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Rabé Marcellin Gnaléi
Laboratory of Pharmacodynamics-Biochemistry UFR Biosciences, Felix HOUPHOUET-BOIGNY University in Abidjan-Cocody, P. O. Box 582 Abidjan 22, Côte d'Ivoire

Calixte Bahi
Laboratory of Pharmacodynamics-Biochemistry UFR Biosciences, Felix HOUPHOUET-BOIGNY University in Abidjan-Cocody, P. O. Box 582 Abidjan 22, Côte d'Ivoire

Prisca Angèle Gui
Laboratory of Pharmacodynamics-Biochemistry UFR Biosciences, Felix HOUPHOUET-BOIGNY University in Abidjan-Cocody, P. O. Box 582 Abidjan 22, Côte d'Ivoire

Wawa Justine Tiekpa
Laboratory of Biotechnology and Valorization of Agroressourses UFR Biological Sciences, University Péléforo GON COULIBALY in Korhogo, P. O. Box 1238, Korhogo, Côte d'Ivoire

Adama Coulibaly
a) Laboratory of Pharmacodynamics-Biochemistry UFR Biosciences, Felix HOUPHOUET-BOIGNY University in Abidjan-Cocody, P. O. Box 582 Abidjan 22, Côte d'Ivoire
b) Laboratory of Biotechnology and Valorization of Agroressourses UFR Biological Sciences, University Péléforo GON COULIBALY in Korhogo, P. O. Box 1238, Korhogo, Côte d'Ivoire

Correspondence:
Rabé Marcellin Gnaléi
Laboratory of Pharmacodynamics-Biochemistry UFR Biosciences, Felix HOUPHOUET-BOIGNY University in Abidjan-Cocody, P. O. Box 582 Abidjan 22, Côte d'Ivoire
Email: gnarabe1976[at]gmail.com

Phytochemical composition and cardioprotective activities of the total aqueous extract of *Phyllanthus muellerianus* leaves in diabetic rats

Rabé Marcellin Gnaléi*, Calixte Bahi, Prisca Angèle Gui, Wawa Justine Tiekpa, Adama Coulibaly

ABSTRACT

The objective of this work is to study the phytochemical composition and cardioprotective activities of the total aqueous extract of *Phyllanthus muellerianus* in diabetic rats. The different chemical groups in the total aqueous extract were evaluated by methods described by Trease and Evans. The cardioprotective activities of total aqueous *Phyllanthus muellerianus* were evaluated in Wistar rats during streptozotocin induced experimental diabetes at a dose of 10 mg / kg bw for 21 days. Glucose, triglycerides, cholesterol, LDL-Cholesterol and HDL-Cholesterol were evaluated after treatment of rats with total aqueous extract *Phyllanthus muellerianus* and Glucidoral, the standard antidiabetic agent. The results obtained showed that the total aqueous extract of *Phyllanthus muellerianus* was very rich in polyphenols, rich in alkaloids and terpenes and moderately rich in flavonoids and quinones did not contain the tannins and saponosides. Blood glucose, triglycerides, total cholesterol and LDL-Cholesterol decreased significantly in diabetic rats treated with *Phyllanthus muellerianus* and Glucidoral®. The concentration of HDL-Cholesterol increased significantly and was then normalized by the total aqueous extract of *Phyllanthus muellerianus*. Treatment with the same extract normalized blood sugar, triglyceride levels and total cholesterol. The total aqueous extract of *Phyllanthus muellerianus*, by lowering triglyceride, total cholesterol and LDL-Cholesterol concentrations, and increasing concentration of HDL-Cholesterol in diabetic rats, could play a cardioprotective role and justify its use in traditional medicine in the treatment of heart diseases.

Keywords: *Phyllanthus muellerianus*, Cardioprotective, Glucidoral, Diabetic rat.

INTRODUCTION

Diabetes is a chronic condition that occurs when the pancreas does not produce enough insulin or when the body is unable to effectively use the insulin it produces. Diabetes is characterized by elevated glucose levels in the blood or hyperglycemia. According to the World Health Organization [1], there is diabetes when fasting blood glucose is greater than or equal to 1.26 g / L twice. Diabetes is a disease considered by WHO as an epidemic whose prevalence has increased dramatically in recent years. In Côte d'Ivoire, the prevalence rate in the general population, which was 5.7% in 2014, increased to 7.5% in 2016 [2]. Diabetes is a major cause of heart disease.

In modern societies, the pharmaceutical industry has managed to develop a whole arsenal of therapy to fight against this disease. In traditional, non-industrialized societies, the medicines available to populations affected by this condition are still not accessible because of their often-high cost. Faced with this desperate situation, more and more sick people are moving towards medicinal plants that are more accessible, efficient and within reach of their purse. Among these plants, *Phyllanthus muellerianus*, a species of African flora, is used in traditional medicine in Africa to treat intestinal disorders, severe dysentery, anemia and toothache.

In this work, we will study the cardioprotective activities of the total aqueous extract of *Phyllanthus muellerianus* in streptozotocin diabetic rats.

MATERIALS AND METHODS

Plant material

The *Phyllanthus muellerianus* leaves used were harvested at Yakassé Mé in the department of Adzopé (Côte d'Ivoire). Harvests were made in the month of September 2016. Authentication of this plant was

made at the National Center of Floristry of the University Felix HOUPHOUET-BOIGNY Abidjan-Cocody where it is registered under the number 1568 of October 18, 1985.

Animal material

White albino rats, male Rattus norgevicus, strain Wistar, genus Musa, were used for this study. These animals were fed with the pellets. They weigh between 162 and 182 g and are two to three months old.

Preparation of the total aqueous extract of *Phyllanthus muellerianus*

The aqueous total extract of *Phyllanthus muellerianus* was prepared according to the method described by Guédé-Guina *et al* [3]. According to this method, 100 g of *Phyllanthus muellerianus* powder were dissolved in two liters (2L) of distilled water. The aqueous mixture was stirred for 48 h at 80 °C using a magnetic stirrer type IKA-MAG RCT. The homogenate obtained was filtered successively twice on hydrophilic cotton, then on büchner with Whatman filter paper 3 mm. The filtrate obtained was evaporated under reduced pressure at a temperature of 50 °C using a Buchi rotary evaporator. The brown evaporates obtained was the total aqueous extract of *Phyllantus muellerianus* [3].

Phytochemical study

The phytochemical study consisted in characterizing the chemical groups present in the total aqueous extract of Phyllanthus muellerianus and likely to possess biological activities. Thus, chemical groups such as alkaloids, polyphenols, flavonoids, quinones, tannins, saponosides, polyterpenes and sterols have been sought by the methods described by Trease and Evans [4].

Search for alkaloids

The search for alkaloids was carried out using Dragendorff and Bouchardat reagents. These two reagents make it possible to highlight the alkaloids but differ in the coloration that the alkaloids take. Alkaloids complex with heavy metals such as bismuth, iodine, mercury and tungsten, and as salts. Thus, they form an orange precipitate with the Dragendorff reagent and a reddish-brown precipitate with that of Bouchardat [4].

Search for polyphenols

The polyphenols were evidenced by the reaction with ferric chloride. Phenols form with ferric chloride (FeCl3) a blue-blackish or green precipitate. The appreciation of this coloration is made with respect to the blue-blackish and green colors printed on ream paper [4].

Search for tannins

The Stiasny reagents (hydrochloric formalin solution) revealed the catechin and gallic tannins. The catechin tannins, in condensed form (non-hydrolyzable), are precipitated in large flakes by heating followed by cooling. The gallic tannins, which are in the form of hydrolyzable glycosides, are hydrolysed after the addition of sodium acetate and then form a blue-blackish precipitate in the presence of ferric chloride [4].

Search for flavonoids

Flavonoids were evidenced by the so-called cyanidin reaction. In alcoholic solution, the flavonic derivatives are colored differently according to their chemical structure. Thus, the flavones give an orange coloring, the flavanols are colored red and the flavonones are red-purplish [4].

Search for terpenes and sterols

The search for terpenes and sterols was carried out by the reaction of Libermann. Sterols and terpenes react with sulfuric acid in the presence of acetic anhydride to form a purple or purple colored complex, turning blue then green. This analysis is done compared to cholesterol as a control. The reagent of Libermann was used for this demonstration [4].

Quinone search

The demonstration of the quinonic substances was carried out using the Borntraeger reagent (ammonia diluted by half). Quinones form with alkaline substances such as ammonia and sodium hydroxide, a complex colored red to purple. The characterization reaction is preceded by acid hydrolysis in order to demonstrate the total quinone substances (free and combined quinone substances) [4].

Search for saponosides

The saponosides were highlighted by the foam production test. In aqueous solution, the saponosides have a very high foam index. They produce a large and persistent foam [4].

Induction and treatment of experimental diabetes

A total of 40 rats, mean weight 172.80 ± 0.80 g, were used for this study. The animals were divided into two groups. A group of 4 rats constituting the control group received distilled water and a group of 36 rats constituting the test group received streptozotocin. Permanent hyperglyceamia was induced in animals by intraperitoneal administration of a single dose of 10 mg / kg bw in solution in 0.1 M citrate buffer pH 4.5. Administration is daily and blood glucose is assessed from day D0 to day D21 using a strip glucose meter. Hyperglyceamia was detected after 6 days and rats with blood glucose level greater than or equal to 1.75 mg / L are considered diabetic after 21 days. These animals now called diabetic group are included in our study. At the end of these 21 days of induction, 24 diabetic rats were selected, divided into six groups with a group that received no treatment and five (5) that were treated with different doses of *Phyllanthus muellerianus* and glucidoral®. 1 ml of each dose was administered daily and regularly to the sick animals by gavage using a cannula. The treatment was done for 7 days. The distribution of groups and treatments were carried out as follows (Table 1).

Table 1: Doses of the aqueous extract of Phyllanthus muellerianus and glucidoral® administered during the treatment of diabetes

Groups	Designation	The doses administered mg / kg bw
1	Non-diabetic control	No dose used
2	Diabetic not treated	No dose used
3	Diabetic treated with aqueous extract	100
4	Diabetic treated with aqueous extract	200
5	Diabetic treated with aqueous extract	300
6	Diabetic treated with glucidoral®	10
7	Diabetic treated with glucidoral®	20

After seven (7) days of treatment, the blood was removed and centrifuged. Serum collected was used for assay, blood glucose, triglycerides, total cholesterol, LDL-Cholesterol and HDL-Cholesterol.

Determination of blood glucose level

Glyceamia was assayed according to Tietz's enzym method [5]. It consists in oxidizing glucose by the enzyme glucose oxidase with production of gluconic diacid and dihydrogen peroxide (H_2O_2).

Determination of lipids

Determination of total cholesterol

Blood cholesterol comes in two forms namely the free form and the ester form. The esterified form is hydrolysed by cholesterol esterase to give the free form and fatty acids. In the presence of cholesterol oxidase, free cholesterol oxidizes to form 4-cholestene-3-one and hydrogen peroxide. Hydrogen peroxide reacts with hydroxybenzoic acid and 4-aminoantipyrine to form red quinoneimine. The absorbance of this color measured at 500 nm is directly proportional to the amount of cholesterol contained in the serum.

Determination of triglycerides

The triglyceride assay was performed using the triglyceride enzymatic method (Glycerol Phosphate Oxidase), which uses the REF 7D74 Glyceride Reagent Kit [6,7]. Triglycerides, following several coupled reactions, give a colored complex whose intensity measured at the spectrophotometer at 500 nm, is proportional to the amount of triglycerides present in the serum.

Determination of LDL and HDL cholesterol

The determination of HDL-Cholesterol and LDL-Cholesterol was carried out using the phosphotungstic reagent associated with magnesium chloride after precipitation of LDL-Cholesterol [7].

Statistical analysis

The statistical analysis of the values and the graphical representation of the data were carried out with Graph Pad Prism 5 software (Microsoft). The average value is accompanied by the standard error on the mean (mean ± SEM). The statistical analysis of the results was performed using the one-way analysis of variances (ANOVA) followed by the Tukey multiple comparison test. P <0.001 is considered significant.

RESULTS

Phytochemical study

The results of the phytochemical study carried out on the aqueous total extract of the leaves of *Phyllanthus muellerianus* are presented in Table 2. The ETA of *Phyllanthus muellerianus* contains the polyphenols, alkaloids, terpenes, sterols, flavonoids and quinones in varying proportions. It does not contain saponosides and tannins (catechins and gallic).

Table 2: Phytochemical studies of the aqueous extract *Phyllanthus muellerianus*

Chemical compounds	Aqueous extract
Alkaloids	++
Polyphenols	++
Catechin tannins	-
Gallic tannins	-
Flavonoids	+
Saponosides	-
Quinones	+
Terpenes	++
Sterols	++

(+): Present (++): Abundant (-): Absent

Evolution of the glyceamia of rats after injection of streptozotocin

The variation in blood glucose levels in healthy and diabetic rats is shown in Figure 1. Mean blood glucose levels in healthy rats were 0.72 ± 0.018 g / L. During diabetes, this blood glucose level varies significantly from 0.72 ± 0.018 g / L to 0.912 ± 0.10 g / L on the 5th day, then to 2.13 ± 0.27 g / L on the 6th day, then to 3, 53 ± 0.01 g / L on the 14th day and finally 3.91 ± 0.01 g / L on the 21st day.

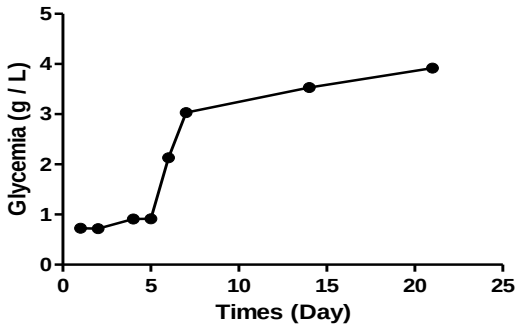

Figure 1: Evolution of blood glucose levels in rats in the test group after streptozotocin injection

Determination of glyceamia after treatment

Figure 2 shows the glyceamia of the rats after seven days of treatment with the aqueous extract of *Phyllanthus muellerianus* and Glucidoral®. The average normal blood glucose value of the rats is 0.72 ± 0.018 g / L. This value increased significantly (P< 0.0001) with the induction of diabetes. It increased from 0.72 ± 0.018 g / L (control value) to 4.06 ± 0.04 g / L (value of untreated diabetic rats). Treatment of diabetic rats with the total aqueous extract of *Phyllanthus muellerianus* at doses of 100, 200, and 300 mg / kg bw and Glucidoral® at doses of 10 and 20 mg / kg bw gave mean blood glucose values, respectively of 2.62 ± 0.06 g / L, 1.86 ± 0.13 g / L, 0.82 ± 0.08 g / L, 0.92 ± 0.05 g / L, respectively and 0.73 ± 0.03 g / L. This treatment significantly decreased (P< 0.0001) the glyceamia of treated rats compared to that of untreated diabetic rats. But the blood glucose levels obtained after treatment with the total aqueous extract of *Phyllanthus muellerianus* at a dose of 100 and 200 mg / kg bw remain extremely superior to that of non-diabetic rats. As for the blood glucose levels obtained with *Phyllanthus muellerianus* at a dose of 300 mg / kg bw and Glucidoral® at a dose of 10 mg / kg bw, they are slightly higher than that of non-diabetic rats but there is no significant difference (P > 0.05). Treatment with the total aqueous extract of *Phyllanthus muellerianus* at a dose of 300mg / kg bw and Glucidoral® at a dose of 20 mg / kg bw brought back the glyceamia of treated rats to normal.

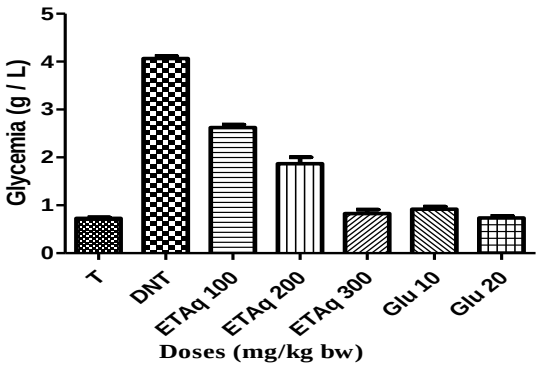

Figure 2: Effects of aqueous extract of *Phyllanthus muellerianus* and Glucidoral® on glyceamia in diabetic rats

Data are expressed as mean ± SEM, (n = 4). T = Group of non-diabetic control rats, DNT = Group of untreated diabetic rats; ETAq= total aqueous extract of *Phyllanthus muellerianus*, ETAq 100 = Group of diabetic rats treated with ETAq at a dose of 100 mg / kg bw, ETAq 200 = Group of diabetic rats treated with ETAq at a dose of 200 mg / kg bw, ETAq 300 = Group of diabetic rats treated with ETAq at a dose of 300 mg / kg bw, Glu 10 = Group of

diabetic rats treated with Glucidoral® at a dose of 10 mg / kg bw and Glu 20 = Group of diabetic rats treated with Gucidoral® at the dose 20 mg / kg bw.

Determination of the lipid profile of diabetic rats

Triglycerides cases in diabetic rats

Figure 3 shows the triglyceride concentrations of diabetic rats treated with the total aqueous extract of *Phyllanthus muellerianus* and Glucidoral®. The normal mean value of the concentration of triglycerides in rats is 1.26 ± 0.03 mg / dL. This value increased significantly (P< 0.0001) with the induction of diabetes. It went from 1.26 ± 0.03 mg / dL (control value) to 2.98 ± 0.01 mg / dL (value of untreated diabetic rats). Treatment of diabetic rats with total aqueous extract *Phyllanthus muellerianus* at doses of 100, 200, and 300 mg / kg. pc and Glucidoral® at doses of 10 and 20 mg / kg bw gave mean triglycerides concentrations, respectively of 2.15 ± 0.02 mg / dL, 1.96 ± 0.02 mg / dL, 1.34 ± 0.004 mg / dL, 1.93 ± 0.05 mg / dL and 1.68 ± 0.07 mg / dL. This treatment significantly decreased (P< 0.0001) the concentration of treated rats compared with untreated diabetic rats. However, the concentrations of triglycerides obtained after total aqueous extract of *Phyllanthus muellerianus* treatment at doses of 100, 200 mg / kg bw and Glucidoral® at doses of 10, 20 mg / kg bw remained significantly higher than in non-diabetic rats. Total aqueous extract of *Phyllanthus muellerianus* at a dose of 300 mg / kg bw did not cause a significant difference in triglycerides concentration compared with non-diabetic rats. Treatment with Total aqueous extract of *Phyllanthus muellerianus* at a dose of 300 mg / kg bw normalized triglycerides concentration of the diabetic rats compared to that of the non-diabetic control rats.

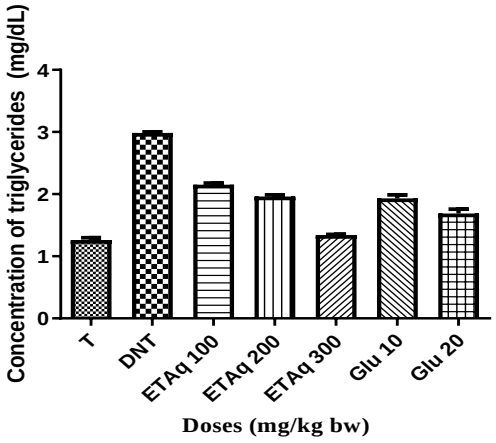

Figure 3: Effects of aqueous extract of *Phyllanthus muellerianus* and Glucidoral® on triglycerides concentration in diabetic rats

Data are expressed as mean ± SEM, (n = 4). T = Group of non-diabetic control rats, DNT = Group of untreated diabetic rats; ETAq= total aqueous extract of *Phyllanthus muellerianus*, ETAq 100 = Group of diabetic rats treated with ETAq at a dose of 100 mg / kg bw, ETAq 200 = Group of diabetic rats treated with ETAq at a dose of 200 mg / kg bw, ETAq 300 = Group of diabetic rats treated with ETAq at a dose of 300 mg / kg bw, Glu 10 = Group of diabetic rats treated with Glucidoral® at a dose of 10 mg / kg bw and Glu 20 = Group of diabetic rats treated with Glucidoral® at the dose 20 mg / kg bw.

Case of total cholesterol

Figure 4 shows total cholesterol concentrations of diabetic rats treated with the total aqueous extract of *Phyllanthus muellerianus* and Glucidoral®. The normal mean value of total cholesterol concentration of the rats is 92.18 ± 0.5 mg / dl. This value increased significantly (P< 0.0001) with the induction of diabetes. It went from 92.18 ± 0.5 mg / dl (control value) to 167.5 ± 3.48 mg / dl (value of untreated diabetic rats). Treatment of diabetic rats with total aqueous extract of *Phyllanthus muellerianus* at doses of 100, 200, and 300 mg / kg bw and Glucidoral® at doses of 10 and 20 mg / kg bw gave mean total cholesterol concentrations, respectively of 116. 30 ± 1.25 mg / dL, 105.7 ± 1.84 mg / dL, 94.43 ± 1.14 mg / dL, 119 ± 0.3 mg / dL and 113.90 ± 0.41 mg / dL. This treatment significantly decreased (P< 0.0001) the total cholesterol concentration of the treated rats compared to that of the untreated diabetic rats. However, total cholesterol concentrations obtained after treatment with total aqueous extract of *Phyllanthus muellerianus* at doses of 100, 200 mg / kg bw and Glucidoral® at doses of 10, 20 mg / kg bw remained significantly higher than non-diabetic rats. As for the concentration of total cholesterol obtained with total aqueous extract of *Phyllanthus muellerianus* 300mg / kg bw, it is slightly higher than that of non-diabetic rats, but there is no significant difference (P > 0.05). Treatment with total aqueous extract of *Phyllanthus muellerianus* at dose of 300 mg / kg bw standardized the concentration of total cholesterol in diabetic rats compared to non-diabetic control rats.

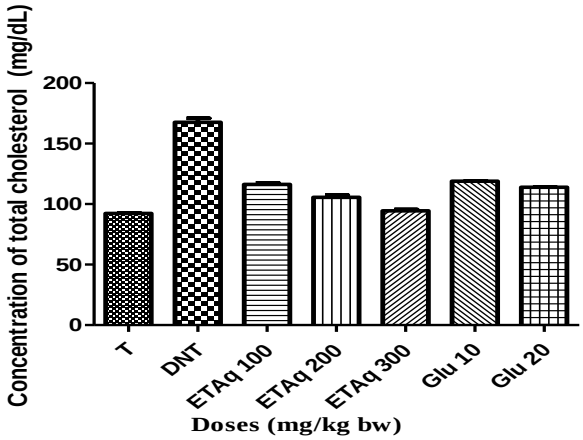

Figure 4: Effects of aqueous extract of *Phyllanthus muellerianus* and Glucidoral® on total cholesterol concentration of diabetic rats

Data are expressed as mean ± SEM, (n = 4). T = Group of non-diabetic control rats, DNT = Group of untreated diabetic rats; ETAq= total aqueous extract of *Phyllanthus muellerianus*, ETAq 100 = Group of diabetic rats treated with ETAq at a dose of 100 mg / kg bw, ETAq 200 = Group of diabetic rats treated with ETAq at a dose of 200 mg / kg bw, ETAq 300 = Group of diabetic rats treated with ETAq at a dose of 300 mg / kg bw, Glu 10 = Group of diabetic rats treated with Glucidoral® at a dose of 10 mg / kg bw and Glu 20 = Group of diabetic rats treated with Glucidoral® at the dose 20 mg / kg bw.

Case of LDL-Cholesterol

Figure 5 shows the LDL-Cholesterol concentrations of diabetic rats treated with the total aqueous extract of *Phyllanthus muellerianus* and Glucidoral®. The normal mean value of the LDL-Cholesterol concentration of the rats is 1.28 ± 0.03 g / L. This value increased significantly (P< 0.0001) with the induction of diabetes. It went from 1.28 ± 0.03 g / L (control value) to 3.49 ± 0.1 g / L (value of untreated diabetic rats). Treatment of diabetic rats with the total aqueous extract of *Phyllanthus muellerianus* at doses of 100, 200, and 300 mg / kg bw and Glucidoral® at doses of 10 and 20 mg / kg. pc gave mean LDL-Cholesterol concentrations, respectively of 1.91 ± 0.02 g / L, 1.69 ± 0.02 g / L, 1.5 ± 0.03 g / L, 2.03 ± 0,03 g / L and 1.89 ± 0.03 g / L. This treatment significantly lowered the LDL-Cholesterol concentration of the treated rats compared to untreated diabetic rats. But none of these doses reduced the LDL-Cholesterol concentration of treated rats to normal.

Case of HDL-Cholesterol

Figure 6 shows the HDL-Cholesterol concentrations of diabetic rats treated with the total aqueous extract of *Phyllanthus muellerianus* and Glucidoral®. The normal mean value of the HDL-Cholesterol concentration of the rats is 0.86 ± 0.006 g / L. This value decreased significantly (P< 0.0001) with the induction of diabetes. It increased from 0.86 ± 0.006 g / L (control value) to 0.11 ± 0.002 g / L (value of untreated diabetic rats). Treatment of diabetic rats with the total aqueous extract of *Phyllanthus muellerianus* at doses of 100, 200, and 300 mg / kg bw and Glucidoral® at 10 and 20 mg / kg bw gave mean HDL-Cholesterol concentrations, respectively of 0.27± 0.02 g / L, 0.55 ± 0.03 g / L, 0.8 ± 0.02 g / L, 0.25 ± 0.01 g / L and 0.36 ± 0.01 g / L L. This treatment dramatically increased the concentration of HDL-

Cholesterol in the treated rats compared to untreated diabetic rats. However, only the 300 mg / kg bw dose of total aqueous extract of *Phyllanthus muellerianus* reduced the concentration of HDL-Cholesterol to a slightly lower level than normal. But this variation is not significant. This variation is not significant.

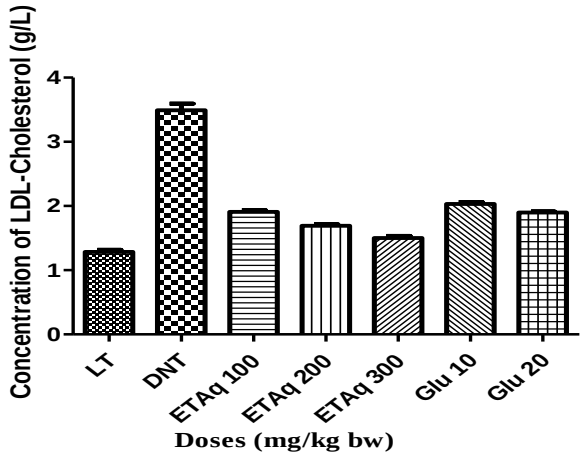

Figure 5: Effects of aqueous extract of *Phyllanthus muellerianus* and Glucidoral® on LDL-C concentration in diabetic rats

Data are expressed as mean ± SEM, (n = 4). T = Group of non-diabetic control rats, DNT = Group of untreated diabetic rats; ETAq= total aqueous extract of *Phyllanthus muellerianus*, ETAq 100 = Group of diabetic rats treated with ETAq at a dose of 100 mg / kg bw, ETAq 200 = Group of diabetic rats treated with ETAq at a dose of 200 mg / kg bw, ETAq 300 = Group of diabetic rats treated with ETAq at a dose of 300 mg / kg bw, Glu 10 = Group of diabetic rats treated with Glucidoral® at a dose of 10 mg / kg bw and Glu 20 = Group of diabetic rats treated with Glucidoral® at the dose 20 mg / kg bw.

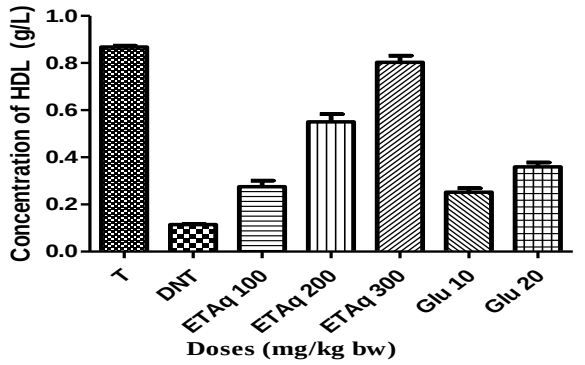

Figure 6: Effects of aqueous extract of *Phyllanthus muellerianus* and Glucidoral® on the HDL-Cholesterol concentration of diabetic rats

Data are expressed as mean ± SEM, (n = 4). T = Group of non-diabetic control rats, DNT = Group of untreated diabetic rats; ETAq= total aqueous extract of *Phyllanthus muellerianus*, ETAq 100 = Group of diabetic rats treated with ETAq at a dose of 100 mg / kg bw, ETAq 200 = Group of diabetic rats treated with ETAq at a dose of 200 mg / kg bw, ETAq 300 = Group of diabetic rats treated with ETAq at a dose of 300 mg / kg bw, Glu 10 = Group of diabetic rats treated with Glucidoral® at a dose of 10 mg / kg bw and Glu 20 = Group of diabetic rats treated with Glucidoral® at the dose 20 mg / kg bw.

DISCUSSION

The phytochemical study of *Phyllanthus muellerianu*s revealed an absence of saponosides and tannins (catechins and gallic) in the aqueous extract from leaves of this plant. However, this study revealed the presence of alkaloids, polyphenols, flavonoids, quinones, terpenes and sterols in the leaves of *Phyllanthus muellerianus*. These results are in agreement with those of Ben-Bala [8] who showed the presence of flavonoids, alkaloids and quinones in the leaf extract of *Phyllanthus muellerianus*.

The results of this work showed a significant increase in blood glucose levels in streptozotocin-diabetic rats. This increase in glyceamia during diabetes is due to streptozocin, which causes a selective cytotoxic effect of β-cells in islets of Langerhans [9,10,11]. In contrast, treatment with total aqueous extract of *Phyllanthus muellerianus* at doses of 100, 200 and 300 mg / kg bw and Glucidoral® at 10 and 20 mg / kg bw of diabetic rats resulted in a significant reduction in blood glucose. The aqueous extract *Phyllanthus muellerianus* at a dose of 300 mg / kg bw and Glucidoral® at a dose of 20 mg / kg bw normalize blood glucose levels in diabetic rats. This reduction of glyceamia of diabetic rats by the aqueous extract of *Phyllanthus muellerianus* would be related to the presence of flavonoids [12,13]. Indeed, flavonoids improve the sensitivity of cells, which reduces the index of type 2 diabetes [14,15]. Glucidoral®'s reduction in glucose levels in diabetic rats is due to its active substance, Carbutamide, which belongs to the sulphonamide hypoglycemic family.

The second part of this work relates to the effect of the aqueous extract of *Phyllanthus muellerianus* on the triglyceride, total cholesterol, LDL-Cholesterol and HDL-Cholesterol levels of streptozocin-diabetic rats in comparison with that of Glucidoral®. Diabetes induced in rats by 10 mg / kg bw Streptozocin is accompanied by strong disturbances in biochemical parameters. Indeed, the results showed that diabetes caused a significant increase in total cholesterol, LDL-Cholesterol, triglycerides and a significant decrease in HDL-Cholesterol. In contrast, treatment with the total aqueous extract *Phyllanthus muellerianus* at doses of 100, 200 and 300 mg / kg bw and Glucidoral® at 10 and 20 mg / kg bw of diabetic rats resulted in a significant reduction in total cholesterol levels, LDL-Cholesterol, triglycerides and a significant increase in HDL-Cholesterol. The total aqueous extract of *Phyllanthus muellerianus* at 300 mg / kg bw normalizes triglyceride, total cholesterol and HDL-Cholesterol levels.

High serum HDL-Cholesterol or "good cholesterol" may reduce the risk of developing atherosclerotic plaques [16]. LDL-Cholesterol or "bad cholesterol" deposits cholesterol on the walls of the arteries. It is then formed, little by little, real plates of fat, called atheromas. A strong presence of LDL-Cholesterol presages a risk of atherosclerosis [17]. This work showed a significant increase in levels of triglycerides, total cholesterol and LDL-Cholesterol and a significant decrease in HDL-Cholesterol levels in diabetic rats. In contrast, treatment of diabetic animals with the total aqueous extract of *Phyllanthus muellerianus* and Glucidoral® significantly reduced serum triglyceride, total cholesterol and LDL-Cholesterol levels. With this same treatment, the serum level of HDL-Cholesterol has increased significantly. Elevated plasma lipid levels, particularly total cholesterol and LDL-Cholesterol, as well as triglycerides observed in diabetic rats, may be the cause of atherosclerosis [18,19,20]. In addition, LDL-Cholesterol is a marker in the diagnosis of myocardial involvement. The alteration of its concentration in the blood accounts for myopathy [21]. HDL-Cholesterol, or good cholesterol, is a diagnostic value for coronary heart disease. Decreasing the serum HDL-Cholesterol concentration during diabetes increases the risk of developing coronary artery disease. A significant decrease in serum HDL-Cholesterol concentration in diabetic rats was observed in this study. These diabetic rats could be exposed to coronary heart disease. Diabetes increases the risk of atherosclerosis, the occurrence of myocardial and coronary heart disease. This hypertriglyceridemia has already been reported by Dhandapani *et al*. [22] who found hypertriglyceridemia in diabetic rats compared to control rats. Total aqueous extract of *Phyllanthus muellerianus* as well as Glucidoral®, whose administration to diabetic animals significantly reduces serum triglycerides, total cholesterol, LDL-Cholesterol, and increases HDL-Cholesterol levels, would intervene by protecting these animals against the risk of atherosclerosis and the occurrence of myocardial and coronary heart disease. Decrease in LDL-Cholesterol concentration and increase in HDL-Cholesterol concentration would be due to polyphenols [23]. In fact, polyphenols prevent cardiovascular disease and fight against hypercholesterolemia [23]. Also, polyphenols are substances that fight against atherosclerosis [24].

CONCLUSION

The objective of this work is to make a phytochemical study and determine cardioprotective activities of the aqueous extract of *Phyllanthus muellerianus* in rats made diabetic with Streptozotocin.

The phytochemical study of *Phyllanthus muellerianus* confirmed by thin layer chromatography revealed an absence of saponosides and tannins (catechins and gallic) in the aqueous extract of the leaves of this plant. However, this study revealed the presence of alkaloids, polyphenols, flavonoids, quinones, terpenes and sterols in the leaves of *Phyllanthus muellerianus*. These chemical elements would be at the origin of the therapeutic virtues of the leaves of *Phyllanthus muellerianus*.

Streptozotocin 10 mg / kg bw, administered by intraperitoneal injection, causes diabetes in rats. This diabetes causes serious disruptions of the biochemical parameters that are glucose, triglycerides, total cholesterol, LDL-Cholesterol and HDL-Cholesterol.

Treatment with total aqueous extract of *Phyllanthus muellerianus* at doses of 100, 200 and 300 mg / kg bw and Glucidoral® at 10 and 20 mg / kg bw diabetic rats significantly decreased the biochemical parameters namely glycemia, triglycerides, total cholesterol, LDL-Cholesterol and increased levels of HDL-Cholesterol. This extract works to protect the heart and may protect against coronary heart disease, atherosclerosis and heart attack.

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