Antidiabetic activity of tannin fraction of Bridelia ferruginea (Benth) leaf extract on fructose-induced diabetic mice

Sabrina Sanvee1, Oudjaniyobi Simalou1*, Gneiny Whad Tchani1, Hèzouwè Kagnou1, Batomayena Bakoma2, Kossi Metowogo3, Kokou Agbékonyi Agbodan4, Kafui Kpegba1

1Laboratoire de Chimie Organique et des Substances Naturelles, Faculté des Sciences, Université de Lomé, 01 BP 1515 Lomé 01, Lomé, Togo
2Faculté des sciences de la santé, Université de Lomé, 01 BP 1515 Lomé 01, Lomé, Togo
3Laboratoire de physiologie/Pharmacologie, Faculté des sciences, Université de Lomé, 01 BP 1515 Lomé 01, Lomé, Togo
4Laboratoire de Génie des Procédés et Ressources Naturelles, Département de Chimie, Université de Lomé, 01 BP 1515 Lomé 01, Lomé, Togo

*Corresponding author: Oudjaniyobi Simalou, Email: jacobsimalou@yahoo.fr

Abstract

Introduction: Bridelia ferruginea is a plant known for its antidiabetic properties. However, few studies on leaf extracts have induced anti-hyperglycemic activity on normal mice subjected to carbohydrate overload. The current study was designed to assess the effect of the leaf extracts' fraction on fructose-induced diabetic mice.

Methods: The in vitro ferric-reducing antioxidant power (FRAP) assay were carried out and the condensed tannins quantified. The vanillin-HCl method was used to characterize the condensed tannins. The antidiabetic effect on fructose-induced diabetic mice was evaluated for 28 days using a fructose-enriched fat diet approach.

Results: The fraction confirmed the antioxidant activity with a reducing power of 800 µg/mL comparable to ascorbic acid at 200 µg/mL. The condensed tannins were estimated at 79.6 ± 3.4 mg catechin equivalent per gram of sample. Significant decreases in blood sugar levels of 6.25% at the 7th day, 11.04% at the 14th day, 12.61% at the 21st day, and 11.35% at the 28th day were obtained in mice treated with the extract dose of 200 mg/kg of body weight, compared to the positive control group. The decreases of 37.11% of triglycerides and 40.16% of total cholesterol were also obtained.

Conclusion: The investigated fraction showed notable antidiabetic activity and might be a good candidate in the treatment of diabetes.

Implication for health policy/practice/research/medical education: The tannin treated fraction of hydro-ethanolic extract of B. ferruginea leaves could be used in the treatment of type 2 diabetes in order to regulate glycaemia and in the decrease of oxidative stress and severe diabetes like hypercholesterolemia.

Material and Methods

Plant material

Fresh leaves of *B. ferruginea* were collected in Danyi-Koumdzavi (7°9’13.5198” N; 0°37’43.55076” E), in Zone IV of Togo on June 9, 2019. The Plant was identified by the Laboratory of Botany and Plant Ecology of “Université de Lomé” and a voucher specimen was kept in the herbarium of the Faculty of Sciences under the number TOGO15511.

Animals

The experimental animals were ICR mice bred and housed in the animal house of the Department of Animal Physiology of the Faculty of Sciences, “Université de Lomé”, in Togo. The mice, weighing 20-30 g, were maintained in metal cages under controlled environmental and standard laboratory conditions (temperature 25±2°C), relative humidity of 44-56%, with a dark and light cycle of 12±1 hours. They were allowed free access to water and standard food. Food was withheld overnight prior to experiments while water was still provided *ad libitum*.

Preparation of the supernatant fraction

The leaves were dried for two weeks at room temperature, powdered and macerated for 72 hours with the ethanol-water (80:20 v/v) under intermittent manual agitation. The solvent was renewed three times (every 24 hours). The resulting macerate was filtered through Whatman filter paper. The filtrate was evaporated *in vacuum* (45°C) (13) and dissolved in an ethanol-water (75:25 v/v) mixture at a ratio of 3 g of extract for 40 mL of solvent. The mixture was centrifuged at 4000 rpm for 15 minutes to obtain two phases: the pellet or precipitate and the supernatant. The supernatant was evaporated and used for the experiments (15).

Study of antidiabetic activity

The method used was inspired by Kadébé et al (15) with some modifications, as described in the next two subsections. The ICR mice were given a mixture of fructose and melted lard *per os* for metabolic syndrome induction.

Preparation of the lard-fructose (L-F) mixture

Six grams of fructose and 1.25 mL of tween 80% were dissolved in 50 mL of distilled water. To the obtained mixture was added 50 mL of melted lard and mixed to get the diet solution.

Bioassay experimental protocol

Twenty-four ICR mice housed in four groups of six were used. The experiment lasted 28 days according to the following protocol:

- **Group 1 (Control):** received 5 mL/kg/d of distilled water for 28 days. From the 15th day to the 28th day, 5 mL/kg/d of distilled water were given 30 minutes in advance;
- **Group 2 (Positive control):** received 5 mL/kg/d of L-F mixture for 28 days. From the 15th day to the 28th day, mice were given 5 mL/kg/d of distilled water 30 minutes before the L-F mixture;
- **Groups 3 and 4 (B. ferruginea 100 and B. ferruginea 200):** received 5 mL/kg/d of L-F mixture for 28 days. From the 15th day to the 28th day, the mice received the supernatant at the doses of 100 mg/kg/d and 200 mg/kg/d, respectively, 30 minutes before administering L-F mixture.

Levels of blood glucose of mice were measured on the 1st day and then each 7 days using a glucometer One Touch Ultra (USA). Mice were weighed each 2 days.

On 26th day, after a 12-hour fast, the mice were subjected to Orally Glucose Tolerance Test (OGTT) by administering orally 4 g of glucose per kg of body weight. Levels of blood glucose were measured at 0, 30, 60, and 120 minutes following glucose administration.

At the end of the experiment on 28th day; the animals were fasted for 12 hours. On the 29th day, the glycaemia of the animals was measured. Blood was taken via the retro orbital sinus after anesthesia (inhalation of diethyl ether) and then centrifuged at 3000 rpm for 10 minutes. Serum was then collected in tubes to assess the contents of triglycerides, cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The abdominal cavity was opened and intra-abdominal fat was collected and weighed.
Determination of condensed tannins

The vanillin-HCl method was used for the condensed tannins content estimation. The method is based on the reaction of vanillin with the ending flavonoid group of condensed tannins and the formation of red complex mixtures. By reacting with vanillin, the tannins give red anthocyanidols. The content of condensed tannins was then determined as described by Julkunen-Titto in 1985 (16).

A volume of 50 μL of the sample was added to 1500 μL of 4% vanillin methanolic solution and mixed vigorously. Then a volume of 750 μL of concentrated HCl was added. The resulting mixture was left in the dark at room temperature for 20 minutes. The absorbance was measured at 550 nm. Ranging concentrations of catechin (0-1000 µg/mL) as standard were used to draw the calibration curve and the results were expressed in mg Catechin Equivalent per gram of sample.

Reducing power activity

The reducing power was measured using FRAP test which is based on the reduction of ferric (Fe³⁺) ions into ferrous (Fe²⁺) ones inducing direct electron transfer. During the experiment, the yellow color changes into pale green and then into blue depending on the content of such antioxidant molecules in the sample. The mixture formed by the reaction is an intense Prussian blue complex, and the absorbance measured; a higher absorbance value indicating a strong reducing power of the sample. So, methanolic solution of the supernatant extract of *B. ferruginea* (100-1000 µg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K₃Fe(CN)₆]. The reaction mixture was well vortexed and incubated at 50°C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was taken and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride (FeCl₃). The absorbance was then recorded at 700 nm using a spectrophotometer. All the measures were done in triplicate with respect to ascorbic acid used as standard (17,18).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.00 (USA) and Microsoft Office Excel 2013 (USA). Data of the effect on abdominal fat and biochemical parameters were subjected to one-way analysis of variance (one-way ANOVA) and those for the anti-hyperglycemic effect processed using the two-way analysis of variance (ANOVA) followed by the Tukey test to compare groups. Results were reported as mean ± standard error on the mean (Mean ± SEM, n=6). Differences were considered significant if *P* < 0.05.

Results

Effect on basal blood glucose levels

The basal glucose under fast showed significant difference in blood glucose levels between control and positive control mice. The increase in positive control group was 17.78% at the 7th day (*P* < 0.001); 24.08% at the 14th day (*P* < 0.001); 25.68% at the 21st day (*P* < 0.001), and 22.87% at the 28th day (*P* < 0.001), compared to the normal control group. Animals under the same diet but treated with *B. ferruginea* extract at a dose of 200 mg/kg showed a reduction in blood glucose levels of 6.25% (*P* < 0.05) at the 7th day, 11.04% (*P* < 0.01) at the 14th day, 12.61% (*P* < 0.01) at the 21st day, and 11.35% (*P* < 0.01) at the 28th day, compared to the positive control. Mice treated with the supernatant extract at 100 mg/kg of body weight showed no significant difference during the experiment (Figure 1A). The area under the blood glucose curve showed a significant increase of 19.84% (*P* < 0.001) of the positive control group compared to the control group, while the area of the *B. ferruginea* 200 group showed significant reduction of 8.85% (*P* < 0.05%) compared to the positive control. This difference was not significant for the *B. ferruginea* 100 group (Figure 1B).

Effect on carbohydrate overload

Administrating 4 g/kg per body weight of glucose increased blood glucose levels in the control group from 5.13 ± 0.59 mmol/L at beginning to 10.17 ± 1.03 at 30 minutes. Blood glucose levels of 7.98 ± 0.76 mmol/L at 60 minutes were then dropped to 5.15 ± 0.45 mmol/L 120 minutes later.

**Figure 1.** Effect of *B. ferruginea* leaf supernatant extracts administration on basal glycaemia of fructose-induced diabetic mice; A- Basal glycaemia evolution over time (28 days); B- Areas under the curves. The values are expressed as means ± SEM: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001 vs. positive control.
Mice fed with L-F diet only, showed a significant increase at 30 minutes: levels of blood glucose increased from 6.4 ± 0.4 mmol/L at beginning up to 18.63 ± 4.03 mmol/L (P < 0.001) 30 minutes later, then reduced to 9.35 ± 0.54 mmol/L and 5.8 ± 0.55 mmol/L, respectively, at 60 minutes and 120 minutes. Significant decreases in blood glucose at 30 minutes in the groups treated with extract at 100 mg/kg and 200 mg/kg per body weight compared to the positive control group were observed. The decreases were 39.96% (P < 0.001) and 42.54% (P < 0.001), respectively, compared to the positive control group (Figure 2A). The areas under the curve showed differences of 27.82% (P < 0.05) for mice under 100 mg/kg of extract and 30.84% for those receiving 200 mg/kg of the fraction (P < 0.05) (Figure 2B).

Effect on abdominal fat
The L-F diet showed a notable effect on the abdominal fat of mice. The L-F diet induced an increase in abdominal fat of 54.51% (P < 0.001) in the positive control group compared to control mice. Moreover, the results showed a significant decrease of 24.44% (P < 0.05) of B. ferruginea group (200 mg/kg) compared to the positive control group. The mice treated with the extract at the dose of 100 mg/kg (B. ferruginea 100) showed no significant difference compared to the Positive control group (Table 1).

Effect on biochemical parameters
Biochemical parameters in the mice of positive control group showed significant increase in triglycerides, total cholesterol, and ALT levels compared to control group. The increases were 37.5% (P < 0.01), 36.96% (P < 0.01), and 28.09% (P < 0.01), respectively, showing that the diet had increasing effect on the three parameters. However, the results showed a decrease of 37.11% (P < 0.01) for triglycerides and 40.16% (P < 0.01) for cholesterol levels of the B. ferruginea 200 group compared to the positive control group. No significant difference in transaminase content was obtained (Table 2).

Condensed tannins content and antioxidant FRAP results
Taking into account that a previous phytochemical screening revealed the presence and the contents of polyphenolic compounds, flavonoid compounds, carbohydrates, and reducing sugars which, were similarly obtained here (results not shown), and the fact that DPPH antioxidant test was performed (14), only additional test results were exhibited here in order to have large view on those aspects. The content of condensed tannins in the supernatant was 79.6 ± 3.4 mg of catechin equivalent per gram. These values were calculated from the linear regression curve:

![Figure 2. Effect of B. ferruginea supernatant extract on overloaded mice; A- Basal blood glucose evolution as a function of time (120 minutes); B- Areas under the curve. The values are expressed as means ± SEM: * P <0.05; ** P <0.01; *** P <0.001 vs. positive control.](http://www.herbmedpharmacol.com)

**Table 1.** Effect of B. ferruginea supernatant fraction on abdominal fat in mice under the lard-fructose diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Positive control</th>
<th>B. ferruginea 100</th>
<th>B. ferruginea 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal fat (g)</td>
<td>0.143 ± 0.029***</td>
<td>0.315 ± 0.112</td>
<td>0.25 ± 0.022</td>
<td>0.238 ± 0.041*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM: * P <0.05; ** P <0.01; *** P <0.001 vs. positive control

**Table 2.** Effect of B. ferruginea supernatant fraction on biochemical parameters in mice under the lard-fructose diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Positive control</th>
<th>B. ferruginea 100</th>
<th>B. ferruginea 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (g/L)</td>
<td>0.4 ± 0.046**</td>
<td>0.64 ± 0.061</td>
<td>0.62 ± 0.049</td>
<td>0.403 ± 0.021**</td>
</tr>
<tr>
<td>Total cholesterol (g/L)</td>
<td>0.828 ± 0.109**</td>
<td>1.313 ± 0.085</td>
<td>0.993± 0.072</td>
<td>0.786 ± 0.094**</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>62.8 ± 6.874**</td>
<td>87.333 ± 1.756</td>
<td>97.667 ± 5.05</td>
<td>85.5 ± 1.723</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>65.2 ± 13.437</td>
<td>128.5 ± 35.89</td>
<td>119.5 ±10.972</td>
<td>104.4 ± 8.738</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM: * P <0.05; ** P <0.01; *** P <0.001 vs. positive control.

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.
OD = 0.0006087°C + 0.02788; R² = 0.9822 with OD meaning optical density, C concentrations in µg/mL, and R² correlation coefficient. In addition, B. ferruginea supernatant extract showed powerful reducing power that exhibited concentration-dependent ability by reducing Fe³⁺. The reduction power of B. ferruginea was 800 µg/mL comparable to that of ascorbic acid at 200 µg/mL (Figure 3).

Discussion
In this study, the supernatant fraction of B. ferruginea leaf extract was administered to L-F induced diabetic mice. The antidiabetic activity was investigated by assessing the effect of the supernatant extract on basal glycaemia, carbohydrate overload, and abdominal fat. In addition, the contents of ALT, AST, total cholesterol, and triglycerides were also evaluated. First, the results showed a significant increase in basal glycaemia, carbohydrate overload, total cholesterol, abdominal fat, and ALT in the positive control group mice due to the high-fructose (L-F) diet (Figures 1 and 2, Tables 1 and 2). These increases showed the diabetogenic effect of the L-F diet in the positive control animals. Indeed, some previous works have shown that fructose might be responsible for hyperglycemia through insulin resistance (4). The hyperglycemia was confirmed in our study by determining the area under the blood glucose curve that measured the total amount of glucose available in the blood during a period of time. Fructose is known to cause hypercholesterolemia (19), hypertriglyceridermia (4,20), and an increase in abdominal fat (21).

Statistical analysis of data of animals under the L-F diet treated with B. ferruginea showed that the plant had a notable anti-hyperglycemic effect in mice treated with B. ferruginea at the dose of 200 mg/kg with a significant decrease in blood glucose levels at the 7th day, the 14th day, the 21st day, and the 28th day compared to the positive control group (Figure 1A). This observation was confirmed by the area under the curve (Figure 1B). The effect on carbohydrate overload was observed at 30 minutes, in animals treated with the extract at 100 mg/kg and 200 mg/kg, with decrease in blood glucose levels of 39.96% (P <0.001) and 42.54% (P <0.001), respectively, compared to the positive control mice. The extract also resulted in decrease in abdominal fat, triglycerides, and total cholesterol. Many works reported a relationship between hyperglycemia in diabetes and oxidative stress, which is characterized by an imbalance between the production of free radicals like peroxides and their enzymatic elimination, as well as antioxidant and non-enzymatic cellular defenses (15). Production of oxygen and lipid reactive species (peroxidation) was found to increase in diabetic patients, and oxidative stress was in particular responsible for the diabetes physiopathology. Moreover, antioxidant treatment has shown significant improvement, and prevented cardiovascular complications (6,15). Therefore, taking into account the past antioxidant tests (14,22), in vitro antioxidant FRAP test was evaluated. In that approach, the reducing FRAP capacity of B. ferruginea extract of 800 µg/mL was found comparable to that of the reference ascorbic acid at 200 µg/mL. Several other works reported antioxidant activities exhibited by various B. ferruginea extracts (22,23). So, the current in vivo antidiabetic activity of B. ferruginea might be supported by the reported proprieties (13,14) of the total phenols, flavonoids, and the current revealed condensed tannins. Antioxidant compounds are capable of granting electrons to reactive radicals, reducing them to stable and non-reactive species (8,24). Indeed, the antioxidant effect of a plant extract is mainly related to phenolic compounds such as flavonoids, phenolic acids, tannins, and phenolic diterpenes (8,25), which in fact were found in the used extract (14). Moreover, a correlation between the content of such compounds and antioxidant activity has been made; the more the content of such phytochemicals, the higher the antioxidant activity (2,14,26). Such antioxidants may prevent the worsening of diabetes by improving antioxidant defense mechanism of beta cells in the pancreas. In addition, these phytochemicals are known to have a wide antidiabetic effect (24,27) and exhibit antimutagenic, anticarcinogenic, and anti-inflammatory properties (8,18).

Conclusion
Bridelia ferruginea supernatant leaves extract was found to have in vivo antidiabetic activity on fructose-induced diabetic mice. The traditional use of B. ferruginea against diabetes could be supported by this study. In fact, previous and current phytochemical screening revealed the presence of phenols, tannins, flavonoids; compounds usually involved in the antidiabetic and antioxidant activities of the plants. The additional antioxidant FRAP test showed that the ferric reducing power at 800 µg/mL of the extract was comparable to that of ascorbic acid at 200 µg/mL. Indeed, Antidiabetic activity in mice treated with...
B. ferruginea extract of 200 mg/kg exhibited a decrease in basal blood glucose levels. In addition, significant decreases in blood glucose levels in an OGGTT at 30 minutes were also obtained. Moreover, obvious decreases were also reached in abdominal fat, triglycerides, and total cholesterol levels. This extract might be considered in treatment of type 2 diabetes in order to regulate glycaemia but also in the decrease of oxidative stress and severe diabetes. Our study only focused on separation by centrifugation; further fractionation and isolation studies must be envisaged in order to isolate some active molecules or fractions.

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Authors’ contributions
SS, OS, and GWT conceived and designed the study. SS, OS, and BB participated in the writing process. SS, HK, and KAA performed general experiments. BB and KM coordinated anti-diabetic experiments. OS, BB, and KK read and approved the final manuscript.

Conflict of interests
The authors declare that they have no conflict of interest.

Ethical considerations
The research was conducted with consideration of ethical issues and obtaining license from the Ethical Committee of the Faculty of Sciences of “Université de Lomé” approved the experimental protocols using World Health Organization guidelines for the care and use of laboratory animals). The protocol has been approved by the Laboratory of Physiology/Pharmacology of “Université de Lomé”, Togo. Principles of laboratory animal care as described in institutional guidelines and ethics of Laboratory of Physiology/Pharmacology of “Université de Lomé” (ref: 001/2012/ CB-FDS-UL) were followed in compliance with the Animal Welfare Act.

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None.

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