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Effect of Total Methanolic Extract of *Chrysophyllum perpulchrum* on Lipemia and Blood Pressure in Rats Made Diabetic by Streptozotocin

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Authors' contributions

This work was carried out in collaboration between all authors. Author BADP carried out the study, the statistical analysis and prepared the manuscript. Author TWJ carried out the technical analysis and prepared various parts of the manuscript. Author BKB provided reagents. Author NJD corrected the English version of the manuscript. Author AJD co-directed the study. All the authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to evaluate the effect of total methanolic extract of *Chrysophyllum perpulchrum* (Sapotaceae) on hyperglycemia, hyperlipidemia and hypertension to diabetic induced rats. This study was conducted at the biochemical pharmacodynamic laboratory of Felix Houphouet Boigny University (Côte d'Ivoire) during the period from August 2015 to February 2016.
Methodology: The plant extract was obtained from the bark of *Chrysophyllum perpulchrum*. These peels were harvested, dried, ground and extracted in methanol (96%). The phytochemical screening permits us to characterize the different components of our plant extract. Then, fifteen

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(15) rats were used to induce diabetes with streptozotocin (55 mg / kg bw) for seven (7) days. These diabetics rats were then treated with two doses of *Chrysophyllum perpulchrum* (200 et 400 mg/kg bw) and Avandia (10 et 20 mg/kg bw) our reference molecule. To assess the effect of *Chrysophyllum perpulchrum*, we determined the levels of insulin, lipids and blood sugar levels throughout treatment.

Results: The results showed that phytochemical analysis of total methanolic extract of *Chrysophyllum perpulchrum* contains alkaloids, flavonoids and polyphenols. After 7 days of treatment with total methanolic extract of *Chrysophyllum perpulchrum*, blood sugar in diabetic rats significantly decreased (P <.001) from 1.93±.05 to 1.40±.00 for the dose to 200 and 1.93±.05 to 1.23±.02 g / L for the dose 400 mg/kg bw. Similarly total methanolic extract of *Chrysophyllum perpulchrum* normalized blood pressure, all of lipids, urea and creatinine previously increased in diabetic rats.

Conclusion: The treatment of diabetic rats with total methanolic extract of *Chrysophyllum perpulchrum* corrects these disruptions and improves resistance against diabetes. This action is related to its chemical composition characterized by the presence of flavonoids and alkaloids. Thus, this extract may play a preventive role in the development of diabetes especially through improvement of carbohydrate and lipid metabolism.

Keywords: Chrysophyllum perpulchrum; diabetes; blood sugar; blood lipids.

1. INTRODUCTION

Diabetes is a metabolic disorder characterized by the inability of the pancreas to produce insulin. This ends in a disorder at the level of the regulation of carbohydrate metabolism from the food and so entrained a hyperglycemia [1].

Despite the advance of hypoglycemic as antidiabetic drugs, WHO today emphasiezes a progressive globale incidence of diabetes. Whereas, 30 millions diabetic cases were recorded in 1985, the figures have increased to 135 millions in 1995, 177 millions in 2000 and 347 millions in 2011. It is estimated that by 2030, 438 millions people will be affected [2,3]. Diabetes and its history are a real public health problem. The coverage and the therapeutic treatment of the diabetes is at present made by strict diets, daily injection of a dose of insulin by moment and the grip of oral antidiabetics. These pharmaceutical treatments are usually expensive, many diabetics are turning to traditional healers who prescribe their treatments based more economic plants [4]. Traditional pharmacopoeia offers a solution within reach of every budget, however, little information is available on the therapeutic potential of these natural diabetes to regulate and control blood glucose in humans. It is therefore necessary to prepare extracts of these antidiabetic plants and test their biological effects for a scientific validation and pharmaceutical purposes.

The objective of this study is the validation of the antidiabetic effect of the total methanolic extract of *C. perpulchrum* (METCp) recognized tradimedecin in the treatment of diabetes.

2. MATERIALS AND METHODS

2.1 Plant Material and Conventional Molecule

C. perpulchrum (Sapotaceae), from the Ivorian pharmacopoeia was used for this study. It was harvested in the forest region of the Central West of Côte d'Ivoire (Issia department). The extract used here was obtained from the bark of the plant. The plant was identified by Prof. AKE-Assi of Botanical Garden of FILIX Houphouet Boigny University in Côte d'Ivoire, where a specimen was filed. Avandia, a conventional molecule used in this study in the treatment of diabetes has been used as a reference for our various antidiabetic tests.

2.2 Animal Model

The rats of the species *Rattus norvegicus*, weighing between 200 and 300 g, were used for this study. They were purchased from the animal farm of the Pasteur Institute of Côte d'Ivoire. Animals maintained in plastic cages with stainless steel covers containing litter of wood shavings renewed every two days throughout the experiment. Rats were allowed to acclimatize for two weeks with access to clean water and standard animal feeds at the experimental site of the National School (ENS) (Abidjan, Côte d'Ivoire). A cycle of light and dark (12 hours each) and a temperature of 25±2°C were maintained in the room.

2.3 Methods

2.3.1 Total methanolic extract preparation

This preparation was performed according to the method described by [5]. Fifty grams (50 g) of ground material of plant bark was mixed with 1.5 L of 96% methanol. The resulting mixture is stirred for 48 hours at room temperature (25°C) using a magnetic stirrer RCT type IKAMAG (Staufen, Germany). Then the mixture was filtered three times on cotton and on Wattman filter paper 3 mm. The filtrate was evaporated at reduced pressure at 40°C using a rotary evaporator Buchi 461 Watter Batch (Strasbourg, France). The resulting powder was used to make the different tests.

2.3.2 Screening phytochimique

The different groups of compounds (sterols, polyterpens, alkaloids, polyphenols, flavonoïds) contained in extracts were highlighted according to methods described by [6].

2.3.3 Induction of diabetes

A total of 18 rats were used for this study. The animals were divided into two groups. A group of 3 rats constituting the control group received distilled water and a group of 15 rats constituting the test batch received streptozotocin. The permanent hyperglycemia was induced in the animals through intra peritoneal (i.p) route once daily for one week. With a single dose of streptozotocin (55 mg/kg bw), dissolved in 0.1 M citrate buffer pH 4.5. Hyperglycemia was detected after 72 hours and rats with higher blood glucose level greater than or equal to 1.75 mg/L after 7 days are considered diabetic. These animals were referred to diabetic rats.

2.3.4 Treatment of diabetic animals

Fifteen (15) rats selected after appearance of diabetes and three (3) healthy rats were divided into six equal lots (3 rats/lot). The sharing out of lots and treatments were performed as follows (Table 1). The treatment of the diabetic rats was performed force-feeding of total methanolic extract of *C. perpulchrum* and Avandia (the reference) during seven days (7 days).

2.3.5 Preparation of samples and preparation of plasma

At the end of treatment, diethyl ether was used to anaesthetize the animals before blood samples were collected through heart puncture into different heparinized tubes. Blood samples are collected in heparinized tubes and then centrifuged at 3000 revolutions / minute for 10 minutes. Plasma was separated in two fractions in eppendorf tubes, one is used for immediate assay such as glucose and the other is placed in the freezer for analyzing lipids namely cholesterol (CHOL), triglycerides (TG), high density lipoproteins (HDL) and low density lipoprotein (LDL). After the sacrifice and the dissection of the animals, one took a sample of the pancreas and was put in the formalin 10% for histological study.

Table 1. Animal groupings/treatment

Groups	Designation	Doses mg / kg bw
01	Healthy control "TS"	No dose used
02	Untreated diabetic "DNT"	No dose used
03	Diabetic treated with METCp I	200
04	Diabetic treated with METCp II	400
05	Diabetic treated with AV I	10
06	Diabetic treated with AV II	20

METCpI: Total methanolic extract of C. perpulchrum (Dose 1); METCpII: Total methanolic extract of C. perpulchrum (Dose 2)
AVI: Avandia (Dose 1)
AVII: Avandia (Dose 2)

2.3.6 Determination of insulin levels in diabetic rats

The change in insulin was determined every other day of the induction of diabetes in rats and after treatment of these animals by antidiabetics (extract and Avandia). This assay was carried out by the chemiluminescent immunometric method, enzymatic solid phase as described by [7]. The solid phase (bead) is coated with anti-insulin murine monoclonal antibodies. A liquid phase consisting of alkaline phosphatase (from calf intestine) associated with anti-sheep polyclonal antibodies insulin, as well as alkaline phosphatase (from calf intestine) associated with anti-insulin murine monoclonal antibodies. The serum of diabetic rats and the reagent was incubated with the coated bead for 60 minutes. Meanwhile, the insulin contained in the sample forms an antibody complex on the bead with anti-insulin murine monoclonal antibodies and, in the

reagent, the anti-sheep insulin polyclonal antibodies associated with enzymes. Finally, chemiluminescent substrate is added to the reaction cup containing the ball and the signal is generated in proportion to the bound enzyme.

2.4 Statistical Analysis of Results

All values were expressed as mean±standard error of mean and the statistical significance between treated and control groups were analyzed by means of Student's t-test. $P < .05$ was considered significant.

3. RESULTS

3.1 Phytochemical Study of the Total Methanol Extract of *C. perpulchrum*

According to the work of [8], the results of phytochemical screening test are reported in the Table 2.

3.2 Effect of Streptozotocin on Blood Glucose, Insulin and Blood Pressure in Rats

The Figs. 1 (A, B and C) represent the variation of blood glucose, insulin and systolic blood pressure (SBP) in the induction of diabetes in rats. The normal value of these parameters identified in the control group at day zero of induction of diabetes is, respectively, .88±.04 mg/L; 1.98 µu/mL and 123.34±1.12 mmHg. The blood glucose values and blood pressure (SBP) rise gradually during induction reaching 1.02 mg/L (blood sugar) and 153.85±.28 mmHg (SBP) in diabetic animals. And insulin down 1.98 µu/mL to 0.98 µu/mL.

3.3 Effect of Total Methanolic Extract of *C. perpulchrum* (METCp) and Avandia on the Insulin Levels in Diabetic Rats

Fig. 2 shows the effect of the total methanolic extract of *C. perpulchrum* (METCp) and Avandia on the insulin levels in diabetic rats. The dosage of insulin showed that there is a significant difference between the untreated diabetic rats (UDIAB) relative to the control group rats on the one hand, and between the diabetic rats treated with the doses of the METCp and Avandia compared to untreated infected animals. Serum insulin level remains very low in UDIAB (.19±.02 µu/mL) compared to the control value (.19±.02 µu/mL). On the other hand, there is no significant difference between diabetic rats group treated by doses of the antidiabetic Avandia a reference compared to diabetic rats (DIAB) before the beginning of the treatment.

Table 3 shows the effect of the total methanolic extract of *C. perpulchrum* and Avandia on blood glucose and lipids in diabetic rats. The administration of the total methanol extract of *C. perpulchrum* to diabetic animals normalizes the values of biochemical parameters previously elevated in diabetes. In fact, blood glucose decreases from 1.93±.05 to 1.40±.00 mg/mL for the dose METCp 200 and 1.93±.05 to 1.23±.02 mg/mL for dose METCp 400. By cons, blood sugar remains high in group UDIAB at l.76±.02 mg/mL compared to the control group. Regarding lipids, serum levels of T CHOL, TG and LDL increase to respective values of .57±.07 to 1.58±.07; .79±.01 to 1.13±.04; .76±.07 to 1.19±.03 mmol/l and HDL decreases from .38±.01 to .11±.02 mmol/l. Treatment of diabetic rats with dose 400 mg/kg bw of METCp for seven days, declining serum levels of CHOLT 1.23±.02, 1.07±TG to .01; LDL of .57±.02 and increase HDL .38±.03 compared to diabetic group (DIAB). Similarly, different doses of Avandia antidiabetic commercially available decreases and normalizes serum values of these same parameters in diabetic rats treated versus untreated diabetic rats (UDIA) (Table 3).

In order verify the biochemical study, histological sections were taken from the pancreas of normal rats, untreated diabetic and diabetic treated with *C. perpulchrum* (Fig. 3). It appears from this study that the normal rats exhibit an islet of Langerhans (LI), a acini (Ac) and blood vessels (Vs). The untreated diabetic rats exhibit a high necrosis of Langerhans cells (NCL) compared to normal rats pancreas. The pancreas of diabetic rats treated with C. *perpulchrum*, showed an islet of Langerhans (LI), a acini (Ac) and blood vessels (Vs) identical normal architecture to those of normal rats (Fig. 3).

Table 2. Results of phytochemical screening

Chemical groups	Polyterpenes	Polyphenols	Alkaloids	Flavonoïds	Sterols
Presence	+	+	+	+	+

(+): presence

Table 3. Effect of METCp and Avandia on blood glucose and lipid levels in diabetic rats

Electrolytic parameters	Control	Diabetes		Treatements			
	T	Diab	UDIAB	METCp 200	METCp 400	AV 10	AV 20
Blood sugar	1.75±.00	1.93±.5***	2.61±.01**	1.40±.0$^{\#\#}$	1.23±.02$^{\#\#\#}$	1.25±.01$^{\#\#\#}$	1.04±.05$^{\#\#\#}$
Cholesterol (mmo/L)	.57±.07	1.58±.07 ***	1.84±,02***	1.43±.01$^{\#\#\#}$	1.26±1.03$^{\#\#\#}$	1.17±.01$^{\#\#\#}$	.96±.08$^{\#\#\#}$
Triglycerides (mmo/L)	.79±.01	1.13±.04***	1.43±,01***	1.17±.01$^{\#\#\#}$	1.07±.01$^{\#\#\#}$	.07±.02$^{\#\#\#}$	.71±.02$^{\#\#\#}$
HDL (mmo/L)	.13±.02	.08±.02***	.08±.01***	.18±.02$^{\#\#\#}$	.38±.03$^{\#\#\#}$	.66±.04*$^{\#\#\#}$	1.07±.04$^{\#\#\#}$
LDL (mmo/L)	.76±.07	1.19±.03 ***	1.41±.04***	.85±.01$^{\#\#\#}$	.57±.02$^{\#\#\#}$	.46±.02$^{\#\#\#}$	.35±.03$^{\#\#\#}$*

Each value represents the mean±SEM, n = 3; (p < .05 ; *** p < .001) significantly different from control group; ###, significantly different from the diabetic group, C: control group; Diab: diabetic group; UDIAB: untread diabetic group; METCp 200: group treated by 200 mg/kg bw of total methanolic extract of C. perpulchrum; lot METCp 400 group treated by 400 mg/kg bw of total methanolic extract of C. perpulchrum; AV 10: group treated by 10 mg/kg bw of avandia AV20: group treated by 10 mg/kg bw of Avandia HDL (high density lipoprotein); LDL: Low density lipoprotein*

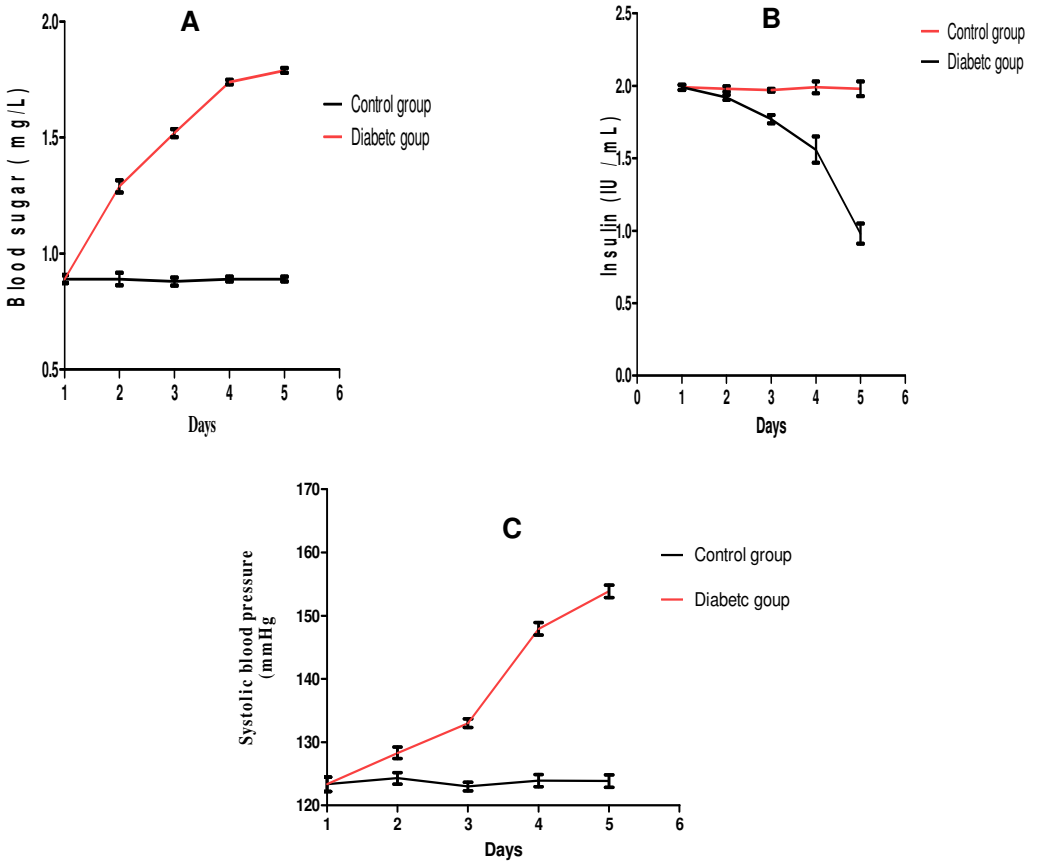


Fig. 1. Comparative change in blood glucose, insulin and blood pressure in diabetic rats

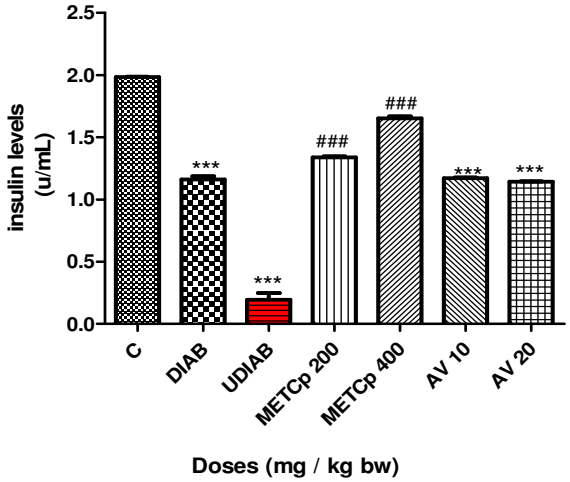
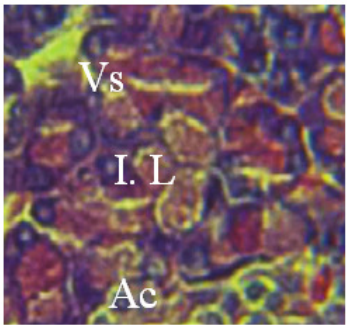


Fig. 2. Insulin change in diabetic rats treated with METCp and Avandia

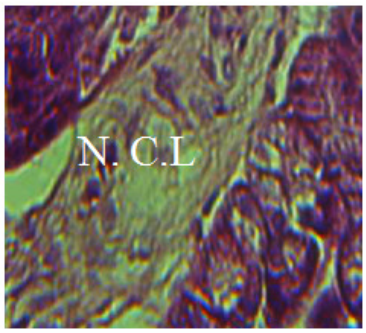
*Each data represents the mean±SEM, n = 3; (*** p < .001) significantly different from control group; ###, significantly different from the diabetic group, C: control group; Diab: diabetic group; UDIAB: untread diabetic group; METCp 200: group treated by 200 mg/kg bw of total methanolic extract of C. perpulchrum; lot METCp 400 group treated by 400 mg/kg bw of total methanolic extract of C. perpulchrum; AV 10: group treated by 10 mg/kg bw of avandia AV20: group treated by 10 mg/kg bw of Avandia*



A

A: Pancreatic section of a normal rat

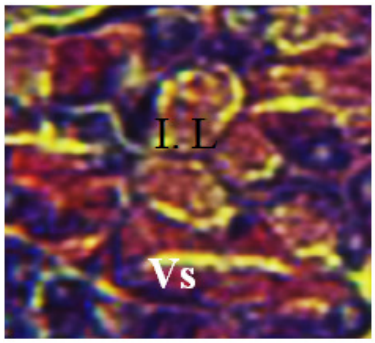
This section presents an islet of Langerhans (LI), an acini (Ac) and blood vessels (Vs) of normal architecture



B

B: Pancreatic section of a diabetic rat

This cut has a high necrosis of Langerhans cells (NCL) compared to normal rat pancreas



C

C: Pancreatic section of a diabetic rat treated by MTECp400

This section presents an islet of Langerhans (LI), a acini (Ac) and blood vessels (Vs).identical normal architecture to those of normal rats

Fig. 3. Selected histological sections of pancreas of control rats (A), untreated diabetic (B) and diabetic treated by 400 mg/kg bw of total methanolic extract of *C. perpulchrum* (C)

4. DISCUSSION

In this study, streptozotocin was used to cause permanent hyperglycemia in rats. Injected into the animal, this substance enters in the pancreatic cells through the glucose transporter and massively destroys the beta cells of the islets of Langerhans with formation of free radicals [9]. Thus, our results (Fig. 3B) showed necrosis of islet cells with destruction of cell populations, including β cells. Insulin is a hormone produced in the β cells of pancreatic islets which promotes uptake and glucose utilization by peripheral tissues [10]. Disturbances of secretion lead to carbohydrate intolerance and diabetes [11]. In contrast, treatment with total methanol extract of *C. perpulchrum* normalizes blood glucose and insulin secretion. Also, the histology of the pancreas of diabetic rats treated showed the presence of some islets of Langerhans. The latter would be responsible for the secretion of insulin and consequently the maintenance of glucose homeostasis. METCp effect on reducing blood sugar levels may be due to the presence of flavonoids [12,13]. These molecules act by enhancing the sensitivity of cells to insulin, which reduces blood glucose [14]. The METCp may have corrected the damage caused by diabetes in the pancreas. Our results show the protective effect of the extract of pancreatic damage in diabetes. Diabetic animals have hypertension and hyperlipidemia. Indeed, the destruction of beta cells of the islets of Langerhans caused by streptozotocin, generated the free radicals [9]. The increase in the vascular production of these free radicals especially superoxide anion is responsible for the subsequent decrease in the bioavailability of NO a vasodilator for smooth muscle cells [15,16]. Reactive oxygen species formed during diabetes are involved in lipid peroxidation which is the oxidation of polyunsaturated fatty acids to saturated fatty acid. In addition, unused fatty acids are in a deleterious way of lipid synthesis, inducing between other, ceramide formation and hyperlipidemia. Moreover, the accumulation of lipids in the myocardium is the source of insulin resistance development leading to a progression of diabetes [17]. METCp normalizes blood pressure and restored lipid imbalance caused by diabetes. The antihypertensive effect of METCp could be related to the presence alkaloids [8]. Under hypertensive condition, alkaloids activate baroreceptors carotid and aortic [18] causing vasodilatation and normalization of blood pressure. Moreover, METCp normalize lipid

levels by inhibition of lipid peroxidation and therefore by the inhibition of oxidation of LDL-c. Our results showed an elevation of serum urea and creatinine in hypertensive animals. Indeed, sustained hypertension is a source of a vascular remodeling characterized by an increase in the thickness of the media, and a reduction in the diameter of the resistance arteries [19,20]. This vasoconstriction, which affects the level of the afferent vessels of the glomeruli, decreases blood flow and glomerular filtration therefore the fluid in the renal tubules [21]. This is characterized by elevated serum levels of urea and creatinine. Thus, hypertension delete could be eventually responsible for kidney disease. METCp normalized serum urea and creatinine by its vasodilator effect exerted by the alkaloids contained in this extract.

5. CONCLUSION

The relationship between diabetes, hypertension and hyperlipidemia is univocal, both in humans as various experimental models. The intraperitoneal injection of streptozotocin in healthy rats, induces disturbances in carbohydrate and lipid metabolism in some organs (pancreas, kidney) function and blood pressure. However the treatment of diabetic rats by total methanol extract of *C. perpulchrum* corrects these disruptions and improves resistance against diabetes. This action of the total methanol extract of *C. perpulchrum* be related to its chemical composition characterized by the presence of flavonoids and alkaloids. Thus, this extract may play a preventive role in the development of diabetes especially through improvement of carbohydrate and lipid metabolism. This justifies its use in traditional environment as an antidiabetic.

ETHICAL APPROVAL

All the experimental procedure were approved by the Ethical Committee of health Sciences, FHB University of Abidjan. These guidelines were in accordance with the European Concil Legislation 87/607/EEC of the protection of experimental animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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