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Hypoglycemic and antihyperglycemic effects of powder and hydro-ethanolic extract of cocoa (*Theobroma cacao*)

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Abstract

Cocoa is one of natural substances rich in minerals and quality metabolites. In Côte d'Ivoire, studies carried out on *Forastero* cocoa from the Abengourou region (Center East region) showed that this variety is richer in polyphenols than those from other Ivorian regions. This work aims to assess hypoglycemic and antihyperglycemic activities of forastero cocoa from this region of Côte d'Ivoire. Hydro-ethanolic extract (HEE) from cocoa and cocoa powder (CoPw) were administered at different doses to normoglycemic rats divided into several groups. For the glucose tolerance test, hyperglycemia was induced to normoglycemic rats by oral administration of glucose at a dose of 4 g/kg bw. Rats were orally treated with HEE, CoPw and glibenclamide before and after hyperglycemia induction. The results obtained show that HEE from cocoa has the best antioxidant activity. In addition, HEE administered at doses of 500, 800 and 1000 mg/kg bw and CoPw at a dose of 800 mg/kg bw induce hypoglycemia. This hypoglycemia is dose-dependent with the 500 and 800 mg/kg bw doses of the HEE from cocoa. Moreover, like glibenclamide (5 mg/kg bw), the doses of 800 and 1000 mg/kg bw of HEE from cocoa as well as CoPw (800 mg/kg bw) significantly reduce the hyperglycemia in post-treated and pre-treated rats. This study therefore reveals that CoPw and HEE from *Forastero* cocoa have good hypoglycemic and anti-hyperglycemic potential. However, HEE leads to a better activity, because it concentrates more the polyphenols, responsible for these pharmacological activities.

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Introduction

In recent years, particular attention has been paid to plants rich in secondary metabolites with antioxidant, anti-inflammatory, anti-ulcer, anti-carcinogenic and anti-mutagenic properties (Queen and Tollefsbol, 2010). Indeed, they own a preventive effect in pathologies that involve the deterioration of cells or the blocking of metabolism by free radicals. As a result, these plants are used in many fields including the food industry, cosmetology, pharmacology (Skandrani *et al.*, 2010) and chemistry (Kalia *et al.*, 2008). Among them is cocoa, fruit of *Theobroma cacao*. Its richness in minerals and quality metabolites has been the subject of numerous studies. These studies focused on the potential benefits of cocoa, in particular its antidiabetic activity (Ruzaidi *et al.,* 2008; Abbe *et al.*, 2009; Djoussé *et al.*, 2011; Olooto *et al.,* 2014 and 2016). Moreover, the work of Grassi *et al.* (2005 and 2008) showed that people whose diet includes dark chocolate have a lower risk of heart disease than those who do not consume it. According to Ala'a *et al.* (2016), compared with people who do not consume dark chocolate, those who consume it have lower insulin resistance. These people also have lower risk markers for heart attacks and liver problems, as well as improved liver function. According to these same authors, the metabolites responsible for these activities are monomers of epicatechins, polyphenols which increase the capacity of beta cells to produce insulin. These results are in agreement with those of Cordero-Herrera *et al.* (2014), who have proved the prevention of type 2 diabetes through the consumption of cocoa. The therapeutic effects of cocoa also include a beneficial action on the intestinal microbiota (Grassi *et al.*, 2005 and 2008).

Moreover, studies conducted in Malaysia have shown that cocoa is rich in phenols and its consumption protects many cells, including those of the pancreas (Ruzaidi *et al.,* 2008). In Côte d'Ivoire, several studies have been carried out on cocoa varieties. Among them, those of Dembélé *et al.* (2018) showed that forastero cocoa from Abengourou region (Center East region of Côte d'Ivoire) is richer in polyphenols than those from other Ivorian terroirs because of the quality of the soil. But, in the current state, no study has been conducted on the antidiabetic activity of Ivorian forastero cocoa. The objective of this work is to evaluate the effect of *Forastero* cocoa (*Theobroma cacao*) from Abengourou in normoglycemic and hyperglycemic rats.

Materials and methods

Plant material

The plant material consists of cocoa beans (*Theobroma cacao*) of the *Forastero* variety. The cocoa pods were harvested in Abengourou, an area located in the East of Côte d'Ivoire.

Animals

One hundred (100) male albino rats, *Rattus norvegicus* of the Wistar strain, weighing 100 to 150 grams, were used in this study. These rats were reared under adequate conditions of hygiene, ambient temperature of 25°C, and in sufficient ventilation. They were housed in cages lined with wood chips where they had free access to water and food. The rats were alternately subjected to 12 hours of light and 12 hours of darkness.

Chemicals

The chemicals used in this study are analytical grade organic solvents and reagents commonly used in the laboratory. Among these products, mention may be made of Folin-Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, ferrozine, vitamin C, iron III chloride ($FeCl_3$), acetic anhydride, gallic acid, sulfuric acid, iron perchloride, sodium acetate and isoamyl alcohol.

Preparation of cocoa powder, aqueous and hydroethanolic extracts

The defatted cocoa powder (CoPw) was prepared according to the method of of Jinap *et al.* (1998), with slight modifications. The non-fermented and dried cocoa beans were roasted for 20 min at 140°C in an oven, then cooled and shelled. The obtained almonds were ground using a blender and the gotten cocoa mass was defatted with hexane using a soxhlet.

The cake obtained was dried at 50°C for 1 h 30 min, finely ground and then sieved in order to obtain the defatted cocoa powder. The aqueous extract (AqE) was prepared by homogenizing 100 g of defatted cocoa powder in 1 L of distilled water using a blender according to the method of Zihiri *et al.* (2003). After six cycles of homogenization, the obtained homogenate was first wrung out in a fabric square and then filtered thrice successively with absorbent cotton and twice with Whatman 3 mm filter paper. The resulting filtrates were concentrated under vacuum at 60°C using a Büchi rotary evaporator. Hydroethanolic extract (HEE) was prepared following the same process using a mixture of ethanol (70%) and water (30%). These two crude extracts were weighed to determine the yield of each extraction. Extracts were stored separately in hermetically sealed jars and kept away from heat, moisture and light.

Phytochemical screening

Phytochemical screening was carried out using appropriate specific reagents whose colorations generated by the tests indicate the presence or absence of the sought compounds. The sought compounds were saponosides, polyphenols, alkaloids, flavonoids, quinones, polyterpenes, steroids and cardiotonic glycosides.

Determination of total phenol contents

Total phenol contents in cocoa extracts were determined by Folin-Ciocalteu reagent (Wood *et al.*, 2002). A volume of 2.5 mL diluted (1/10) Folin-Ciocalteu reagent was added to 0.5 mL of extract. The mixture was kept for 2 min in the dark at room temperature and 2 mL of calcium carbonate solution (75 $g.L^{-1}$) was added. The mixture was then incubated for 10 min in a water bath at 50°C and rapidly cooled. Absorbance was measured at 760 nm. A calibration line was established with gallic acid at different concentrations. Analyses were carried out in triplicate, and polyphenol concentration was expressed as milligram equivalent of gallic acid per gram of dry extract (mg Eq GA/g dry extract).

Determination of antioxidant activity

Assessment of *in vitro* antioxidant activity of cocoa extracts was carried out by measuring the anti-radical activity through the DDPH test and the measurement of the chelating power of iron through the FRAP test.

DPPH radical scavenging test

DPPH• is characterized by its ability to produce stable free radicals. This stability is due to the delocalization of free electrons within the molecule. The presence of these DPPH• radicals generates a dark violet color. The reduction of DPPH• radicals by an antioxidant results in a yellow color (Molyneux, 2004). The color change can be monitored spectrophotometrically at 517 nm.

Briefly, 2 mL of a methanolic solution of DPPH (100 µM) was mixed with 1.5 mL of different dilutions of the cocoa extracts (0-200 µg/mL). The resulting mixture was then incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm against a control consisting of 2 mL DPPH solution and 1.5 mL methanol. A concentration range (0-100 µg/mL) for vitamin C prepared under the same operating conditions was used as a reference. The percentage inhibition (PI) of DPPH radicals was calculated according to the below formula.

$$PI = \frac{A_0 - A_1}{A_0} \times 100$$

PI: Percentage inhibition (%)

A_0: absorbance of DPPH solution in the absence of extract (blank)

A_1: absorbance of DPPH solution in the presence of extract (assay)

Concentration of cocoa extracts or vitamin C responsible for 50% inhibition of DPPH radicals (IC_{50}) was determined from the graph representing percentage DPPH inhibition as a function of concentration of extracts and vitamin C.

Reduction power measurement

The FRAP test is based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). This method assesses the reducing power of antioxidants present in a mixture by their ability to reduce ferric tripyridyltriazine (Fe^{3+}-TPTZ) to ferrous ion (Fe^{2+}-TPTZ) at acid pH (Ou *et al.*, 2001). Therefore, Fe^{2+} can be assessed by measuring and monitoring the increase in cyan blue color density in the reaction medium at 593 nm (Chung *et al.*, 2002).

For this purpose, a fresh solution of FRAP reagent (10 mM) was prepared by mixing 2.5 mL of 2,4,6-tris (2-pyridyl) 1,3,5-s-triazine (TPTZ) (10 mM) with 2.5 mL of $FeCl_3$ solution (20 mM) and 2.5 mL of sodium acetate buffer solution (300 mM, pH 3.6). Next, 3500 µL of FRAP reagent was added to 140 µL of cocoa extracts dissolved in methanolic solution. After 30 min incubation at room temperature and protected from light, absorbance was read at 593 nm. Trolox was used as control. A calibration line was established with the following concentrations of Trolox: 1; 0.5; 0.25; 0.125; 0.0625 and 0.031 mg/mL.

Assessment of hypoglycemic activity

Thirty (30) male rats were distributed in 6 groups of 5 rats. These animals were fasted for 12 h. Then, their initial blood sugar (t_0) was measured using a glucometer (ACCU CHEK) and reactive strips according to the method of Gunjan *et al.* (2013). After cleaning the tail of each rat with alcohol, it was cut using a blade and a drop of blood was placed on a reactive strip inserted into the glucometer. This device automatically displays the blood sugar value. Subsequently, for each group of rats, a single dose of the hydro-ethanolic extract from cocoa (HEE) or cocoa powder (CoPw) was administered to them orally. The constitution of the groups is as follows:

Group 1: normoglycemic control rats having received distilled water (1 mL/100 g bw);
Group 2: rats treated with glibenclamide (reference substance) at a dose of 5 mg/kg bw;
Groups 3, 4 and 5: rats treated with HEE from cocoa at 500, 800 and 1000 mg/kg bw respectively;
Group 6: rats treated with CoPw at a dose of 800 mg/kg bw.

After the treatment of animals, blood glucose was again measured every 30 min, for 2 h 30 min, and the percentage reduction in blood glucose compared to the initial blood glucose was calculated according to the formula below.

$$\text{ReduceX(t)(\%)} = \frac{\text{Gly(t)} - \text{Gly(t}_0\text{)}}{\text{Gly(t}_0\text{)}} \times 100$$

ReduceX(t)(%): percentage reduction of blood sugar at time (t); Gly(t): blood sugar at time (t); Gly(t_0): initial blood sugar.

Glucose tolerance test

Glucose tolerance is the body's ability to metabolize glucose. This test which aims to check the sensitivity of cells to endogenous insulin, involves insulin secretion, utilization of glucose by peripheral tissues and stimulation of the autonomic nervous system (N'Doua *et al.*, 2015).

Blood sugar measurement in post-treated rats

Thirty-five (35) male rats were distributed in 7 groups of 5 animals. These animals were fasted for 12 h. Their initial blood sugar (t_0) was measured using a glucometer (ACCU CHEK) and reactive strips. Subsequently, a glucose solution (4 g/kg bw) was administered orally. Thirty (30) minutes later, the rats' blood sugar was again determined. Then, HEE from cocoa at different doses (500, 800 and 1000 mg/kg bw), CoPw (800 mg/kg bw) and glibenclamide (5 mg/kg bw) were orally administered (N'Doua *et al.*, 2015). The constitution of the groups is as follows:

Group 1: normoglycemic control rats having received distilled water (1 mL/100 g bw);
Group 2: hyperglycemic control rats having received 4 g/kg bw of glucose, then distilled water (1 mL/100 g bw) 30 min later;
Group 3: rats having received 4 g/kg bw of glucose, then glibenclamide (5 mg/kg bw) 30 min later;
Groups 4, 5 and 6: rats having received 4 g/kg bw of glucose, then HEE from cocoa at 500, 800 and 1000 mg/kg bw respectively 30 min later;
Group 7: rats having received 4 g/kg bw of glucose, then CoPw (800 mg/kg bw) 30 min later.

The blood sugar of the rats in each group was subsequently measured at 30 min intervals for 3 hours. Finally, the percentages of induction and reduction of induced hyperglycemia were determined according to the following formulas:

$$\text{IHyp(t)(\%)} = \frac{\text{HyGly} - \text{Gly(t}_0\text{)}}{\text{Gly(t}_0\text{)}} \times 100$$

$$\text{RHyp(t)(\%)} = \frac{\text{Gly(t)} - \text{HyGly}}{\text{HyGly}} \times 100$$

IHyp(t)(%): percentage of hyperglycemia induction at time (t); RHyp(t)(%): percentage of hyperglycemia reduction at time (t); HypGly: peak value of induced

hyperglycemia in animals; Gly(t): blood sugar value measured at time (t); Gly(t_0): initial blood sugar value measured at time (t_0).

Blood sugar measurement in pretreated rats

The sampling is the same as that carried out in post-processing. However, the different groups of rats received different doses of HEE from cocoa (500, 800 and 1000 mg/kg bw), CoPw (800 mg/kg bw) or glibenclamide (5 mg/kg bw) 30 min before administration of glucose (4 g/kg bw). The initial blood sugar of rats was measured just before the administration of the test substances. The constitution of the groups is as follows:

Group 1: normoglycemic control rats having received distilled water (1 mL/100 g bw);
Group 2: hyperglycemic control rats having received distilled water (1 mL/100 g bw) then, 4 g/kg bw of glucose 30 min later;
Group 3: rats having received glibenclamide (5 mg/kg bw), then 4 g/kg bw of glucose 30 min later;
Groups 4, 5 and 6: rats having received HEE from cocoa at 500, 800 and 1000 mg/kg bw respectively and 4 g/kg bw of glucose 30 min later;
Group 7: rats having received cocoa powder at a dose of 800 mg/kg bw, then 4 g/kg bw of glucose 30 min later.

The blood sugar of rats in each group was measured at 30 min intervals for 3 h. The values obtained were compared with the initial blood sugar and with that of rats of the control groups. The percentages of induction and reduction of induced hyperglycemia were determined according to the following formulas:

$$\text{IHyp(t)(\%)} = \frac{\text{HyGly} - \text{Gly}(t_0)}{\text{Gly}(t_0)} \times 100$$

$$\text{RHyp(t)(\%)} = \frac{\text{Gly(t)} - \text{HyGly}}{\text{HyGly}} \times 100$$

IHyp(t)(%) : Percentage of hyperglycemia induction at time (t); RHyp(t)(%): Percentage of reduction of hyperglycemia at time (t); HypGly: peak value of induced hyperglycemia in animals; Gly(t): blood glucose value measured at time (t); Gly(t_0): initial blood sugar value at time (t_0).

Analysis and expression of results

Data analysis was performed using Graph Pad Prism software Version 8.4.3 (686). The results were expressed as means plus or minus standard error from the mean (M±SEM). The comparison of the variance of the means of the different experimental groups was made with one-way ANOVA followed by Tukey's multiple comparison test at the 5% threshold. The difference between the means is significant when $p < 0.05$. Graph Pad Prism software. Version 8.4.3 (686) was also used to plot the graphics.

Results

Total phenol contents

The total phenol content of *forastero* cocoa extracts was determined from the linear regression equation $Y= 11.4x + 0.005$; $R^2 = 0.9939$. This content was expressed in mg equivalent of gallic acid per gram of dry matter (mg Eq GA/g of dry extract). Fig. 1 presents the total phenol content of CoPw, AqE and HEE from cocoa. HEE from cocoa has a higher content of total phenol in the order of 1391 ± 194.1 mg Eq GA/g of dry extract. Regarding AqE from cocoa and CoPw, total phenol contents are respectively 313.4 ± 23.98 and 761.9 ± 26.89 mg Eq GA/g of dry extract. These contents are lower than that of HEE from cocoa. Statistical analysis reveals that the values are significantly different from each other.

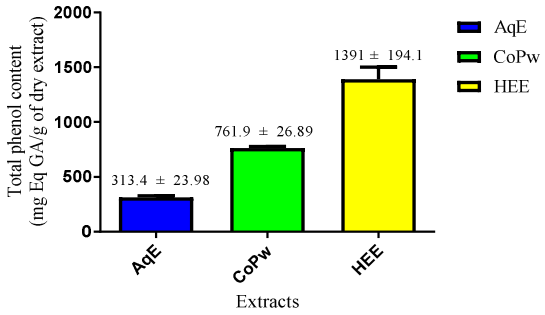

Fig. 1. Total phenol contents of cocoa extracts
AqE: aqueous extract from cocoa, HEE: Hydroethanolic extract from cocoa; CoPw: Cocoa powder

Scavenging activity

Fig. 2 shows that all extracts have ability to scavenge free radical DPPH. The inhibition percentage of free radical DPPH is proportional to the concentration of extracts.

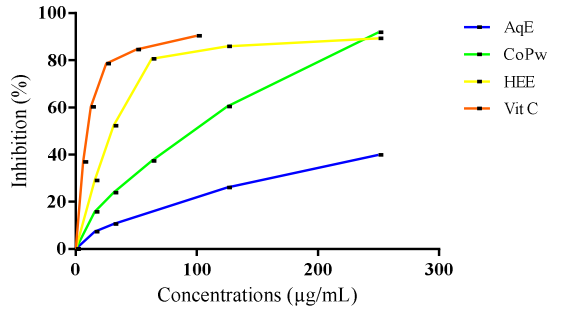

Fig. 2. Evolution of DPPH radical inhibition according to the concentration of extracts
AqE: aqueous extract from cocoa, HEE: Hydroethanolic extract from cocoa; CoPw: Cocoa powder

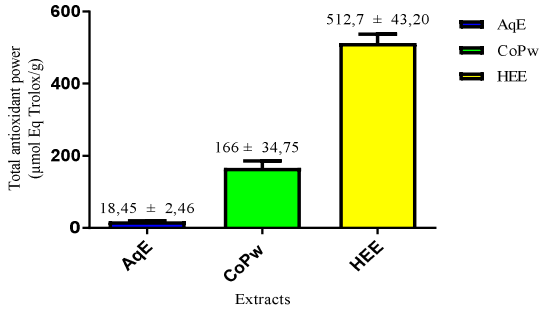

Fig. 3. Reducing power of different cocoa extracts
AqE: aqueous extract from cocoa, HEE: Hydroethanolic extract from cocoa; CoPw: Cocoa powder

At a concentration of 100 µg/mL, the inhibition percentage of vitamin C was 90.8% while those of AqE, HEE and CoPw were 21.8%, 84.8% and 52.8% respectively. Regarding the IC_{50}, the values obtained are 7.83 ± 0.09; 143.3±2.5; 24.03 ± 0.26 and 68.98 ±0.5 µg/mL respectively for vitamin C, AqE, HEE and CoPw (Table 1). Statistical analysis of the data shows that IC_{50} values are significantly different. The lower the IC_{50}, the higher the extract has antioxidant activity. Thus, in ascending order of antioxidant activity, we obtain: vitamin C, HEE, CoPw and AqE.

Table 1. IC_{50} values of the different extracts

Extracts	AqE	CoPw	HEE	Vit C
IC_{50} (µg/mL)	143.3 ±2.5	68.98 ±0.5	24.03 ± 0.26	7.83 ± 0.09

AqE: aqueous extract from cocoa, HEE: Hydroethanolic extract from cocoa; CoPw: Cocoa powder; Vit C: Vitamin C

Reducing power of cocoa powder and extracts

The reducing power of the forastero cocoa extracts was determined using the linear regression equation Y= 1.14x + 0.093; R^2= 0.9966. Results were expressed in µmol equivalent of Trolox per gram of dry extract (µmol Eq Trolox/g of dry extract). The values obtained are respectively 18.45±2.46; 166 ± 34.75 and 512.7 ± 43.20 µmol Eq Trolox/g for AqE, CoPw and HEE (Fig. 3). These results show that HEE has the highest reducing power followed by CoPw and finally AqE which has the lowest reducing power. Thus, HEE has the best antioxidant activity among the three cocoa products analyzed.

Effects of HEE from cocoa and CoPw on blood sugar of normoglycemic rats

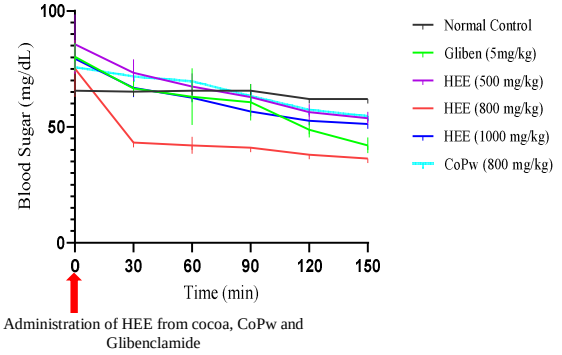

Fig. 4. Evolution of glycemia as a function of time in normoglycemic rats treated with HEE from cocoa, CoPw and glibenclamide

Normal control: Normoglycemic controls, Gliben (5 mg/kg): Glibenclamide 5mg/kg bw, HEE (500 mg/kg): Hydro-ethanolic extract at a dose of 500 mg/kg bw, HEE (800 mg/kg): Hydro-ethanolic extract at a dose of 800 mg/kg bw, HEE (1000 mg/kg): Hydro-ethanolic extract at a dose of 1000 mg/kg bw; CoPw (800 mg/kg): Cocoa powder at a dose of 800 mg/kg bw

The variation in blood sugar following oral administration of doses of 500, 800 and 1000 mg/kg bw of HEE, CoPw (800 mg/kg bw), glibenclamide (5 mg /kg bw) and distilled water (control group) to nomoglycemic rats is illustrated on Fig. 4. The blood sugar of the control rats having received distilled water did not vary significantly ($p > 0.05$), because it went from 65.60 ± 0.24 to 62.00 ± 1.24 mg/dL. In the treated rats, the results show that for all the

substances tested (HEE, CoPw, glibenclamide), the blood sugar levels of rats drop progressively throughout the experiment. Indeed, the reference hypoglycemic substance (glibenclamide) produced 66.60 ± 3.15 mg/dL, either 16.96% to 42.00 ± 3.34 mg/dL, either 47.63% reduction of blood sugar compared with rats initial value, respectively at 30 min and 2h 30 min after treatment (Fig. 4, Table 2). It is more active than 500 and 1000 mg/kg bw doses of HEE from cocoa and CoPw, but less active than 800 mg/kg bw dose of HEE from cocoa.

In addition, the results reveal that the lowest performance was obtained with cocoa powder, which generates respectively at 30 min and 150 min, 71.80 ± 1.71 mg/dL, either 5.28% of reduction percentage and 54.60 ± 0.24 mg/dL, either 27.97 % of reduction percentage. In contrast, the strongest activity was generated by the 800 mg/kg bw dose of the HEE from cocoa. Indeed, at different blood sugar measures (30, 60, 90, 120 and 150 min after treatment), HEE 800 mg/kg bw induced respective reduction percentages of 42.55% (43.20 ± 1.31 mg/dL); 44.15 %; 45.48%; 49.47% and 51.60% (36.40 ± 1.12 mg/dL). The results also show that 30 min after administration of the different substances, the level of reduction in blood sugar induced by HEE (42.55%) is significantly higher than that of glibenclamide (16.96%). This better performance of HEE was maintained until the end of the experiment.

Table 2. Reduction percentage on blood sugar of cocoa extract and glibenclamide in normoglycemic rats

Reduction Percentage in blood sugar	Time after administration of HEE, CoPw and glibenclamide (min)					
	0	30	60	90	120	150
Gliben (5 mg/kg bw)	0.00	16.96	21.45	24.44	39.15	47.63
HEE (500 mg/kg bw)	0.00	14.25	21.26	26.64	34.11	37.15
HEE (800 mg/kg bw)	0.00	42.55	44.15	45.48	49.47	51.60
HEE (1000 mg/kg bw)	0.00	15.87	21.16	28.72	33.75	35.52
CoPw (800 mg/kg bw)	0.00	5.28	8.18	16.36	24.27	27.97

Gliben (5 mg/kg): Glibenclamide 5mg/kg bw, HEE (500 mg/kg): Hydro-ethanolic extract at a dose of 500 mg/kg bw, HEE (800 mg/kg): Hydro-ethanolic extract at a dose of 800 mg/kg bw, HEE (1000 mg/kg): Hydro-ethanolic extract at a dose of 1000 mg/kg bw; CoPw (800 mg/kg): Cocoa powder at a dose of 800 mg/kg bw

Effects of HEE from cocoa and CoPw on induced hyperglycemia in post-treated rats

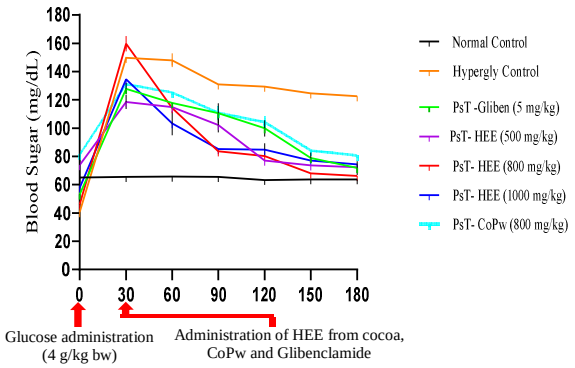

Fig. 5. Evolution as function of time of blood sugar in post-treated hyperglycemic rats with HEE from cocoa, CoPw and glibenclamide

Normal control: Normoglycemic control, Hypergly control: Hyperglycemic control, PsT-Gliben (5mg/kg): Post-treated group with glibenclamide at a dose of 5mg/kg bw, PsT-HEE (500 mg/kg): Post-treated group with the hydro-ethanolic extract at a dose of 500 mg/kg bw, PsT-HEE (800 mg/kg): Post-treated group with the hydro-ethanolic extract at a dose of 800 mg/kg bw, PsT-HEE (1000 mg/kg): Post-treated group with the hydro-ethanolic extract at a dose of 1000 mg/kgbw; PsT-CoPw (800 mg/kg): Post-treated group with cocoa powder at a dose of 800 mg/kg bw

The results reveal that blood sugar evolution curve is almost linear in the normoglycemic control rats, thus showing that their blood sugar varies very little. Compared with that of control rats, blood sugar of treated rats increased significantly and reached a peak, 30 min after the administration of glucose (Fig. 5). At time T60, either 30 min after the treatment with different tested substances, blood sugar of all animals gradually decreases. This low is very moderate with hyperglycemic control rat group, but quick with all other treated groups.

Table 3. Induction and reduction percentage of induced hyperglycemia in post-treated rats

Induction and reduction percentage of glucose-induced hyperglycemia 4 g/kg bw	Time after administration of glucose, HEE, CoPw and glibenclamide (min)					
	30	60	90	120	150	180
Hypergly Control	268.97	-1.20	-12.55	-13.62	-16.82	-18.16
Gliben (5mg/kg bw)	140.23	-7.82	-13.62	-21.91	-38.18	-43.82
HEE (500 mg/kg bw)	60.43	-3.04	-13.68	-34.97	-37.84	-38.85
HEE (800 mg/kg bw)	246.96	-28.45	-47.62	-49.75	-57.39	-58.52
HEE (1000 mg/kg bw)	132.87	-23.18	-36.70	-37.15	-42.64	-44.73
CoPw (800 mg/kg bw)	61.58	-4.57	-15.55	-20.58	-35.98	-38.41

Hypergly Control: hyperglycemic controls, Gliben (5 mg/kg): Glibenclamide 5mg/kg bw, HEE (500 mg/kg): Hydro-ethanolic extract at a dose of 500 mg/kg bw, HEE (800 mg/kg): Hydro-ethanolic extract at a dose of 800 mg/kg bw, HEE (1000 mg/kg): Hydro-ethanolic extract at a dose of 1000 mg/kg bw; CoPw (800 mg/kg): Cocoa powder at a dose of 800 mg/kg bw

The treatment of hyperglycemic rats with the different substances led in each group to strong and progressive decrease in blood sugar to low values equivalent to that of normal control rats, after 2 h 30 min. For each group, the level of reduction are variable and more or less strong depending on the substance used, compared with the highest value of induced hyperglycemia. Thus, with glibenclamide, the blood sugar level has passed from 127.80 ± 4.00 to 118.40 ± 4.99 mg/dL, either 7.82% of reduction at T60 (Table 3). In treated groups with HEE (500, 800 and 1000 mg/kg bw) and CoPw, blood sugar levels respectively decreased from 118.4 ± 4.99 to 114.8 ± 2.57 mg/dL; 159.6 ±5.47 to 114.2 ± 3.23 mg/dL; 134.6 ± 2.06 to 103.4 ± 8.61 mg/dL and 131.2 ± 2.95 to 125.2 ± 1.77 mg/dL. The strongest decrease (-45.4 mg/dL) was obtained with HEE at 800 mg/kg bw. From there, this dose generated the best performance until the end of the experiment (150 min), where the blood sugar level (66.2 ± 2.53 mg/dL) reached a value equivalent to that of normoglycemic control rats. Apart from HEE from cocoa (800 mg/kg bw), all the other substances tested generated gradual reductions until the end of the treatment period. However, the final blood glucose values did not equal that of the blood glucose of normoglycemic rats. In contrary to the groups treated with cocoa extracts, the blood sugar decreases very slowly in hyperglycemic control group.

Effects of HEE from cocoa and CoPw on induced hyperglycemia in pretreated rats

The variations in the blood sugar of rats following oral administration of the different doses of HEE from cocoa, CoPw, glibenclamide and distilled water (control group) as function of time are presented in Fig. 6.

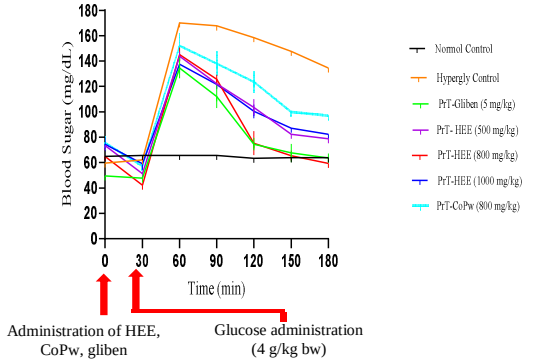

Fig. 6. Evolution as function of time of blood sugar in pretreated hyperglycemic rats with HEE from cocoa, CoPw and glibenclamide

Normal control: Normoglycemic control, Hypergly control: Hyperglycemic control, PrT-Gliben (5mg/kg): Pretreated group with Glibenclamide at a dose of 5 mg/kg bw, PrT-HEE (500 mg/kg): Pretreated group with the hydro-ethanolic extract at a dose of 500 mg/kg bw, PrT-HEE (800 mg/kg): Pretreated group with the hydro-ethanolic extract at a dose of 800 mg/kg bw, PrT-HEE (1000 mg/kg): Pretreated group with the hydro-ethanolic extract at a dose of 1000 mg/kg bw; PrT-CoPw (800 mg/kg): Pretreated group with cocoa powder at a dose of 800 mg/kg bw

The results reveal that the blood sugar evolution curve is almost linear with normoglycemic control rats, showing that their blood sugar varies very little. With treated rats, blood sugar curve shows 3 phases. For all the doses, the blood sugar decreases from To to T30 after the administration of cocoa extracts.

Table 4. Induction and reduction percentage of induced hyperglycemia in pretreated rats

Induction and reduction percentage of glucose-induced hyperglycemia 4 g/kg bw	Time after administration of HEE, CoPw, glibenclamide and glucose (min)					
	30	60	90	120	150	180
Hypergly Control	4.36	185.57	-1.41	-6.93	-13.28	-20.92
Gliben (5 mg/kg bw)	-3.63	171.37	-16.79	-44.58	-49.78	-52.75
HEE (500 mg/kg bw)	-29.97	95.91	-15.02	-27.96	-42.70	-45.20
HEE (800 mg/kg bw)	-34.88	124.07	-13.36	-48.07	-54.96	-59.09
HEE (1000 mg/kg bw)	-20.43	84.95	-11.63	-27.03	-36.63	-40.12
CoPw (800 mg/kg bw)	-24.34	101.32	-9.33	-18.79	-34.30	-36.27

Hypergly Control: hyperglycemic controls, Gliben (5 mg/kg): Glibenclamide 5mg/kg bw, HEE (500 mg/kg): Hydro-ethanolic extract at a dose of 500 mg/kg bw, HEE (800 mg/kg): Hydro-ethanolic extract at a dose of 800 mg/kg bw, HEE (1000 mg/kg): Hydro-ethanolic extract at a dose of 1000 mg/kg bw; CoPw (800 mg/kg): Cocoa powder at a dose of 800 mg/kg bw

From T30 to T60, blood sugar increases sharply after glucose administration, and from T60 to T180, a gradual decrease of blood sugar is observed. The best reduction profile was generated by HEE from cocoa (800 mg/kg bw) followed by glibenclamide, HEE (500 mg/kg), HEE (1000 mg/kg) and CoPw (800 mg/kg bw). In contrary with treated groups, the blood sugar decreases very slowly with hyperglycemic control group. Table 4 presents the reductions percentage of glucose-induced hyperglycemia.

Discussion

This work is the result of the evaluation of the hypoglycaemic and antihyperglycaemic activity of powder and hydro-ethanolic extract of cocoa (*Theobroma cacao*) on the glycemia of normoglycemic rats and rats subjected to the glucose tolerance test.

The result of total polyphenol contents reveals that hydroethanolic extract (HEE) from cocoa contains 1.8 times more than cocoa powder (CoPw) and 4.4 times more than aqueous extract (AqE). This would be due to the nature of the solvent (ethanol-water mixture) used in terms of polarity and chemical composition. Indeed, the ethanol-water mixture is not only more polar, polyoxygenated, but also contains an organic molecule. As a result, the final chemical composition of the mixture is closer to that of polyphenols than water which is a mineral solvent (Bushra *et al.*, 2009). Thus, many organic, polar and polyoxygen molecules are more soluble in ethanol-water mixture and relatively less soluble in water. These results also show that hydroethanolic extraction allowed a better concentration of active substances in HEE.

In their work carried out in 2018, Dembélé *et al.* have obtained polyphenol contents of 830 ± 6.89 and 820 ± 2.80 mg Eq GA/g of dry extract in *forastero* and *mercedes* varieties from Abengourou and 720 ± 10 and 710 ± 14.80 mg Eq GA/g of dry extract in *forastero* and *mercedes* varieties from Divo. Compared to these values, the polyphenol content of HEE obtained in the present study is higher (1391 ±194.1 mg Eq GA/g of dry extract). This difference in polyphenol contents is due to several factors including the places of harvest (soil quality, climate), methods of processing the beans (fermentation or not), roasting processes of beans, methods of extractions and cocoa variety (Gbogbri, 2020).

Regarding antioxidant activity, the results show that aqueous (AqE) and hydroethanolic (HEE) extracts from cocoa and cocoa powder (CoPw) have capacity to reduce DPPH free radical. However, HEE has a higher activity than CoPw which itself is more active than AqE. Indeed, the comparison of their activity on the base of their IC_{50} reveals that HEE is 5.96 times more active than AqE and 2.87 times more active than CoPw. This could be explained by the high content of phenolic compounds in HEE. These results are similar to those of Faisal *et al.* (2015) and Dembélé *et al.* (2018). This assertion is confirmed by previous studies which have shown that there is a correlation between the content of phenolic compounds in an extract and its antioxidant activity (Oulai *et al.*, 2019). In addition, according to Guilland (2011), the antioxidant effect can also be enhanced by the presence of various vitamins (C, E and group B), minerals and trace elements.

In sum, overall antioxidant activity must be considered taking into account the interactions of all substances that can act in this way.

Measuring the reducing power of the ferric ion is the second method used to evaluate antioxidant activity. The results reveal that compared with CoPw and AqE, HEE from cocoa has better reducing power. These results confirm those obtained with DPPH test and are consistent with those of Dembélé *et al.* (2018). Thus, HEE from cocoa has better antioxidant activity than the other two cocoa products. Regular consumption of cocoa would help fight against oxidative stress and related diseases.

Furthermore, the hypoglycemic and antihyperglycemic effects of HEE and CoPw were evaluated in normoglycemic and hyperglycemic rats. In normoglycemic control rats, the results reveal an almost linear evolution profile of blood sugar whose values are between 65.6 ± 0.24 and 62.00 ± 1.22 mg/dL. This evolution shows that in normal animals, blood sugar varies very little. It is an evolution of blood sugar compatible with the normal life of animals. This result is consistent with those obtained by N'Doua *et al.* (2015). Compared with normoglycemic control rats, the results show that for all the substances tested (HEE, CoPw, glibenclamide), the blood sugar of treated rats gradually decreased throughout the experiment. These three substances therefore have a hypoglycemic effect.

The detailed analysis reveals that the reference substance (glibenclamide) resulted in a rate of reduction in blood sugar of 47.63% compared with its initial value. It is more active than HEE from cocoa at doses of 500 and 1000 mg/kg bw, but less active than 800 mg/kg bw dose of HEE. This reduction in blood sugar in rats treated with the reference substance compared with normal control rats demonstrates that glibenclamide is a hypoglycemic molecule.

This confirms that the product tested is active. These results are similar to those of N'Doua *et al.* (2015) and Etame-Loe *et al.* (2018), who demonstrated the hypoglycemic activity of glibenclamide at a dose of 10 mg/kg body weight in rats.

The analysis also reveals that for all these activities, the lowest performance was obtained with CoPw, which generates at the 150th min, a reduction in sugar levels of 54.60 ± 0.24 mg/dL. This cocoa powder is therefore 19.66% less active than glibenclamide. In contrast, the highest activity was generated by 800 mg/kg bw dose of HEE from cocoa. This extract is more active than glibenclamide (5 mg/kg bw). Indeed, at the different blood sugar dosages (30, 60, 90, 120 and 150 min), HEE 800 mg/kg bw induced respective reduction percentages of 42.55%; 44.15%; 45.48%; 49.47% and 51.60%. The results also show that 30 min after the administration of the different substances, the rate of blood sugar reduction induced by HEE (42.55%) is significantly higher than that of glibenclamide (16.96%). This better performance of HEE is maintained until the end of the experiment. This is an excellent performance, given that the product is not purified. The good activity of HEE can be explained by the presence of polyphenols in this extract.

According to Kobayashi *et al.* (2000) and Johnston *et al.* (2005), the mechanisms by which polyphenols reduce plasma glucose have been widely established, particularly their effects on muscle and intestine. The mechanisms could involve activation of the synthesis of glucose isoform 1 transporter protein (GLUT1), activation of phosphatidylinositol 3-kinase (PI3K) in muscle cells and inhibition of facilitated absorption of glucose into gut and inhibition of sodium-glucose dependent transporter type 1 (SGLT1) in intestinal cell lines. This experiment reveals above all that for the same dose of substance, HEE from cocoa concentrates more hypoglycemic substances than cocoa powder. The dose 800 mg/kg bw is therefore the efficient dose of the HEE and 1000 mg/kg bw dose would saturate. The hypoglycemic activity of HEE and CoPw at different doses is due to their richness in compounds such as terpenoids, polyphenols (flavonoids, tannins), saponosides, and alkaloids.

Indeed, substances such as polyphenols are generally known to have hypoglycemic effects (N'Doua *et al.*, 2015). These results are consistent with those of Ruzaidi *et al.* (2005) and Olasope *et al.* (2016 and 2017) who demonstrated the hypoglycemic activity of aqueous and hydroethanolic extracts of cocoa beans. The results also reveal that the hypoglycemic activity of HEE is not dose-dependent.

Moreover, the high polyphenol content of HEE compared with CoPw would be responsible for its greater hypoglycemic activity. Thus, the present study showed that HEE from cocoa and CoPw contain hypoglycemic substances because they act like glibenclamide (5 mg/kg bw) which is a hypoglycemic sulphonamide. According to Gebreyohannis *et al.* (2014), sulfonylureas induce hypoglycemia in normoglycemic rats by stimulating insulin production by pancreatic beta cells, thus promoting glycogen storage in the liver.

Regarding the evaluation of the antihyperglycemic activity of different substances in post-treated rats, all groups were treated with an anhydrous glucose solution at 4 g/kg bw, with the exception of the normoglycemic control group. Results of this test reveals that compared with normoglycemic control rats, the blood sugar of all treated rats increased significantly and reached peaks 30 min after glucose administration. After 30 min, the blood sugar of all animals gradually decreased. In the hyperglycemic control group, this decreased is very moderate, but it is rapid in all the other groups. The result obtained in the hyperglycemic control rats shows that the organism of these rats is capable of regulating blood sugar on its own without exogenous intake of hypoglycemic substances. But, the decreased in blood sugar is slow.

The treatment of hyperglycemic rats with the different substances generated in each group a very strong and progressive reduction in blood sugar to low values equivalent to that of the normal control rats, after 2 hours 30 min. Compared with value of induced hyperglycemia, the blood sugar of rats given glibenclamide (5 mg/kg bw) reached 71.80 ± 4.98 mg/dL, either reduction rate of 43.82%. Those of the animals having received HEE from cocoa at doses of 500, 800 and 1000 mg/kg bw were respectively 72.40 ± 2.46 mg/dL; 66.20 ± 2.53 mg/dL, 74.40 ± 2.63 mg/dL, either reduction percentages of 38.85%; 58.52% and 44.73%. On the other hand, the blood sugar of rats treated with CoPw reached 81.00 ± 3.00 mg/dL at the end of the experiment, either 38.41% reduction. These results reveal that the rate of blood sugar reduction generated by HEE at a dose of 800 mg/kg bw is significantly higher than that of glibenclamide and CoPw. It therefore appears that HEE is more active in reducing hyperglycemia and the dose 800 mg/kg bw is the effective dose.

In pretreated rats, results reveal that compared with normal control rats, there are three phases in the evolution of curves. A first phase located between T0 and T30 where blood sugar decreases after administration of the substances tested, a second phase where blood sugar increases significantly after the induction of hyperglycemia, with peaks reached at T60, and a third phase located between T60 and T180, where blood sugar decreases significantly. Concerning the evolution of blood sugar in hyperglycemic control group, results reveal that between T60 and T90, the drop of blood sugar is very slight, but beyond T90, this drop is progressive with a slow evolution comparatively with all groups processed. The minimum level reached is 134.60 ± 2.24 mg/dL, either a reduction of 20.92% at T180. These data once again confirm that apart from any exogenous substances, the situation of postprandial hyperglycemia in a healthy individual can self-regulate. These results corroborate those of Katz *et al.* (1983) and Mosora *et al.* (1976). These authors showed that after a meal rich in carbohydrates, the net synthesis of hepatic glycogen in non-diabetic people represented 28°363.7 g where 1 to 2 g were incorporated into triglycerides in adipose tissue and approximately 50 g were stored in the form of glycogen in muscle.

This drop of blood sugar has also been observed with the other substances. Like glibenclamide, all doses of HEE from cocoa and cocoa powder produced significant reductions of induced hyperglycemia. Among the cocoa extracts, the 800 mg/kg bw dose of HEE was the most effective. The result shows that between T60 and T90, glibenclamide which generated a reduction rate of 16.79% is more effective than HEE from cocoa (800 mg/kg bw) whose reduction rate is 13.36 %. From T90 to T120, HEE (800 mg/kg bw) becomes more efficient and generates a reduction of blood sugar at 48.07% against 44.58% for glibenclamide. HEE from cocoa (800 mg/kg bw) retains this better performance until the end of the experiment where the reduction rate is 59.09% against 52, 75% for glibenclamide. This is a good performance, because HEE is not yet a purified product. CoPw was the least effective substance. This confirms that the hydroethanolic extraction allowed bettering concentrating the antihyperglycemic substances of cocoa. Moreover, this better hypoglycemic and antihyperglycemic activity of HEE compared to that of CoPw would be explained on the one hand, by the higher polyphenol content in HEE than in cocoa powder and on the other hand, by the better antioxidant activity of HEE.

The comparative analysis of evolution of blood sugar in post-treated and pre-treated rats reveals that the amplitude of the induced hyperglycemia is less in rats pretreated with the three doses of HEE from cocoa than in rats post-treated. This phenomenon was not observed with glibenclamide and cocoa powder. Thus, pretreatment with HEE from cocoa prevents a strong elevation of hyperglycemia amplitude. Like glibenclamide, HEE and CoPw also induce a significant decrease in glucose-induced hyperglycemia. Therefore, these extracts have hypoglycemic and antihyperglycemic effects. These results corroborate those of Ruzaidi *et al.* (2008) and Olasope *et al.* (2016 and 2017) who demonstrated the hypoglycemic activity of cocoa. The similar effects of HEE from cocoa and CoPw with those of glibenclamide on blood sugar suggest that these extracts are antihyperglycemic substances.

Thus, hypoglycemia and reduction of hyperglycemia observed in post-treated and pre-treated rats with HEE from cocoa and CoPw could be explained by an improvement blood sugar regulation in rats, either by stimulating of insulin secretion by the pancreas (N'Doua *et al.*, 2015), or by increasing of peripheral use of glucose in presence of extract (Yasodha *et al.*, 2008) or by storage of glucose in liver through glycogenesis. These substances could also act by inhibiting of glucose absorption in intestine.

Conclusion

This study showed that HEE from cocoa and CoPw have a good hypoglycemic and anti-hyperglycemic potential, which justifies the virtues of cocoa and its use by populations. Polyphenols, saponosides and terpenoids present in these products derived from forastero cocoa would be responsible for these activities. However, the best antioxidant, hypoglycemic and antihyperglycemic activities were generated by HEE at a dose of 800 mg/kg bw. In fact, this extract more concentrated polyphenols such as flavonoids and catechin tannins responsible for these pharmacological effects.

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References

Abbe MMJ, Amin I, Pei Pei C, Muhajir H, Syed HSK. 2009. Effects of cocoa extract containing polyphenols and methylxanthines on biochemical parameters of obese-diabetic rats. Journal of the Science of Food and Agriculture **89**, 130-137.

Ala'a A, Nicolas S, Georgina EC, Merrill FE, Saverio S. 2016. Daily chocolate consumption is inversely associated with insulin resistance and liver enzymes in the Observation of Cardiovascular Risk Factors in Luxembourg study. British Journal of Nutrition **115**(9), 1661-1668.

Bushra S, Farooq A, Muhammad A. 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules **14**(6), 2167-2180.

Chung YC, Chang CT, Chao WW, Lin CF, Chou ST. 2002. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. Journal of Agricultural and Food Chemistry **50**, 2454-2458.

Cordero-Herrera I, Martín MÁ, Goya L, Ramos S. 2014. Cocoa flavonoids attenuate high glucose-induced insulin signaling blockade and modulate glucose uptake and production in human HepG2 cells. Food Chemical Toxicology **64**, 10-19.

Dembélé A, Yapo KD, Kouassi K, Kambou PS, Djaman AJ, N'guessan JD. 2018. Effects of genotype, soil and technological treatment on chemical composition and antioxidant activity of cocoa beans produced in Cote d'Ivoire. Indian Journal of Research in Pharmacy and Biotechnology **6**(1), 16-24.

Djoussé L, Hopkins PN, North KE, Pankow JS, Arnett DK, Ellison RC. 2011. Chocolate consumption is inversely associated with prevalent coronary heart disease: The National Heart, Lung, and Blood Institute Family Heart Study. Clinical Nutrition, **30**(2), 182-187.

Etame LG, Yinyang J, Okalla EC, Makondo BV, Ngaba GP, Mpondo ME, Dibong SD. 2017. Étude de la toxicité aigüe et subaigüe de l'extrait au vin des graines de *Carica papaya* Linn. Journal of Applied Biosciences **120**, 12077-12085.

Faisal A, Yazan R, Amin I, Norhaizan ME. 2015. Identification of phenolic compounds in polyphenols-rich extract of Malaysian cocoa powder using the HPLC-UV-ESI-MS/MS and probing their antioxidant properties. Journal of Food Sciences and Technology **52**(4), 2103-2111.

Gbogbri G-F. 2020. Impact de la fermentation sur les propriétés antioxydantes, anti-inflammatoires et immunomodulatrices du cacao. Thèse de doctorat. Université de Montpellier, France, 134-138.

Gebreyohannis T, Shibeshi W, Asres K. 2014. Effects of Solvent Fractions of *Caylusea abyssinica* (Fresen.) Fisch. & Mey. on Blood Glucose Levels of Normoglycemic, Glucose Loaded and Streptozotocin-induced Diabetic Rodents. Journal of Natural Remedies **14**, 67-75.

Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, Blumberg JB, Ferri C. 2008. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. Journal of Nutrition **138**(9), 1671-1676.

Grassi D, Lippi C, Necozione S, Desideri G, Ferri C. 2005. Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. American Journal of Clinical Nutrition **81**(3), 611-614.

Guilland J-C. 2011. Les interactions entre les vitamines A, D, E et K: synergie et/ou compétition. Oilseeds and fats Crops and Lipids **18**(2), 59-67.

Gunjan B, Krishnendu A. 2013. Hypoglycemic activity of ethanolic extract *Astraeus hygrometricus* (PERS.) MORG In alloxan-induced diabetic mice. International Journal of Pharmacy and Pharmaceutical Sciences **5**(1), 0975-1491.

Jinap S, Wan Rosli WI, Russly AR, Nordin LM. 1998. Effect of roasting time and temperature on volatile component profiles during nib roasting of cocoa bean. Journal of the Science of Food and Agriculture **77**, 441-448.

Johnston K, Sharp P, Clifford M, Morgan L. 2005. Dietary polyphenols decrease glucose uptake by human intestinal Caco-2 cells. FEBS Lett **579,** 1653-1657.

Kalia K, Sharma K, Singh HP, Singh B. 2008. Effects of extraction methods on phenolic contents and antioxidant activity in aerial aarts of *Potentilla atrosanguinea* Lodd. and quantification of its phenolic constituents by RP-HPLC. Journal of Agricultural and Food Chemistry **56**, 10129-10134.

Katz LD, Glickman MG, Rapoport S, Ferrannini E, DeFronzo RA. 1983. Elimination splanchnique et périphérique du glucose oral chez l'homme. Diabète, 32675-9.

Kobayashi Y, Suzuki M, Satsu H, Arai S, Hara Y, Suzuki K. 2000. Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. Journal of Agricultural *and* Food Chemistry **48**, 5618-5623.

Molyneux P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology **26**(2), 211-219.

Mosora F, Lefèbvre P, Pirnay F, Lacroix M, Luyckx A, Duchesne J. 1976. Evaluation quantitative de l'oxydation d'une charge de glucose exogène à l'aide de13C-glucose. Métabolisme, 251575-82.

N'doua LAR, Abo KJC, Aoussi S, Gbogbo M, Yapo AP, Ehile EE. 2015. Effets hypoglycémique et antihyperglycémique de l'extrait éthanolique 70 % de racines de *Rauvolfia vomitoria afzel* (Apocynaceae). European Scientific Journal **11**(6), 177-190.

Olasope MT, Fadupin TG, Olubamiwa OO, Jayeola OC. 2016. Glucose-lowering Potential of Cocoa Powder Intake- An Avenue for Positive Management of Diabetes Mellitus. British Journal of Medicine & Medical Research **16**(2), 1-7.

Olasope MT, Fadupin TG, Olubamiwa O, Jayeola OC, Soetan OK. 2017. Haematological and anti-diabetic effects of cocoa powder on alloxan-induced diabetic albino rats. World Journal of Pharmaceutical and Medical Research **3**(3), 1-6.

Olooto EW, Onakomaya AO, Ezima NE, Akasoro SK, Aderinola AA. 2016. Aqueous Cocoa Powder Extract Moderates Physical, Biochemical, Hematological Parameters and Total Antioxidant Capacity in Alloxan Diabetic Wistar Albino Rat. Asian Journal of Biotechnology **8**, 1-7.

Olooto WE, Ogundahunsi OE, Banjo TA, Salau BA, Amballi AA, Ajani EO, Onakomaya OA. 2014. Hypoglycemic and modifying effect of aqueous cocoa powder extract on diabetic-induced histologic changes in the pancreas of alloxan diabetic rats. Annals of Biological Sciences **2**(1), 10-18.

Ou B, Hampsch-Woodill M, Prior RL. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry **49**(10), 4619-4626.

Oulai A, Ibrahima C, Koffi NE, Anin LA, Adima AA. 2019. Composition phytochimique, nutritionnelle et activité antioxydante des feuilles de baobab de Côte d'Ivoire. Journal of Applied Biosciences **141,** 14391-99.

Queen BL, Tollefsbol TO. 2010. Polyphenols and Aging. Current Aging Science **3**, 34-42.

Ruzaidi A, Abbe M, Amin I, Nawalyah AG, Muhajir H, Pauliena MBSMJ, Muskinah MS. 2008**.** Hypoglycaemic properties of Malaysian cocoa (*Theobroma cacao*) polyphenols-rich extract. International Food Research Journal **15**(3), 305-312.

Ruzaidi A, Amin I, Nawalyah AG, Hamid M, Faizul HA. 2005. The effect of Malaysian cocoa extract on glucose levels and lipid profiles in diabetic rats. Journal of Ethnopharmacology **98,** 55-60.

Ruzaidi MMA, Abbe JMM, Amin I, Nawalyal GA, Muhajir H. 2008. Protective effect of polyphenol-rich extract prepared from Malaysian cocoa (*Theobroma cacao*) on glucose levels and lipid profiles in streptozotocin-induced diabetic rats. Journal of the Science of Food and Agriculture **88**, 1442-1447.

Skandrani I, Boubaker J, Bhouri W, Limem I, Kilani S, Sghaier MB, Neffati A, Bouhlel I, Ghedira K, Chekir-Ghedira L. 2010. Leaf extracts from *Moricandia arvensis* promote antiproliferation of human cancer cells, induce apoptosis, and enhance antioxidant activity. Drug and Chemical Toxicology, **33**(1), 20-27.

Wood JE, Senthilmohana ST, Peskinb AV. 2002. Antioxidant activity of procyanidin containing plant extracts at different pHs. Food Chemistry **77**(2), 155-161.

Yasodha KJ, Jayaveera KN, Ravindra RK, Rupesh K, Raghavendra D. 2008. Anti-diabetic activity of aqueous extract of *Talinum juncifolium* in rats. Pharmacologyline **2**, 198-206.

Zihiri GN, Kra AM, Guédé-Guina F. 2003. Evaluation de l'activité antifongique de *Microglossa pyrifolia* (LARMACK) O. Kunze (Asteraceae) « PYMI » Sur la croissance *in vitro* de *Candida albicans*. Médecine Pharmacie Africaine **17**, 11-18.