wt); group 4 (EGC) and group 5 (EGCG) received respectively epigallocatechin (10 mg/kg wt) and epigallocatechin 3-O gallate (10 mg/kg wt); group 6 (MET) received metformine (50 mg/kg).

Drugs and vehicle were administered daily by gavage for 21 days, and water and food intake were recorded. During the experimentation, blood glucose level was measured on the first, 7 th, 14th and 21st day after 12 h fasting using Free style papillon Glucometer.

Oral glucose tolerance test (OGTT) in STZ induced diabetic mice

OGTT was performed after 21 day treatment, during which mice were fed with normal diets. Mice were fasted over night; glucose (2 g/kg) was fed 30 min after administration of drugs. Blood was withdrawn from tail-vein at 0, 30, 60 and 120 min after glucose loading. Blood glucose level was measured immediately using Free style papillon Glucometer.

Estimation of biochemical parameters

Mice were anesthetized with pentobarbital (50 mg/kg i.p) and blood was collected by heart puncture and centrifuged at 3000 *g* for 15 min and the plasma was aliquoted and frozen for blood glucose, plasma total cholesterol (Ch), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT) level determination using commercial kit (Biomerieux,Marcy l'Etoile, France).

Insulin concentrations were determined from frozen plasma samples using the Rat and mice Insulin Enzyme Immunoassay Kit (SPI-BIO, Montigny Le Bretonneux, France).

The index of insulin resistance was estimated by homeostasis model assessment (HOMA) according to the following formula: HOMA-IR= Insulin (mUI/L) x Plasma glucose (mmol/ L)/22.4 (Matthews et al., 1985).

Statistical analysis

GraphPad Prism 5.00 (USA) software was used to process the results. They are expressed as mean value with the standard error of the mean (M ± E.S.M). These results are analyzed using the variance analysis (ANOVA) followed by the Tukey posttest, to compare the batches. The materiality threshold is set at $P < 0.05$.

RESULTS

Effect of substances on STZ-induced diabetic mice blood glycaemia during the experiment mice

The anti-hyperglycemic effect of EGC and EGCG was evaluated in STZ-induced diabetic rats. Blood glucose level was measured in normal and experimental rats on days 0, 7, 14, 21 of drug treatment. Streptozotocin administration (100 mg/kg) led to over 2.8 fold elevation of glycemia in a time-dependent manner ($p<0.001$) compared to normal mice. STZ-induced diabetic mice

Table 1. Effect of substances on blood sugar level during the experiment.

Groups	Blood glucose (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
Normal Control	81±3.1	86±2.8	88±36	85±31
Diabetic control	226±20	241±17###	282±0.8###	317±25###
EA	213±12	222±24	210±13***	225±26**
EGC	247±8.6	239±35	215±15**	221±13**
EGCG	264±18	271 ±16	248±10*	205±10 ***
MET	250±25	218±13	208±9***	182±24***

Data were expressed as value with the standard error of the mean (M ± E.S.M, n = 8) and evaluated by ANOVA followed by Tukey's test at 5% *P < 0,05 ; **p < 0,01; ***P<0,001 (vs DC); ## P < 0,01 ; ### P < 0,001 (vs C).

Table 2. Effect of substances on oral glucose tolerance test in STZ-induced diabetic mice.

Groups	Blood glucose (mg/dl)			
	0 min	30 min	60 min	120 min
Normal Control	85±3.1	146±2.8	180±3.6	162±3.6
Diabetic control	317±25	441±17###	382±8	412±8
EA	225±26	321±34 **	291±26 **	278±9
EGC	221±13	399±25*	345±15**	285±18
EGCG	205±10	341 ±16***	318±10***	220±15***
MET	182±24	318±13**	249±9**	190±11**

Data were expressed as value with the standard error of the mean (M ± E.S.M, n = 8) and evaluated by ANOVA followed by Tukey's test at 5% *P < 0,05 ; **p < 0,01; ***P<0,001 (vs DC); ## P < 0,01 ; ### P < 0,001 (vs C).

treated respectively with ethyl acetate fraction (50 mg/kg wt), EGC (10 mg/kg wt) and EGCG (10 mg/kg wt) for 3 weeks showed a significant (p<0.001) decrease in glycemia compared to diabetic control group. Normal control mice did not show any alteration in their glycemia through the duration of the experiment significantly (Table 1).

Oral glucose tolerance test

Administration of glucose (2 g/kg,) produced significant increase in blood sugar level of normal control mice. Treatment with EGC, EGCG, and metformin significantly reduced blood glucose level at 30 min, 60 min and 120 min compared to diabetic control mice (Table 2).

Effect of substances on plasma biochemical parameters

At the end of the study, fasting blood sugar level of STZ-diabetic control (271±16 mg/dl) was high compared to normal control group (129±34 mg/dl) significantly (p < 0.001).

Ethyl acetate fraction, catechins and metformin treated groups showed significant (p < 0.001) decrease of glycaemia over 21 days of treatment compared to STZ-diabetic control group. Plasma triglycerides and total cholesterol levels at the end of the study were significantly (p < 0.001) higher in the STZ-diabetic control group (167±13; 131±4.9 mg/dl) than in normal control group (121±5.6; 117±3.9). Treated groups showed a significant (p < 0.01) reduction of plasma cholesterol and triglycerides level, neither EGCG treated group. AST level was significantly (p <0.001) higher in diabetic control group (286±42 UI/L) compared to normal control group (114±7.3 UI/L), only metformin treated group showed a significant (p <0.01) reduction of plasma AST.

Plasma insulin concentrations were significantly lower in diabetic control group (0.47±0.5 ng/ml) compared to normal control group (0.85±0.2 ng/ml) but only EGCG and metformin treated groups showed significant increase of plasma insulin concentrations compared to diabetic control group (p < 0.01) (Table 3).

DISCUSSION

EGC and EGCG were tested in diabetic mice. To induce

Table 3. Effect of catechins on plasma biochemical parameters, insulin index in control, diabetic and treated mice.

Parameters	NC	DC	EA	EGC	EGCG	MET
Plasma glucose (mg/dl)	129±34	271±16$^{###}$	228±36***	218±21	197±16	144±28***
Insulin (ng/ml)	0.85±0.2	0.47±0.05$^{##}$	0.42±0.8	0.37±0.5	0.59±0.3	0.54±0.3
AST (UI/L)	114±7.3	286±42$^{###}$	155±15**	238±41	127±1.6**	166±21**
ALT (UI/L)	64±6.8	71 ±6.1	48±10*	65±10	67±6.2	63±7.7
TG (mg/dl)	121±5.6	157±13$^{##}$	118±9**	112±24**	115±8.2	93±5**
Ch (mg/dl)	117±3.9	131±4.9$^{##}$	130±8.7**	110±10*	126±11	104±10**

Data were expressed as value with the standard error of the mean (M ± E.S.M, n = 8) and evaluated by ANOVA followed by Tukey's test at 5% *P < 0,05 ; **p < 0,01; ***P<0,001 (vs DC); ## P < 0,01 ; ### P < 0,001 (vs C).

diabetes *in vivo*, Streptozotocin were used, a molecule produced by *Streptomyces achromogenes*; it is a substance with antineoplastic, oncogenic and diabetogenic activities (Like and Rossini, 1976). It destroys selectively pancreatic-cells by oxidative stress (Szkudelski, 2001; Long-Ze, 2008). Streptozotocin induces type 1 or Type 2 diabetes depending on administered dose (Islam and Loots, 2009). Multiple low-dose of STZ leads to diabetic rats resembling type 1 diabetes in humans characterized by insulitis with accumulation of inflammatory cells and degranulation of cells. A single high-dose administration of STZ causes toxicity to cells, with inflammation free islet lesions and degranulation, which is like type 2 diabetes (Islam and Loots, 2009).

In this study, a single high-dose administration of STZ significantly induced hyperglycemia accompanied by hypoinsulinemia. Oral administration of catechins and metformin for 21 days induced a marked anti-hyperglycemic activity in STZ-induced-diabetic mice by reducing glycemia and showing a significant improvement in glucose tolerance.

This effect can be the result of intestinal glucose absorption with extra pancreatic action including the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process.

Hypercholesteremia and hypertriglyceridemia are factors seen in the development of atherosclerosis and coronary heart disease which are some complications of diabetes (Ananthan et al., 2003). Catechins and metformin significantly reduced serum triglycerides and total cholesterol in STZ-diabetic mice. Thus, it is reasonable to conclude that catechins of *B. ferruginea* could modulate blood lipid abnormalities.

Liver is the vital organ of metabolism, detoxification, storage and excretion of toxic agents and their metabolites. ALT and AST are markers of liver function (Ohaeri, 2001). An increase in the activities of ALT and AST in plasma might be due to the leakage of these enzymes from the liver cytosol into the blood stream which gives an indication of hepatotoxic effect of STZ Ramesh et al. (2010). Treated diabetic mice showed a reduction of these enzymes activities in plasma compared to the diabetic untreated mice and consequently alleviated liver damage caused by STZ-induced diabetes. Significant reductions in the activities of these enzymes in treated diabetic mice indicated the hepato protective role in preventing diabetic complications.

Some authors indicate also that catechins are hypoglycaemic properties and act to control diabetes (Kao et al., 2000; Mai and Chuyen, 2007). Catechins are powerful antioxidants; increase the sensitivity of cells to insulin, inhibit the lipogenic enzymes and fat absorption, (Thielecke and Boschmann, 2009; Roghani and Tourandokht, 2010; Cherniack, 2011; Rains et al., 2011; Sae-tan et al., 2011). These data confirms our hypothesis that catechins are responsible for the activity of the ethyl acetate fraction and suggest the mechanism by which this fraction is useful in the treatement of type 2 diabetes.

Conclusion

This study suggests that catechins can be some of *B. ferruginea* active molecules. EGC and EGCG tested improved blood sugar level and glucose tolerance in STZ induced diabetes. This confirm that catechins of *B. ferruginea* root bark are responsible for the antidiabetic activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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