Wakouba an Antidiabetic Plant Extract with Protective Effect against Cardiovascular and Nephropathies Complications in Streptozotocin-Induced Diabetic Rats

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Abstract

Background: Diabetes is a disease characterized by hyperglycemia that is often the cause of complications in people with diabetes. The modern antidiabetic drugs generally used do not prevent these complications in these diabetic subjects. Wakouba regenerates β cells of Langerhans, restores insulin production and normalizes blood glucose. Wakouba, could also be a panacea to fight against diabetes and its complications.

Aims: The objective of this study is to evaluate the protective role of Wakouba in streptozotocin-diabetic rats (STZ) by studying its effect on catalase activity and nitric oxide (NO) levels in their body during induced diabetes.

Materials and Methods: The rats were made diabetic with Streptozotocin 55 mg / kg bw and treated with Wakouba at 1000 and 2500 mg / kg bw. NO rate was evaluated in the rat aorta by the Frouin method. The enzymatic activity of catalase was evaluated in the homogenates of the kidney and the heart by the spectrophotometric method of Elia.

Results: During diabetes, the level of NO decreases significantly from 0.51 ± 0.02mM to 0.35 ± 0.01mM in the diabetic rat lot. Treatment of diabetic rats with Wakouba significantly increased (p <0.05) this NO rate until normalization. This NO value varies from 0.35 ± 0.01 mM to 0.45 ± 0.02 mM and then to 0.50 ± 0.03 mM. Treatment with Glibenclamide (Daonil) does not normalize this level of NO which varies from 0.35 ± 0.01mM to 0.37 ± 0.102mM.

Regarding the activity of catalase, during diabetes, this activity increases significantly (p <0.05). It evolves from 22.23 ± 0.01mM to 32.13 ± 0.03mM in the kidney and from 13.29 ± 0.02mM to 27.12 ± 0.01mM in the heart. But treatment of diabetic rats with Wakouba at 1000 and 2500mg / kg bw brings these values of catalase back close to the normal. These

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values decreased significantly ($p <0.05$) from 32.13 ± 0.03 mM to 24.14 ± 0.02 mM in the kidney and from 27.12 ± 0.01 mM to 17.26 ± 0.03 mM in the heart. However, with glibenclamide treatment we noted significant decreases in catalase activity from 32.13 ± 0.03 mM to 27.34 ± 0.04 mM in the kidney and from 27.12 ± 0.01 mM to 25.12 mM in the heart. Wakouba treatment has a better effect than Glibenclamide treatment; because the statistical analysis gives significantly more difference, in terms of catalase activity with Wakouba, than the values obtained with Glibenclamide treatment.

**Conclusion:** In streptozotocin-induced diabetic rats, Wakouba would restore NO-producing cells and restore their integrity to re-synthesize NO, which will again play a full physiological role in maintaining the nervous system, cardiovascular, genitourinary, digestive and immune. Wakouba would also be able to fight against insulin resistance in diabetic rats by restoring the production of NO. Wakouba would therefore act as protective and curative substances that neutralize the harmful activities of certain exogenous and endogenous agents such as ROS by sequestration, thanks to its affinity for the latter, thus reducing their toxic effect in the body.

**Keywords:** Wakouba; Fight; Diabetes Complications

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**Abbreviations:** WHO: World Health Organization; CVA: Cardiovascular strokes; IPCI: Pasteur Institute of Côte d'Ivoire; LDC: Low Density Cholesterol; HDC: High Density Cholesterol.

**Introduction**

Natural products play an important role in the discovery of new drugs. Nowadays, nearly 50% of the therapeutic agents used, come from natural sources (animals, plants, fungi, algae, etc.) and less than 10% of the plant species have been studied for their biological activities [1,2].

According to the World Health Organization (WHO), unlike modern care, traditional medicine is accessible, available and popular, as about 80% of africans use it for their health needs. And traditional medicine is to be promoted to fight against diseases [3].

The acute complications of diabetes mellitus lead frequently to admission to emergency department and intensive care unit. The severity of these complications makes the knowledge of their pathophysiology essential to the proper conduct of their treatment. Clinical signs may be related to either metabolic abnormalities or degenerative complications [4]. In diabetic patients, the state of chronic hyperglycemia is responsible for specific complications affecting micro vessels, retinopathy and nephropathy, as well as the nerves, it is neuropathy.

It also participates in the development and complications of arterial lesions that are grouped under the term macroangiopathy. Diabetes mellitus can lead to complications that are currently serious because they are difficult to prevent and treat [4].

Indeed, diabetes can damage: the heart: 50% of diabetes die of a cardiovascular disease (mainly heart disease and stroke) [5,6]. And people with diabetes are two to four times more prone to cardiovascular risk [7]. Cardiovascular strokes (CVA) are twice as common in people with diabetes and hypertension than in hypertensives only [7]. Diabetic retinopathy is an important cause of blindness and occurs as a result of damage to the small blood vessels of the retina that builds up over time. After 15 years of diabetes, nearly 2% of subjects become blind and 10% have serious visual impairment. About 10 to 20% of people with diabetes die of kidney disease. And 50% are affected by diabetic neuropathy that follows nerve damage. Nephropathy only occurs in the 30 to 40% of diabetics, because of a predisposition, may be genetic; it is the most serious manifestation of microangiopathy, because it leads to kidney disease.

This work aims to evaluate some protective effects of Wakouba, an antidiabetics extract from a plant which restores blood and insulin level by restoring the integrity of the Beta cells of Langerhans, in the fight against diabetes complications in diabetic induced rat [5].
Material and Methods

Plant

The slings of the oil palm or Elaeis guineensis Jacq were used. These slings were harvested in Sassandra (south-west) of Côte d'Ivoire between March 2017. They were washed with distilled water, cut into small pieces and dried in the open at room temperature 26° to 30°C. They were subsequently ashed with a muffle furnace at a temperature of 400°C. These ashes were used in the preparation of Wakouba salt.

Preparation of the Extract

The Wakouba salt was prepared according to the method described by Guédé-Guina, et al. [8]. According to this method, 100 g of Elaeis guineensis Jacq ash was collected and dissolved in 1 L of distilled water. The aqueous mixture was homogenized for 2 hours at laboratory temperature (25-30°C) using an IKA MAG magnetic stirrer (USA). The homogenate obtained was filtered twice on hydrophilic cotton and once on 3 mm whatman paper. The collected filtrate was evaporated in an oven at 60°C. The evapora in gray crystals obtained was codified Wakouba which was used in this study as salt extracted from the oil palm.

Induction of Diabetes

The mice of the species Rattus norvegicus of strain Wistar, whose average weight varies between 200 and 220 g, were used for the antidiabetic study. These rats, provided by the Pasteur Institute of Côte d'Ivoire (IPCI), were acclimatized for 3 weeks in order to harmonize their physiological state. Eighteen mice were divided into two batches. A batch of 3 mice as control batch, received distilled water and the other test batch of 15 mice received streptozotocin. Permanent hyperglycemia was induced in animals by subcutaneous daily administration of a single dose of 55 mg / kg bw in solution of 0.1 M citrate buffer pH 4.5. Hyperglycaemia was detected after 72 hours. And after 7 days of induction, mice with a blood glucose level greater or equal to 1.75 mg / L are considered diabetic. These animals now constitute the Diabetic batch.

Evaluation of the Activity of Catalase

Catalase was assessed in the homogenates of various organs such as the heart and kidney by the spectrophotometric method used by Elia, et al. [9]. Indeed, catalases are responsible for the degradation of H2O2 in H2O and O2. The assessment method therefore consists in measuring the decrease of the absorbance linked to the disappearance of the hydrogen peroxide substrate of the enzyme. This decrease in hydrogen peroxide, proportional to the activity of catalase, is determined as follows. In a quartz measuring vessel, containing a substrate solution composed of 1 mL of phosphate buffer (0.1 M KH2PO4, pH 7.4), 0.950 mL of H2O2 (0.019 M), 0.025 mL of the source was added enzymatic (organ homogenate). The reaction was followed by recording the absorbance at 560 nm every minute for 2 min.

Evaluation of NO Activity

The NO assessment was carried out by the method of Frouin et al. which is based on the determination of the total nitrite in the sample to be tested [10]. To do this, 2 granules of cadmium were added in 6 test tubes each containing 100 μL of aortic homogenate. The mixture was incubated at 37°C in a water bath for 90 minutes. Then, 100 μL of the Griess reagent and 100 μL of distilled water were added to the tubes. After 10 min incubation at room temperature (30°C), followed by centrifugation at 10,000 rpm for 10 min, the supernatant was recovered and the optical density was determined spectrophotometer (Genesys Thermo Spectronic). At 570 nm.

Statistics Analysis

Analysis of variance (ANOVA) using data were represented as mean ± standard deviation, with n representing the number of different separate experiments. Statistical significance of the values was analyzed using an analysis of variance (ANOVA) followed by multiple comparison tests by Tukey-Kramer. P values less than 0.05 were considered significant.

Results

Induction of Diabetes by Streptozotocin in Rats

| Batches | Désignation                  | Administered doses mg/kg bw |
|---------|------------------------------|-----------------------------|
| 1       | Healthy rats « TS »          | None                        |
| 2       | Non treated Diabetic rats « DNT » | None                     |
| 3       | Diabetics rats treated by Wakouba I (WAK1) | 1000                    |
| 4       | Diabetics rats treated by Wakouba II (WAK2) | 2500                    |
| 5       | Diabetics rats treated by Daonil I (DAO1) | 10                      |
| 6       | Diabetics rats treated by Daonil II (DAO II) | 20                      |

Table 1: Different doses of Wakouba and Daonil to rats.
Figure 1: Variation of insulin and blood glucose levels during diabetes induction by streptozotocin (STZ).

Each bar represents the mean ± SEM, n = 4; a: Significantly different from the healthy lot at p <0.01; b

The production of Insulin decreased regularly during the induction of diabetes by streptozotocin (Figure 1) this decrease is accompanied by a significant increase of blood glucose level (Figure 1).

For healthy mice, insulin level was 3.48 ± 0.47 IU / mL prior to any treatment and the blood glucose level value of healthy mice was 0.86 ± 0.03 g / L. After induction of diabetes by injection of streptozotocin to healthy mice we noticed a significant decrease of insulin level from 3.48 ± 0.47 IU / mL to 2.28 ± 0.05 IU / mL within 7 days, and to 0.47 ± 0.07 UI/mL at day 14 for untreated diabetic rats. This shows respectively a significant decrease of 34.58% and 86.40%. At the same time blood glucose level value raised up to 3.64 ± 0.10 g / L, i.e a significant increase of 322.87% compared to healthy mice.

**Evaluation of the variation of NO Levels in the Aorta of Diabetic Rats Induced and Treated with different Doses of Wakouba and Glibenclamide (Daonil)**

Table 1 shows the evolution of NO levels in diabetic, untreated diabetic rats and diabetic rats treated with Wakouba and Glibenclamide (Daonil).

|          | DIAB | DIAB NT | WAK1 | WAK2 | DAO1 | DAO2 |
|----------|------|---------|------|------|------|------|
| T        | 0.51±0.02 | 0.35±0.01 | 0.22±0.01 | 0.45±0.02 | 0.5±0.03 | 0.31±0.02 | 0.37±0.02 |

Table 2: Evolution of NO value during the treatment of diabetic rats with Wakouba (a natural vegetable substance) and Daonil (a hypoglycemic substance of reference).

Figure 2: The variation of the level of NO in the samples of diabetic rats, during the treatment of these rats with Wakouba and Glibenclamide (DAONIL).
Each bar represent the mean ± SEM, n=3. Letters (a, b, c, d) represent statistical significance. All the groups are compared together. Means with different letters are significantly different (p < 0.05).

WAK1: group treated with 'Wakouba' at dose of 1000 mg/kg/wc; WAK2, group treated with 'Wakouba' at dose of 2500 mg/kg/wc; DAO10, batch treated with Daonil at dose of 10 mg/kg/wc; DAO20, batch treated with Daonil at dose of 20 mg/kg/wc.

Figure 2 shows the variation of the NO level in the samples of diabetic rats, during the treatment of these rats with Wakouba and Glibenclamide (DAONIL). In normal control (T) rats that were not treated with streptozotocin, the value of NO measured was 0.51 ± 0.02 mM. However, after the streptozotocin treatment, we observed in diabetic rats, a decrease in NO level from 0.51 ± 0.02 mM to 0.35 ± 0.01 mM in the control patient; a very significant decrease of 31.37%. This NO value decreases considerably by more than half and goes from 0.51 ± 0.02 mM to 0.22 ± 0.01 mM; either a more significant decrease of 56.86%; when diabetic rats are not treated. However, with Wakouba treatment, the NO level increased from 0.35 ± 0.01 mM to 0.45 ± 0.02 mM, i.e., a significant increase of 28.57% for an administered dose of Wakouba 1000 mg/kg/bw. This level of NO is normalized and goes from 0.35 ± 0.01 mM to 0.50 ± 0.03 mM, i.e., a growth of 42.86% for one-dose treatment of Wakouba administered at 2500 mg/kg/day pc. In fact, we do not statistically record a significant difference between the NO level of healthy control rats (0.51 ± 0.02 mM) and that of diabetic rats treated with Wakouba (0.5 ± 0.03 mM). Which means that Wakouba has normalized the NO rate and, it should be noted that, for the treatment of diabetic rats at different doses of Glibenclamide (Daonil), changes in the level of NO are not significant.

And these NO values in the aorta varies from 0.35 ± 0.01 mM to 0.31 ± 0.02 mM and from 0.35 ± 0.02 mM to 0.37 ± 0.02 mM, respectively, with Daonil. Statistically, we recorded a significant difference between the NO level of Glibenclamide-treated rats (0.37 ± 0.02 mM) and that of healthy control rats (0.5 ± 0.03 mM).

Evaluation of the Activity of Catalase in the Different Organs (Kidney and Heart) of Diabetic Rats Induced and Treated with Wakouba and Glibenclamide (Daonil)

| Catalase Activity | T     | DIAB  | DIAB NT | WAK1  | WAK2  | DAO1  | DAO2  |
|-------------------|-------|-------|---------|-------|-------|-------|-------|
| Kidney            | 22.23±0.01 | 32.13±0.03 | 53.09±0.02 | 28.53±0.01 | 24.14±0.02 | 30.45±0.02 | 27.34±0.02 |
| Heart             | 13.29±0.02 | 27.12±0.01 | 42.14±0.02 | 24.04±0.02 | 17.26±0.03 | 27.03±0.47 | 25.12±0.02 |

Table 3: Evolution of catalase activity in kidney and heart during treatment of diabetic rats with Wakouba, and Glibenclamide (Daonil).

Figure 3: Evolution of catalase activity in the kidney during treatment of diabetic rats with Wakouba, and Glibenclamide (Daonil).
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Each bar represent the mean ± SEM, n=3. Letters (a, b, c, d, e, f, g) represent statistical significance. All the groups are compared. Means with different letters are significantly different (p < 0.05).

Figure 4: Evolution of catalase activity in the heart during treatment of diabetic rats with Wakouba, and Glibenclamide (Daonil)

Each bar represent the mean ± SEM, n=3. Letters (a, b, c) represent statistical significance. All the groups are compared. Means with different letters are significantly different (p < 0.05).

WAK1: group treated with 'Wakouba' at dose of 1000 mg/kg/pc; WAK2, group treated with 'Wakouba' at dose of 2500 mg/kg/pc; DAO10, batch treated with Daonil at dose of 10 mg/kg/pc; DAO20, batch treated with Daonil at dose of 20 mg/kg/pc.

Table 3 and Figures 3 & 4 show the variation of catalase activity in the kidney and heart of these diabetic, untreated and diabetic diabetic rats treated with Wakouba and Glibenclamide. According to this table, in non-diabetic healthy rats, the measured catalase activities are respectively 22.23 ± 0.01 mM and 13.29 ± 0.02 mM in the kidney and in the heart.

But after induced diabetes in these rats with streptozotocin, the activity of catalase increases significantly from 22.23 ± 0.01mM in the kidney to a value of 32.13 ± 0.03mM; ie an increase of 44.53%. In the heart, this increase of catalase activity doubles at 104.06% from an initial value of 13.29 ± 0.02 mM in healthy subjects to a value of 27.12 ± 0, 01mM in diabetic rats. These values increased and reached 53.09 ± 0.02 mM for kidney and 42.14 ± 0.02 mM for heart in untreated diabetic rats; ie increases of 138.82% and 217.08% respectively.

Whatever the organ, we note that catalase activity increases significantly in induced diabetic rats compared to healthy control rats (T).

The treatment of diabetic rats with Wakouba at a dose of 1000 mg / kg / pc caused a significant decrease in the catalase activity from 32.13 ± 0.03 to 28.53 ± 0.01 mM in the kidney and from 27.12 ± 0.01Mm to 24.04 ± 0.02mM in the the heart; ie significant decreases respectively of 11.20% and 11.36%. For doses of Wakouba administered at 2500 mg / kg / bw, we note more significant decrease of catalase activity. This activity increased from 32.13 ± 0.03mM to 24.14 ± 0.02mM in the kidney; ie a significant decrease of 24.87%. This decrease of catalase activity is also significantly recorded in the heart and goes from 27.12 ± 0.01mM to 17.26 ± 0.03mM; ie a decrease of 36.36% of its activity. This same decrease of catalase activity is also significantly observed with Daonil treatment. However, we note a significant difference of catalase activity between treatment with Wakouba and treatment with Glibenclamide (Daonil). Decreases of catalase activity in diabetic rats treated with Glibenclamide (Daonil) decreased from 32.13 ± 0.03mM to 30.45 ± 0.02mM in the kidney for an administered dose of 10mg / kg / bw and from 27.12 ± 0.01mM to 27.03 ±
0.47 mM in the heart; ie respective decreases of 5.23% in the kidney and 0.44% in the heart. With higher doses of Glibenclamide (Daonil at 20 mg/kg/bw) we noted significant decreases in catalase activity from 32.13 ± 0.03 mM to 27.34 ± 0.02 mM in the kidney and from 27.12 ± 0.01 mM to 25.12 ± 0.02 mM in the heart; either respective decreases of catalase activity of 14.91% in the kidney and 7.37% in the heart.

Discussion

Variation of NO levels in the Aorta of Diabetic Rats Treated with Different Doses of Wakouba and Glibenclamide (Daonil)

In the normal control rats (T), the rate of NO measured is 0.51 ± 0.02 mM. On the other hand, the induction of diabetes in these rats led to a significant decrease of the NO value which passed from 0.51 ± 0.02 mM to 0.35 ± 0.01 mM in the rat Diabetic control; then from 0.35 ± 0.01 mM to 0.22 ± 0.01 mM in untreated diabetic rats, ie decreases of 31.37% and 56.86%, respectively (Table 2).

Nitric oxide (NO) is a gas whose synthesis has been described in mammals in 1985 [11]. Apart from macrophages, several types of cells have been recognized as capable of secreting NO from NO synthase II: endothelial cells, smooth muscle cells, fibroblasts, epithelial (renal and pulmonary) cells, cardiac myocyte, chondrocytes and hepatocytes [12]. And endogenous synthesis of NO has also been shown in the endothelial cell from different preparations (endothelium isolated, endothelial cells in culture) [13,14]. It can therefore be deduced that this variation of NO would be related to its quantity produced in the various organs of these diabetic rats. Therefore, this decrease in NO recorded in diabetic rats should be due to a decrease of the production of NO in the cell during diabetes. In cells, the NO synthesized plays a role in the physiology of many systems: nervous, cardiovascular, genitourinary, digestive and immune. This synthesized NO is also involved in vasodilation and maintenance of vascular integrity in case of aggression [15,16].

According to some authors, radicals released by neutrophils alter endothelial cells and contribute to increase vascular permeability in endotoxin, ischemia, ethanol or nonsteroidal anti-inflammatory lesions [17]. The overproduction of free radicals leads, during diabetes, to imbalances called oxidative stress, the consequence of which is often the appearance of irreversible damage to the cells.

This reduction of NO production in diabetic rats, therefore, would be the cause of cell dysfunction during diabetes; because most studies in humans show an important role of NO availability decrease in endothelial dysfunction related to hypertension [18]. Therefore Diabetes induced with streptozotocin would have created a dysfunction in the cells involved in the production of NO. As a result, these would no longer be able to synthesize NO normally, hence this decline of NO levels found in these diabetic rats. And this decrease of NO leads to an attack of endothelial cells that can no longer maintain their vascular integrity. This vascular endothelial dysfunction is pharmacologically characterized by the absence of an NO-dependent vasodilator response to acetylcholine [19,20]. The reduced NO would no longer be sufficient to play effectively, in these induced diabetic rats, its role in the physiology of the nervous, cardiovascular, genitourinary, digestive and immune systems [21]. However, it is important to emphasize that it has been clearly established that the production of NO in humans is considerable, and not negligible, in the immune defense mechanisms against certain types of protozoa, bacteria, fungi and viruses [22]. It has been shown that the induction of iNOS, nitric oxide synthase which produces NO is involved in the mechanism of inflammation, during an aggression of the body by an external agent, and the development of several pathologies including asthma, arthritis, multiple sclerosis, metabolic and neurodegenerative diseases, and cancer [23,24].

However, with the treatment of diabetic rats by Wakouba, the NO level increases significantly from 0.35 ± 0.01 mM to 0.45 ± 0.02 mM, ie a growth of 28.57% for a dose administered of Wakouba at 1000 mg/kg/pc. This level of NO is normalized and goes from 0.35 ± 0.01 mM to 0.50 ± 0.03 mM, ie a growth of 42.86% for treatment at a dose administered of Wakouba at 2500 mg/kg/bw. It could then be said that Wakouba administered to diabetic rats at doses of 1000 mg/kg/bw and 2500 mg/kg/bw would contain substances to restore damaged NO-producing cells in diabetic rats. Therefore Wakouba would allow these cells to regain their integrity to synthesize the NO that will play again their role in the physiology of the nervous, cardiovascular, genitourinary, digestive and immune systems. It could then be affirmed that Wakouba would participate, in streptozotocin-induced diabetic rats, of the protection and restoration of the nervous, cardiovascular, genitourinary, and digestive and immune systems. Wakouba would protect the nervous, cardiovascular, genitourinary, digestive and immune systems, and also protect the liver, kidney, vessels, genital tract and heart in...
these diabetic rats. Unlike Wakouba, administration of Glibenclamide (Daonil) to diabetic rats do not significantly increase NO production. Glibenclamide (Daonil) would therefore not allow NO-producing cells to restore and maintain their physiological integrity.

In addition, according to Professor Furchgott, the role of the NO-producing enzyme, iNOS, in the relationship between obesity and diabetes has also been demonstrated. The abnormal rate of nitric oxide produced by the action of this enzyme hinder the action of insulin, preventing glucose from entering the muscles; what scientists have called the insulin resistance syndrome that sets the table for type II diabetes [21]. So if Wakouba helps to restore the normal rate of NO, Wakouba would also be able to fight against insulin resistance in diabetics by restoring the production of NO in that case. According to Mahfouz, insulin resistance is a consequence of a decrease of the ability of peripheral target tissues to respond correctly to circulating normal insulin levels [25].

According to some authors, insulin resistance would intervene:
1- With a decrease of the number of insulin receptors that have been observed in adipocytes isolated from obese subjects [26]. According to this theory Wakouba could act directly or indirectly by a mechanism that would increase the number of insulin receptors in adipocytes of obese subjects.

2- Or with an increase of the expression of the PI3-kinase p85α regulatory subunit, which explains some cases of insulin resistance, including that related to obesity [27-29]. Indeed, the increase of p85α expression would change the stoichiometry between free p85 and the p85-p110 complex and prevent the p85-p110 complex from binding with IRS1 and thus propagating the insulin signal [30]. Then Wakouba, in fighting against insulin resistance, could also act against an increase of the expression of the regulatory subunit of PI3-kinase p85α. Wakouba would thus restore the alterations of the post-receptor insulin pathway to fight against the appearance of resistance to muscle insulin.

**Catalase Activity in the Kidney and in the Heart during Treatment of Diabetic Rats with Wakouba, and Glibenclamide (Daonil)**

Table 3 shows that in non-diabetic healthy rats, the catalase activity measured in the kidney is $22.23 \pm 0.01$ mM and $13.29 \pm 0.02$ mM in the heart. But after the induction of diabetes in these rats with streptozotocin, the activity of catalase increased significantly from 44.53% and goes from $22.23 \pm 0.01$mM to $32.13 \pm 0.03$mM in the kidney. And in the heart, this same increase doubled in catalase activity of 104.06% from $13.29 \pm 0.02$mM to $27.12 \pm 0.01$mM in diabetic rats. These values grew up respectively to 138.82% and 217.08%. Studies show that catalase is an enzyme that plays an important role in protecting cells against the toxic effects of hydrogen peroxide [31]. This enzyme is widely distributed in our body, with a high concentration in the liver, kidneys and erythrocytes and its expression is regulated at different levels; i.e. the transcriptional, post-translational and translational level [32]. The increase of catalase activity may be due to the high production of Reactive Oxygen Species (ROS) [33-35]. It can therefore be deduced that diabetes induced in rats would have increased quantity of ROS. Diabetes would have caused an increase of ROS in the body of diabetic rats [36]. Among the factors involved in the dysfunction of the vascular endothelium, oxidative stress plays a preponderant role. ROS are involved in the occurrence of endothelial complications. And so the body, naturally reacts to this aggression by activating more and more catalase to eliminate these ROS.

On the other hand, after the treatment given to diabetic rats at the Wakouba dose of 1000mg / kg / bw, we observed significant decreases of the activity of catalase respectively from $32.13 \pm 0.03$mM to $28.53 \pm 0.01$ mM in the kidney and $27.12 \pm 0.1$ mM at $24.04 \pm 0.02$ mM in the heart; i.e., respective decreases of 11.20% and 11.36%. For doses of Wakouba administered at 2500 mg / kg / bw, we observed more significant decrease of catalase activity from $32.13 \pm 0.03$ mM to $24.14 \pm 0.02$ mM in the kidney; i.e. recorded decrease of 24.87%. This decrease of activity is significantly recorded in the heart and goes from $27.12 \pm 0.01$mM to $17.26 \pm 0.03$mM; i.e., a decrease of 36.36% of its activity. Treatment with Wakouba has a much better effect on catalase activity than glibenclamide (Daonil). It can be deduced that administration of Wakouba would reduce the amount of ROS in the organs of diabetic rats, resulting in decreased activity of catalase in these organs. Wakouba would therefore act as protective substances such as albumin, which neutralizes the harmful activities of certain exogenous and endogenous agents by sequestration, thanks to its affinity for the latter, thus reducing their toxic effect in the body [37].

**Conclusion**

Our study reveals that Wakouba would restore NO production in diabetic rats during treatment. By this restorative activity of Wakouba on the production of NO, Wakouba thus plays a protective role in the nervous,
cardiovascular, genito-urinary, digestive and immune systems. Wakouba would therefore have a protective role on the liver, the kidney, the vessels, the genital tract and the heart of these diabetic rats. Wakouba would also be able to fight against insulin resistance of diabetics’ rats. Indeed restoring the production of NO, Wakouba:
- Would increases the number of insulin receptors in adipocytes of obese subjects.
- Would act against an increase of the expression of the regulatory subunit PI3-kinase p85α.

Wakouba would therefore restore the alterations of the post-receptor insulin pathway to fight against the appearance of resistance to muscle insulin. Wakouba would also fight insulin resistance by restoring NO production, since NO would facilitate the binding of insulin to its cellular receptor and it would contribute to insulin-induced vasodilation [38,39]. Moreover, according to Sydow et al., increased inhibition of NOS may contribute to the development of endothelial dysfunction and hypertension [40]. Besides, insulin-resistant individual’s exhibit impaired insulin vasodilation in skeletal muscle and decreased glucose uptake by insulin.

This study also revealed that the administration of Wakouba in diabetic rats induced would reduce the quantity of ROS in their bodies, which would explain the decline of catalase activity in the organs of these diabetic rats treated with Wakouba. Wakouba is therefore a protective substance that neutralizes the harmful activities of certain exogenous and endogenous agents by sequestration, thanks to its affinity for the latter, reducing then their toxic effect in the body. It is now accepted that high concentrations of glucose in extra and intracellular media induce oxidative stress, defined as an imbalance in the balance between pro-oxidants and antioxidants [41]. According to authors, the cellular over expression of antioxidant enzymes, such as catalase, is a response to oxidative stress following high glucose levels [42].

These studies on Wakouba should be deeper by testing its effects. We need to test the effects of wakouba on the blood level of total cholesterol, Low Density Cholesterol (LDC), High Density Cholesterol (HDC) and triglyceride on rats suffering from diabetic complications.

**Ethical Approval**

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

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