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Original Research Article

TITLE: EFFECT OF METANOLIC TOTAL EXTRACT OF *CHRYSOPHILLUM PERPULCHRUM* ON LIPEMIA, AND BLOOD PRESSURE IN RATS MADE DIABETIC BY STREPTOZOTOCIN.

ABSTRACT

Introduction: According to WHO, diabetes is a serious public health problem by the World progressive impact. In addition, there is a significant correlation between hyperglycemia, hypertension and dyslipidemia. African traditional medicine offers an alternative to conventional antidiabetic whose cost for the treatment is expensive. The objective of this study is to evaluate the effect of methanolic total extract of *Chrysophyllum perpulchrum* on hyperglycemia, hyperlipidemia and hypertension in diabetic rats made

Material and Methodology: The plant extract was obtained from the bark of *Chrysophyllum perpulchrum*. These peels were harvested, dried, ground and extracted in methanol. After the chemical screening, the extract (200 and 400 mg / kg body weight (bw)) was administered at a dose of 55 mg/kg bw.

Results: The results showed that triphytochimique of methanolic total extract of *Chrysophyllum perpulchrum* contains alkaloids, flavonoids, polyphenols. After 7 days of treatment with methanolic total extract of *Chrysophyllum perpulchrum*, blood sugar in diabetics significantly decreased ($P < 0.001$) of 1.93 ± 0.05 to 1.40 ± 0.00 for the dose to 200 and 1.93 ± 0,05 to 1.23 ± 0.02 g / L for the dose 400 mg/kg bw. Similarly methanolic total extract of *Chrysophyllum perpulchrum* normalizes blood pressure, all of lipids, urea and creatinine previously brought to the increase in diabetic rats.

Conclusion: The treatment of diabetic rats by methanol total 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

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alkaloids. Thus, this extract may play a preventive role in the development of diabetes especially through improvement of carbohydrate metabolism, lipid.

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

INTRODUCTION

Diabetes is a metabolic disorder characterized by the inability of the pancreas to produce insulin. Which results in a disorder in the regulation of carbohydrate metabolism from power thereby resulting in hyperglycemia (Ducorps 1997). Despite the advance of hypoglycemic as antidiabetic drugs, WHO today emphasizes a progressive global incidence of diabetes. Indeed, 30 millions diabetics listed in 1985, the figures are respectively 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 (Zhou *et al*., 2009; Danaei *et al*, 2011). Diabetes and its history are a real public health problem. Care and therapeutic treatment of diabetes is currently done by strict diets, the daily injection of insulin by the time oral antidiabetic intake. But these pharmaceutical treatments are usually expensive, many diabetics are turning to traditional healers who prescribe their treatments based more economic plants (Talkmore 2015). Traditional pharmacopoeia and 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 lighting for pharmaceutical purposes.

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

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II. Material and methods

1. Plant Material

C. perpulchrum, 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 Botanical Garden of FILIX Houphouet Boigny University in Côte d'Ivoire, where a specimen was filed.

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 a 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.

3. Methods

3.1. Methanolic total extract preparation

This preparation is performed according to the method described by Bidie *et al* (2008). Indeed, 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 is filtered three times on cotton and on Wattman filter paper 3 mm. The filtrate was evaporated at reduced

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pressure at 40° C using a rotary evaporator Buchi 461 Watter Batch (Strasbourg, France). The resulting powder was used to make the different tests.

3.1.1. 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. Of a single dose (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 or equal to 1.75 mg /L after 7 days are considered diabetic. These animals are called Diabetic lot now included in our study.

3.1.2. Treatment of diabetic animals

Fifteen (15) rats selected after onset of diabetes and three (3) healthy rats were divided into five groups of three rats each. The distribution of group and treatments were as follows (Table I):

3.1.3. Preparation of samples and the 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 is 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 blood collection, animals were sacrificed by carefully

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decapitation under light ether anesthesia and pancreas was removed into 10 % formal saline contained in a Petri dish.

3.1.4. Insulin dosage in diabetic animals

The change in insulin was determined every other day of the induction of diabetes in rats and after treatment of these animals by antidiabetics. This assay was carried out by the chemiluminescent immunometric method, enzymatic solid phase as described in Raufman, *et al.*, (1992). The solid phase (bead) is coated with anti-insulin murine monoclonal antibodies. A liquid phase consisting of alkaline phosphatase (calf intestine) associated with polyclonal antibodies anti-sheep insulin, as well as alkaline phosphatase (calf intestine) associated with anti-insulin murine monoclonal antibodies. The serum of diabetic rats and the reagent are 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.

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.

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III.RESULTS

1. Phytochemical Study of the total methanol extract of *C. perpulchrum*

According to the work of Bidie *et al.* (2000), the results of phytochemical screening test are reported in the table II.

2. Effect of streptozotocin on blood glucose, insulin and blood pressure in rats.

The Figures I (A, B and C) represents 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, 0.88 ± 0.04; 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 (blood sugar) and 153.85 ± 0.28 mmHg (SBP) in diabetic animals. And insulin down 1.98 µu/mL to 0.98 µu/mL.

2. Effect of methanolic extract total of C. perpulchrum (METCp) and avandia on insulin diabetic rats

Figure 2 shows the effect of the methanolic extract total of *C. perpulchrum* (METCp) and Avandia on insulin 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 rate remains very low in UDIAB (0.19 ± 0.02 µu/mL) compared to the control value (0.19 ± 0.02 µu/mL). On the other hand there is no significant difference between diabetic rats group treated by doses of the antidiabetic Avandia a commercially available compared to diabetic rats (DIAB).

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Table III shows the effect of the methanolic total extract of *C. perpulchrum* and the 'AVANDIA on blood glucose and lipids in diabetic rats. The administration of the methanol total 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 ± 0.05 to 1.40 ± 0.00 for the dose METCp 200 and 1.93 ± 0.05 to 1.23 ± 0.02 mg/mL for dose METCp 400 against blood sugar remains high in group UDIAB at 1 2.61 ± 0.01 mg / mL compared to the control group. Regarding lipids, serum levels of T CHOL, TG, LDL and increase to respective values of 0.57 ± 0.07 to 1.58 ± 0, 07; 0.79 ± 0.01 to 1.13 ± 0.04; 0.76 ± 0.07 to 1.19 ± 0.03 mmol / l and HDL decreases from 0.38 ± 0.01 to 0.11 ± 0.02 mmol / L. Treatment of diabetic rats the dose 400 mg / kg bw of METCp for seven days, declining serum levels of CHOLT 1.23 ± 0.02, 1.07 ± TG to 0.01; LDL of 0.57 ± 0.02 and increase HDL 0.38 ± 0.03 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 III).

IV. dicussion

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 (Akbarzadeh et al., 2007). Thus, our results 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 promotes uptake and glucose utilization by peripheral tissues (Sambo, 2005). Disturbances of secretion lead carbohydrate intolerance and leads to diabetes (Amadou, 2006). In contrast, treatment with methanol total extract of *C. perpulchrum* **(MTECp)** to diabetic animals normalizes blood glucose and insulin secretion. Also, the histology of the pancreas of such diabetic rats treated showed the presence of some islets of Langerhans. The

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latter would be responsible for the secretion of insulin and consequently the maintenance of glucose homeostasis. MTECp effect on reducing blood sugar levels may be due to the presence of flavonoids (N'Diaye et al 2008. Olagbende-Dada and al., 2011). These molecules act in effect by enhancing the sensitivity of cells to insulin, which reduces blood glucose (Lean et al. 1999). The **MTECp** would 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 generated by streptozotocin, causes the free radicals (Akbarzadeh et al., 2007). The increase in the vascular production of these free radicals especially superoxide anion ensuing is responsible for the subsequent decrease in the bioavailability of NO a vasodilator for smooth muscle cells (Kerr, 1999 and Dash, 2001). 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 the formation of ceramide and hyperlipidemia. Moreover, the accumulation of lipids in the myocardium is the source of insulin resistance development leading to a progression of diabetes (Manios, 2000). **MTECp** normalizes blood pressure and restored lipid imbalance caused by diabetes. The antihypertensive effect of **MTECp** could be related to the presence alkaloids (Bidie et al., 2011). At a hypertension, alkaloids activate baroreceptors carotid and aortic (Kjolby et al., 2005) causing vasodilatation and normalization of blood pressure. Moreover, **MTECp** 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 (Intengan 1999 Higashiyama et al., 2007). This vasoconstriction, which

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affects the level of afferent glomerular decreases blood flow and glomerular therefore fluid filtration in the renal tubules (Baluchenjadmojard and Rghani, 2003). This is characterized by elevated serum levels of urea and creatinine. Thus, hypertension not deal could be eventually responsible for kidney disease. ETMCp normalize serum urea and creatinine by its vasodilator effect exerted by the alkaloids contained in this extract.

V. 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 carbohydrate metabolic disturbances, lipid, in the functioning of some organs (kidney, pancreas) and blood pressure. However the treatments of diabetic rats by methanol total extract of *C.perpulchrum* corrects these disruptions and improves resistance against diabetes. This action of the methanol total 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 metabolism, lipid. 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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301

302 **Tableau I**: animal groupings/treatment

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groups	Désignation	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 I	400
05	diabetic treated with AV	10
06	diabetic treated with AV	20

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305 Table II: Results of phytochemical screening

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chemical groups	polyterpenes	**Polyphénols**	alkaloids	**Flavonoïds**	**Stérols**
Présence	+	+	+	+	+

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308 (+) : présence

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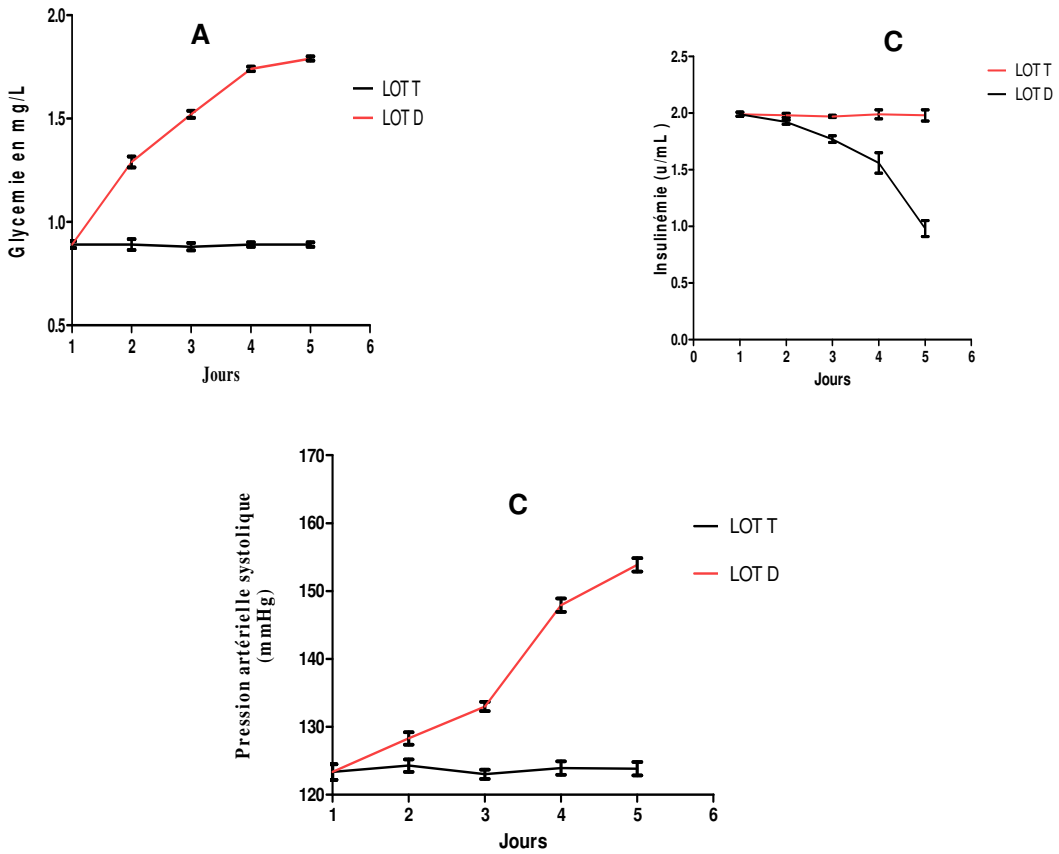

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313 **Figure I** : Comparative Change in blood glucose, insulin and blood pressure
314 in diabetic rats

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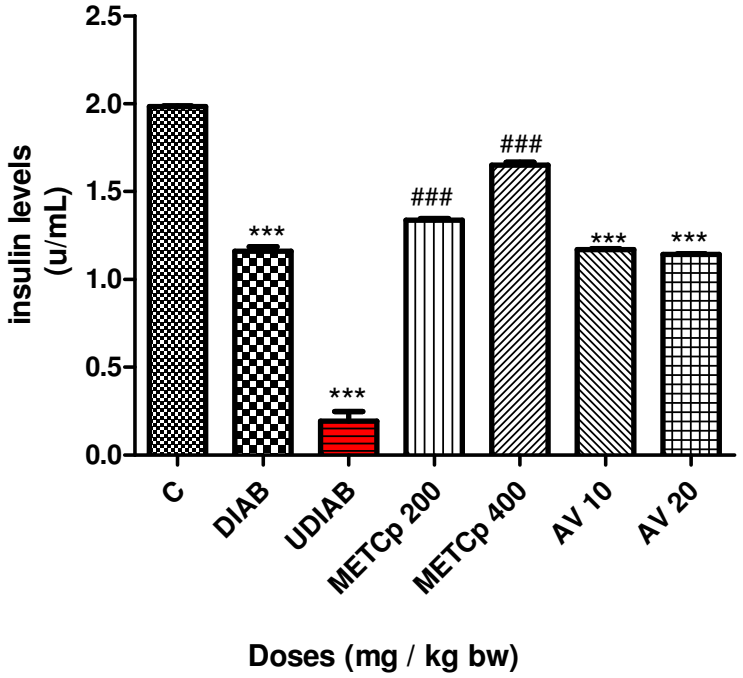

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318 **Figure 2**: nsulin change in diabetic rats treated with METCp and Avandia

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320 *Each data represents the mean ± SEM, n = 3; (*** p < 0.001) significantly different from control group; ###,*
321 *significantly different from the diabetic group, C: control group; Diab: diabetic group; UDIAB: untread*
322 *diabetic group; METCp 200: group treated by 200mg/kg bw of methanolic total extract of C. perpulchrum; lot*
323 *METCp 400 group treated by 400 mg/kg bw of methanolic total extract of C. perpulchrum; AV 10: group*
324 *treated by 10 mg/kg bw of avandia AV20: group treated by 10 mg/kg bw of Avandia*

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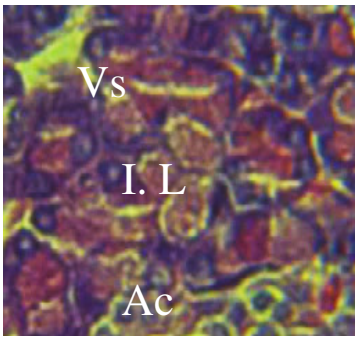
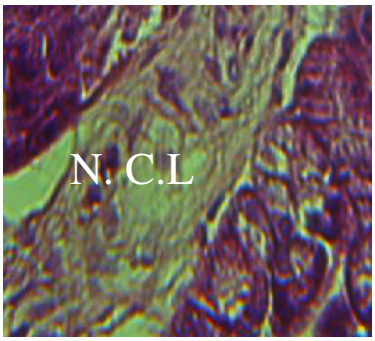


A: Pancreatic section of a normal rat

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

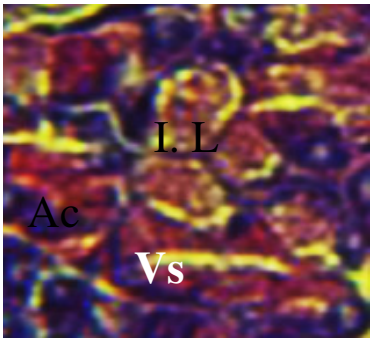
B



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.

Figure 3: selected Photographies of pancreas of control rats (A), untreated diabetic (B) and diabetic treated by 400 mg/kg bw of methanolic extract total of C. perpulchrum(C)

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350 **Tableau III**: Effet de l'**ETMCp** et de l'AVANDIA sur la glycémie et la lipidémie chez les
351 rats diabétiques

Electrolytic parameters	**control**	**Diabetes**		**Treatements**			
	T	**Diab**	**UDIAB**	**METCp 200**	**METCp 400**	**AV 10**	**AV 20**
Glycémie	1,75 ± 0,00	**1,93 ± 0,05*****	**2,61± 0,01****	1,40 ± 0,00##	1,23 ± 0,02###	1,25± 0,01###	1,04±0,05###
Cholestérol (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###
Triglycérides (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 ± 0,01*****	0,18 ± 0,02###	0,38 ± 0,03###	0,66 ± 0,04*###	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###*

352 Each value represents the mean ± SEM , n = 3 ; (* $p < 0.05$; *** $p < 0.001$) *) significantly different from*
353 *control group; ###, significantly different from the diabetic group, C: control group; Diab: diabetic group;*
354 *UDIAB: untread diabetic group; METCp 200: group treated by 200mg/kg bw of methanolic total extract of C.*
355 *perpulchrum; lot METCp 400 group treated by 400 mg/kg bw of methanolic total extract of C. perpulchrum;*
356 *AV 10: group treated by 10 mg/kg bw of avandia AV20: group treated by 10 mg/kg bw of Avandia*
357 HDL (high density lipoprotein); LDL: Low density lipoprotein