

**ANTIOXIDANT ACTIVITIES OF THE TOTAL AQUEOUS EXTRACT OF  
*PHYLLANTHUS MUELLERIANUS* LEAVES IN DIABETIC RATS****Gnaléi Rabé Marcellin<sup>1\*</sup>, Bahi Calixte<sup>1</sup>, GUI Prisca Angèle<sup>1</sup>, Tiekpa Wawa Justine<sup>2</sup> and Coulibaly Adama<sup>1,2</sup>**<sup>1</sup>Laboratory of Pharmacodynamics-Biochemistry UFR Biosciences, Felix Houphouët-Boigny University in Abidjan-Cocody, P. O. Box 582 Abidjan 22, Côte d'Ivoire.<sup>2</sup>Laboratory of Biotechnology and Valorization of Agroressources UFR Biological Sciences, University Péléforo GON Coulibaly in Korhogo, P. O. Box 1238, Korhogo, Côte d'Ivoire.**\*Corresponding Author: Gnaléi Rabé Marcellin**

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**ABSTRACT**

Diabetes mellitus is accompanied by oxidative stress and free radical production by various mechanisms. This oxidative stress is involved in the aggravation of the disease as well as in the occurrence of chronic complications related to diabetes. The objective of the present work is to study the antioxidant activity of the total aqueous extract of *Phyllanthus muellerianus* in diabetic rats. Diabetes is induced by a single daily dose of 10 mg / kg bw streptozotocin (STZ) for twenty-one (21) days. The animals were then treated for seven (7) days with a dose of different concentrations of the total aqueous extract (ETAq) of *Phyllanthus muellerianus* leaves (100, 200 and 300 mg / kg bw) and Glucidoral® (Glu) (10 and 20 mg / kg bw), a standard antidiabetic agent. After treatment, these rats were sacrificed, the liver was removed for the determination of superoxide dismutase and catalase activities, and the concentration of malondialdehyde. The induction of diabetes decreased the activities of superoxide dismutase and catalase, and increased blood glucose and malondialdehyde concentration in the liver of diabetic rats. In contrast, treatment with the aqueous total extract (ETAq) of *Phyllanthus muellerianus* and Glucidoral® (Glu) increased the activities of superoxide dismutase and catalase, and decreased blood glucose and concentration of malondialdehyde in the liver of these animals. The results showed that the total aqueous extract (ETAq) of *Phyllanthus muellerianus* leaves can protect cells from the oxidative stress that accompanies diabetes.

**KEYWORDS:** *Phyllanthus muellerianus*, antioxidant activity, glucidoral, diabetic rats.**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<sup>[19]</sup>, 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.<sup>[2]</sup> Diabetes mellitus is accompanied by oxidative stress and free radical production. This causes the development of the disease by disrupting insulin secretion, promoting insulin resistance and chronic complications associated with it.

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 to treat intestinal disorders, severe dysentery, anemia and toothache.

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

**II- MATERIALS AND METHODS****II. 1- Plant material**

The *Phyllanthus muellerianus* leaves used were harvested at Yakassé Mé in the Adzopé department (Ivory Coast). Harvests were made in the month of September 2016. Authentication of this plant was made at the National Center of Floristry (CNF) of the University Felix HOUPHOUËT-BOIGNY Abidjan-Cocody where it is registered under the number 1568 of October 18, 1985.

## II. 2- Animal material

White albino rats, male *Rattus norvegicus*, 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.

## II. 3- Preparation of the total aqueous extract of *Phyllanthus muellerianus*

The aqueous total extract (ETAq) of *Phyllanthus muellerianus* was prepared according to the method described by Guédé-Guina et al.<sup>[9]</sup> 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 evaporate obtained was the total aqueous extract of *Phyllanthus muellerianus*.<sup>[9]</sup>

## II. 4-Induction and treatment of experimental diabetes

A total of 40 rats, mean weight  $172.80 \pm 0.80$  g, were used for this study. The animals were divided into two

lots. A group of 4 rats constituting the control group received distilled water and a group of 36 rats constituting the test group received streptozotocin (STZ). Permanent hyperglycaemia 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. Hyperglycaemia 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<sup>®</sup>. 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 the groups and the treatments were carried out as follows (Table 1).

**Table 1: Doses of the aqueous extract of *Phyllanthus muellerianus* and glucidoral<sup>®</sup> 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 <sup>®</sup>	10
7	Diabetic treated with glucidoral <sup>®</sup>	20

After seven (7) days of treatment, the blood was collected for the determination of blood glucose. The liver was removed, milled and homogenized in a Mac Even solution after the rats were sacrificed. After centrifugation of the ground material obtained, the supernatant was recovered for the determination of malondialdehyde and the determination of the enzymatic activities which are the activity of superoxide dismutase (SOD) and that of catalase.

## II. 5- Determination of glycaemia

Glycaemia was assayed according to Tietz's enzyme method.<sup>[16]</sup> It consists in oxidizing glucose by the enzyme glucose oxidase with production of gluconic diacid and dihydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).

## II. 5- Activity of the SOD

SOD activity was assayed by the Tetrazonium Blue Nitro Test. In fact, the nitro blue tetrazonium (NBT) is reduced by NADPH in the presence of the superoxide anion (O<sub>2</sub> • <sup>-</sup>) to give a dark purple chromophore.<sup>[17]</sup> While SOD eliminates the superoxide anion (O<sub>2</sub> • <sup>-</sup>), the intensity of

purple chromophore staining will be proportional to the activity of SOD in the medium.

## II. 6- Activity of catalase

The activity of catalase was assayed in livers homogenates by the spectrophotometric method used by Elia et al.<sup>[7]</sup>

## II. 7-Determination of malondialdehyde (MDA)

The dosage of MDA in the liver was performed according to the Ohkawa spectrophotometric method.<sup>[14]</sup> This method uses thiobarbituric acid (TBA).

## II. 8- 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.

### III-RESULTS

#### III. 1- Evolution of the glycaemia 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  $0.72 \pm 0.018$  g / L. During diabetes, this blood glucose level varies significantly from  $0.72 \pm 0.018$  g / L to  $0.912 \pm 0.10$  g / L on the 5th day, then to  $2.13 \pm 0.27$  g / L on the 6th day, then to  $3.53 \pm 0.01$  g / L on the 14th day and finally  $3.91 \pm 0.01$  g / L on the 21st day.

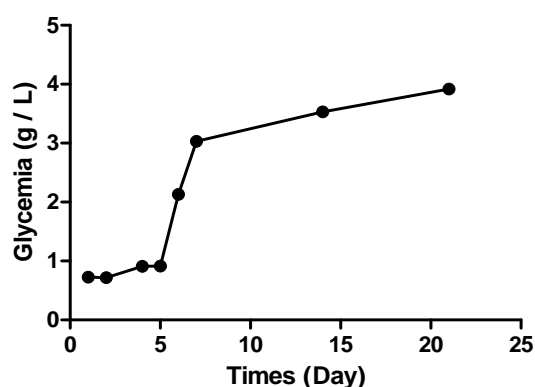


Figure 1: Evolution of blood glucose levels in rats in the test group after streptozotocin injection.

#### III. 3-Determination of glycaemia after treatment

Figure 2 shows the glycaemia of the rats after seven days of treatment with the aqueousextract of *Phyllanthus muellerianus* and Glucidoral® (Glu). The average normal blood glucose value of the rats is  $0.72 \pm 0.018$  g / L. This value increased significantly ( $P < 0.0001$ ) with the induction of diabetes. It increased from  $0.72 \pm 0.018$  g / L (control value) to  $4.06 \pm 0.04$  g / L (value of untreated diabetic rats). Treatment of diabetic rats with *Phyllanthus muellerianus* ETAq at doses of 100, 200, and 300 mg / kg bw and Glucidoral® (Glu) at doses of 10 and 20 mg / kg bw gave mean blood glucose values, respectively of  $2.62 \pm 0.06$  g / L,  $1.86 \pm 0.13$  g / L,  $0.82 \pm 0.08$  g / L,  $0.92 \pm 0.05$  g / L and  $0.73 \pm 0.03$  g / L. This treatment significantly decreased ( $P < 0.0001$ ) the glycaemia of treated rats compared to that of untreated diabetic rats. But the blood glucose levels obtained after treatment with ETAq 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 ETAq at a dose of 300 mg / kg bw and Glu 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 ETAq at a dose of 300mg / kg bw and Glu at a dose of 20 mg / kg bw brought back the glycaemia of treated rats to normal.

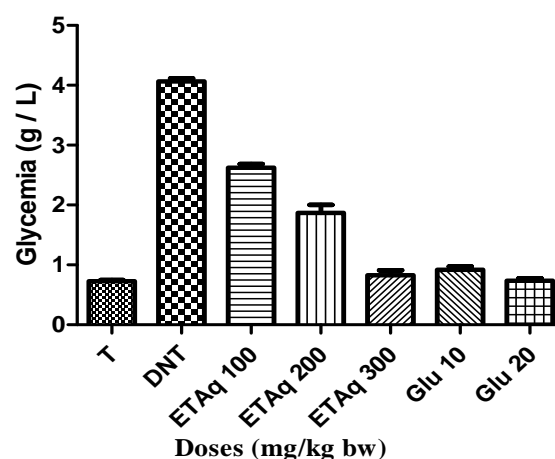


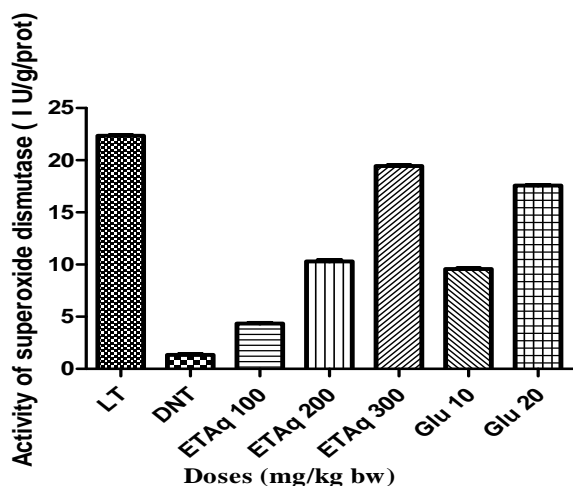
Figure 2: Effects of aqueous extract of *Phyllanthus muellerianus* and Glucidoral® on glycaemia in diabetic rats.

Data are expressed as mean  $\pm$  SEM, ( $n = 4$ ). T = Group of non-diabetic control rats, DNT = Group of untreated diabetic rats; 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 Glu at a dose of 10 mg / kg bw and Glu 20 = Group of diabetic rats treated with Glu at the dose 20 mg / kg bw

#### III. 3-Determination of oxidative stress markers in the liver of diabetic rats

##### III. 3.1. Activity of superoxide dismutase (SOD) in the liver

Figure 3 shows the activities of the superoxide dismutase in the liver of diabetic rats treated with the aqueous extract of *Phyllanthus muellerianus* and Glucidoral® (Glu). The normal mean value of superoxide dismutase activity in rat liver is  $22.35 \pm 0.04$  IU / g / prot. This value decreased significantly ( $P < 0.0001$ ) with the induction of diabetes. It increased from  $22.35 \pm 0.04$  IU / g / prot to  $1.33 \pm 0.06$  IU / g / prot (value of untreated diabetic rats). Treatment of diabetic rats with ETAq 100, 200, and 300 mg / kg bw and Glu 10 and 20 mg / kg bw gave superoxide dismutase activity, respectively of  $4.35 \pm 0.06$  IU / g / prot,  $10.30 \pm 0.12$  IU / g / prot,  $19.45 \pm 0.06$  IU / g / prot,  $9.56 \pm 0.07$  IU / g / prot and  $17.65 \pm 0.04$  IU / g / prot. This treatment significantly increased the activity of the superoxide dismutase in the liver of the treated rats compared to that of the untreated diabetic rats. But none of these doses reduced the activity of superoxide dismutase in the liver of normal-treated rats.

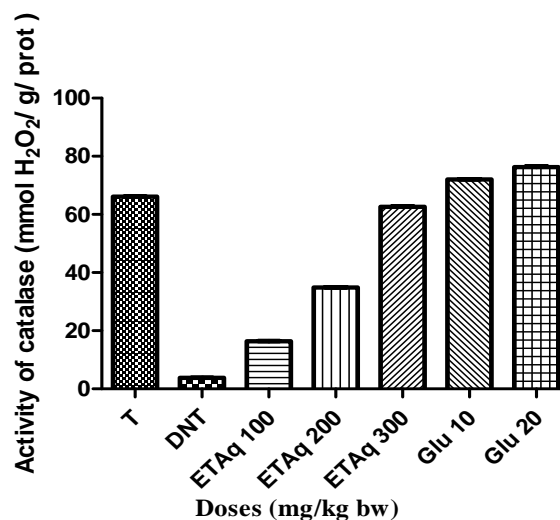


**Figure 3:** Effects of the aqueous extract of *Phyllanthus muellerianus* and Glucidoral® on the activity of superoxide dismutase in the liver of diabetic rats.

Data are expressed as mean  $\pm$  SEM, (n = 4). T = Group of non-diabetic control rats, DNT = Group of untreated diabetic rats; 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 Glu at a dose of 10 mg / kg bw and Glu 20 = Group of diabetic rats treated with Glu at the dose 20 mg / kg bw

### III. 3. 2- Activity of catalase in the liver

Figure 4 shows the catalase activities in the liver of diabetic rats treated with the aqueous extract of *Phyllanthus muellerianus* and Glucidoral® (Glu). The normal mean value of catalase activity in rat liver is  $66.10 \pm 0.06$  mmol  $H_2O_2$  / g / prot. This value decreased significantly ( $P < 0.0001$ ) with the induction of diabetes. It increased from  $66.10 \pm 0.06$  mmol  $H_2O_2$  / g / prot to  $3.76 \pm 0.06$  mmol  $H_2O_2$  / g / prot (value of untreated diabetic rats). Treatment of diabetic rats with ETAq 100, 200, and 300 mg / kg bw and Glu 10 and 20 mg / kg bw yielded mean catalase activities, respectively of  $16.36 \pm 0.06$  mmol  $H_2O_2$  / g / prot,  $34.90 \pm 0.04$  mmol  $H_2O_2$  / g / prot,  $62.66 \pm 0.08$  mmol  $H_2O_2$  / g / prot,  $72.05 \pm 0.04$  mmol  $H_2O_2$  / g / prot and  $76.32 \pm 0.1$  mmol  $H_2O_2$  / g / prot. This treatment significantly increased catalase activity in the liver of the treated rats compared to untreated diabetic rats. Only Glucidoral® at doses of 10 and 20 mg / kg bw have significantly higher catalase activity than normal ( $P < 0.0001$ ).

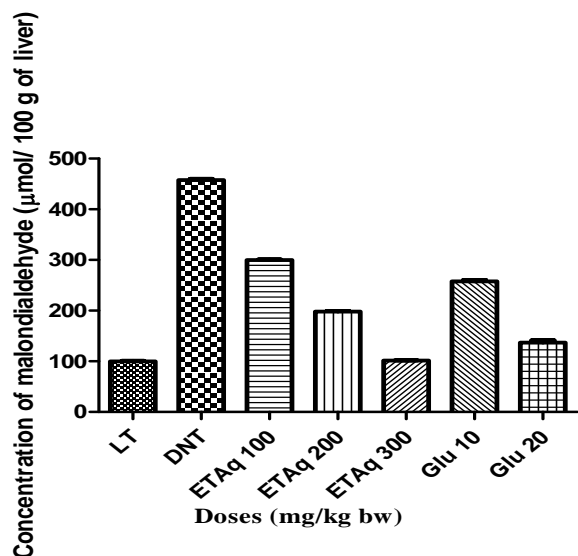


**Figure 4:** Effects of aqueous extract of *Phyllanthus muellerianus* and Glucidoral® on catalase activity in the liver of diabetic rats.

Data are expressed as mean  $\pm$  SEM, (n = 4). T = Group of non-diabetic control rats, DNT = Group of untreated diabetic rats; 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 Glu at a dose of 10 mg / kg bw and Glu 20 = Group of diabetic rats treated with Glu at the dose 20 mg / kg bw.

### III. 3.3- Determination of hepatic malondialdehyde (MDA)

Figure 5 shows the concentration of MDA on the liver of diabetic rats treated with the aqueous extract of *Phyllanthus muellerianus* and Glucidoral® (Glu). The normal mean value of the MDA concentration is  $99.73 \pm 1.23$   $\mu$ mol / 100 g liver. This value increased significantly ( $P < 0.0001$ ) with the induction of diabetes. It increased from  $99.73 \pm 1.23$   $\mu$ mol / 100 g of liver to  $457.7 \pm 2.48$   $\mu$ mol / 100 g of liver (value of untreated diabetic rats). Treatment of diabetic rats with ETAq 100, 200, and 300 mg / kg bw and Glu 10 and 20 mg / kg bw gave mean MDA concentrations, respectively of  $300 \pm 1.18$   $\mu$ mol / 100 g liver,  $198.10 \pm 1.26$   $\mu$ mol / 100 g liver,  $101.3 \pm 0.45$   $\mu$ mol / 100 g liver and  $137 \pm 4.40$   $\mu$ mol / 100 g of liver. This treatment significantly decreased the concentration of MDA in the liver of the treated rats compared to that of the untreated diabetic rats. Only *Phyllanthus muellerianus* ETAq at 300 mg / kg bw reduced the hepatic MDA concentration of treated rats to normal.



**Figure 5: Effects of aqueous extract of *Phyllanthus muellerianus* and Glucidoral® on malondialdehyde in the liver of diabetic rats.**

Data are expressed as mean  $\pm$  SEM, (n = 4). T = Group of non-diabetic control rats, DNT = Group of untreated diabetic rats; 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 Glu at a dose of 10 mg / kg bw and Glu 20 = Group of diabetic rats treated with Glu at the dose 20 mg / kg bw.

#### IV-DISCUSSION

The results of this work showed a significant increase in blood glucose levels in streptozotocin-diabetic rats. This increase in glycaemia during diabetes is due to streptozocin, which causes a selective cytotoxic effect of  $\beta$ -cells in islets of Langerhans.<sup>[1,5,20]</sup> In contrast, treatment with 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.pc and Glucidoral® at a dose of 20 mg / kg bw normalize blood glucose levels in diabetic rats. This decrease of glycaemia of diabetic rats by the aqueous extract of *Phyllanthus muellerianus* would be due to flavonoids<sup>[10,12]</sup> present in this plant.<sup>[3]</sup> Indeed, flavonoids improve the sensitivity of cells, which reduces the index of type 2 diabetes.<sup>[8,10]</sup> Glucidoral®'s reduction in glucose levels in diabetic rats is due to its active substance, Carbutamide, which belongs to the sulphonamide hypoglycaemic family.

The induction of diabetes in this study caused a decrease in the enzymatic activities of superoxide dismutase (SOD) and catalase in the liver of diabetic rats. In contrast, the treatment of diabetic rats with the total

aqueous extract of *Phyllanthus muellerianus* at doses of 100, 200 and 300 mg / kg bw and with Glucidoral® at doses of 10 and 20 mg / kg bw significantly increased the activity of these enzymes. The decrease in enzymatic activities of superoxide dismutase (SOD) and catalase is thought to be due to oxidative stress and the production of free radicals that accompany diabetes. The aqueous total extract (ETAq) of *Phyllanthus muellerianus* as well as Glucidoral®, whose administration to diabetic animals significantly increases the enzymatic activities of superoxide dismutase (SOD) and catalase, would intervene by increasing the activities of these enzymes. This would fight stress and protect liver cells. The increase in enzymatic activities of superoxide dismutase (SOD) and catalase is due to flavonoids, present in the total aqueous extract of *Phyllanthus muellerianus* leaves<sup>[3]</sup>, which have both anti-inflammatory and antioxidant effects.<sup>[18]</sup> Also, this antioxidant activity is due to the ability of *Phyllanthus muellerianus* to increase serum levels of antioxidant enzymes including superoxide dismutase, glutathione peroxidase and catalase as well as vitamins E and C.<sup>[6]</sup>

With regard to malondialdehyde (MDA), the results showed a significant increase in its concentration in livers of diabetic rats compared to those of non-diabetic control rats. The results of work by other authors such as El Ghouli et al.<sup>[6]</sup> and Chaudhry et al.<sup>[4]</sup> are in agreement with ours because they showed significant lipid peroxidation in the liver (p < 0.05) of diabetic rats compared to control rats. This lipid peroxidation in the liver may be the result of the action of streptozotocin which is a generator of free radicals<sup>[11]</sup> and the resulting hyperglycemia of experimental diabetes. In addition, the treatment of diabetic rats with the total aqueous extract of *Phyllanthus muellerianus* at doses of 100, 200 and 300 mg / kg bw and with Glucidoral® at doses of 10 and 20 mg / kg bw resulted in a reduction in hepatic MDA compared to untreated diabetic rats (p < 0.0001). Treatment with the aqueous total extract of *Phyllanthus muellerianus* at a dose of 300 mg / kg bw normalized malondialdehyde (MDA) liver levels in diabetic rats compared to control rats. This shows that the total aqueous extract of *Phyllanthus muellerianus* has limited lipid peroxidation to a relatively normal level. These data are consistent with the results obtained by El Ghouli et al.<sup>[6]</sup> who found that decoction of *Zygodium album* reduced lipid peroxidation in the pancreas of diabetic rats by 35% but significantly (p < 0.05). This antioxidant effect could be explained, on the one hand, by the presence of antioxidant flavonoids in the aerial parts of *Phyllanthus muellerianus*, on the other hand, by the ability of *Phyllanthus muellerianus* to increase the serum concentrations of antioxidant enzymes including superoxide dismutase, glutathione peroxidase and catalase as well as vitamins E and C.<sup>[6]</sup>

## V-CONCLUSION

The objective of this work was to study the antioxidant activities of the aqueous extract of *Phyllanthus muellerianus* in rats made diabetic with Streptozotocin.

Diabetes induction with streptozotocin at a dose of 10 mg / kg bw for 21 days caused disruption in diabetic rats. Enzyme activities of SOD and catalase decreased significantly. Glucose and MDA concentrations have increased. In contrast, after seven days of treatment with ETAq 100, 200, and 300 mg / kg.pc and Glu 10 and 20 mg / kg bw, SOD and catalase enzyme activities increased significantly. ETAq at the dose of 300mg / kg. pc normalized catalase activity. Treatment decreased and normalized glucose and MDA concentrations with ETAq at a dose of 300 mg / kg bw.

SOD and catalase are antioxidant enzymes. Increased activity of these enzymes and decreased concentration of MDA by *Phyllanthus muellerianus* ETAq in diabetic rats indicates that this plant has antioxidant activity. This would allow it to act by protecting the liver and other vital organs.

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