World Journal of Medical Sciences 14 (1): 01-07, 2017
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2017.01.07

Valorization of a Hybrid Coffee of Cote D'ivoire: Arabusta: Effects on Lipidemia and Blood Pressure in Streptozotocin Diabetic Rats

[1]Cassime Tiémoko, [1]Alain Dit Philippe Bidie, [1]Adou Francis Yapo,
[2]Wawa Justine Tiekpa, [1]N'guessan Jean David and [1]Alico Joseph Djaman

[1]Laboratoire de pharmacodynamie-Biochimique,
UFRBiosciences Université Félix Houphouet Boigny, 22 BP582 Abidjan 22 Côted'Ivoire
[2]Université Péléforo Gon Coulibaly, Bp: 1328 Korhogo, Côte d'Ivoire

Abstract: According to the WHO, diabetes is a serious public health problem due to its progressive worldwide impact. In addition, there is a significant correlation between hyperglycemia, hypertension and dyslipidemia. Cross-sectional studies have shown a decrease in the prevalence of type 2 diabetes among coffee consumers. The objective of this study is to compare the effects of two coffee varieties (Canephora and Arabusta) on blood sugar, lipidemia and blood pressure in diabetic rats. Coffee extracts were supplied by the National Agronomic Center of Côte d'Ivoire (CNRA-CI). These products were subjected to aqueous extraction. After the chemical screening, the extract (400 and 800 mg/kg bw) was administered to normoglycemic albino Wistar rats rendered diabetic by subcutaneous administration of streptozotocin at a dose of 55 mg/kg bw. The results showed that both coffee varieties contain caffeine, chlorogenics acid with more chlorogenics acid compounds in the Arabusta variety. After 15 days of treatment with the two varieties of coffee, blood sugar levels in diabetics decreased significantly ($P < 0.001$) from 1.33 ± 0.02 to 1.15 ± 0.03 g/L for the dose 400 mg/kg bw and 1.33 ± 0.02 to 1.07 ± 0.005g/L for the dose 800 mg/kg bw for the Arabusta variety. Compared to the Robusta variety at the same doses, 1.33 ± 0.02 to 1.28 ± 0.01 and 1.33 ± 0.02 to 1.23 ± 0.06 g/L respectively were obtained. In addition, Arabusta better normalizes blood pressure, total lipids, urea, creatinine and antioxidant parameters increased in diabetic rats compared to Robusta.

Key words: Robusta · Arabusta · Diabetes · Glucose · Lipidemia

INTRODUCTION

Diabetes is caused by the relative inability of the pancreas to respond to increased metabolic demands and to compensate for insulin resistance. This phenomenon is accompanied by a quantitative and qualitative defect of insulin secretion correlated with hyperglycemia. Thus, the consequent functional impairment of the pancreatic cells leads to the development of diabetes [1]. As a result, there has been a rapid increase in the prevalence of this disease and its prevention has become a paramount concern for public health [2]. In Africa, its prevalence rate has increased to 5.1% in 2014 and could double by 2035 if nothing is done[3]. Diabetes and its antecedents are thus a real public health problem. [4,5] limited caloric intake to prevent or correct overweight, adequate fiber intake, preference for complex carbohydrates, Saturated fats and regular physical activity. Smoking cessation and moderate drinking have also shown a protective effect[6]. The use of drug therapies is only a second option, considered when lifestyle changes prove insufficient [5].

After the water, coffee is one of the most consumed drinks in the world. Its use is accompanied by different physiological responses affecting the cardiac, digestive, cerebral, renal, pulmonary and endocrine systems [7]. These various actions are, for the most part, attributed to caffeine. The harmful role of high consumption of coffee on the risk of cardiovascular accidents has long been the subject of considerable controversy, although it seems that the most recent studies do not find any significant association [8]. Besides caffeine, this drink contains many other substances, including chlorogenic acid and various

Corresponding Author: Bidie Alain Dit Philippe, Laboratoire de pharmacodynamie-Biochimique, UFR Biosciences Université Félix Houphouet Boigny, 22 BP582 Abidjan 22 Côted'Ivoir. E-mail: alphbidie@yahoo.fr.

antioxidants, which could be responsible for some favorable metabolic changes. Cross-sectional studies almost all demonstrate a protective effect of coffee on the risk of developing diabetes, either in the general population or in particular subgroups [9].

Côte d'Ivoire, 3rd African coffee producer [10], takes first place for the Robusta. Given the economic threats of the market that favors quality coffees such as Arabica, it is necessary to diversify coffee production in Côte d'Ivoire (low altitude). Thus, the efforts of researchers to improve the coffee produced in Ivory Cost were oriented towards obtaining an interspecific hybrid between the two cultivated species. This is how the Arabusta was made Côte d'Ivoire. It produces a very good quality coffee (good taste, caffeine content less than 2% of the DM), but its productivity per hectare remains low, which makes it impossible to popularize it before the development of its agronomic value. Numerous studies are currently being carried out in Côte d'Ivoire by the National Center for Agronomic Research (CNRA) to solve this problem.

The objective of this study is the valuation of hybrid coffee: Arabusta by comparing its antidiabetic effects with those of Robusta produced in Côte d'Ivoire.

MATERIALS AND METHODS

Plant Material and Conventional Molecule: It consists of packs of decaffeinated ground coffee of the species Robusta and Arabusta, a hybrid variety. These samples were provided by the National Center for Agronomic Research of Côte d'Ivoire (CNRA-CI).

Acarbose, a conventional molecule used in the treatment of diabetes has been used as a reference substance for our various antidiabetic tests.

Animal Model: The rats of the Rattusnorvegicus strain of Wistar strain weighing between 200 and 300 g were used for this study. They were provided by the laboratory breeding farms of animal physiology of the Félix Houphouët Boigny University of Côte d'Ivoire. Animals maintained in plastic cages with stainless steel covers containing litter of wood shavings renewed every two days throughout the experiment. Rats were allowed to acclimatize for two weeks with access to clean water and standard animal feeds at the experimental site of the National School (ENS) (Abidjan, Côte d'Ivoire). A cycle of light and dark (12 hours each) and a temperature of 25 ± 2°C were maintained in the room.

Methods

Analysis of Chlorogenic Acid and Caffeine by HPLC: The components of levels in the coffee brews (chlorogenic acid and caffeine) were determined according to the method described by Vitorino *et al*. [11]. Quantitative analysis was performed by high performance liquid chromatography (HPLC).

Preparation of the Infusion: The coffee extracts were prepared according to the method described by Lima *et al*. [12]. Thus, to 100 mL of distilled water at 90 ° C. contained in a beaker, 10 g of coffee powder are added. The doses used are functions of the daily consumption of coffee for an adult. This consumption is 350 mL for a person of 70 kg (10%) or 500 mg/kg of p c.

Induction of Diabetes: For the induction of diabetes, the experimental protocol used is that described by [13]. Indeed, 24 rats were used and the animals were divided into two batches. A batch of 3 rats constituting the control batch received distilled water and a batch of 21 rats constituting the test batch received streptozotocin. Permanent hyperglycemia was induced in animals by subcutaneous administration of a single dose of 55 mg/kg in solution in 0.1 M citrate buffer pH 4.5. Hyperglycemia was detected after 72 hours and rats with blood glucose level greater than or equal to 1.33 g/L were considered diabetic after 8 days. These animals now referred to as the Diabetic Batch are included in our study.

Treatment of Diabetic Animals: The treatment of the animals was carried out according to the method described by Bidié *et al*. [13], slightly modified. Thus, the twenty-one (21) rats selected after the onset of diabetes and the three (3) healthy rats were divided into eight equal lots (3 rats / lot). The distribution of batches and treatments were carried out as follows (Table 1):

Table 1: Distribution of batches and treatments

Groups	Designation	doses mg / kg bw
01	healthy control "T"	No dose used
02	Untreated diabetic "UDIAB"	No dose used
03	Diabetic treated with Rob I	400
04	Diabetic treated with Rob II	800
05	diabetic treated with Ara I	400
06	diabetic treated with Ara II	800
07	diabetic treated with Aca I	50
08	diabetic treated with Aca II	100

Rob I: Robusta bw (Dose 1) Rob II: Robusta bw (Dose 2)
Ara I: Arabusta bw (Dose 1) Ara II: Arabusta bw (Dose 2)
Aca I: Acarbose (Dose 1) Aca II: Acarbose (Dose 2)

Preparation of Samples and Plasma: At the end of the treatment, the rats are sacrificed in the morning on an empty stomach under anesthesia with diethyl ether. Blood samples were taken for the different batches. The blood samples collected in the tubes without anticoagulant were centrifuged at 3000 rpm for 10 min. The sera collected, stored at -20 ° C, were used to measure blood glucose, liver and kidney parameters (ALT, AST, creatinine and urea), lipid parameters (TG CHOL, HDL and LDL).

Dosage of Insulin in Diabetic Animals: The variation in insulinaemia was determined every two days of induction of diabetes in rats and after treatment of these animals with antidiabetics. This assay was carried out by the immunometric, enzymatic, solid-phase chemiluminescent method as described by Raufman *et al*. [14].

Statistical Analysis of Results: The data obtained are analyzed statistically using the computer program STATISTICA 7.1 by analysis of variance (ANOVA). Each time a significant difference ($p < 0.05$) is revealed, the ANOVA test is completed by the Tukey post ANOVA test, in order to identify the variable (s) with very significant differences from the values Witnesses.

RESULTS

Analysis of Chlorogenic Acid and Caffeine by HPLC of the Two Coffee Varieties: The results of the HPLC analysis are presented in Table 2. Bioactive compounds, such as chlorogenic acid (CGA) and caffeine were identified by comparison of the sample retention times with standards. The amount of each compound present was quantified using an external standard calibration. The retention times for the chlorogenic acid and caffeine standards, were 9 and 10 min, respectively.

Table 2: Concentrations of chlorogenic acid (CGA) and caffeine in the coffee brews (Percentage by mass relative to the dry matter)

	Concentration (en g/100g) en MS	
Composants	*Robusta*	*Arabusta*
Caféine	2,7	1,96
Acides chlorogéniques	7	9

Effect of Streptozotocin on Blood Glucose and Blood Pressure in Rats: Figure 1 (A and B) shows the variation in blood sugar and systolic blood pressure (SBP) during induction of diabetes to rats. The normal value of these parameters observed in the control batch at day zero of the induction of diabetes were 1.06 ± 0.02 g / L and 123.66 ± 3.36 mmHg, respectively. Blood sugar values and SBP rose gradually during induction to 1.33 ± 0.02 g / L (glucose) and 152.79 ± 1.69 mmHg (SBP) in diabetic animals.

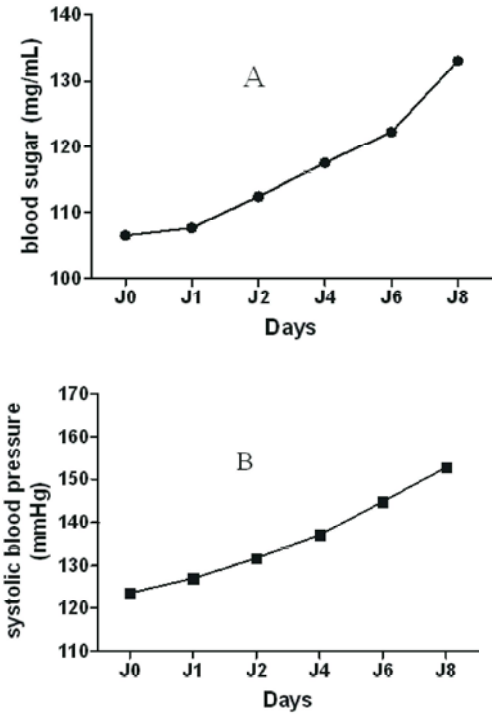

Fig. 1: Changes in blood glucose and blood pressure in diabetic rats
A: blood sugar
B: blood pressure

Effect of Arabusta, Robusta, Acarbose on Insulinemia of Diabetic Rats: Figure 2 shows the effect of Arabusta, Robusta, Acarbose (reference molecule) on diabetic rat insulinemia.

The insulin dosage showed that there was a significant difference between untreated diabetic rats (UDIAB) versus healthy control (T) rats on the one hand and between diabetic rats treated with doses of Arabusta, Robusta and Acarbose compared to untreated sick animals. The serum insulin level remains very low in the UDIAB (0.08 ± 0.01 µU/mL) compared to the control value (0.35 µU / mL). On the other hand there is no significant difference between diabetic rats treated with doses of Acarbose a commercially available antidiabetic versus diabetic rats (DIAB).

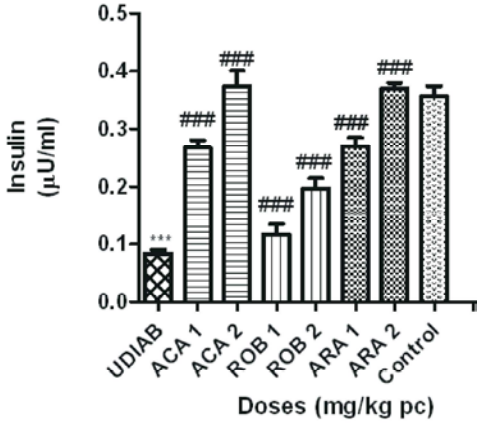

Fig 2: Insulin change in diabetic rats treated with Arabusta, Robusta and Acarbose

Each data represents the mean ± SEM, n = 3; (*** $p < 0.05$) significantly different from control group; ###, significantly different from the diabetic group, C: control group; DT: diabetic group; UDIAB: untread diabetic group; ROB 1: group treated by 400mg/kg bw of Robusta extract; lot ROB 2: group treated by 800mg/kg bw of Robusta extract; ARA1: group treated by 400mg/kg bw of Arabusta extract; ARA 2: group treated by 800mg/kg bw of Arabusta extract; ACA 1: group treated by 50 mg/kg bw of acarbose; ACA 2: group treated by 100 mg/kg bw of acarbose.

Effect of Coffee Extracts (Robusta and Arabusta) and Acarbose on the Biochemical Parameters of Diabetic Rats: Table 3 shows the effect of coffee extracts (Robusta and Arabusta) and Arcabose (reference substance) on the biochemical parameters in diabetic rats. The treatment of animals with diabetes by Arabusta better normalizes the values ??of biochemical parameters previously elevated during diabetes compared to Robusta. Indeed, blood sugar decreases from 1.33 ± 0.02 to 1.15 ± 0.03 and from 1.33 ± 0.02 to 1.07 ± 0.005 g/L for the doses of 400 and 800 mg / kg of Arabusta whereas for the same doses the blood sugar values ??decreased respectively from 1.33 ± 0.02 to 1.28 ± 0.01 g/L and 1.33 ± 0.02 at 1.23 ± 0, 06 g/L for the Robusta. On the other hand, blood sugar remains very high in the UDIAB batch at 1.53 ± 0.03 g/L compared to the control batch (T). In addition, kidney and liver parameters (urea, creatinine, ASAT and ALAT) decreased to the respective values of 1.26 ± 0.54 to 0.54 ± 0.03 g/L; from 0.46 ± 0.03 to 0.26 ± 0.02 g/L; from 169.1 ± 0.22 to 144.79 ± 4.03 U/I and from 153.6 ± 2.1 to 124.07 ± 1.58 U/I at the 800 mg / kg dose of Arabusta. For the same dose of Robusta, these values ??are respectively 1.26 ± 0.54 and 0.75 ± 0.05 g/L; From 0.46 ± 0.03 to 0.28 ± 0.95 g/L; 169.1 ± 0.22 to 155.79 ± 4.15 U/I and 153.6 ± 2.11 at 142.93 ± 0.17 U/I. Concerning the antioxidant parameter, malondialdehyde (MDA) decreases to 0.71 ± 0.05 to 0.54 ± 0.02 nmol/g for the dose of 800 mg/kg of Arabusta and 0.71 ± 0.05 to 0.62 ± 0.1 nmol/g for the same dose of Robusta. As for lipids, serum levels of CHOLT, TG and LDL increase to 1.44 ± 0.04 to 2.11 ± 0.03 mmol/L, respectively, from 0.56 ± 0.02 to 1.09 ± 0.01 mmol/L, 0.35 ± 0.05-1.90 ± 0.07 mmol/L and HDL decreased from 0.69 ± 0.07 to 0.43 ± 0.02 mmol/L. Treatment with Arabusta at a dose of 800 mg / kg to 15-day diabetic rats decreased serum CHOLT levels to 1.46 ± 0.04 mmol/L, TG at 0.47 ± 0.02 mmol/L; of LDL at 0.33 ± 0.01 mmol/L and increase HDL at 0.79 ± 0.05 mmol/L relative to the diabetic lot (Diab). Treatment with Robusta at the same dose lowers these CHOLT serum levels to 1.63 ± 0.04 mmol/L, TG to 0.74 ± 0.02 mmol/L, LDL at 0.37 ± 0.023 mmol/L and increase HDL to 0.57 ± 0.02 mmol/L, relative to the diabetic lot (Diab). Similarly, the different doses of Acarbose, a commercially available anti-diabetic, decreased and standardized the serum values ??of these same parameters in treated diabetic rats compared to untreated rats (UDIAB).

DISCUSSION

In this study, streptozotocin was used to cause hyperglycaemia. Indeed, the injection of this substance causes the mass destruction of the â cells of the Langerhans islets located in the pancreatic cells with formation of free radicals [15]. This destruction of â-cells decreases and stabilizes the production of insulin. This results in non-penetration of glucose into cells and consequently hyperglycemia. On the other hand, the treatment of diabetic animals by coffee extracts normalizes the glycemia and the secretion of insulin. This normalization is better achieved with Arabusta than with Robusta (1.07 ± 0.29 vs 1.23 ± 6.19 g/L for blood glucose and 0.37 ± 0.01 vs 0.19 ± 0.03 µU/mL for insulin). These findings are consistent with those obtained by Gaafar *et al*. [16] in a study of diabetic rats who concluded that supplemented to the diet of experimental diabetic groups significantly increased the insulin concentration in plasma compared to the diabetic group and the same with normal control group. In addition, during the course of diabetes, the balance between the production of free radicals and antioxidants can be broken, resulting in an oxidative

Table 3: Effect of Arabusta, Robusta and Acarbose on electrolytic parameters levels in diabetic rats

		Diabetes		Treatments					
Electrolytic parameters	Control	DIAB	UDIAB	ACA 1	ACA 2	ROB 1	ROB 2	ARA 1	ARA 2
Blood sugar (g/L)	1.06±0.02	1.33±0.02 ***	1.53±0.03***	1.25±0.03 ###	1.05±0.04 ###	1.28±0.01 ####	1.23±0.06 ###	1.15±0.03 ###	1.07±0.005 ###
Insulinémie (µU/ ml)	0.35±0.03	0.14±0.011***	0.08±0.02***	0.26±0.02 ###	0.37±0.04 ###	0.11±0.02 ###	0.19±0.03 ###	0.27±0.02	0.37±0.01 ###
SBP (mmHg)	123.66±3.36	152.79±1.69***	133.21±2.10***	124.81±1.89 ###	133.21±2.10 ###	124.81±1.89	147.41±2.15	140.75±1.33	136.93±2.18
Cholesterol (mmol/L)	1.44±0.04	2.11±0.03***	2.64±0.58***	1.74±0.03 ###	1.54±0.03 ###	1.84±0.03 ###	1.63±0.04 ###	1.64±0.02 ###	1.46±0.04 ###
Triglycerides (mmol/L)	0.56±0.02	1.09±0.01***	1.55±0. 01***	0.80±0.02 ###	0.48±0.01 ###	0.96±0.017 ###	0.74±0.02 ###	0.47±0.017 ###	0.47±0.026 ###
HDL (mmol/L)	0.69±0.07	0.43±0.02	0.33±0.03***	0.48±0.06 ###	0.68±0.05 ###	0.47±0.06 ###	0.57±0.02 ###	0.47±0.06 ###	0.79±0.05 ###
LDL (mmol/L)	0.35±0.05	1.39±0.07***	2.51±2.55***	1.17±0.02 ###	0.75±0.21 ###	1.4±0.05 ###	0.37±0.02 ###	1.25±0.025 ###	0.33±0.01 ###
MDA (nmol/g)	0.56±0.06	0.72±0.05***	0.89±0.09***	0.68±0.05 ###	0.59±0.03 ###	0.67±0.04 ###	0.62±0.1 ###	0.63±0.04 ###	0.54±0.02 ###
GSH (nmol/g)	0.61±0.02	0.87±0.020***	1.10±0.07	0.73±0.02 ###	0.58±0.07 ###	0.83±0.02 ###	0.79±0.02 ###	0.72±0.01 ###	GSH (nmol/g)
ASAT (U/L)	143.66±5.70	169.1±0.22***	194.48±5.80***	154.95±3.87 ###	142.61±2.99 ###	162.3±1.24 ###	155.79±4.15 ###	155.04±3.12 ###	144.79±4.03 ###
ALAT (U/L)	125.59±4.003	153.6±2.11***	188.16±1.14***	152.94±14.20 ###	125.46±2.09 ###	147.26±3.80 ###	142.93±0.17 ###	137.22±3.77 ###	124.70±1.58 ###
Serum UREA (g/L)	0.56±0.025	1.26±0.54***	1.50±0.14***	0.93±0.02 ###	0.68±0.01 ###	1.233±0.015 ###	0.7533±0.049 ###	1.040±0.026 ###	0.5467±0.04 ###
CREATININE (g/L)	0.24±0.01	0.46±0.03 ***	0.62±0.02***	0.34±0.03 ###	0.27±0.01 ###	0.38±0.01 ###	0.28±0.01 ###	0.31±0.01 ###	0.26±0.02 ###

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; ROB 1: group treated by 400mg/kg bw of Robusta extract ; ROB 2: group treated by 800 mg/kg bw of Robusta extract; ARA 1: group treated by 400mg/kg bw of Arabusta extract;ARA 2: group treated by 400mg/kg bw of Arabusta extract ACA 1: group treated by 50 mg/kg bw of acarbose; ACA2: group treated by 100 mg/kg bw of acarbose.* HDL (high density lipoprotein); LDL: Low density lipoprotein; ASAT: serum aspartate aminotransferase ALAT: alanine aminotransferase

stress that, through a series of events, disrupts the cellular functions leading to hepatic necrosis, for example. In our study, there was a significant increase in levels of plasma malondialdehyde (MDA) which is a marker of lipid oxidation and reduced-form glutathione (GSH). In this reduced form, glutathione is an antioxidant molecule that protects cells from free radicals in diabetic rats relative to the control group. Treatment with coffee extracts (Robusta and Arabusta) significantly reversed these changes with a more pronounced effect on the Arabusta extract. Therefore, it is possible that the mechanism of hepato-protection may be due to their antioxidant activity. Thus, chlorogenic acids (ACGs), the major constituents of coffee, appear to be endowed with various properties with favorable metabolic potential [17]. They possess, in particular, a high antioxidant power, that is to say they neutralize the free radicals which would damage the cells, which has the effect of reinforcing the immune defenses [18]. They also act by improving the sensitivity of the body's cells to insulin, which reduces blood glucose [19]. In addition, the unused fatty acids enter into a deleterious pathway of lipid synthesis, inducing, inter alia, the formation of ceramide and hyperlipidemia. Moreover, the accumulation of these lipids in the myocardium is the source of the development of resistance to insulin leading to a progression of diabetes [20]. Of the two coffee extracts, only Arabusta normalizes blood pressure better and restores lipid imbalance caused by diabetes compared to Robusta (124.34 ± 1.24 vs 140.75 mmHg). Indeed, coffee is a beverage that includes a wide range of components including chlorogenic acids in particular, which have antihypertensive effects [21]. This explains the decrease in the systolic pressure of 151.5 ± 1.43 to 124.55 ± 1.24 mmHg observed with Arabusta extract with a high chlorogenic acid content compared to that of Robusta. Moreover, Arabusta would normalize lipidemia by inhibition of lipid peroxidation and thus by inhibition of the oxidation of LDL-c. Our results are in agreement with those of Kempf *et al.* [22] who reported that coffee consumption appears to have beneficial effects on subclinical inflammation and HDL cholesterol. Our results showed an increase in serum urea and creatinine levels in hypertensive animals. Indeed, sustained arterial hypertension is a source of vascular remodeling characterized by an increase in the thickness of the media and a decrease in the diameter of the arteries of resistance [23,24]. This vasoconstriction, which affects the afferent vessels of the glomeruli, decreases the glomerular blood flow and consequently the fluid filtration in the renal tubules [25].This is characterized by a high serum level of urea and creatinine.

CONCLUSION

Diabetes, due to its frequency and complications, is a disease that is of increasing concern to populations and health professionals. The consumption of coffee is associated, in many prospective studies, with a decrease in the risk of developing a DT2. Thus, carbohydrate, lipid and blood pressure metabolic disturbances were better re-established by the Arabusta extract, a hybrid coffee compared to the Robusta extract. This action of Arabusta is therefore linked to its chemical composition characterized by the presence mainly of chlorogenic acids

which are very good antioxidants with a higher content compared to the Robusta. This other study could allow us to advise the consumption of Arabusta coffee to our populations as an interesting non pharmacological approach for not only prevention but especially the treatment of high blood pressure and diabetes.

REFERENCES

1. Scheen, A.J., Van and L.F. Gaal, 2005. Le diabète de type 2 au cœur du syndrome métabolique: plaidoyer pour une prise en charge globale. Rev Med Liège, 60: 566-571.
2. Spellman, C.W., 2010. Pathophysiology of type 2 diabetes: targeting islet cell dysfunction. The journal of the American Osteopathic Association, 110: S2-S7.
3. Anonyme, 1., 2014. FID: Atlas du diabète, 6e édition.
4. Schulze, M.B. and Hu, F.B. 2005. Primary prevention of diabetes: what can be done and how much can be prevented? Annu Rev Public Health, 26: 445-467.
5. Scheen, A.J, M.R. Letiexhe and Ph. Ernest, 2003. Prévention du diabète de type 2: style de vie ou médicaments? Rev Med Liège, 58: 206-210.
6. Magis, D., I. Geronooz and A.J. Scheen, 2002. Tabagisme, insulino-résistance et diabète de type 2. Rev Med Liège, 57: 575-581.
7. Higdon, J.V. and B. Frei, 2005. Coffee and health: a review of recent human research. Crit Rev Food Sci Nutr, 46: 101-123.
8. Ranheim, T. and B. Halvorsen, 2005. Coffee consumption and human health--beneficial or detrimental?--Mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and type 2 diabetes mellitus. Mol. Nutr. Food Res, 49: 274-284.
9. Van Dam, R.M. and F.B. Hu, 2005. Coffee consumption and risk of type 2 diabetes.A systematic review. JAMA, 294: 97-104.
10. Anonyme, 2. 2015. OIC: rapport sur le marché du Café-mars
11. Vitorino, M.D., A.S. França, L.S. Oliveira and M.L.A. Borges, 2001. Metodologias de obtenção de extrato de café visando à dosagem de compostos não voláteis. Rev Bras Armazenamento., 26: 17-24.
12. Lima, A.R., R.G.F.A. Pereira, S.A. Abrahão, S.M.S. Duarte and F.B.A. Paula, 2010. Compostos bioativos do café: atividade antioxidante in vitro do café verde e torrado antes e após a descafeinação. Quim Nova, 33: 20-24.
13. Bidié, A.D.P., W.J. Tiekpa, K.B. Bla, J.D. N'guessan and A.J. Djaman, 2016. Effect of Total Methanolic Extract of Chrysophyllum perpulchrum on Lipemia and Blood Pressure in Rats Made Diabetic by Streptozotocin.nternational Journal of Biochemistry Research & Review, 13(2): 1-9.
14. Raufman, J.P.1., L. Singh, G. Singh and J. Eng, 1992. Truncated glucagon-like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas. Identification of a mammalian analogue of the reptilian peptide exendin-4. J. Biol. Chem., 267(30): 21432-21437.
15. Akbarzadeh, A., D. Norouzian, R. Mehrabim, S.H. Jamshidi, A. Farhangi, V.A. Allah, S.M.A. Mofidiant and R.B. Lame, 2007. Induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistry, 22(2): 60-64.
16. Gaafar, M., H.E. Ahmed, El-Ghamery and F.S. Mahmuod, 2013. Effect of Green and Degree of Roasted Arabic Coffee on Hyperlipidemia and Antioxidant Status in Diabetic Rats. Adv. J. Food Sci. Technol., 5(5): 619-626.
17. McCarty, M.F., 2005. A chlorogenic acid-induced increase in GLP-1 production may mediate the impact of heavy coffee consumption on diabetes risk. Med. Hypotheses, 64: 848-853.
18. Kim, K., 2001. A pharmaceutical composition having chlorogenic acid as active component for preventing or treating the declining of male reproduction capability, n° WO0166101.
19. Pimentel, G.D., J.C. Zemdegs, J.A. Theodoro and J.F. Mota, 2009. Does long-term coffee intake reduce type 2 diabetes mellitus risk? Diabetol Metab Syndr; 1: 6.
20. Manios, Y., 2010. Development of a lifestyle-diet quality index for primary schoolchildren and its relation to insulin resistance: the Healthy Lifestyle-Diet Index. Eur J Clin Nutr., 64(12): 1399-406.
21. Mubarak, A., C.P. Bondonno, A.H. Liu, M.J. Considine, L. Rich and E. Mas, 2012. Acute effects of chlorogenic acid on nitric oxide status, endothelial function and blood pressure in healthy volunteers: a randomized trial. J. Agric Food Chem., 60: 9130-6.
22. Kempf, K., C.I. Herder, H. Erlund, S. Kolb, M. Martin, W. Carstensen, J. Koenig, S. Sundvall, Bidel S. Kuha and T. Jaakko, 2010. Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: A clinical trial. Am. J. Clin. Nutr., 91(4): 950-957.

23. Intengan, H.D., 1999. Mechanics and composition of human subcutaneous resistance arteries in essential hypertension. Hypertension, 33: 569-574.
24. Higashiyama, H., 2007. Histopathological study of time course changes in interregnal aortic banding-induced left ventricular hypertrophy of mice. Int. J. Exp. Pathol., 88(1): 31-8.
25. Baluchenjadmojard, T. and M. Rghani, 2003. Beneficial effect of aqueous garlic extract on the vascular reactivity of streptozotocin-diabetic rats. Journal of Ethnopharmacology, 85: 139-144.