

Antioxidative activity of Wakouba, a salt extracted from *Elaeis guineensis* Jacq in streptozotocin-induced diabetic rats

ABSTRACT

Background: Oxidative stress plays a major role in the chronic complications of diabetes, high blood pressure, cancer etc. free radicals such as superoxyde anions, hydrogen peroxides cause severe cell damage. The use of plants is increasingly recommended to treat diseases related to oxidative stress.

Aims: This work aims to evaluate the antioxidant properties of Wakouba, a salt extracted from *Elaeis guineensis* Jacq on biochemical markers of oxidative stress.

Place and Duration of Study: Pharmacodynamie-biochemical UPR, Biology and Health Laboratory and Department of Radiology, Services Institute of Medical Sciences (SIMS), Services Hospital Lahore, between March 2017 and July 2018.

Materials and Methods: Diabetes was induced in rats *Rattus norvegicus* Wistar by streptozotocin 55 mg / kg bw. The biochemical parameters such as insulin and glycemia, the activities and the level of markers of oxidative stress such as superoxide dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) in the aorta, heart and the kidney were determined in the absence and presence of different doses of Wakouba (1000 and 2500 mg / kg bw) and Glibenclamide, reference product at 10 and 20 mg / kg bw.

Results: The results showed that the administration of streptozotocin at 55 mg / kg bw in rats caused a significant drop ($P<0.05$) in insulin production followed by a significant increase ($P<0.05$) in blood glucose. Similarly, during diabetes, the activities and levels of oxidative stress markers (SOD, CAT and MDA) increase significantly ($P<0.05$). Treatment of diabetic rats with Wakouba at 1000 and 2500 mg / kg bw normalizes insulin production, blood sugar levels, SOD and CAT activities and MDA levels in the aorta, heart and kidneys of these rats. the same results were obtained with Glibenclamide at 10 and 20 mg / kg bw.

Conclusion: Wakouba, a salt extracted from *Elaeis guineensis* Jacq, lowers and normalizes the activities of SOD, CAT and the level of MDA which are markers of oxidative stress in rats made diabetic by streptozocin. Wakouba also normalizes insulin production and blood sugar levels in diabetic rats. Wakouba would have antioxidant properties coupled with antidiabetic properties, which would justify its use in traditional medicine for the treatment of diabetes.

Keywords: *Wakouba*; oxidative stress; oxidative stress markers, *Antioxidative*

INTRODUCTION

Oxidative stress (SO) is an imbalance between prooxidants and antioxidants leading to a disruption of cell control and redox signaling and/or molecular damage"[1]. There are three main families currently known as reactive species: reactive oxygen species (ROS), chlorinated oxidants (RCS) and reactive nitrogen species (RNS). They are composed largely of free radicals (O_2^- , HO, NO, .) and non-radical molecules (O_2 , H_2O_2 , ONOOH, ...) [2]. Oxidation reactions are habitual and useful in our cells. They produce free radicals involved in physiological and pathological processes, in particular the development of cardiovascular diseases [3]. SO is involved in the occurrence of experimental diabetes [4]. The imbalance in oxidation reactions is either due to an exaggerated production of free radicals or from an alteration of the defense mechanisms. Free radicals can cause oxidative

damage to lipid membranes, DNA, proteins that lead to cellular dysfunction and tissue damage. They cause changes in the activities of the anti-oxidant enzymes (superoxide dismutase, Catalase etc.) that support the antioxidant defense system [5]. In the case of diabetes, oxidative stress can have several origins, such as the excessive production of reactive oxygen species (ROS) from glucose self-oxidation or the non-enzymatic reaction between glucose and circulating proteins, resulting in advanced glycation products (AGE) [6]. There are also exogenous sources such as photochemical pollutants, tobacco, drugs and ionizing radiations, penetrating the body through breathing, feeding or mucous membranes. Oxidative stress, a very important factor is involved in the genesis of diseases. In conditions of chronic hyperglycemia, several mechanisms such as the polyol pathway and glycation may be responsible for the production of free radicals. The valorization of natural substances for the treatment of diseases is a growing concern in many countries. WHO has recommended safety and efficacy studies of herbal medicines to standardize their use and integrate them into conventional health care systems [7]. Ethno pharmacological studies focuses on the experimental validation of healing properties of traditional plants [8]. This work is to evaluate anti oxidative activity of a natural extract called Wakouba traditionally used to treat metabolic affections such as diabetes and high blood pressure.

2. MATERIALS AND METHODS

2.1 Chemicals

Thiobarbituric acid (TBA) and Streptozotocin (STZ) were procured from Sigma Chemical Company (St Louis, United State of America), while Trichloroacetic acid (TCA) has by Merck (Darmstadt, Germany), Glucose by Prolabo, (France), Glibenclamide by Sanofi Winthrop Industry (France). All other chemicals were of analytical grade.

2.2 Plant Material

Wakouba was prepared according to the method described by Zirihi [9], using the slings of *Elaeis guineensis* Jack. These slings of *Elaeis guineensis* were harvested, washed, carved into approximate 1 cm length pieces, and dried at room temperature of 25-30°C for four weeks. Afterwards, the dried leaves pieces were incinerated into a muffle furnace at 400°C until white ashes. Thereafter, one hundred grams (100 g) of ash was dissolved into one liter (1 L) of distilled water and homogenized for 2 h at room temperature (25-30°C) using a magnetic stirrer. The homogenate has then undergone filtration two times upon a cotton wool followed by another filtration on Whatman paper. The final filtrate was evaporated in an oven at 60°C to obtain the *Wakouba* salt.

2.3 Animal Treatment

Eighteen male and femal Wistar rats (170–210 g) aged between 10 and 12 weeks were procured from the animal house at Pasteur Institute in Côte d'Ivoire. Rats were kept in plastic cages in a ventilated room under controlled laboratory conditions of normal light–dark cycle (12 h light/dark) and temperature (25 ± 2°C). The animals had access to food and water *ad libitum*. Experimental design and animal handling were executed according to the guidelines in Guide for the Care and Use of Laboratory Animals. After 2-weeks of acclimatization period in order to harmonize their physiological state, rats were randomly assigned to six different groups of three animals each. Group I rats, served as the control, orally received 1 mL/kg body weight (bwt) of distilled water, and the other goup rats (Group II at Group VI)

were orally administered streptozotocin at a dose of 55 mg/kg bwt. Hyperglycemia was detected 72 hours after administration of streptozotocin and after 7 days diabetes was established. Diabetic animals in groups III and IV received orally Wakouba dissolved in physiological serum, at doses of 1000 and 2500 mg / kg bwt while those in groups V and VI received orally Glibenclamide at 10 and 20 mg / kg bwt. All treatments were given for 21 days. Twenty-four hours after the last treatment, the rats were anaesthetized using diethyl ether and killed by cervical dislocation. The aorta, heart and kidney were excised and processed for biochemical assays after been cleared from other adhering tissues.

2.4 Biochemicals assays

2-4-1 Evaluation of antioxidative activity of Wakouba

2.4.1-1 Determination of Superoxide Dismutase (SOD) activity

The method of Van [10] was used in determining SOD activity in the rats organs homogenates (aorta/heart). The reaction was initiated with the addition of 2 mL of the reaction mixture (sodium cyanide at 2.10^{-5} M; NBT solution at $1.76.10^{-4}$ M, EDTA at 6.10^{-3} M; 2.10^{-6} M riboflavin; 10^{-2} M methionine and 3 mg NADPH) at five (5) μ L of organ homogenate (aorta/heart). The mixture was then irradiated with a 15 watt lamp for 10 min. The absorbance of each tube was determined at 560 nm against a blank.

2.4.1-2 Determination of catalase (CAT) activity

The activity of CAT was measured by the method [11]. A substrate solution composed of 1 mL of phosphate buffer (KH_2PO_4 , 0.1 M, pH 7.4), 0.950 mL of H_2O_2 (0.019 M), was added 0.025 mL of organ homogenate (kidney/heart). The reaction was followed by recording the absorbance at 560 nm every minute for 2 min. The CAT activity was expressed as nmol/mg protein.

2.4.1-3 Evaluation of MAD concentration

MDA rate was evaluated according to the methode [12]. To do this, 0.5 mL of 20% trichloroacetic acid (TCA) and 1 mL of 0.67% TBA were added to 0.5 mL of the liver homogenate. The mixture was heated at 100°C for 15 min, cooled and then added with 4 mL of n-butanol After centrifugation at 3000 rpm for 15 min, the optical density of the supernatant was read with a spectrophotometer at 530 nm. The MDA rate was expressed as nmol per gram of wet tissue.

2.5 Statistics Studies

One-way analysis of variance was used for the statistical analysis after which multiple comparisons were carried out by Tukey's test. The results were expressed as the mean \pm standard deviation (SD) and values with $p < 0.05$ were considered statistically significant. Data were analysed using GraphPad Prism version 6.49.

3. RESULTS

3.1 Induced diabetes by STZ

Hyperglycemia was detected 72h (Three days) after STZ administration. Results are showing that insulin production decreases significantly ($P < 0.05$) while blood glucose level increases significantly after diabetes induction with STZ (**fig. 1**)

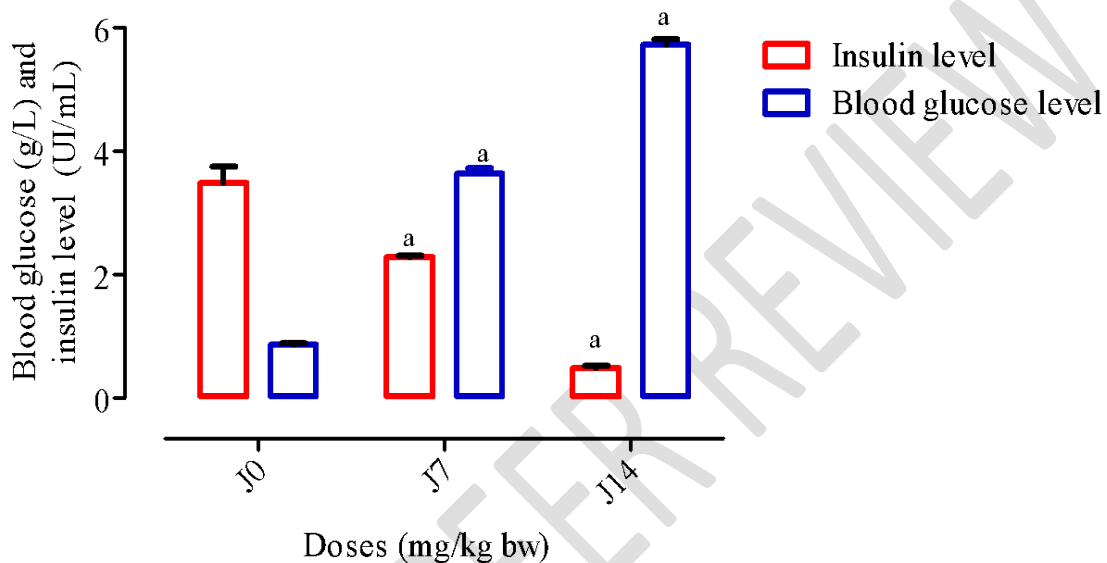


Figure 1 : Variation of insulin and blood glucose rate after diabetes induction

Each bar represents the mean \pm SEM, $n = 3$. Letter (a) represent statistical significance. a: significantly different from the healthy batch at $p < 0.01$.

3.2 Effects Wakouba on oxidative stress parameters on induced diabetics rats with STZ

3.2.1 Effects Wakouba on the Superoxide dismutase (SOD) activity in diabetic rats

The activity of superoxide dismutase (SOD) in the aorta and heart increased significantly ($p < 0,05$) in the diabetic rats compared the control group (**Fig. 2 and 3**). In the control rat group (T), the activity of SOD was $1,19 \pm 0,08$ U/ng of protein in aorta (**Fig. 2**) and $2,32 \pm 0,04$ U/ng of protein in the heart (**Fig.3**). After administration of STZ, these values increased significantly ($p < 0,05$) from 1.19 ± 0.08 U/ng of protein to 2.03 ± 0.09 U / ng of protein in the aorta and from 2.32 ± 0.04 U/ng of protein at 3.41 ± 0.04 U/ng of protein in the heart. Treatment

of diabetic rats with Wakouba at doses of 1000 and 2500 mg/kg bw resulted in a significant reduction until normalization of superoxide dismutase (SOD) activity compared to the group of untreated rats. Glibenclamide, a reference product at 10 and 20 mg / kgpc partially reduced the activity of SOD in the aorta and in the heart.

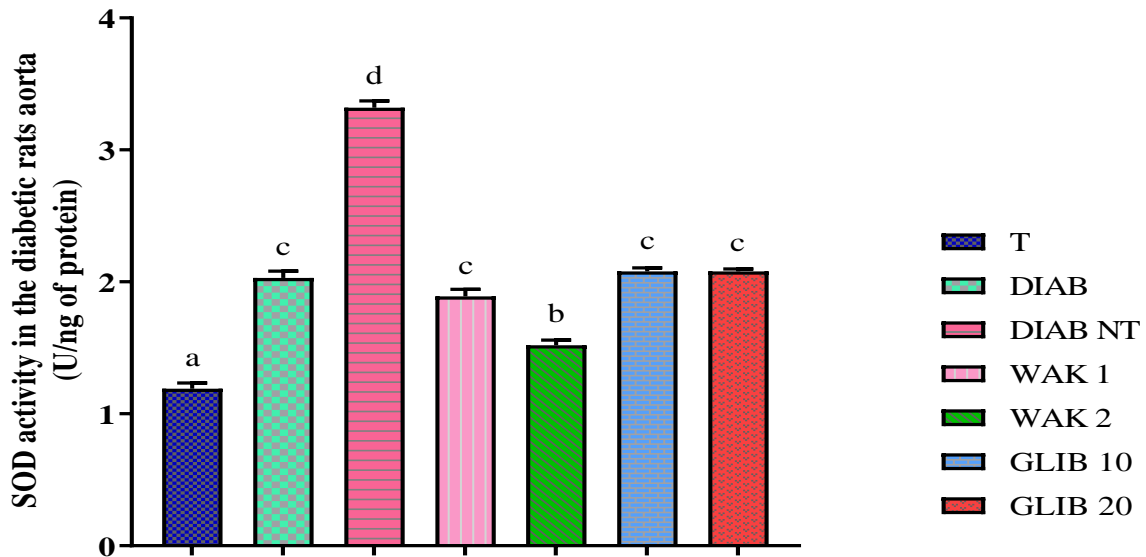


Figure 2 : Effects Wakouba on the activity of SOD in the aorta of induced diabetic rats' treatment

Each bar represents the mean \pm SEM, $n = 3$. Letters (**a**, **b**, **c**, **d**) represent statistical significance. Means with different letter are significantly different; and mean with same letter are not significantly different. ($p < 0,05$). **T** : Control batch ; **DIAB** : Initial diabetic rat ; **DIAB NT** : Non Treated Diabetic rat, **WAK 1**: Rats treated with Wakouba' dose of 1000 mg/kg bw; **WAK 2** : Rats treated with Wakouba' dose of 2500 mg/kg bw; **GLIB 10** : Rats treated with Glibenclamide of 10 mg/kg bw; **GLIB 20** : Rats treated with Glibenclamide of 20 mg/kg bw.

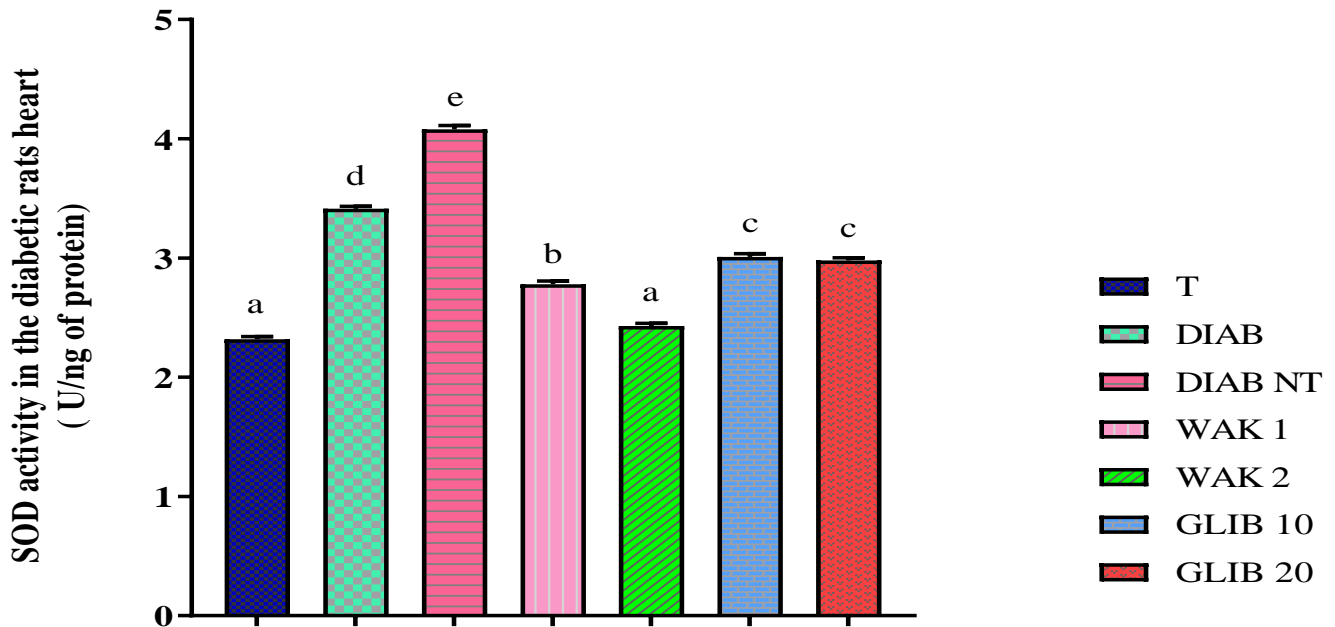


Figure 3 : Effects Wakouba on the activity of SOD in the heart of induced diabetic rats' treatment

Each bar represents the mean \pm SEM, $n = 3$. Letters (a, b, c, d,e) represent statistical significance. Means with different letter are significantly different; and mean with same letter are not significantly different. ($p < 0,05$). **T** : Control batch ; **DIAB** : Initial diabetic rat ; **DIAB NT** : Non Treated Diabetic rat, **WAK 1**: Rats treated with Wakouba' dose of 1000 mg/kg bw; **WAK 2** : Rats treated with Wakouba' dose of 2500 mg/kg bw; **GLIB 10** : Rats treated with Glibenclamide of 10 mg/kg bw; **GLIB 20** : Rats treated with Glibenclamide of 20 mg/kg bw.

3.2.2- Effects Wakouba on Catalase (CAT) activity in diabetic rats

Figures 4 and 5 represent effects of Wakouba and glibenclamide on catalase (CAT) activity in the kidney and heart of diabetic rats. **The administration of Streptozotocin (STZ) alone significantly increased CAT activities in rat kidney and heart when compared with the control ($p < 0.05$).** Administration of Wakouba at 1000 and 2500 mg / kg bwt restores and normalizes SOD activity when compared with the **control ($p < 0.05$).** **Glibenclamide partially.** Glibenclamide partially restores CAT activity in kidneys and heart compared to control.

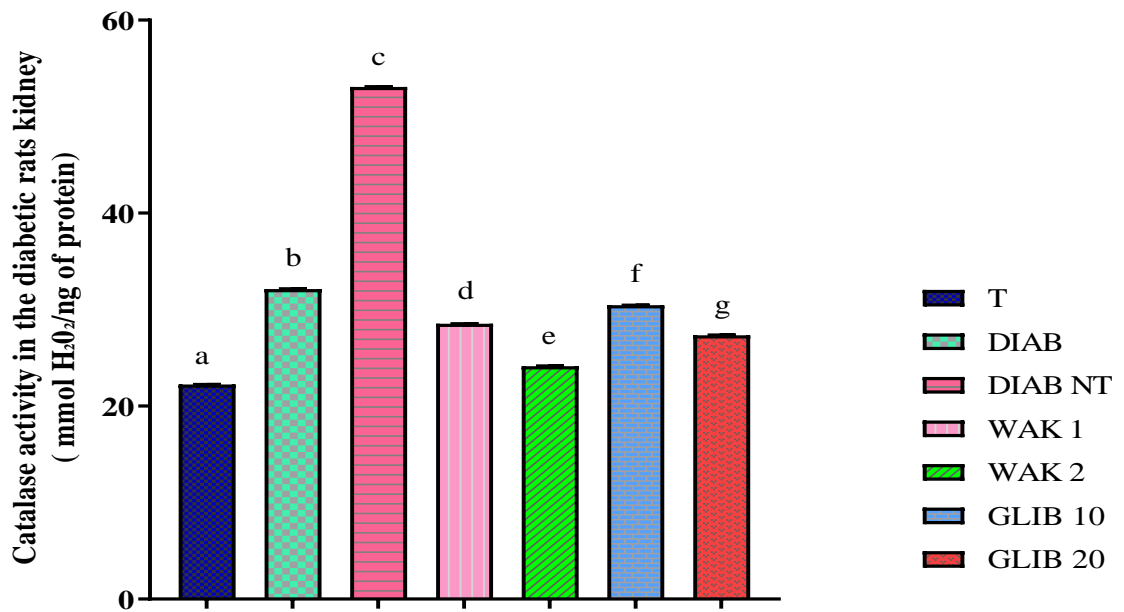


Figure 4 : Effects Wakouba on the CAT's activity in the kidney of induced diabetic rats' treatment

Each bar represents the mean \pm SEM, $n = 3$. Letters (a, b, c, d,e,f,g) represent statistical significance. Means with different letter are significantly different; and mean with same letter are not significantly different. ($p < 0, 05$). **T** : Control batch ; **DIAB** : Initial diabetic rat ; **DIAB NT** : Non Treated Diabetic rat, **WAK 1**: Rats treated with Wakouba' dose of 1000 mg/kg bw; **WAK 2** : Rats treated with Wakouba' dose of 2500 mg/kg bw; **GLIB 10** : Rats treated with Glibenclamide of 10 mg/kg bw; **GLIB 20** : Rats treated with Glibenclamide of 20 mg/kg bw.

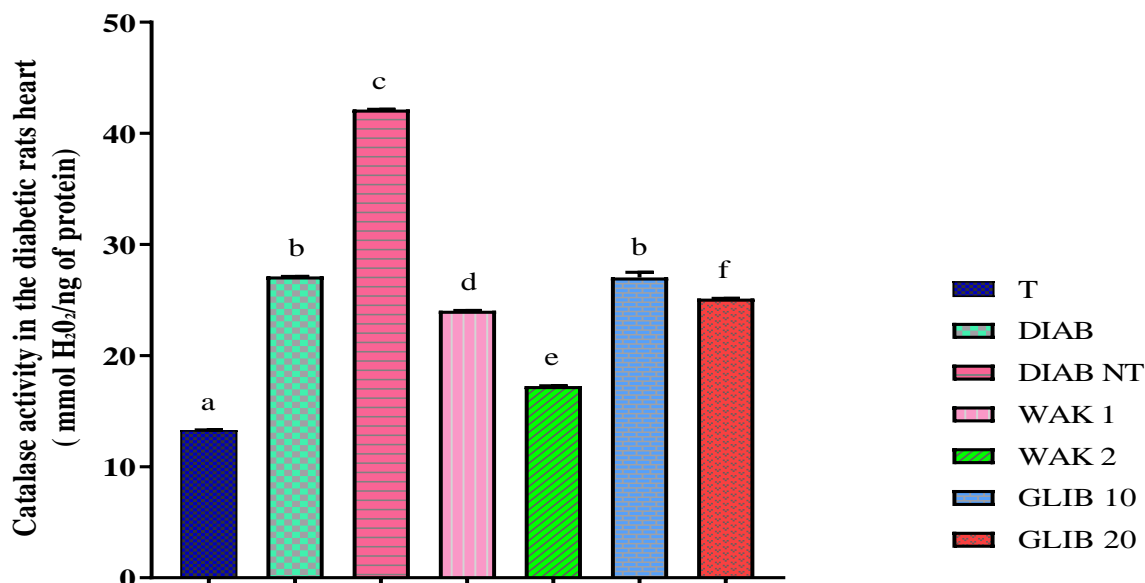


Figure 5: Wakouba effects on the CAT's activity in the heart of induced diabetic rats' treatment

Each bar represents the mean \pm SEM, $n = 3$. Letters (a, b, c, d, e, f, g) represent statistical significance. Means with different letter are significantly different; and mean with same letter are not significantly different. ($p < 0,05$).

T : Control batch ; *DIAB* : Initial diabetic rat ; *DIAB NT* : Non Treated Diabetic rat, *WAK 1*: Rats treated with Wakouba' dose of 1000 mg/kg bw; *WAK 2* : Rats treated with Wakouba' dose of 2500 mg/kg bw; *GLIB 10* : Rats treated with Glibenclamide à dose of 10 mg/kg bw; *GLIB 20* : Rats treated with Glibenclamide à dose of 20 mg/kg bw.

3.2.3 Effects Wakouba on Malondialdehyde (MAD) level in diabetic rats'

The variation in the level of lipid peroxidation products in the kidneys and the heart of diabetic rats treated and not treated with Wakouba at 1000 and 2500 mg / kg-bw and Glibenclamide at 10 and 20 mg / kg bwt are shown in Figures 6 and 7 .

The administration of Streptozotocin (STZ) alone significantly increased CAT activities in rat kidney and heart when compared with the control ($p < 0.05$). Administration of Wakouba at 1000 and 2500 mg / kg bwt restores and normalizes SOD activity when compared with the control ($p < 0.05$). **Glibenclamide partially.** Glibenclamide partially restores CAT activity in kidneys and heart compared to control.

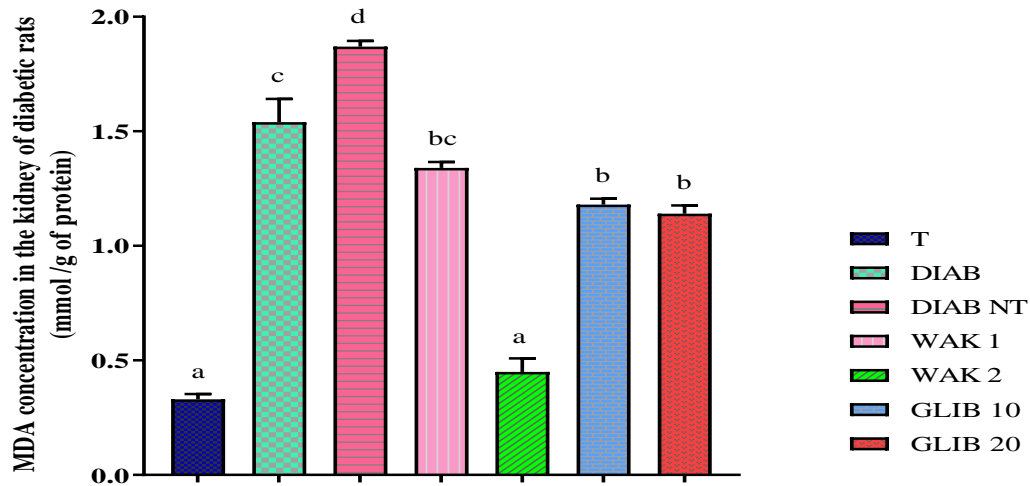


Figure 6: Effects of Wakouba on the variation of the MDA level of the kidney of diabetic rats Each bar represents the mean \pm SEM, $n = 3$. Letters (a, b, c, d) represent statistical significance. Means with different letter are significantly different; and mean with same letter are not significantly different. ($p < 0,05$). **T** : Control batch ; **DIAB** : Initial diabetic rat ; **DIAB NT** : Non Treated Diabetic rat, **WAK 1**: Rats treated with Wakouba' dose of 1000 mg/kg bw; **WAK 2** : Rats treated with Wakouba' dose of 2500 mg/kg bw; **GLIB 10** : Rats treated with Glibenclamide of 10 mg/kg bw; **GLIB 20** : Rats treated with Glibenclamide of 20 mg/kg bw.

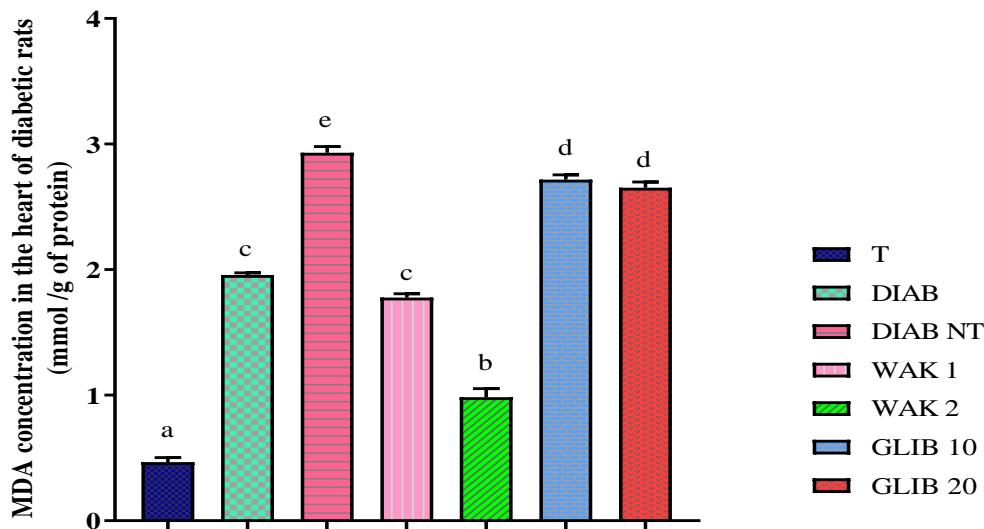


Figure 7: Effects of Wakouba on the variation of the MDA level of the heart of diabetic rats

Each bar represents the mean \pm SEM, $n = 3$. Letters (a, b, c, d) represent statistical significance. Means with different letter are significantly different; and mean with same letter are not significantly different. ($p < 0,05$). **T** : Control batch ; **DIAB** : Initial diabetic rat ; **DIAB NT** : Non Treated Diabetic rat, **WAK 1**: Rats treated with Wakouba' dose of 1000 mg/kg bw; **WAK 2** : Rats treated with Wakouba' dose of 2500 mg/kg bw; **GLIB 10** : Rats treated with Glibenclamide of 10 mg/kg bw; **GLIB 20** : Rats treated with Glibenclamide of 20 mg/kg bw.

4. DISCUSSION

In conditions of chronic hyperglycemia, several mechanisms such as the polyol pathway and glycation may be responsible for the production of free radicals. In addition to these pathways, other mechanisms such as glucose self-oxidation and activation of NADPH vascular oxidation may be the source of oxidative stress in diabetes [13].

The subject with diabetes thus exhibits an overproduction of free radicals on the one hand, and on the other hand, a decrease in antioxidants, which generates this state of oxidative stress at the origin of micro and macroangiopathies [14]. Hyperglycemia inducing the increase in glycation terminal products is a metabolic aggression that impairs cellular and humoral immune functions. This condition leads to overproduction of free radicals and development of diabetes complications [15]. Cellular damage caused by oxidative agent is variable in intensity, proportional to their rate of production and duration of action. They can be:

- transitory (defense mechanism by destruction of pathogenic bacteria);
- moderate chronic (inflammatory syndrome) characterizing various pathologies: vascular (Diabetes, Atherosclerosis, Cardiomyopathy), neurodegenerative (Alzheimer's, Parkinson's), rheumatoids (Arthritis, Amyotrophic Sclerosis), Bronchopulmonary (Asthma, Respiratory Syndrome, Pulmonary Fibrosis, Emphysema), Gastrointestinal (Colitis, Crohn's Disease);
- Acute chronicles (cell destruction) by necrosis and apoptosis (Cancers) [16].

In addition to conventional diabetes treatments (sulfonylureas, biguanides, insulin), complementary therapy to anti-oxidants was quickly initiated [17]. This study evaluated the effects of Wakouba on variation in malondialdehyde (MDA) levels, catalase activity (CAT), and superoxide dismutase (SOD) activity. This study revealed a significant increase in the level of malondialdehyde in the kidneys and heart in untreated diabetic rats. This increase is associated with an equally significant increase in the enzyme activity of superoxide dismutase and catalase in the heart, kidneys and aorta in these rats. MDA, a reactive aldehyde, is one of many reactive electrophilic species that cause toxic stress in cells and form covalent protein adducts called Advanced Lipoxidation End Products (FTAs), analogous to advanced glycation end products (AGE) [18]. The increase in the level of MDA in the heart and kidneys of diabetic rats leads to damage to these organs. Treatment with Wakouba has resulted in a significant decrease in MDA levels in both heart and kidneys. Wakouba would therefore have trapped the free radicals produced after the induction of diabetes in rats and thus reduce the risk of toxic stress in these organs. Antioxidant enzymes such as superoxide dismutase and catalase are the first level of defense against free radicals by controlling their formation and proliferation. SOD catalyzes the dismutation of superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), making it potentially less dangerous (Dringen and *al.*, 2005) [19]. Catalase works in synergy with SOD by accelerating the dismutation of hydrogen peroxide into water and molecular oxygen, thus protecting cells from the toxic effects of hydrogen peroxide. The increase in SOD and catalase activities in diabetic rats may be due to the high production of reactive oxygen species such as superoxide anion and hydrogen peroxide (Iannone, 2010) [20]. These two enzymes, in their role as the body's primary protection against these reactive species, had to increase their activity at the same time to neutralize them. After treatment of rats with Wakouba, SOD and catalase activities decreased significantly. The decline in enzyme activity could also be explained by the inhibition of the production of reactive species or their neutralization by Wakouba.

5. CONCLUSION

Pharmacological studies show that Wakouba in addition to having antidiabetic activity also has antioxidant properties that result in limiting the effects of oxidative stress and the potentiation of the activity of antioxidant enzymes namely superoxide dismutase and catalase to eliminate oxidative Agent. Wakouba could have also neutralized the oxidative agent produced in unhealthy rats by the administration of STZ.

CONSENT

It Is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors. COMPETING INTERESTS Authors have declared that no competing interests exist.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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