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Restoration of Insulin Productionin Induced Diabetic Rats, by Wakouba an Extract of Local Plant

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Authors' contributions

This work was carried out in collaboration between all authors. Author KF designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author TJ and Author BC managed the analyses of the study. Authors CA and LB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Wakouba is an extract of a medicinal plant used by the local populations of Côte d'Ivoire for the treatment of diabetes
Aims: Develop a new drug from the medicinal plants of our country Cote d'Ivoire that will be able to treat emerging diseases like diabetes.
Materials and Methods: The antidiabetic effects of wakouba were evaluated in Mice. The decrease as well as the production of insulin were determined by the enzymatic immunometric chemiluminescent method of Raufman and *al.* in 1992.Blood glucose was also assessed by the Tietz enzymatic method in 1987. Diabetes was induced with streptozotocin (STZ).Histopathological sections studies of the pancreas were made according to the method used by Agbor and et al.
Results: The results show that the production of insulin decreases significantly during the diabetes whereas the same rate increases during the treatment of the diabetic animals by Wakouba. At the

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same time, blood glucose decreases and normalizes. Besides, Wakouba restores the integrity of the Beta cells of Langerhans cells which had been destroyed during the induction of diabetes by streptozotocin.

Conclusion: Wakouba restores the integrity of the Beta cells of Langerhans cells which had been destroyed during the induction of diabetes by streptozotocin. Wakouba may be responsible for conversion of pancreatic α cells into pancreatic β cells. This may be confirmed by further researches.

Keywords: Wakouba; restoration; insulin; Glycemia.

1. INTRODUCTION

Noncommunicable diseases, also known as chronic diseases, are long-term diseases. Of the 57 million deaths that occurred globally in 2008, 36 million – almost two thirds – were due to NCDs, comprising mainly cardiovascular diseases, cancers, diabetes and chronic lung diseases [1]. Noncommunicable diseases (NCDs) kill 40 million people each year, equivalent to 70% of all deaths globally. Noncommunicable diseases (NCDs) are one of the major health and development challenges of the 21st century, in terms of both the human suffering they cause and they inflict on the socioeconomic fabric of countries, particularly low- and middle-income countries.

Diabetes is an important public health problem, one of four priority noncommunicable diseases (NCDs) targeted for action by world leaders.

Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population.

The International Diabetes Federation (IDF) forecasts a 55% increase by 2035, ie 592 million diabetics; an alarming number that is set to reach 640 million by 2040 [1,2].

Diabetes and obesity have reached such proportions worldwide we're talking about pandemic [3].

Diabetes and its complications bring about substantial economic loss to people with diabetes and their families, and to health systems and national economies through direct medical costs and loss of work and wages. While the major cost drivers are hospital and outpatient care, a contributing factor is the rise in cost for analogue insulins [4].

The treatment of diabetes is difficult and expensive. The molecules proposed by modern medicine are not accessible to the entire poor population. This population for treatment is increasingly oriented towards the plants or medicines offered by traditional healers. This attitude of the population is favored by tradition; but also by the richness of the Ivorian flora in medicinal plants.

Ethnobotanical studies with healers have identified a plant used in the traditional treatment of diabetes (Unpublished result). This plant called *Elaeis Guineensis* was used to prepare a codified salt called Wakouba.

The aim of this work is to evaluate the restoration of insulin production by Wakouba in diabetic rats.

This study focused on evaluating the rate of insulin and the level of glucose in the blood during induced diabetes. Then following up their evolution during the treatment of diabetic rats by different doses of Wakouba.

Finally, histological sections were performed on Langerhans Beta cells at different stages of the disease and its treatment with Wakouba.

2. MATERIALS AND METHODS

2.1 Plant

The slings of the oil palm or *Elaeis guineensis Jack* 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 26º to 30ºC.). They were subsequently ashed with a muffle furnace at a temperature of 400ºC. These ashes were used in the preparation of Wakouba salt.

2.2 Preparation of the Extract

The Wakouba salt was prepared according to the method described by Guédé-Guina and et al. [5].

According to this method, 100 g of *Elaeis guineensis Jacq* ash was collected and dissolved in 1L of distilled water. The aqueous mixture was homogenized for 2 hours at laboratory temperature (25-30ºC.) 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 60ºC. The evapora in gray crystals obtained was codified Wakouba which was used in this study as salt extracted from the oil palm.

2.3. Induction of Diabetes

The rats 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 rats 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.

2.4 Evaluation of the Antidiabetic Activity of Wakouba

The fifteen rats rendered diabetic were divided into 5 batches of 3 rats and treated with oral doses of Wakouba and Daonil (Reference substance). The treatment was carried out as follows:

- The untreated diabetic batch (DNT) received no treatment.
- The batches of diabetic rats WAK1 and WAK2 respectively received the doses of Wakouba at 1000 and 2500 mg / kg bw.
- The batches DAO1 and DAO 2 received Daonil at the respective doses of 10 and 20 mg / kg bw.

At the end of the treatment, the fasted rats were sacrificed in the morning by gentle decapitation (which prevents animals from experiencing pain)

The blood samples of the different batches are collected in heparinized tubes and then centrifuged at 3000 rpm for 10 minutes. The plasma then is separated in two or three fractions in Eppendorf tubes, and used for the determination of blood glucose and insulin level.

The blood glucose level was determined by the enzymatic method as used by Tietz, [6]; and insulin level by the immunometric, enzymatic, solid-phase chemiluminescent method as described by Raufman and et al. [7].

DAONIL is the trade name of an oral antidiabetic used in the treatment of diabetes. Its active ingredient is glibenclamide which is a powerful hypoglycemic agent. Daonil contains 5mg of glibenclamide.

The tablets were first made into powder, then dissolved in water and administered to the rats was done by gave (orally).

The quantity of glibenclamide administered to each animal by weight is less than the dose that could induce significant side effects.

Table 1. Different doses of Wakouba and Daonil to rats

Batches	Désignation	Administrated doses mg/kg bw
01	Healthy rats « TS »	None
02	Non treated Diabetic rats « DNT »	None
03	Diabetics rats treated by Wakouba I (WAK1)	1000
04	Diabetics rats treated by Wakouba II (WAK2)	2500
05	Diabetics rats treated by Daonil I (DAO1)	10
06	Diabetics rats treated by (Daonil II DAO II)	20

2.5 Histological Study of the Pancreas of Diabetic Mice Treated with Wakouba

This study is carried out according to the method used by Agbor and et al. [8]. Histopathological sections of the pancreas were made prior to the induction of diabetes, then during the disease; and after the treatment of the sick rats by Wakouba. The resulting cuts were observed using an olympus photon microscope (USA). The photographs were taken using an Olympus (USA) camera mounted on the microscope. Representative photographs have been selected for histopathological analysis.

2.6 Statistics Studies

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 0.01 were considered significant.

3. RESULTS

3.1 Induction of Diabetes by Streptozotocin in Mice

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

For healthy rats, insulin level was 3.48 ± 0.47 IU / mL prior to any treatment and the blood glucose level value of healthy rats was 0.86 ± 0.03 g / L. After induction of diabetes by Injection of streptozotocin to healthy rats we noticed a significant decrease of insulin level from 3.48 ± 0.47 IU / mL to 2.28 ± 0.05 IU / mL within 7days, and to 0.47 ± 0.07 UI/mL at day14 for untreated diabetic rats. That 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, ie a significant increase of 322.87% compared to healthy rats.

3.2 Wakouba Effects on Diabetic Mice's Insulin and Blood Glucose Level

The variation of insulin and blood glucose levels in the treatment of diabetic rats by Wakouba (1000 and 2500 mg / kg bw) and Daonil (10 and 20 mg / kg bw) are summarized in Fig. 2. The insulin level of the untreated diabetic rats had reached 0.42 ± 0.08 IU / mL while the blood glucose had increased to 5.89 ± 0.06 g / mL at the same time.

Oral administration of Wakouba at different doses of 1000 and 2500 mg / kg bw (respectively WAK1& WAK2)resulted in a restoration of insulin level from 2.28 ± 0.05 IU / mL to 2.61 ± 0.02 IU / mL and 3.01 ± 0.03 IU / mL in treated rats. Which means an increase of 14.49% and 32.21% of insulin level. At the same time these different doses of Wakouba at 1000 and 2500 mg / kg bw

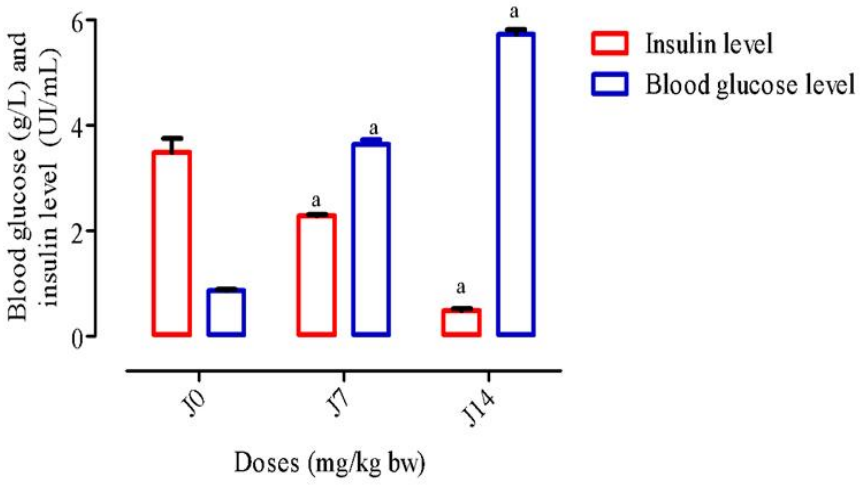

Fig.1. Variation of insulin and blood glucose levels during diabetes induction by streptozotocin (STZ)

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

normalized blood glucose level and leads respectively to a significant decrease of this blood glucose level from 3,64±0,14 to 1,76 ± 0,04 g/L(WAK1); and from 3,64±0,14 to 0,97 ± 0,02 g/L(WAK2) ie respective decrease of 51.60% and 73.42% compared to diabetic rats. Insulin levels in diabetic rats treated with Wakouba (1000 and 2500 mg / kg bw) increased significantly until normalization. The same results were obtained with Daonil, a reference product. It should be noted that Wakouba at a dose of 2500 mg / kg bw restored insulin levels by 32.21% diabetic rats.

3.3 Histological Sections of Rat's Pancreas

Figs. 3a, 3b and 3c show aspects of histological sections of the pancreas of non-diabetic rats (control mice) untreated diabetic rats and diabetic rats treated with Wakouba (1000 mg / kg bw). The histological section of the pancreas of the non-diabetic rats shows islets of Langerhans containing β-cells while that carried out on the untreated diabetic rat's pancreas shows destruction of these islets of Langerhans and β-cells. The histological section performed on the diabetic rats treated with Wakouba (1000 mg / kg of body weight) shows islets of Langerhans reconstituted with restored β cells.

Fig. 3a shows that in healthy rats, the structure and the cell composition of the islets of Langerhans show no abnormalities. The islets of Langerhans are perfectly organized with a nucleus inside which we note the presence of a chromatin and a nucleolus, a plasma membrane which surrounds the cytoplasm. The acini are well individualized.

Table 2. Effect of Wakouba on Insulin and blood glucose level

	Healthy rats Jo	Diabetics J7	Diabetics non treated J14	WAK1	WAK2	DAO 10	DAO 20
Insulin Level	3,48±0,47	2,28±0,05	0,42±0,08	2,61±0,02	3,01±0,03	2,96±0,04	3,51±0,34
Blood Glucose Level	0,86±0,03	3,64±0,14	5,89±0,06	1,76±0,04	0,97±0,02	2,35±0,27	0,95±0,04

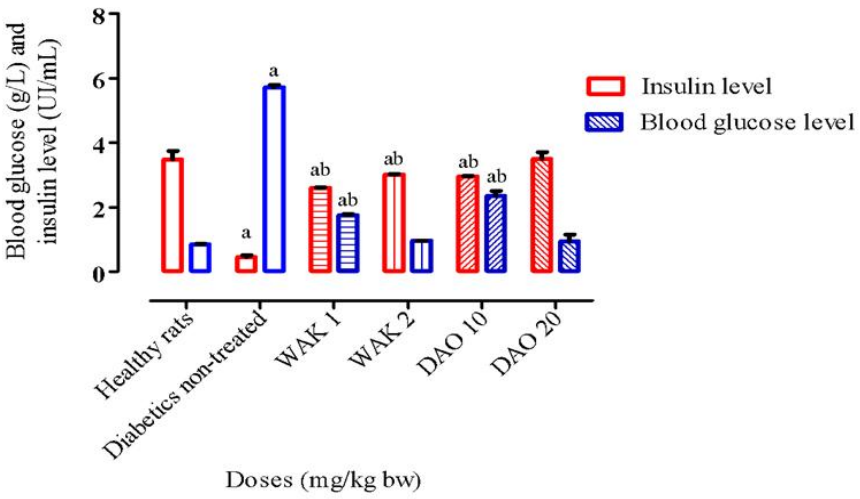

Fig. 2. Evolution of insulin and blood glucose levels during treatment of animals with Wakouba, (a natural vegetable substance) and by Daonil (a substanceHypoglycemic of reference)

Each bar represents the mean ± SEM, n = 4; a: Significantly different from the healthy lot at p <0.05; b: Significant difference compared with diabetic non-treated lot at p <0.01.; ab: Significantly different from the healthy and diabetic non-treated lots at 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

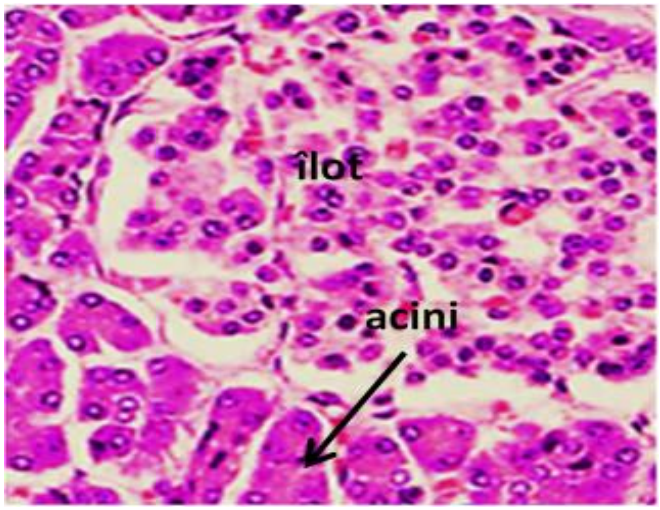

Fig. 3a. Histological section of non-diabetic rat's pancreas

In Fig. 3a we can see that for untreated diabetic rats, the pancreas presents islets of Langerhans with characteristic alterations that are lesions and cellular necrosis (nc) following the action of streptozotocin. These islets are narrowed, the cells inside are almost completely destroyed. At the acini level, the basic structure of the cells is disorganized.

Fig.3c for the diabetic rats treated with Wakouba show a totally reconstituted pancreas with a structure identical to that of healthy control rats. The islet cells of Langerhans are completely restored. The cells as well as the acini lobes have a structure similar to that of the control.

4. DISCUSSION

In this work, the chemical destruction of pancreatic β-cells of islets of Langerhans by streptozotocin (STZ) at 55 mg / kg bw (lots of diabetic rats) resulted into a significant decrease up to the cessation of the production of insulin (Fig. 1). In the control group of healthy non-diabetic animals, the average insulin level is 3.48 ± 0.47 IU / mL. This rate decreases and passes to 0.42 ± 0.08 IU / mL for the lot of untreated diabetic animals.

Compared with the non-diabetic control rats, the decrease of insulin production in the diabetic rat

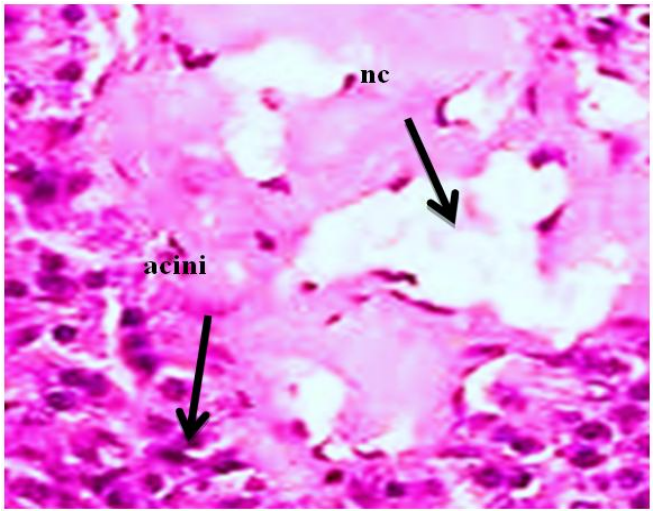

Fig. 3b. Histological section non-treated diabetic rat's pancreas

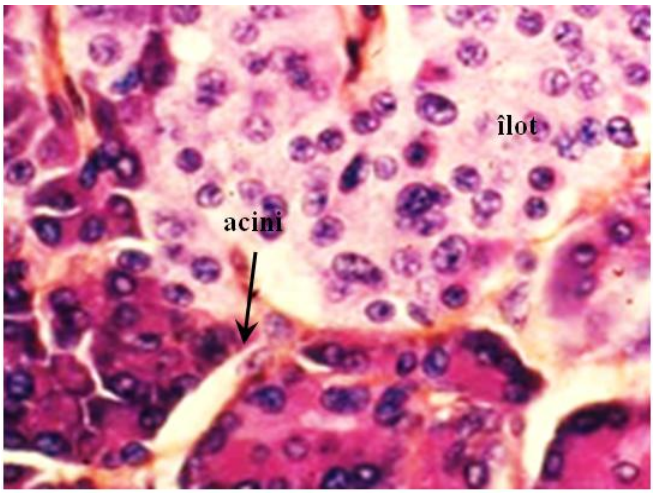

Fig. 3c. Histological section of the pancreas of a diabetic rats treated with Wakouba (1000 mg / kg bw).

lot was accompanied by a significant increase of glucose called hyperglycemia (Fig. 1), which increased from 0.86 ± 0.03 g / mL (batch of healthy control animals) to 5.89 ± 0.06 g / mL (lot of untreated diabetic animals). The variation in blood glucose should be related to the presence of insulin in the blood.

These results are in agreement with those of Cook and Taborsky, [9] and (Fajan,) [10]. According to these authors, insulin is a hypoglycemic hormone essential to the survival of a person. Its total absence, as in the case of diabetes of type 1 which is due to the destruction of β-cells in islets of Langerhans, leads to a hyperglycemia coupled with a toxic concentration of circulating ketone bodies. A situation that results in death without the help of exogenous supply of insulin. Insulin regulates blood glucose levels through several mechanisms:

1- It increases intracellular glucose uptake and its utilization by insulin-sensitive tissues by stimulating glucose transport and glycolysis. It increases also the level of plasma fatty acids;
2- It lowers the release of glucose by the liver by inhibiting glycogenolysis and gluconeogenesis.It also stimulates glycogenesis and glycolysis.

The treatment of diabetic rats with Wakouba at doses of 1000 and 2500 mg / kg bw and Daonil , a glybenclamide, taken as reference product at doses of 10 and 20 mg / kg bw causes two effects:

1- Regeneration of pancreatic β-cells in islets of Langerhans (Histological sections 3a, b and c)
2- Restoration of the secretion of insulin and normalization of blood sugar.

The Quantity of glibenclamide actually received per animal if refers to the average weight of each animal is significantly less than the limit quantity.

These results are in agreement with those obtained by Collombat et al., [11].; Thorel et al., [12]. Thorel et al [12], by inducing the destruction of almost all (99%) pancreatic β-cells in transgenic rats, were able to reprogram and convert bi-hormonal pancreatic α cells (Glucagon and insulin) into functional β pancreatic cell. Similarly, Collombat et al [13], by activation of a gene named Pax4, transformed the α pancreatic cells of Langerhans islet into β pancreatic cells capable to produce functional insulin. Collombat and Mansouri, [14]. used a pharmacological substance, a food supplement, γ-aminobutyric acid (GABA) to transform pancreatic α cells into pancreatic β cells.

Wakouba is a natural substance from vegetable, its mechanism of action is not yet clearly and completely elucidated. But we assume that Wakouba contains active components with hypoglycemic action. The hypoglycemic action of Wakouba would be indirect and would go through a regeneration of pancreatic β cells process. Wakouba, like γ-aminobutyric acid (GABA), could stimulate the transformation of pancreatic α cells to produce functional

pancreatic β cells capable to produce insulin that can fully play its dual role of hypoglycemic and regulation of glucose.

It was also evident during our work that the restoration of insulin is accompanied by a significant reduction of the level of blood glucose level that normalizes in diabetic rat treated with Wakouba. In the case of our study, Wakouba would therefore cancel the production of pancreatic α cells and program them into pancreatic β cells.

5. CONCLUSION

Insulin is a hypoglycemic hormone. Its lack of production or its absence is at the origin of chronic hyperglycemia or diabetes. The β cells of the islets of Langerhans are responsible for the endogenous production of insulin. The destruction of these cells by the STZ is causing the disappearance or the fall of the production of the insulin. Wakouba at doses of 1000 and 2500mg / kg bw restored insulin production in hyper glycemic rats. The mechanisms by which Wakouba restores the endogenous production of insulin remain to be elucidated by subsequent work.

And we assume that this restoration should pass either by the regeneration of pancreatic β cells which produce insulin; either by the conversion of pancreatic α cells into pancreatic β cells. However we notice that the insulin produced by the regenerated pancreatic β cells retains all its functional properties which allow it to play its full role of hypoglycemic hormone.

Our next work will consist in studying the effect of Wakouba, an extract of plant, on the transformation of pancreatic α cells into pancreatic β cells.

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.

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