

EFFECT OF TOTAL METHANOLIC EXTRACT OF *CHRYSOPHYLLUM PERPULCHRUM* ON LIPEMIA, AND BLOOD PRESSURE IN RATS MADE DIABETIC BY STREPTOZOTOCIN

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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 (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 $1.93 \pm .05$ to $1.40 \pm .00$ for the dose to 200 and $1.93 \pm .05$ to $1.23 \pm .02$ 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.

19

20 Keywords: *Chrysophyllum* *perpulchrum*, diabetes, blood sugar, blood lipids.

21

22 1. INTRODUCTION

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

26 Despite the advance of hypoglycemic as antidiabetic drugs, WHO today emphasiez a
27 progressive globale incidence of diabetes. Whereas, 30 millions diabetic cases were
28 recorded in 1985, the figures have increased to 135 millions in 1995, 177 millions in 2000
29 and 347 millions in 2011. It is estimated that by 2030, 438 millions people will be affected
30 ([2]; [3]). Diabetes and its history are a real public health problem. The coverage and the
31 therapeutic treatment of the diabetes is at present made by strict diets, daily injection of a
32 dose of insulin by moment and the grip of oral antidiabetics. These pharmaceutical
33 treatments are usually expensive, many diabetics are turning to traditional healers who

34 prescribe their treatments based more economic plants ([4]). Traditional pharmacopoeia
35 offers a solution within reach of every budget, however, little information is available on the
36 therapeutic potential of these natural diabetes to regulate and control blood glucose in
37 humans. It is therefore necessary to prepare extracts of these antidiabetic plants and test
38 their biological effects for a scientific validation and pharmaceutical purposes.
39 The objective of this study is the validation of the antidiabetic effect of the total methanolic
40 extract of *C. perpulchrum* (METCp) recognized tradimedecin in the treatment of diabetes.

41

42 2. MATERIAL AND METHODS

43 2.1. Plant Material and conventional molecule

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

50

51 2.2. Animal model

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

59

60

61 **2.3. Methods**

62 **2.3.1. Total methanolic extract preparation**

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

70

71 **2.3.2. Screening Phytochimique**

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

74

75 **2.3.3. Induction of diabetes**

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

84

85 **2.3.4. Treatment of diabetic animals**

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

91 **Tableau 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

92

93 METCpI: Total methanolic extract of *C. perpulchrum* (Dose 1)

94 METCpII: Total methanolic extract of *C. perpulchrum* (Dose 2)

95 AVI: Avandia (Dose 1)

96 AVII: Avandia (Dose 2)

97

98 2.3.5. Preparation of samples and preparation of plasma

99 At the end of treatment, diethyl ether was used to anaesthetize the animals before blood
 100 samples were collected through heart puncture into different heparinized tubes. Blood
 101 samples are collected in heparinized tubes and then centrifuged at 3000 revolutions / minute
 102 for 10 minutes. Plasma was separated in two fractions in eppendorf tubes, one is used for
 103 immediate assay such as glucose and the other is placed in the freezer for analyzing lipids
 104 namely cholesterol (CHOL), triglycerides (TG), high density lipoproteins (HDL) and low

105 density lipoprotein (LDL). After the sacrifice and the dissection of the animals, one took a
106 sample of the pancreas and was put in the formalin 10 % for histological study.

107 **2.3.6. Determination of insulin levels in diabetic rats**

108 The change in insulin was determined every other day of the induction of diabetes in rats
109 and after treatment of these animals by antidiabetics (extract and Avandia). This assay was
110 carried out by the chemiluminescent immunometric method, enzymatic solid phase as
111 described by [7]. The solid phase (bead) is coated with anti-insulin murine monoclonal
112 antibodies. A liquid phase consisting of alkaline phosphatase (from calf intestine) associated
113 with anti-sheep polyclonal antibodies insulin, as well as alkaline phosphatase (from calf
114 intestine) associated with anti-insulin murine monoclonal antibodies. The serum of diabetic
115 rats and the reagent was incubated with the coated bead for 60 minutes. Meanwhile, the
116 insulin contained in the sample forms an antibody complex on the bead with anti-insulin
117 murine monoclonal antibodies and, in the reagent, the anti-sheep insulin polyclonal
118 antibodies associated with enzymes. Finally, chemiluminescent substrate is added to the
119 reaction cup containing the ball and the signal is generated in proportion to the bound
120 enzyme.

121

122 **2.4. Statistical analysis of results**

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

126

127 **3. RESULTS**

128 **3.1. Phytochemical Study of the total methanol extract of *C. perpulchrum***

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

131

132 **Table 2:** Results of phytochemical screening

133

chemical groups	polyterpenes	Polyphenols	alkaloids	Flavonoïds	Sterols
Presence	+	+	+	+	+

134

135 (+): presence

136

137

138 **3.2. Effect of streptozotocin on blood glucose, insulin and blood pressure in** 139 **rats.**

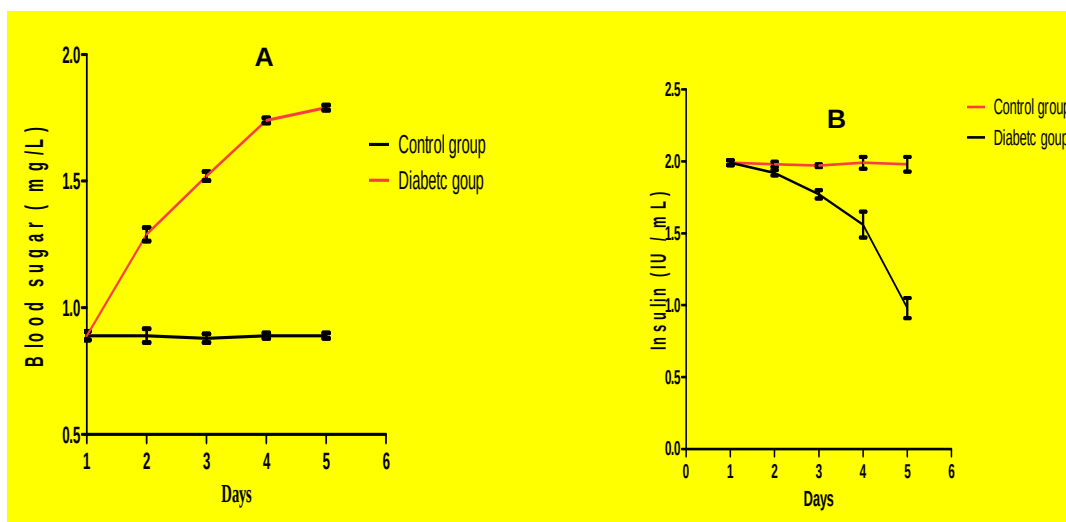
140 The Figures 1 (A, B and C) represent the variation of blood glucose, insulin and systolic
141 blood pressure (SBP) in the induction of diabetes in rats. The normal value of these
142 parameters identified in the control group at day zero of induction of diabetes is,
143 respectively, $.88 \pm .04$ mg/L; $1.98 \mu\text{u/mL}$ and 123.34 ± 1.12 mmHg. The blood glucose
144 values and blood pressure (SBP) rise gradually during induction reaching 1.02 mg/L (blood
145 sugar) and $153.85 \pm .28$ mmHg (SBP) in diabetic animals. And insulin down $1.98 \mu\text{u/mL}$ to
146 $0.98 \mu\text{u/mL}$.

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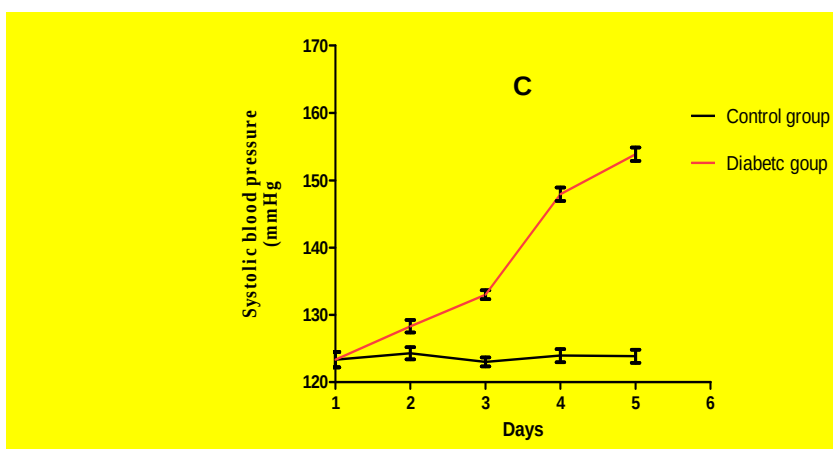
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153 **Figure I : Comparative Change in blood glucose, insulin and blood pressure in diabetic rats**

154

155 **3.3. Effect of total methanolic extract of *C. perpulchrum* (METCp) and avandia**
 156 **on the insulin levels in diabetic rats**

157 Figure 2 shows the effect of the total methanolic extract of *C. perpulchrum* (METCp) and
 158 Avandia on the insulin levels in diabetic rats. The dosage of insulin showed that there is a
 159 significant difference between the untreated diabetic rats (UDIAB) relative to the control
 160 group rats on the one hand, and between the diabetic rats treated with the doses of the
 161 METCp and Avandia compared to untreated infected animals. Serum insulin level remains
 162 very low in UDIAB ($.19 \pm .02 \mu\text{u/mL}$) compared to the control value ($.19 \pm .02 \mu\text{u/mL}$). On the

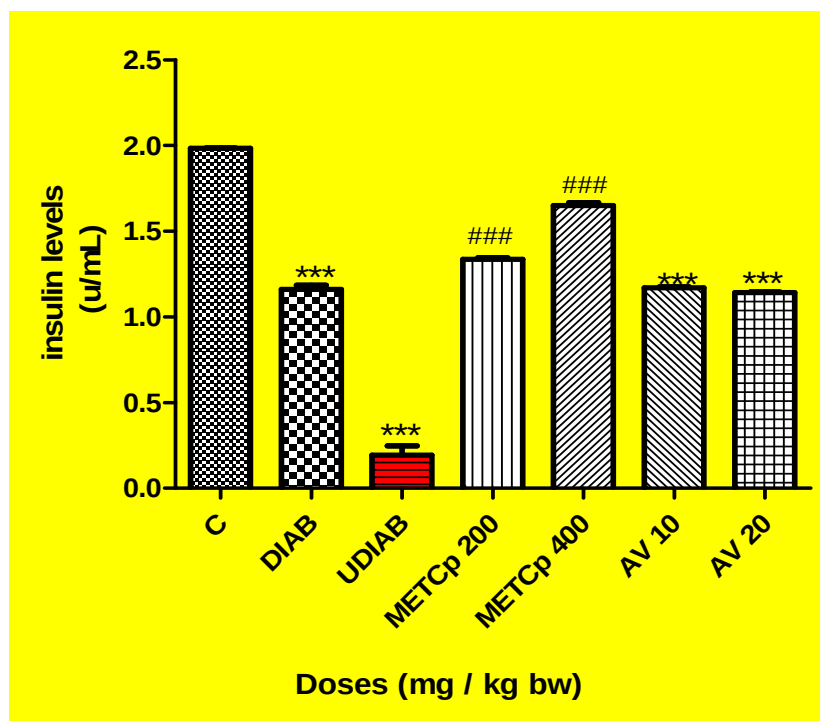
163 other hand, there is no significant difference between diabetic rats group treated by doses of
164 the antidiabetic Avandia a reference compared to diabetic rats (DIAB) before the beginning
165 of the treatment.

166 Table III shows the effect of the total methanolic extract of *C. perpulchrum* and Avandia on
167 blood glucose and lipids in diabetic rats. The administration of the total methanol extract of
168 *C. perpulchrum* to diabetic animals normalizes the values of biochemical parameters
169 previously elevated in diabetes. In fact, blood glucose decreases from $1.93 \pm .05$ to $1.40 \pm$
170 $.00$ mg/mL for the dose METCp 200 and $1.93 \pm .05$ to $1.23 \pm .02$ mg/mL for dose METCp
171 400. By cons, blood sugar remains high in group UDIAB at $1.76 \pm .02$ mg/mL compared to
172 the control group. Regarding lipids, serum levels of T CHOL, TG and LDL increase to
173 respective values of $.57 \pm .07$ to $1.58 \pm .07$; $.79 \pm .01$ to $1.13 \pm .04$; $.76 \pm .07$ to $1.19 \pm .03$
174 mmol/l and HDL decreases from $.38 \pm .01$ to $.11 \pm .02$ mmol/l. Treatment of diabetic rats with
175 dose 400 mg/kg bw of METCp for seven days, declining serum levels of CHOLT $1.23 \pm .02$,
176 $1.07 \pm$ TG to $.01$; LDL of $.57 \pm .02$ and increase HDL $.38 \pm .03$ compared to diabetic group
177 (DIAB). Similarly, different doses of Avandia antidiabetic commercially available decreases
178 and normalizes serum values of these same parameters in diabetic rats treated versus
179 untreated diabetic rats (UDIA) (Table III).

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

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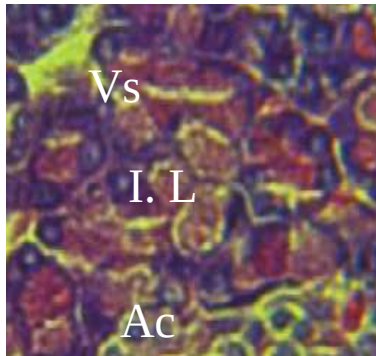
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Figure 2: insulin change in diabetic rats treated with METCp and Avandia

Each data represents the mean $\pm$ SEM, $n = 3$; (***) 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 200mg/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

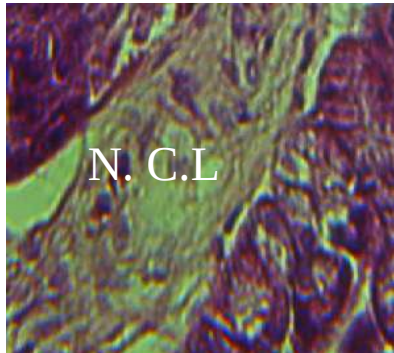


A: Pancreatic section of a normal rat

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

202

B

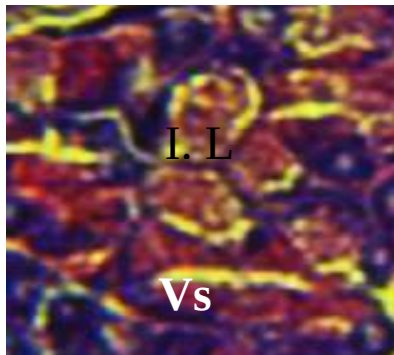


B: Pancreatic section of a diabetic rat

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

203

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.

204

205 **Figure 3:** Selected histological sections of pancreas of control rats (A), untreated diabetic

206 (B) and diabetic treated by 400 mg/kg bw of total methanolic extract of

207 *C. perpulchrum*(C)

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Tableau 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±0.00	1.93 ± 0.5***	2.61 ± 0.01**	1.40 ± 0.0 ^{###}	1.23 ± 0.02 ^{###}	1.25 ± 0,01 ^{###}	1.04 ± 0.05 ^{###}
Cholesterol (mmo/L)	0.57±0.07	1.58 ± 0.07 ***	1.84 ± 0,02***	1.43 ± 0.01 ^{###}	1.26 ± 1.03 ^{###}	1.17 ± 0.01 ^{###}	0.96 ± 0.08 ^{###}
Triglycerides (mmo/L)	0.79 ± 0.01	1.13 ± 0.04***	1.43 ± 0,01***	1.17 ± 0.01 ^{###}	1.07 ± 0.01 ^{###}	0.07± 0.02 ^{###}	0.71 ± 0.02 ^{###}
HDL (mmo/L)	0.13 ± 0.02	0.08±0.02 ***	0.08±0.0 1***	0.18±0.0 2 ^{###}	0.38±0.0 3 ^{###}	0.66±0.0 4* ^{###}	1.07 ± 0.04 ^{###}
LDL (mmo/L)	0.76 ± 0.07	1.19 ± 0.03 ***	1.41 ± 0.04***	0.85 ± 0.01 ^{###}	0.57 ± 0.02 ^{###}	0.46 ± 0.02 ^{###}	0.35 ± 0.03 ^{###} *

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 200mg/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.

227 4. Dicussion

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

254 ([8]). Under hypertensive condition, alkaloids activate baroreceptors carotid and aortic ([18])
255 causing vasodilatation and normalization of blood pressure. Moreover, METCp normalize
256 lipid levels by inhibition of lipid peroxidation and therefore by the inhibition of oxidation of
257 LDL-c. Our results showed an elevation of serum urea and creatinine in hypertensive
258 animals. Indeed, sustained hypertension is a source of a vascular remodeling characterized
259 by an increase in the thickness of the media, and a reduction in the diameter of the
260 resistance arteries ([19]; [20]). This vasoconstriction, which affects the level of the afferent
261 vessels of the glomeruli, decreases blood flow and glomerular filtration therefore the fluid in
262 the renal tubules ([21]). This is characterized by elevated serum levels of urea and
263 creatinine. Thus, hypertension delete could be eventually responsible for kidney disease.
264 METCp normalized serum urea and creatinine by its vasodilator effect exerted by the
265 alkaloids contained in this extract.

266

267 5. Conclusion

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

278

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280
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283 identification of *Chrysophyllum perpulchrum* (Sapotaceae).

284

285 **COMPETING INTERESTS**

286
287 **THE AUTHORS DECLARE THAT THEY HAVE NO COMPETING INTERESTS.**

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291 **AUTHORS' CONTRIBUTIONS**

292 **Bidié AP:** Carried out the study, the statistical analysis and prepared the manuscript

293 **Tiekpa WJ:** Carried out the technical analysis and prepared various parts of the manuscript

294 **Bla KB:** Provided reagents

295 **Djaman AJ:** Co-directed the study

296 All the authors read and approved the final manuscript.

297

298 **Ethical approval**

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

302

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