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Journal of Applied Biotechnology
ISSN 2327-0640
2024, Vol. 11, No. 1

Evaluation of Antidiabetic Effect of Aqueous Extract of *Imperata Cylindrica* (Poaceae) Roots in Wistar Rats

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Journal of Applied Biotechnology
ISSN 2327-0640
2024, Vol. 11, No. 1

Received: June 20, 2024 Accepted: August 24, 2024 Published: August 29, 2024

Doi: 10.5296/jab.v10i1.22209 URL: https://doi.org/10.5296/jab.v10i1.22209

Abstract

Diabetes, which has become a major public health problem, is treated by certain plants in our regions, including Imperata cylindrica. The aqueous extract of the dried roots of this plant was obtained by infusion. The various chemical groups in the total aqueous extract were evaluated using using color reaction characterization. Rats made diabetic by injection of a single dose (50mg/kg bw) of streptozotocin were treated with different doses of the extract compared with glibenclamide. Treatment began 28 days after injection and lasted 28 days. Blood glucose and glycated hemoglobin (HbA1c) were measured before streptozotocin injection, before and after treatment with our extract, and one week after cessation of treatment. Histopathological sections of the pancreas were then taken.

The phytochemical screening study carried out on the roots of this plant showed that it contains sterols, polyterpenes, polyphenols, flavonoids, alkaloids and saponosides. Blood glucose levels in normal rats ranged from 0.902±0.009 g/L to 1.013±0.00 g/L and glycated hemoglobin from 3.377±0.130% to 3.87±0.186%. Untreated diabetic rats blood glucose and glycated hemoglobin increased to 3.26±0.26 g/L and 9.583±0.322% at D63 respectively. One week after discontinuation of treatment, blood glucose values increased with glibenclamide and remained normal in batches treated with the extract. Histological sections showed reconstituting cells. The extract normalizes blood glucose and lowers glycated hemoglobin, justifying its traditional use in the treatment of diabetes.

Keywords: glycemia, glibenclamide, *Imperata cylindrica*, glycated hemoglobin

1- Introduction

Diabetes is a metabolic disease characterized by chronic hyperglycemia due either to insufficient insulin production by the β-cells of the Langherhans islets, or insulinopenia, or to poor utilization of the insulin produced, or insulin resistance, or both (Barakat et al., 2010). It is an asymptomatic, non-transmissible disease that is responsible for many deaths worldwide. According to the World Health Organization, the number of people with diabetes rose from 108 million in 1980 to 422 million in 2014 with a prevalence rate ranging from 4.7% to 8.5% worldwide (WHO, 2016). In 2017; 3.2 to 5 million deaths were due to diabetes, or 10.7% of global mortality (IDF 2017). Managing diabetes is prohibitively expensive. According to estimates by the International Diabetes Federation (IDF), spending on this pathology rose from 232 billion us dollars in 2007 to 727 billion us dollars in 2017 for the 20 to 79 age group. In Côte d'Ivoire, according to IDF data, the prevalence of diabetes was estimated at 4.9% in 2016 (IDF 2014), and the cost of treatment per sufferer was 164 US dollars, or nearly 90,000

CFA francs per month. In addition to hygienic and dietary measures, the medicines available to the population are not accessible to all and are very expensive (Fourrier and Seidowsky, 2010). For their health problems, people in developing countries like Côte d'Ivoire turn to plants for treatment. Our approach consisted in carrying out an ethnobotanical survey among traditional healers to identify the plants used to treat diabetes. Among these plants, *Imperata cylindrica* caught our attention. Its leaves have anti-carcinogenic, analgesic, antipyretic, anti-inflammatory and antioxidant properties (Rinah et al., 2021). It is also used to treat high blood pressure (Mak-Mensah et al., 2010). The aim of this study was to evaluate the activity of the aqueous extract of this plant's roots on variations in blood glucose and glycated hemoglobin in rats made diabetic by streptozotocin.

2- Materials and methods

2-1-Material

2-1-1- Plant material

The plant material consisted of *Imperata cylindrica* (Poaceae) roots harvested in the Agnéby Tiassa region, precisely at N'Douci (a town 117 km from Abidjan). The plant was identified at the National Floristics Center at the Université Felix Houphouët-Boigny de Cocody using analytical flora.

2-1-2- Animal material

Witsar rats *(Rattus norvegicus*) weighing 224±6.65 g were used for this study. They were kindly donated by the vivarium of the Ecole Normale Superieure (ENS) d'Abidjan and were fed with pellets supplied by IVOGRAIN, Abidjan (Côte d'Ivoire), with free access to water.

2-2- Methods

2-2-1- Preparation of aqueous extract

Imperata cylindrica roots were pulverized using a binatone grinder to obtain a fine powder. The aqueous extract of *Imperata cylindrica* roots was prepared according to the method described by Koffi N'guessan **(**koffi et al., 2009**)**. According to this method, 100 g of this powder were infused in one (1) liter of boiling water for 15 (fifteen) minutes. The resulting solution was filtered three times on absorbent cotton and once on Wattman n°1 filter paper, then oven-dried at 55°C to obtain a powder: the total aqueous extract (Impecy).

2-2-2- Physicochemical characterization of the aqueous extract of Impecy

The chemical groups present in the aqueous extract of *Imperata cylindrica* roots were identified by the classical methods of **(**Houghton and Raman, 1998) using color reaction characterization (Table 1). Solutions showing positive reactions indicate the presence of the highlighted chemical groups in the extract.

2-2-3- Study of antidiabetic activity

2-2-3-1. Induction of diabetes

Total of 40 rats with an average weight of 224±6.65 g were used for this study. The animals were divided into two batches. A control batch of 3 rats received distilled water and a test batch of 37 rats received streptozotocin (STZ). Permanent hyperglycemia was induced in the animals by intraperitoneal administration of a single 50 mg/kg bw dose of streptozotocin (STZ) in solution in distilled water **(**Singh et al., 2005). Hyperglycemia was detected after 7 days, and rats with blood glucose levels greater than or equal to 1.75 g/L were considered diabetic after 28 (twenty-eight) days (Gnaléi et al., 2019). After 28 (twenty-eight) days of induction, 15 diabetic rats were retained.

Table 1: Specific reagents and reactions for phytochemical screening

Chemical groups		**Specific reagents**	**Characteristic reactions**
Sterols and polyterpenes		Libermann (acetic anhydride-H2SO4)	Appearance of a purple or violet ring at interphase, turning blue and then green
polyphenols		Ferric chloride	Greenish or blue-black color
Phenolic compounds	tannins	Stiansy reaction ($FeCl_3$)	Greenish or blue-black color
	flavonoids	Cyanidine reaction	Pinkish-orange, purplish-pink or red colouration
Quinonic compounds		Bornträger-UV reagent	Intense inflorescence
Alkaloids		Dragendorff reagent	Reddish-brown precipitate
		Bouchardat reagent	
Saponosides		Determination of foam index (IM*)	Positive test if IM>100 intense foam

(Houghton and Raman, 1998)

2-2-3-2. Animal treatment

A set of 18 (eighteen) rats were divided into 6 batches of 3 rats each.

Batch 1: normoglycemic control rats given distilled water (10 ml/kg/bw)

Batch 2: rats treated with glibenclamide at 10 mg/kg/bw.

Batch 3: untreated diabetic rats

Batch 4: diabetic rats treated with Impecy at 200 mg/kg/bw.

Batch 5: diabetic rats treated with Impecy at 500 mg/kg/bw.

Batch 6: diabetic rats treated with Impecy at 1000 mg/kg/bw.

One (1) mL (v/p) of each dose was regularly administered daily to sick animals by gavage through a cannula. Treatment lasted four weeks. On day 0, day 28 (twenty-eight), day 56 (fifty-six) and day 63 (sixty-three) of treatment, the animals were weighed and anesthetized, and a caudal amputation blood sample was taken in a red-capped tube and a violet-capped tube. The red-capped tube was centrifuged using a JOUAN centrifuge at 3,000 rpm for 5 min. The serum obtained was stored at - 20˚C for blood glucose determination **(**Tietz et al., 2006) The purple-capped tube was used for HbA1c determination **(**Golsdtein et al., 1986**)**. Treatment began on day 28 (twenty-eight) th and was stopped on day 56 (fifty-six) th. At the end of the treatment, the pancreas was isolated and fixed in 10% formalin for histological studies.

3- Statistical Analysis

Results were analyzed using Graph Pad Prism 8.0.2 (Microsoft, USA). Means were compared using analysis of variance (ANOVA TWO WAY). Differences between means were determined using Dunnett's comparison test. Differences were considered significant when p-value was less than 0.05 ($p < 0.05$). Means were expressed as mean ± standard deviation of the mean.

Means were determined with replicates.

n = 3

4- Results

4-1- Phytochemical sorting

The results of the triphytochemical study of the aqueous extract of *Impera cylindrica* are presented in Table 2. This extract contained sterols, polyterpenes, polyphenols, flavonoids, alkaloids and saponosides, but no tannins or quinonic substances.

Table 2: Metabolites detected in *Imperata cylindrica* aqueous extract

Chemical compounds	Results
Sterols/Polyterpenes	+
Polyphenols	+
Flavonoids	+
Tannins	-
Quinonic substances	-
Alkaloids	+
Saponosides	+

4-2-Variation in blood glucose and glycated hemoglobin in normal rats over time

Figure 1 shows the variation in blood glucose and HbA1c values over time. Blood glucose levels in normal rats were 0.902±0.009 g/L at D0; 1.013±0.013 g/L at D28; 0.957±0.032 g/L at D56 and 0.963±0.015 at D63. Hba1c values were as follows: 3.54±0.075% at D0; 3.377±0.130% at D28; 3.87±0.186% at D56 and 3.767±0.079%. No significant difference observed p (> 0.05).

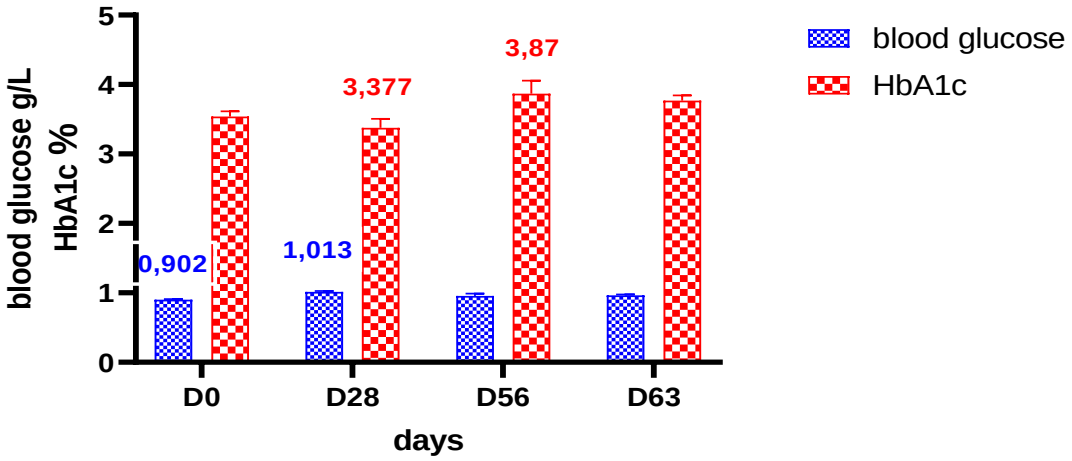


Figure 1: Changes in blood glucose and HbA1c levels in normal rats

4-3- Variation in blood glucose and HbA1c levels of untreated diabetic rats over time

Administration of streptozotocin at 50 mg/kg bw significantly ($p<0.0001$) increased the blood glucose and HbA1c levels of rats in the negative control group from 0.902±0.014 at D0 to 3.26±0.015 g/L at D63 for blood glucose and from 3.377±0.130% at D0 to 9.583±0.322% at D63 for HbA1c **(**Figure 2).

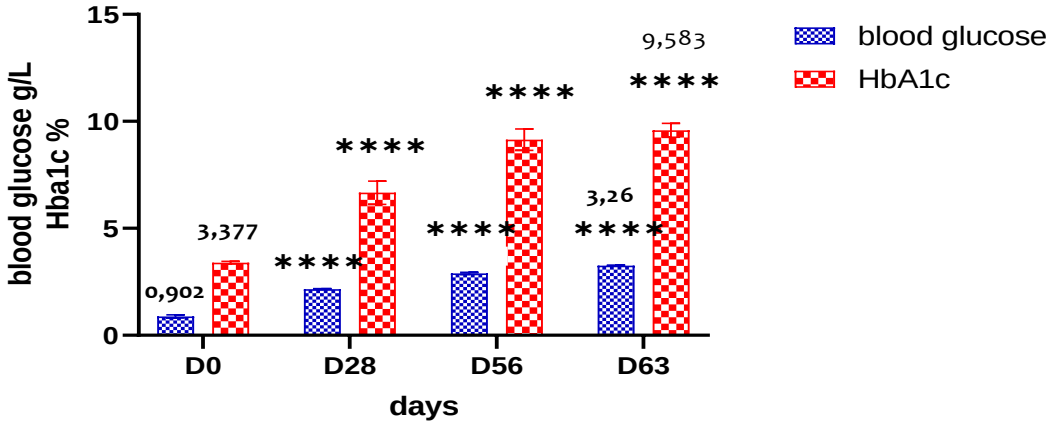


Figure 2: Evolution of blood glucose levels in untreated diabetic rats

Journal of Applied Biotechnology
ISSN 2327-0640
2024, Vol. 11, No. 1

4-4- Variation in blood glucose and HbA1c levels in diabetic rats treated with glibenclamide and different concentrations of aqueous extract of Imperata cylindrica roots

The blood glucose and HbA1c values of diabetic rats treated with 10 mg/kg bw glibenclamide and with the aqueous extract of *Imperata* cylindrica roots at different doses are shown in figure 3. At D0, normal blood glucose was 0.902±0.009 g/L and HbA1c 3.377±0.130%. During diabetes induction, blood glucose and HbA1c values increased significantly ($p<0.0001$). They yielded 2.16±0.015 g/L for blood glucose and 6.309±0.12% for HbA1c at D28.

Treatment of diabetic rats with 10 mg/kg bw of glibenclamide and different doses of aqueous extract of *Imperata cylindrica* roots significantly ($p<0.0001$) reduced blood glucose levels until normalization. Blood glucose values fell from 2.16±0.15 g/L at D28 to 0.663±0.026 g/L; 1.05±0.029 g/L; 0.95±0.04 g/L and 1.213±0.013 g/L for batches 2, 4, 5 and 6 respectively (figure 3). The glycated hemoglobin value of rats in the reference batch fell significantly ($p=0.0332$) compared with their value at D28, but the fall was not significant for batches 4, 5 and 6 at D56 (figure 4). One week after discontinuation of treatment, blood glucose levels remained normal at the various doses of aqueous extract of Imperata cylindrica, whereas a rise was observed with glibenclamide (the reference substance).

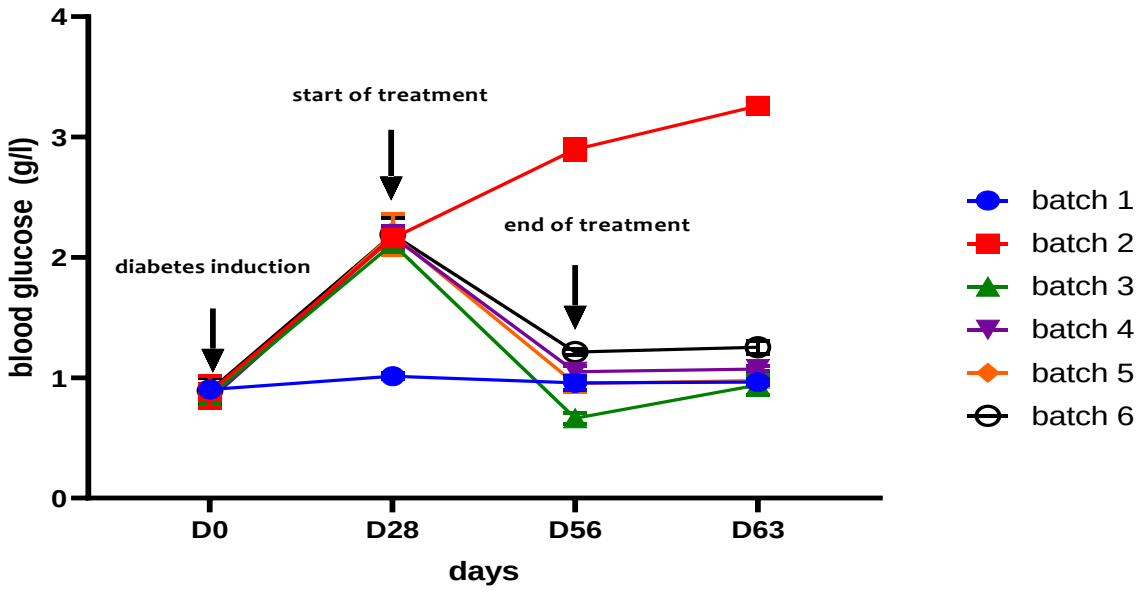


Figure 3: Effects of Impecy and glibenclamide on hyperglycemia in streptozotocin-intoxicated rats

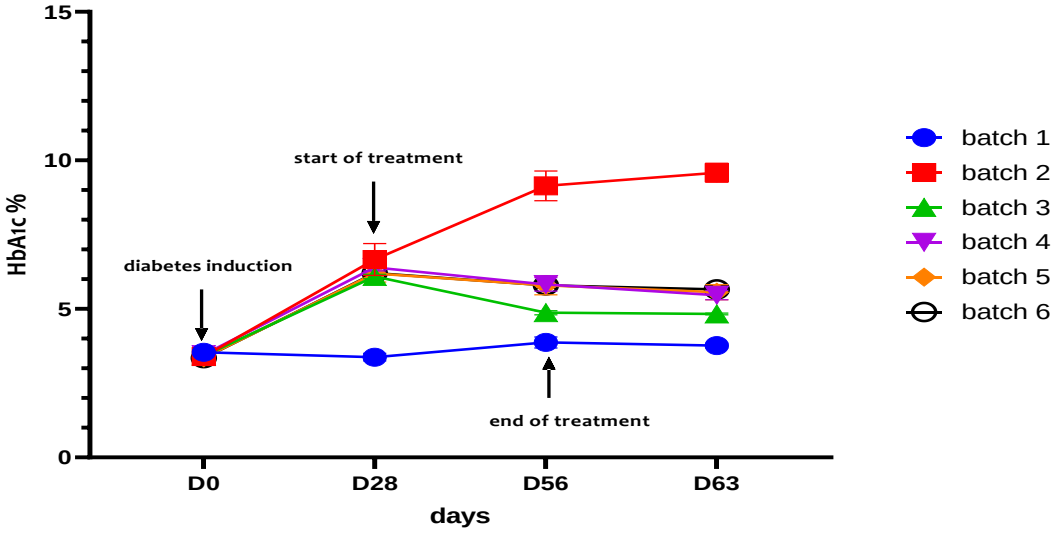


Figure 4: Effects of Impecy and glibenclamide on glycated hemoglobin in streptozotocin-intoxicated rats.

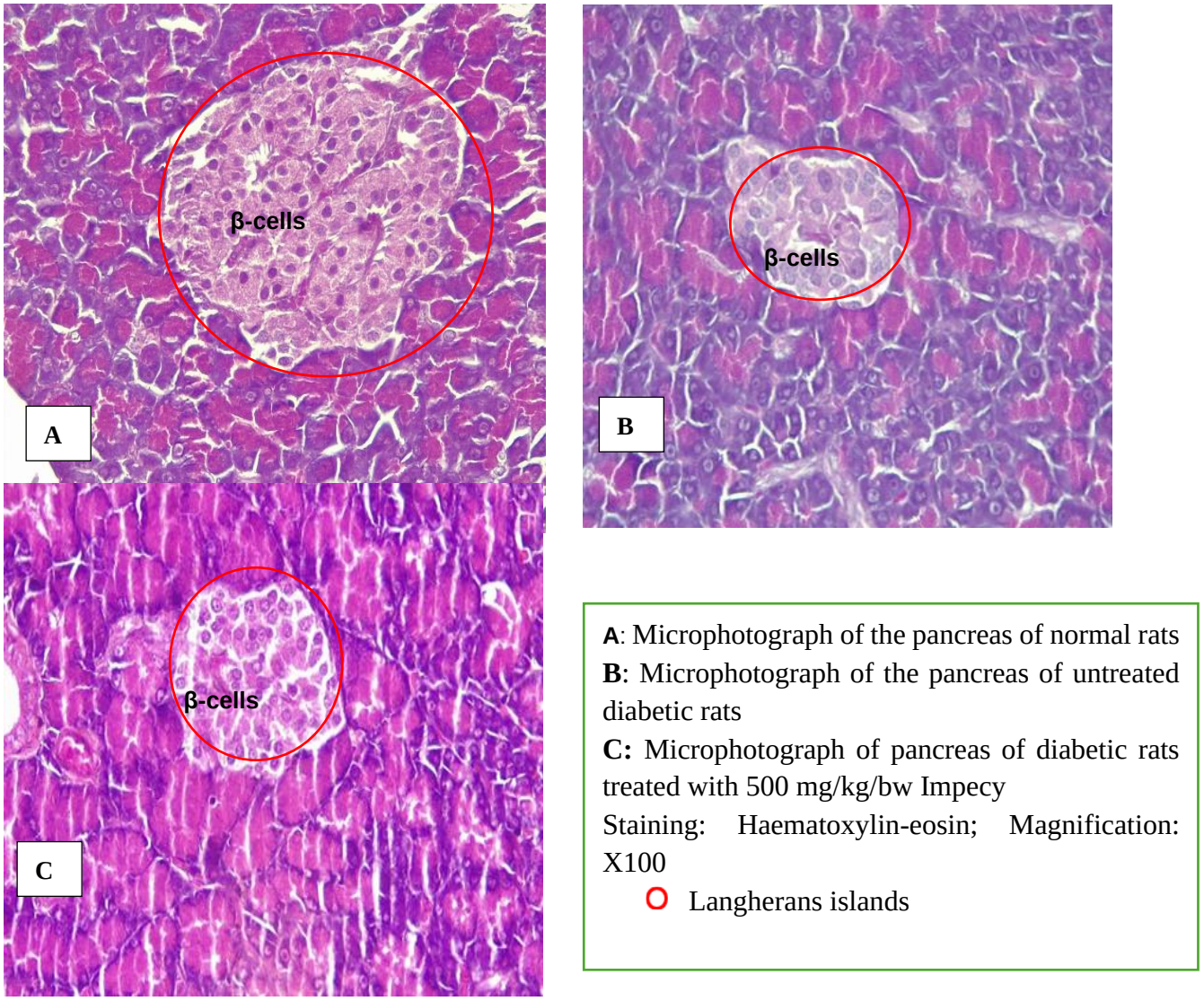
4-5- Histological sections of rat pancreas

Figures 5A, 5B and 5C show histological aspects of pancreas sections from non-diabetic rats (control rats), untreated diabetic rats and diabetic rats treated with Impecy (500 mg/kg bw). The histological section from the pancreas of non-diabetic rats showed islets of Langerhans containing β-cells, while that from the pancreas of untreated diabetic rats showed destruction of these islets and β-cells. The histological section from diabetic rats treated with Impecy (500 mg/kg body weight) shows islets of Langerhans with partially restored β-cells.

Figure 5A shows that in healthy rats, the structure and cellular composition of islets show no abnormalities. Islets are perfectly organized, with a nucleus containing chromatin and a nucleolus, and a plasma membrane surrounding the cytoplasm. The acini are well individualized.

In Figure 5B, we can see that in untreated diabetic rats, the pancreas shows islets of Langerhans with characteristic lesions and cell necrosis (nc) following the action of streptozotocin.

In Figure 5C, diabetic rats treated with Impecy show a partially reconstituted pancreas with a structure close to that of healthy control rats. Islet cells are partially restored. Both cells and acini lobes have a structure similar to that of the control.



A: Microphotograph of the pancreas of normal rats
B: Microphotograph of the pancreas of untreated diabetic rats
C: Microphotograph of pancreas of diabetic rats treated with 500 mg/kg/bw Impecy
Staining: Haematoxylin-eosin; Magnification: X100
O Langherans islands

Figure 5: Microphotograph of the pancreas of Wistar rats

5- Discussion

Imperata cylindrica is a plant used by traditional practitioners in the treatment of diabetes in Côte d'Ivoire. The present work was carried out to evaluate the anti-diabetic activities of the aqueous extract of *Imperata cylindrica* (Impecy) roots. Phytochemical screening revealed the presence of sterols, polyterpenes, flavonoids, alkaloids and saponosides in the extract. However, the extract does not contain tannins or quinone substances. Saponosides, flavonoids, terpenes and alkaloids were also identified by Krishnaiah **(**Krishnaiah et al., 2009) in the aqueous extract of *Imperata cylindrica* leaves. These authors also report the presence of tannins and cardiac glycosides in *Imperata cylindrica* leaf extracts. According to Abayomi **(**Abayomi, 1996), this can be explained by the fact that a plant's composition of secondary metabolites varies according to geographical location, the organ sampled, the time of sampling and storage conditions.

Blood glucose levels in normal rats ranged from 0.902±0.009 g/L at D0 to 1.013±0.013 g/L at D28, 0.95±0.031 g/L at D56 and 0.96±0.014 g/L at D63. These results are similar to those of Gohi parfait Kahou Bi **(**Gohi et al., 2016**)** who obtained blood glucose values ranging from 0.95 g/L to 1.02 g/L in normal rats. In the study of antidiabetic activity, diabetes was induced by intraperitoneal administration of streptozotocin (STZ) at 50 mg/kg bw. According to Anderson **(**Anderson et al., 1974**)**, streptozotocin is a nitrosated glucosamine with a selective cytotoxic effect on islet β-cells. STZ, once injected, enters β-cells via the GLUT 2 transporter and causes cell necrosis via DNA alkylation. This observation was made on the pancreases of rats injected with streptozotocin in our experiment (Figure 5B). This destruction of β-cells, responsible for insulin secretion, results in reduced secretion of this hypoglycemic hormone, leading to hyperglycemia **(**Elsner et al., 2000). Indeed, Herrera **(**Herrera et al., 2011**)** and Ibeh (Ibeh and Ezeaja, 2011) noted that after one week of diabetes induction, blood glucose levels in rats increased by 30% and 40% respectively. This proves that streptozotocin induces hyperglycemia in these animals, reflecting experimental diabetes following partial β-cell necrosis **(**Lenzen, 2008**)**.

This chronic hyperglycemia leads to increased glucose binding to proteins, and thus to an increase in glycated hemoglobin. HbA1c is a glycated hemoglobin formed by the attachment of a glucose molecule to the N-terminus of at least one hemoglobin A beta chain. It is the reference parameter for monitoring diabetic patients. Its use is currently reserved for monitoring diabetes.

Treatment with aqueous total extract of *Imperata cylindrica* roots at doses of 200, 500 and 1000 mg/kg/bw and glybenclamide at a dose of 10 mg/kg bw resulted in a significant reduction ($p<0.0001$) to normal blood glucose levels. On the other hand, aqueous extract at different doses partially reduced glycated hemoglobin levels after one month. The same results were observed with glibenclamide, a hypoglycemic reference substance. This may be explained by our extract's ability to partially regenerate the damaged pancreas. These results are in line with those obtained by Collombat (Collombat et al., 2009) and Thorel (Thorel et al., 2010**).** Indeed, by inducing the destruction of almost all (99%) pancreatic β cells in transgenic rats, these authors were able to reprogram and convert bi-hormonal (Glucagon and insulin) pancreatic α cells into functional pancreatic β cells. Similarly, Collombat **(**Collombat et al., 2009), by activating a gene called Pax4, transformed islet pancreatic α cells into pancreatic β cells capable of producing functional insulin. Collombat **(**Collombat and Mansouri, 2009) were able to transform pancreatic α cells into pancreatic β cells by injecting rats with a pharmacological substance γ-aminobutyric acid (GABA).

Impecy has an anti-diabetic effect, lowering blood sugar levels in streptozotocin-induced diabetic rats to normal levels. In fact, this plant could be involved in inducing the production of islet β-cells capable of producing insulin to lower blood sugar levels.

Impecy, like γ-aminobutyric acid (GABA), could stimulate the transformation of pancreatic α cells to produce β cells capable of producing insulin, which can fully play its dual role of hypoglycemic and glucose regulator.

6- Conclusion

Islet β-cells are responsible for the endogenous production of insulin, the body's hypoglycemic hormone. The destruction of these cells by streptozotocine has led to the disappearance or fall in insulin production. Impecy at doses of 200 and 500 mg/kg bw partially restored islet β-cells. The mechanisms of action of Impecy in β-cell regeneration remain to be elucidated. And we assume that this restoration should involve either the regeneration of insulin-producing pancreatic β-cells; or the conversion of pancreatic α-cells into pancreatic β-cells. However, insulin produced by regenerated β-cells retains all its functional properties, enabling it to play its full role as a hypoglycemic hormone. Our next step will be to study the effect of Impecy, a plant extract, on the transformation of pancreatic α cells into pancreatic β cells.

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Acknowledgments

Not Applicable.

Funding

Not Applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Macrothink Institute.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.