



Effect of *Beilschmiedia obscura* on the prevention of high fat/high sucrose diet induced metabolic syndrome on male Albino Wistar rats

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ABSTRACT

Introduction: *Beilschmiedia* (Lauraceae) is a pantropical genus of about 287 species, distributed in tropical Asia and Africa used in traditional medicines to cure many diseases. This study aimed to explore biological properties of *Beilschmiedia obscura* (*B. obscura*) on the prevention and management of metabolic syndrome (MetS) features induced by High Fat/High Sucrose (HF/HS) diet in rats as therapeutic option.

Methods: MetS was induced after administration of HF/HS diet followed by administration of *B. Obscura* powder at 5% or 10% for 21 days, while the control group received a chow diet and distilled water and the positive control group received the HF/HS diet and distilled water. At the end of the experiment, rats were sacrificed; the parameters of lipid profile, markers of oxidative stress, antioxidant status were evaluated.

Results: HF/HS diet successfully induced weight gain, oxidative stress and lipid profile disorders from rats. Treatment with powder of *B. obscura* at 10% than the 5% showed a reduction of body weight in treated groups and, anti-hyperlipidemic effect by improving lipid profile parameters. Triglycerides, Total cholesterol and LDL cholesterol levels were lower ($p < 0.05$) and HDL-cholesterol levels higher in the treated groups compared to positive control. Inhibition of lipid peroxidation, and improvement protein thiols levels and catalase activity were also observed in treated groups.

Conclusion: This study revealed that *B. obscura* whole plant was efficient in reducing biomarkers involved in metabolic syndrome and could efficiently help in its management by preventive effect.

1. Introduction

The incidence of cardiometabolic disorders is increasing all over the world with close to 1/3 of death attributed to cardiovascular diseases [1]. Africa, earlier colonized by proliferation of infectious diseases, is nowadays also facing high mortality and morbidity rate, because of rapid urbanization, changes in eating habits and lifestyles among populations. These modifications lead to metabolic syndrome (MetS). MetS is defined as constellation of interconnected physiological, biochemical, clinical and metabolic factors which directly increase the risk of cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM) with high mortality [2]. Although the causes of MetS are not fully understood, but it plays a central role of visceral adiposity and insulin resistance (IR) involved in the pathophysiology. Also, obesity and T2DM are associated

with systemic oxidative stress, adipokine imbalance and reduced anti-oxidant defenses, leading to dyslipidemia such as hypercholesterolemia, hypertriglyceridemia which are closely related to vascular disease and hepatic steatosis [3]. The availability of animal models mainly rodents that mimics human MetS when fed on carbohydrate- and fat-rich dietary components have been used to induce the signs and symptoms of MetS [4]. In fact, high level of caloric intake has been associated with many diet-induced complications, including MetS, hypertriglyceridemia, CVD and non-alcoholic fatty liver disease (NAFLD) making the *in vivo* assays possible [5].

Drugs currently used in the treatment of MetS apart from the known side effects, fail to be efficacious on all the individual components at the same time. For instance, the drugs currently used as antidiabetic medicines include synthetic agents such as biguanides, thiazolidinediones, insulin sensitizers and insulin showed considerable side effects like

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Abbreviations

| | |
|---------|--------------------------------------|
| CSF | Cerebrospinal fluid |
| CVD | Cardiovascular disease |
| FFA | Free fatty acid |
| HDL-c | High density lipoprotein cholesterol |
| HEE | Hydroethanolic extract |
| HF/HS | High fat/high sucrose |
| HMG-CoA | Hydroxy-3-methylCoA |
| LDL-c | Low density lipoprotein cholesterol |
| MDA | Malondialdehyde |
| MetS | Metabolic syndrome |
| T2DM | Type 2 diabetes mellitus |
| TC | Total cholesterol |
| TG | Triglycerides |

hypoglycemia, drug resistance, dropsy and weight gain as well as limited hypolipidemic, anti-inflammatory and antioxidant activities [6]. These drugs also have diverse and variable effects on hypertension and other individual components of MetS [7]. Efforts are being made to search new effective, cheaper and more available drugs or drug combination to face this growing public health challenge. The alternative treatment based on the use of plants, herbs spices and other functional foods with better effectiveness and lower toxicity, accepted as valuable resources for primary healthcare by the World Health Organization (WHO) are being explored [8].

Many plants from Cameroonian pharmacopeia tested on animal models including *Cucurbitaceae* seeds (protein-rich) were reported to have hypoglycaemic activity in diabetic animals [9]. A more recent study showed that *Tetrapleura tetraptera* spice demonstrates the anti-insulin resistance, antilipidemic, anti-obesity, hypotensive and anti-inflammatory properties in the management of MetS such as obesity, T2DM and hypertension [10]. Also, it has been demonstrated that food fibers consumption, like Fenugreek and gum guar have shown benefit effects in management of obesity and diabetes advised within the framework of food balance and for their therapeutic properties [11]. *Irvingia gabonensis*, *Hibiscus esculentus*, other sources of soluble fibers usually intervene in the management of obesity and the diabetes [12].

Beilschmiedia (Lauraceae) is a pantropical genus of about 287 species, distributed in tropical Asia and Africa. The bark and leaves of some *Beilschmiedia* species are used in traditional medicine [13]. Recently *B. obscura* was demonstrated to be efficacious against infectious diseases caused by Gram-negative bacteria [14]. This work aimed at evaluating the effect of *B. obscura* powders on HF/HS diet induced MetS on rats.

2. Material and methods

2.1. Collection, identification and preparation of powder of plant

The seeds of *B. obscura* were purchased at the Abong Mbang market in the East region of Cameroon. The material collected was identified at the national herbarium with a voucher number 2102/SRFR.

The seeds collected were washed, dried and crushed with a blender (PHILIPS) until the powder was obtained.

2.2. Design and experimental protocol

The study was carried out with twelve week old male Albino Wistar rats, weighing average 200g. The rats were randomly assigned in four (4) groups of five (5) rats each, with free access to water and food *ad libitum*. After one week acclimatization, rats were randomly assigned:

- Negative Control: receiving a chow diet;

- Positive Control (PC): receiving HF/HS diet,
- Assay 1: receiving HF/HS diet and powder of *B. obscura* 5%
- Assay 2: receiving HF/HS diet and powder of *B. obscura* 10%.

HF/HS diet was prepared according to the mixture described in Table 1 [15].

All groups received a daily treatment every morning in experimental diet. In order to follow the variation of the body weight, animals were weighed once per week using a sensitive scale. After 21 days of experimentation, overnight fasting rats were sacrificed by cervical dislocation under anesthesia with diethyl-ether and blood was collected in EDTA tubes for plasma and erythrocyte hemolysates preparation and stored at -20°C until use. Heart, liver and kidney were collected, carefully washed and rinsed with ice-cold saline (0.9% NaCl) for the preparation of homogenates.

2.3. Biochemical analysis

2.3.1. Measurement of lipid profile parameters

For evaluation of lipid profile, total cholesterol (TC), total triglycerides (TG) and total HDL-cholesterol (HDL-c) were estimated using standard kits (*Chronolab*). Low Density Lipoprotein Cholesterol (LDL-c) concentration was calculated using a Friedwald formula [16]: $\text{LDL-c} = \text{TC} - (\text{HDL-c} + \text{TG}/5)$.

2.3.2. Evaluation of oxidative stress markers

2.3.2.1. Lipid peroxidation: Lipid peroxidation was estimated as lipid hydroperoxides [17] and thiobarbituric reactive substance (TBARS) by measuring the pink colored chromophore formed by the reaction of thiobarbituric acid with malondialdehyde (MDA) [18].

2.3.2.2. Protein thiols: The amino acids containing thiol groups and sulphur are the most susceptible site for ROS action. The protein thiols content was estimated by method based on the development of a yellow color when DTNB was added to compounds containing sulphhydryl groups [19].

2.3.2.3. Catalase activity: Catalase is one of the most antioxidant enzyme which decomposes hydrogen peroxide to water and oxygen. Catalase activity was assayed using hydrogen peroxide as substrate. The activity was expressed in U/g protein. The CAT unit (UCAT) is defined as the enzyme concentration required converting 1 mmol of hydrogen peroxide in 1 min [20].

2.4. Statistical analysis

Results were expressed as mean \pm standard error mean. Statistical analysis was carried out using Statistical Package for Social Science (SPSS) 16.0 for Windows. The normality of value was checked using Kolmogorov-Smirnov. The difference between different groups of treatment was analyzed by ANOVA (One-way) following Turkey's post test. Results were significant for $p < 0.05$.

Table 1
Composition of high fat/high sucrose diet.

| Composition | Chow diet % | High fat/high sucrose diet (%) |
|---------------|-------------|--------------------------------|
| Corn starch | 53 | 35 |
| Sucrose | 14.74 | 20 |
| Proteins | 19.25 | 15 |
| Lipids | 5.7 | 20 |
| Vitamins | 0.3 | 1.5 |
| Mineral salts | 3.21 | 3.5 |
| Fibers | 3.8 | 5 |
| Energy | 3.40 | 4.64 |

3. Results

3.1. Effect of *B. obscura* on body weight of HF/HS diet fed rats

The gain of body is an indicator weight of effect of HF/HS on the development and management of obesity. Feeding animals with HF/HS diet triggered an increase in body weight in the positive control group compared to the negative control. Reduction of body weight was observed in all the groups treated with supplementation of *B. obscura* compared to the positive control and the negative control. A 10% supplementation was very efficient in reduction body weight from day 5 to day 21 (1.77%) (Fig. 1).

3.2. Effect of *B. obscura* on plasma blood lipids and oxidative stress markers on HF/HS diet induced *MetS* on rats

Administration of *B. obscura* powder supplementation (5% and 10%) with HF/HS diet efficiently reduced TC, TG and LDL-c and increase HDL-c levels. In addition, *B. obscura* was more efficient at 10% supplementation compared to control and groups treated at 5% (Table 2).

In terms of cardiovascular risk, the HF/HS diet created an increase in atherosclerotic risk markers. A supplementation of *B. obscura* led to a decrease in cardiovascular risk. There was a significant decrease of the atherogenic index (TC/HDL-c; TG/HDL-c; log TG/HDL-c) with supplementation (Table 3).

3.3. Effect of *B. obscura* on plasma and tissue oxidative stress markers on HF/HS diet induced *MetS* rats

The administration of HF/HS diet increased MDA levels compared in the positive control group compared to the negative control group. Supplementation of deleterious diet by powder of plant (10%) protects against lipid peroxidation and reduced MDA levels. In addition, supplementation of deleterious diet by powder of plant (5% and 10%) increased enzymatic (catalase activity) and non-enzymatic (protein thiols) antioxidant systems (Table 4).

Administration of HF/FS diet caused an increase of cardiac and renal secondary products of the lipid peroxidation in the positive control group. Associated with it, there was a decrease in the markers of non-enzymatic antioxidant system and an increase of catalase activity in the liver and kidneys. The increase of lipid peroxidation markers with supplementation has been observed. The beneficial effects with cardiac and kidney protein thiols markers were noted. Also, catalase activity decreased in the liver and heart but increased in the kidney (Table 4).

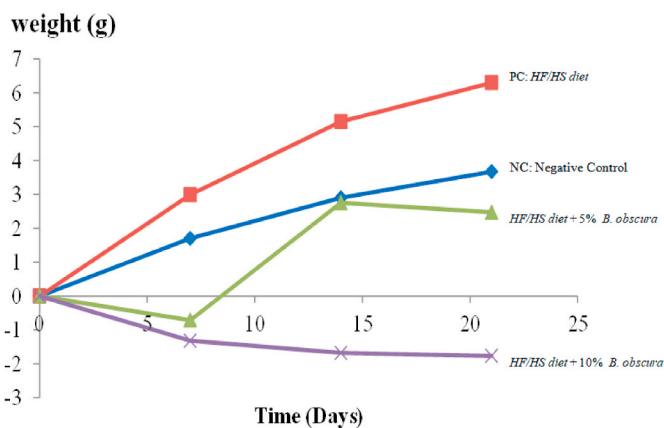


Fig. 1. Effect of supplementation of *B. obscura* whole plant on body weight of rats fed with HF/HS diet
PC: positive Control, NC: Negative Control, HF/HS: high fat/high sucrose.

Table 2

Effect of *B. obscura* on plasma blood lipids and oxidative stress markers on HF/HS diet induced diabetic rats.

| Biomarkers | Groups | | | |
|---------------|------------------|--------------------------------|-----------------------------------|------------------------------------|
| | Negative control | Positive control (HF /HS diet) | HF /HS diet +5% <i>B. obscura</i> | HF /HS diet +10% <i>B. obscura</i> |
| TC (mg/dL) | 119.70 ± 5.02 | 163.80 ± 8.07 ^{*,a} | 123.80 ± 18.74 ^b | 112.60 ± 6.45 ^b |
| TG (mg/dL) | 45.33 ± 2.33 | 80.60 ± 9.48 ^{*,a} | 67.20 ± 5.62 ^b | 54.40 ± 3.37 ^b |
| HDL-c (mg/dL) | 70.50 ± 3.50 | 36.40 ± 1.99 ^{*,a} | 57.60 ± 6.18 ^b | 50.20 ± 6.86 ^c |
| LDL-c (mg/dL) | 39.60 ± 5.24 | 111.20 ± 9.58 ^{*,a} | 52.76 ± 23.68 ^b | 51.52 ± 10.63 ^b |

Values shown are mean ± SEM (n=5) * = p<0.05 significant between negative and positive control; a,b,c = p<0.05 significant between positive control and different groups treated by *B. obscura*.

Table 3

Preventive effect of *B. obscura* on cardiovascular risk.

| Biomarkers | Groups | | | |
|------------|------------------|--------------------------------|-----------------------------------|------------------------------------|
| | Negative control | Positive control (HF /HS diet) | HF /HS diet +5% <i>B. obscura</i> | HF /HS diet +10% <i>B. obscura</i> |
| CT/HDL | 1.70 ± 0.09 | 4.58 ± 0.41 ^{*,a} | 2.39 ± 0.66 ^b | 2.46 ± 0.45 ^b |
| TG/HDL | 0.64 ± 0.04 | 2.22 ± 0.24 ^{*,a} | 1.23 ± 0.18 ^b | 1.20 ± 0.22 ^b |
| log TG/HDL | 0.19 ± 0.02 | 0.34 ± 0.05 ^{*,a} | 0.07 ± 0.02 ^b | 0.05 ± 0.02 ^b |

Values shown are mean ± SEM (n=5) * = p<0.05 significant between negative and positive control; a,b = p<0.05 significant between positive control and different groups treated by *B. obscura*.

Table 4

Effect of *B. obscura* on tissue oxidative stress markers on high fat/high sucrose diet induced diabetic rats.

| Organs Biomarkers | Negative control | Positive control (HF /HS diet) | HF /HS diet +5% <i>B. obscura</i> | HF /HS diet +10% <i>B. obscura</i> | |
|---------------------------------|------------------|--------------------------------|-----------------------------------|------------------------------------|---------------------------|
| MDA (µmol/L) | Plasma | 1.40 ± 0.09 | 6.85 ± 0.25 ^{*,a} | 6.02 ± 0.43 ^a | 4.93 ± 0.44 ^b |
| | Liver | 2.01 ± 0.05 | 0.40 ± 0.04 ^{*,a} | 0.41 ± 0.03 ^a | 0.39 ± 0.04 ^a |
| | Heart | 3.71 ± 0.03 | 6.26 ± 0.16 ^{*,a} | 7.18 ± 0.31 ^b | 7.34 ± 0.55 ^b |
| | Kidney | 2.39 ± 0.16 | 6.68 ± 0.59 ^{*,a} | 9.45 ± 0.90 ^b | 7.52 ± 0.85 ^c |
| Protein thiols (µmol/g protein) | Plasma | 5.00 ± 0.41 | 5.25 ± 0.33 ^a | 7.00 ± 0.66 ^b | 8.42 ± 0.08 ^c |
| | Liver | 4.64 ± 0.06 | 2.81 ± 0.40 ^{*,a} | 2.43 ± 0.34 ^a | 2.46 ± 0.47 ^a |
| | Heart | 4.18 ± 0.23 | 3.65 ± 0.72 ^a | 4.17 ± 0.61 ^a | 3.11 ± 0.84 ^a |
| | Kidney | 3.50 ± 0.09 | 1.76 ± 0.41 ^{*,a} | 2.37 ± 0.44 ^b | 1.51 ± 0.29 ^a |
| Catalase activity (U/g protein) | Plasma | 2.10 ± 0.01 | 5.30 ± 1.53 ^{*,a} | 2.96 ± 0.42 ^b | 13.27 ± 0.59 ^c |
| | Liver | 1.85 ± 0.06 | 5.63 ± 1.73 ^{*,a} | 3.89 ± 1.20 ^a | 5.92 ± 0.91 ^a |
| | Heart | 2.76 ± 0.19 | 2.12 ± 0.31 ^a | 1.20 ± 0.45 ^b | 1.28 ± 0.22 ^b |
| | Kidney | 1.66 ± 0.01 | 2.54 ± 0.84 ^a | 0.98 ± 0.28 ^b | 3.56 ± 0.98 ^c |

Values shown are mean ± SEM (n=5) * = p<0.05 significant between negative and positive control; a,b,c = p<0.05 significant between positive control and different groups treated by *B. obscura*.

4. Discussion

The current study was investigated the protective effect of powder of *Beilschmiedia obscura* on metabolic syndrome (MetS) induced by high fat/high sucrose diet on rats. MetS is a range of cardiovascular factors risk increasing the development of diabetes, cardiovascular diseases. The treatment of MetS is difficult as result of side effects associated to treatment of each of its individual components. To solve the problem, the search of optional treatments such as remedies from herbal medicines is still a great challenge [21]. Certain secondary metabolites of the plants have shown beneficial effects by decreasing the rate of plasma glucose and lowering lipid profile in MetS acting therefore at different levels of etiology of the syndrome. Several studies have shown that treatment by antioxidants prevent and decrease the risks of the complications and other individual components of MetS. In this context, a recent interest was considered to use of the bioactive compounds of plants as tools [7].

HF/HS diet used is a model of induction of MetS in animal model that mimics Human feature of the disease. This diet leads to increase body weight, dyslipidemia and other features of MetS and is recommended for his animal studies. The mechanism by which fructose increases weight is likely via its ability to stimulate hunger, block satiety responses, and reduction in resting energy expenditure in overweight and obese subjects. Henceforth, weight gain observed in this study was driven primarily by increased energy intake from fat and reduced metabolism rate as a consequence of high fructose from sucrose intake induced leptin resistance in rats [10].

The significant increase of weight could be explained by the fact that carbohydrates are known to induce hyperinsulinemia, state which increases the accumulation of fat by activation of lipoprotein lipase of adipocytes [22]. The reduction of body weight observed with 10% supplementation of *B. obscura* could result from the inhibition of the lipoprotein lipase. Indeed, *B. obscura* is a food fiber and many studies revealed that consumption of dietary fibers is associated to a reduction of body weight [23]; and can also affect satiety which results in the reduction of dietary intake. These results corroborate those obtained on *Iringia gabonensis* [12].

The assessment of the lipid profile became crucial via its use in the treatment/management of MetS and several CVD [24]. Many studies reported that the cardiovascular complications associated with MetS, diabetes are due to disturbances in lipid metabolism [ref]. The results of this study showed that HF/HS diet led not only to a significant increase in glycemia and body weight, but also led to an increase ($P < 0.05$) in TC, TG and LDL-c but with a reduction in HDL-c levels in accordance to findings of [25]. In fact, hyperlipidemia and related-tissue steatosis are among the most characteristic features of T2DM and MetS.

These are also two major risk factors that contribute to the pathogenesis of cardiovascular diseases. According to literature, administration of HEE of *Tetrapleura tetraptera* (200 mg/kg) reduced the serum level of TG, TC, Free Fatty Acid (FFA) and increase that of the HDL-c [10]. This result confirms the hypolipidemic activity of *B. obscura*. The hyperlipidemia observed can be explained by insulin deficiency which inhibits the 3-hydroxy 3-methyl glutamyl coA reductase (HMG-coA reductase) involved in the biosynthesis of cholesterol [26]. Diet supplementation with *B. obscura* improves these parameters as shown in Table 1 with an increase of HDL-c, a reduction of TC, TG and LDL-c in treated groups. High levels of these markers increase their susceptibility to oxidation by the free radicals [27] thus producing oxidized LDL-c endowed with pro-atherogenic properties. This improvement of lipid profile could be attributed to the presence of saponins [28]; and soluble fibers contained in the plant. In fact, because of its viscosity, *B. obscura* could bind to bile salts interrupting the entero-hepatic cycle, promoting elimination of exogenous and endogenous cholesterol.

The rise in TC and the LDL-c is associated to an increase of cardiovascular risk in a curvilinear way. A low concentration of HDL-c can be considered as an additional risk factor, whereas high concentration of

HDL-c is a protection factor. Compared to the positive control, supplementation of *B. obscura* induced a reduction in the cardiovascular risk in treated groups.

Studies showed that a HF/HS diet led to an increase cholesterolemia and triglyceridemia. Hypercholesterolemia increases levels of reactive oxygen species which can initiate the lipid peroxidation and damage in DNA, thus leading to carcinogenesis and cellular death if the antioxidant system is unbalanced [29]. In the present study, a significant increase ($p < 0.05$) in MDA has been noted in HF/HS rats as well as a significant increase of hydroperoxides. These high levels of MDA could be due to the oxidative damage at the cellular level. Moreover, during the lipid peroxidation, MDA are generally most abundant, very reactive with respect to thiols and amines groups. These peroxidative compounds bind to proteins, deteriorating cellular homeostasis and thus inducing apoptosis [30].

Supplementation of *B. obscura* decreases these markers by their antioxidant capacity. Similar results were observed which implied that CSFs had beneficial anti-diabetic effects by regulating the lipid metabolism and eliminating the oxygen radicals, which protected the organism's metabolism and repaired the antioxidant capacity [7]. Furthermore, the protein thiols intervene to reinforce the principal antioxidant system of the body which is glutathione when this one is overflowed by the oxidized agents. The depletion of protein thiols and catalase activity could then reflect and justify the increase in their use to fight against the oxidized agents [31].

5. Conclusion

B. obscura used as whole plant supplement at 10% in HF/HS diet is able to reduce body weight, glycaemia, improved markers of antioxidant status, correct the lipid profile and reduce the cardiovascular risk. Therefore, *B. obscura* powders can be used in the prevention and management of MetS and its complications. Thus it could be used as functional food to prevent or slow the progression of T2DM and its complications through a reduction in glycaemia, blood lipids and oxidative stress markers. However, further studies are required to assess the mechanisms of antidiabetic activities of this plant and included two additional group receiving diet of either HF or HS.

Data availability

The authors can provide the data of this research article on request.

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Credit authorship contribution statement

Florine Essouman Mbappe: Conceptualization, Methodology; performed the experiments; Writing – original draft. Ferdinand Lanvin Edoum Ebouel: Methodology; Formal analysis. Fils Armand Ella: Formal analysis; Writing – original draft. Bruno Dupon Ambamba Akamba: Performed the experiments. Jules Kanga Nanhah: Performed the experiments. Innocent Guado: Conceptualization, Methodology, Writing – original draft. Judith Laure Ngondi: Conceptualization, Methodology, Writing – original draft.

Ethical approval

The study was approved by the Animal Ethics Committee of the Faculty of Sciences, University of Yaoundé 1, Cameroon.

Declaration of competing interest

Authors have no conflicting interests.

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