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Original Article

Safety and antihyperglycemic activity of "Pancréa free," an antidiabetic remedy formulated by an Ivorian traditional practitioner

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ABSTRACT

Diabetes remains a public health problem due to the limitations of conventional treatments. Traditional medicine offers an alternative. "Pancréas Free" is a remedy produced by an Ivorian traditional practitioner to treat diabetes. This study aimed to evaluate the safety and antihyperglycemic activity of "Pancréas Free."

The safety of Pancréas Free was evaluated according to OECD 423 and OECD 407 guidelines. The hypoglycemic risk of Pancréas Free at doses of 4.5, 9, and 18 mg/kg was investigated. The anti-hyperglycemic activity of the same doses was evaluated in an induced-hyperglycemia animal model. Blood glucose levels of rats were checked before hyperglycemia was induced and then every hour for 4 hours.

'Pancréas Free' did not cause any signs of clinical toxicity or lethality up to 2000 mg/kg. When administered once daily for 28 days, "Pancréas Free" also showed no signs of clinical, hematological, or biochemical toxicity. "Pancréas Free" did not cause a significant decrease in fasting blood glucose levels. "Pancréas Free" caused a non-significant reduction in induced hyperglycemia. This decrease was more pronounced from the third hour for doses of 4.5 mg/kg and 18 mg/kg, and from the second hour at the dose of 9 mg/kg.

Pancréas Free demonstrates good clinical, hematological, renal, and hepatic tolerance as well as low antihyperglycemic activity without hypoglycemic risk. Its combined use, according to the traditional practitioner's protocol, could justify its use in the treatment of diabetes.

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Introduction

Diabetes remains a challenge for healthcare systems worldwide. In 2021, approximately 537 million adults had diabetes. This figure is expected to reach 783 million by 2045 if adequate measures are not taken [1]. Type 1 and type 2 diabetes are the most common forms [2]. In Côte d'Ivoire, the prevalence of diabetes in 2017

was approximately 6.2% of the adult population [3]. This situation is attributed to several factors, including rapid urbanization, changes in lifestyles and diets, and an aging population [4]. Diabetes-related complications, such as cardiovascular disease, neuropathy, and nephropathy, represent a significant burden on the Ivorian healthcare system [5-7]. Therefore, effective diabetes management requires an integrated approach that includes lifestyle changes, medication, and, in some cases, alternative therapies. Among alternative approaches, herbal remedies and natural products have gained popularity. Approximately 80% of the Ivorian population uses traditional medicine for various health care needs, including diabetes treatment [8]. Studies have shown that several plants have antihyperglycemic properties, making them potential candidates for diabetes management [9, 10]. These traditional remedies are often chosen because of their accessibility, affordability, and cultural trustworthiness [11]. However, the safety and efficacy of these plants must be scientifically proven in order to ensure their safe integration into modern treatment protocols [12]. In this context, a combination of herbal remedies from an Ivorian traditional practitioner, called "Diabetes Free" and "Pancrea Free," was the subject of an observational study on Ivorian diabetics and showed promising results [13]. Other studies on the clinical and biological tolerance, as well as the antihyperglycemic activity and hypoglycemic risk of this combination, showed lethality at 1000 mg/kg, nevertheless, at therapeutic doses, good clinical and biological tolerance [14] and antihyperglycemic activity without hypoglycemic risk was observed [15]. In order to identify the role of each remedy, the aim of this study was to evaluate the safety and antihyperglycemic activity of "Pancréa Free" alone.

Material

'Pancreas-free' remedies

This remedy was directly collected from the traditional practitioner in April 2024. It is available in form of white tablets. It contains four plants, namely 100 mg of ginseng root bark (Panax ginseng) and 150 mg of gurmar leaves (Gymnema sylvestre).

Animals

The animals used in this study were Wistar rats (Rattus norvegicus), approximately 3 months old and weighing between 132 and 234 g. The rats were kept in Plexiglas cages with stainless steel lids and equipped with feeding bottles. The temperature and humidity in the laboratory complied with the standards for experimental facilities (T= 22˚C ± 3˚C and 50-60% humidity), with a light cycle of 12 hours of light and 12 hours of darkness. The rats were given a diet of food pellets and had access to drinking water ad libitum.

Solvents and reagents

The powders, solutions, solvents and reference substances used to conduct the study were as follows: Glucose powder (D-glucose), a 0.9% sodium chloride (NaCl) solution, distilled water and 5 mg of glibenclamide (Daonil®, Sanofi Laboratory) as a reference substance.

Other equipment

The equipment used in this work included a scale, a SYSMEX XN-1000 automated analyzer (for hematological analysis), a COBAS C-311 automated analyzer (for biochemical analysis), a centrifuge (ROTANTA 460), a mortar, a pestle, beakers, feeding tubes, red and purple cap blood collection tubes, cotton wool, Pasteur pipettes, clean gloves, a lab notebook, markers, a glucometer, test strips, scalpel blades, a spatula, a watch glass, and syringes.

Methods

Evaluation of the safety of "Pancréas free"

Acute toxicity

The acute toxicity of the remedies was evaluated according to OECD method 423. Twenty-four female Wistar rats were used as test animals. The animals were divided into two groups of three animals each and were put into fasting for eight hours with free access to water. The administration of "Pancréas free" solutions was done in four successive series. In each series, three female rats received doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 1000 mg/kg on the first day. Each series was carried out in parallel with a control group receiving NaCl under the same conditions. The animals were monitored daily for 14 days for the following signs of toxicity: apathy, excitement, breathing difficulties, excessive grooming, rloss of appetite, oral bleeding, abdominal pain, coma, diarrhea, convulsions, and death [16].

Subacute toxicity (OECD 407)

The doses used were determined in our previous study: 4.5 mg/kg, 9 mg/kg/day and 18 mg/kg [14, 15]. A tablet containing 100 mg of garlic extract from the "Pancreas Free" remedy was homogenized in 55 ml of physiological water to obtain a concentration of 1.9 mg/ml, a volume of 10 ml/kg administered to rats corresponds to a dose of 19 mg/kg. From this 1.9 mg/ml stock solution, a dilution to one-half (½) was carried out to obtain two daughter solutions at 0.95 mg/ml and 0.475 mg/ml, corresponding to doses of 9 mg/kg and 4.5 mg/kg respectively. Twenty-four rats (12 females and 12 males) were put into fasting for 8 hours prior to experimentation and divided into 4 groups of six rats per group (3 males and 3 females). The animals were given doses by oral route once daily as follows: group 1 (control): received NaCl at a volume of 10 ml/kg; group 2: received "Pancreas Free" at a dose of 4.5 mg/kg; group 3: was given "Pancreas Free" at a dose of 9 mg/kg ; group 4: was administered "Pancreas Free" at a dose of 18 mg/kg. The animals were observed and weighed every other day for 28 days for signs of toxicity, such as apathy, excitement, breathing difficulties, refusal of food, excessive grooming, bleeding from the mouth, abdominal pain, diarrhoea, convulsions, coma and death. On day 29, the rats were anaesthetised with ether-ethyl. Blood was collected by puncture from the retro-orbital sinus. A blood sample was collected in two tubes, one containing violet-capped EDTA for the determination of hematological tolerance parameters (Complete Blood Count), and the other a dry red-capped tube for the determination of hepatic and renal parameters (AST, ALT, urea, creatinine). Analyses were performed at the Institut Pasteur de Côte d'Ivoire, CHU Cocody [17].

Hypoglycemic risk

The test consisted in measuring the influence of "Pancréas Free" on basal glycemia, i.e. outside hypoglycemia, in order to predict its safety of use [18]. Four (4) groups of 6 rats per group were put into fasting for 8 hours before experimentation. Fasting blood glucose levels were measured using a "HumaSens 2.0" glucometer. The animals were then given 10 ml/kg of the various solutions by oral route, as follows: group1, NaCl; groups 2, 3 and 4, "Pancreas Free" at doses of 4.5mg/kg, 9mg/kg and 18mg/kg respectively. After administration, blood glucose levels were also measured at 1h, 2h, 3h and 4h.

Evaluation of the antihyperglycemic activity of "Pancréas free"

Principle

The test consisted in measuring the effect of "Pancreas Free" on hyperglycemia induced by oral glucose overload. An antihyperglycemic substance prevents hyperglycemia in rats. Otherwise, hyperglycemia is observed in rats [18].

Procedure

Five (5) groups of 6 rats per group were put into fasting for 8 hours prior to experimentation. Fasting blood glucose levels were measured by taking blood samples from the tail vein and analyse them using a Hummas glucometer. The animals were given by oral route a volume of 10ml/kg as follows: group 1: NaCl; group 2: glibenclamide at a dose of 10mg/kg; group 3, 4 and 5, "Pancreas Free" at doses of 4.5mg/kg, 9mg/kg and 18mg/kg respectively. 30 min later, they all received a glucose overload of 10g/kg and blood glucose levels were measured every hour for 4h.

Data processing and analysis

Results were expressed as mean ± standard deviation. Graphical representation was performed using Graph Pad Prism software version 8.0.2. Means were compared by using the analysis of variance (ANOVA) test at a significance level of $\alpha = 5\%$.

Results

Safety of "Pancréas free" use

Acute toxicity

Figure 1 illustrates the variation of rat body weights over 14 days after a single-dose administration of "Pancreas free" at 2000 mg/kg. The weights of rats in the control group and those in the treated group followed the same evolutionary kinetics ($p>0.05$). No other signs of clinical toxicity or lethality were observed at this dose.

Subacute toxicity

Clinical toxicity

Weight trends in rats given "Pancreas free" at 4.5 mg/kg, 9 mg/kg and 18 mg/kg (Fig. 2) show similar kinetics to those of control rats ($p>0.05$). During 28 consecutive days of administration, "Pancreas free" did not affect rat body weight. No other signs of clinical toxicity or lethality were observed at these doses.

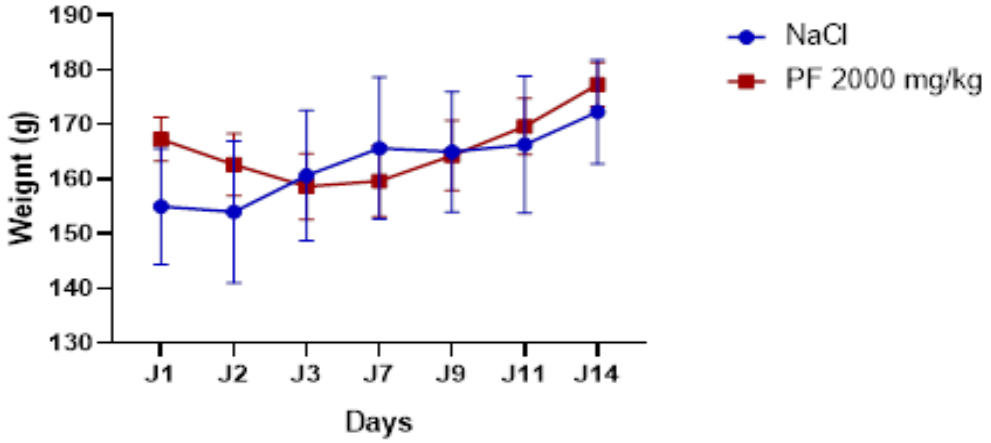

Figure 1: 14-day weight monitoring of rats given 'Pancreas free' at 2000 mg/kg. Kruskal Wallis test, p>0.05

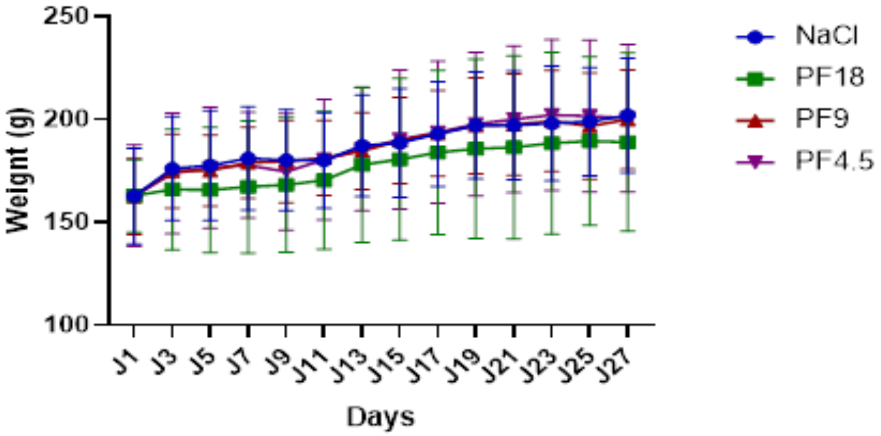

Figure 2: Weight monitoring of rats given Pancreas free for 28 consecutive days. Kruskal Wallis test, p>0.05

Hematological toxicity

The effect of "Pancreas free" on red blood cell (A), hemoglobin (B), white blood cell (C) and platelet counts (D) is shown in figure 3, which shows that administration of "Pancreas free" at 4.5 mg/kg, 9 mg/kg and 18 mg/kg for 28 consecutive days produced no significant disturbance in these hematological parameters (p>0.05). However, compared to the control group, there was a non-significant decrease in red blood cells and hemoglobin at 4.5 mg/kg, and a non-significant decrease in white blood cells at all doses (p>0.05). Other hematological parameters (neutrophils, monocytes, basophils, lymphocytes) were not significantly affected (p>0.05).

Liver and kidney toxicity

Figure 4 shows the effect of "Pancréas free" on transaminase activity (ASAT, ALAT). The evolution of ALAT and ASAT activity in rats treated with "Pancréas free" over 28 days was similar on that in rats given normal saline (p>0.05).

Figure 5 shows the effect of 28-day administration of "Pancreas free" on rat uremia (A) and creatinemia (B). It shows that administration of "Pancreas free" at doses of 4.5 mg/kg, 9 mg/kg and 18 mg/kg did not significantly affect uremia and creatinemia in rats compared to the control group (p>0.05).

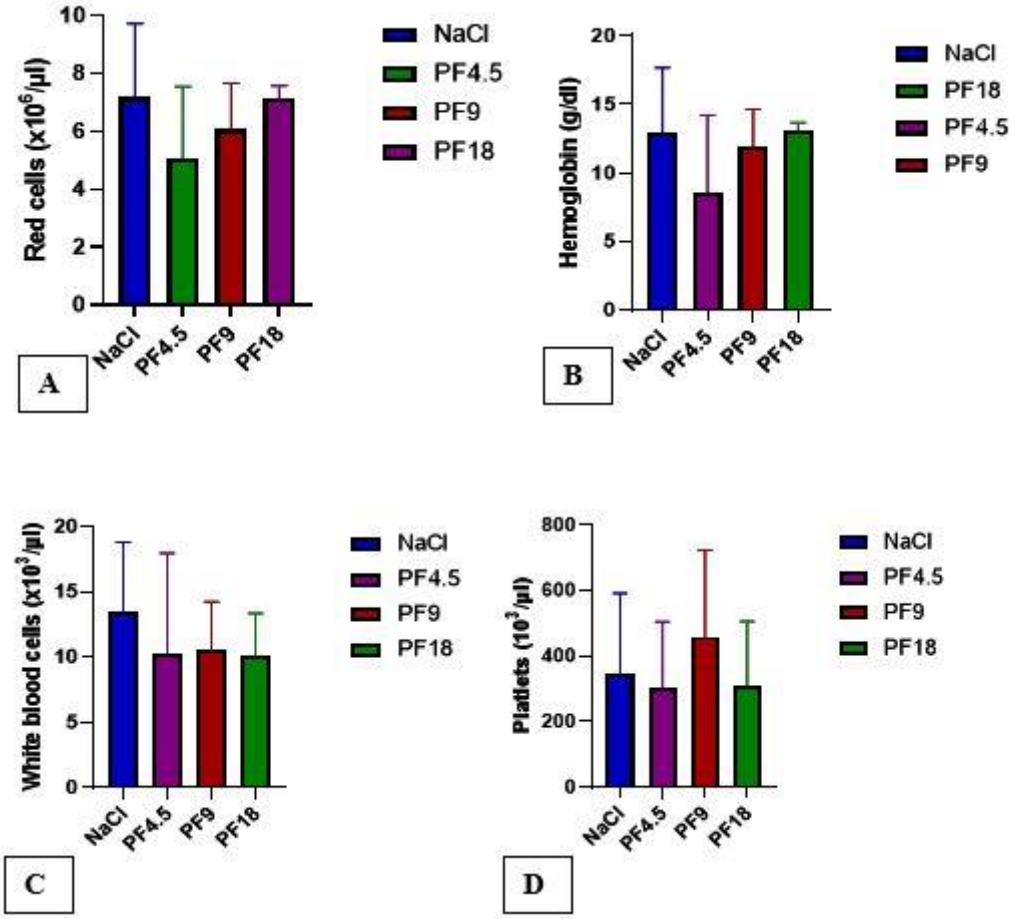

Figure 3: Effect of Pancreas free on hematological parameters: A (red blood cells), B (hemoglobin), C (white blood cells) and D (platelet counts). Kruskal Wallis test, p>0.05; PF="Pancreas free".

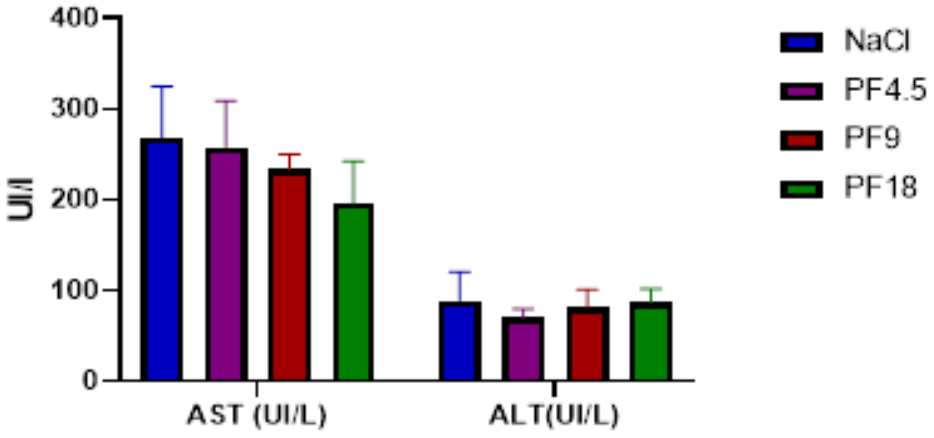

Figure 4: Effect of PF on rat transaminases. Kruskal Wallis test, p>0.05; PF: Pancreas free

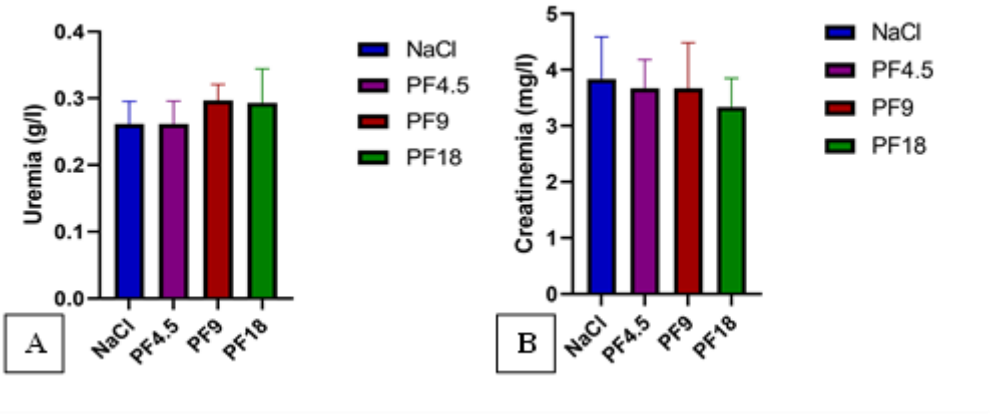

Figure 5: Effect of PF on rat uremia and creatinemia. Kruskal Wallis test, p>0.05; PF="Pancreas free".

Hypoglycemic risk

Figure 6 shows the effect of "Pancreas Free" at different doses on fasting blood glucose levels. During the 4h observation period, "Pancreas Free" at a dose of 4.5 mg/kg had little effect on fasting blood glucose levels in rats (p >0.05). At a dose of 9 mg/kg, there was a significant decrease in fasting blood glucose at 2 hours post-dosing (p = 0.01), but this hypoglycemia come to normal at the 3rd and 4th hours. "Pancreas free" at 18 mg/kg had no effect on fasting blood glucose levels in rats (p >0.05).

Antihyperglycemic activity

Figure 7 shows the effect of "Pancreas Free" at different doses on the prevention of orally induced hyperglycemia. "Pancreas Free at doses of 4.5 mg/kg and 18 mg/kg produced a non-significant reduction in glucose-induced hyperglycemia from the 3rd hour. At a dose of 9 mg/kg, there was also a non-significant drop in hyperglycemia from the 2nd hour, whereas glibenclamide, the standard antihyperglycemic drug in this study, was effective in preventing hyperglycemia during the 4 hours of observation (p<0.05).

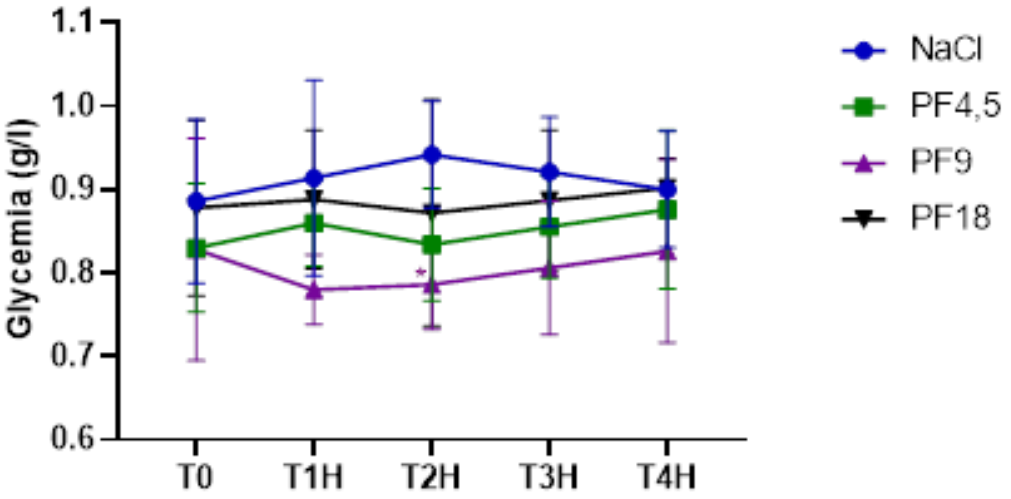

Figure 6: Effect of Pancreas Free on fasting blood glucose levels. Kruskal Wallis test, *p = 0.05; PF= "Pancreas free".

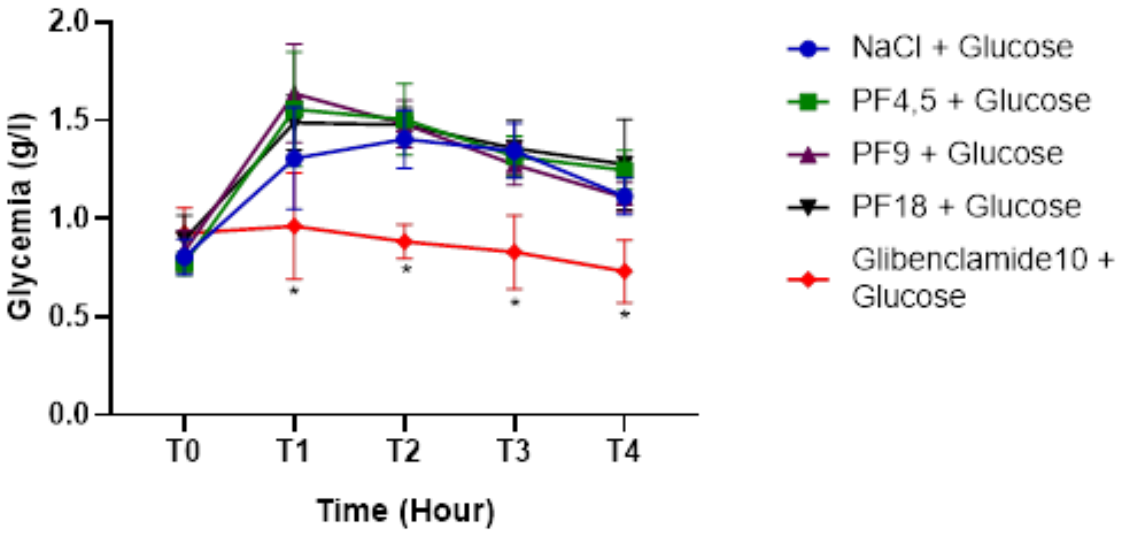

Figure 7: Anti-hyperglycemic effect of Pancreas free. Kruskal Wallis test, *p = 0.05; PF= "Pancreas free".

Discussion

The aim of this study was to assess the safety of "Pancreas free", an anti-diabetic remedy from an Ivorian traditional practitioner, in combination with "Diabetes free" for the treatment of diabetes in laboratory rats.

Safety was assessed according to OECD 423 acute toxicity [16] and OECD 407 sub-acute toxicity [17] guidelines, as well as by investigating the hypoglycemic risk [18] of "Pancréas Free" at doses of 4.5 mg/kg, 9 mg/kg and 18 mg/kg.

Assessment of the acute toxicity of "Pancréas Free" revealed no signs of toxicity in animals up to a dose of 2000mg/kg. However, the combination of "Pancréas free" and "Diabète free" caused death of rats at a dose of 1000 mg/kg [14].

The partial composition of "Pancréas free" includes ginseng and gurmar. Toxicity studies were carried out on both ingredients. Studies on the toxicity of ginseng in rats revealed an LD50 beyond the dose of 5000 mg/kg [19, 20]. Lee et al. also demonstrated that ginseng at doses of 5, 10 and 15 g/kg caused no mortality, behavioural changes or abnormal clinical signs [21]. Another study by Park et al. on laboratory rats also showed that gurmar did not significantly alter body weight [22].

The mortality observed in animals receiving the combination of "Pancréas free" and "Diabète free" at 1000 mg/kg [14] could therefore be attributed to a harmful interaction between the different components of the two remedies or to additional components in the second remedy. concerning subacute toxicity, administration of "Pancréas free" at doses of 4.5 mg/kg, 9 mg/kg, and 18 mg/kg for 28 consecutive days did not cause any signs of clinical, biological, or biochemical toxicity in laboratory rats. Pancréas free therefore has good clinical, hematological, hepatic, and renal tolerance. The combination of Pancréas free and Diabète free showed the same tolerance profile [14].

Ginseng and gurmar, components of Pancréas free, have also been studied for subacute toxicity. Chan et al. showed that administration of ginseng for 4 weeks at doses of 500 mg/kg, 1000 mg/kg, and 2000 mg/kg did not cause any weight abnormalities or signs of clinical or biological toxicity in animals [19]. Similarly, Lee et al. showed that ginseng did not cause any significant changes in body weight or impairment in renal or hematological functions in mice [21]. Regarding gurmar, Shiyovich et al. found no evidence of liver toxicity in laboratory animals [23]. However, Pothuraju et al. showed that gurmar caused a significant decrease in body weight in rats [24]. Furthermore, Raji et al. reported that at doses of 300 mg/kg and 600 mg/kg, gurmar caused weight loss and significant disturbances in some biochemical parameters (ASAT, ALAT, urea, creatinine), without causing any hematological toxic effects [20]. This difference in observation could be explained by the use of lower doses in the study. The assessment of the hypoglycemic risk of "Pancréas free" showed that this traditional remedy did not significantly

interfere with fasting blood glucose levels. These results are similar to the results obtained in our previous study, which indicated that the combination of "Pancréas free" and "Diabète free" increases a hypoglycemic risk [15]. The absence of hypoglycemic risk associated with "Pancréas free" could be attributed to its ginseng and gurmar content. Indeed, Vuksan et al. [25] demonstrated that ginseng can improve insulin sensitivity and promote glucose absorption by the body's cells without causing hypoglycemia. Wei et al. [26] also showed that the hypoglycemic risk of ginseng was more pronounced in diabetic subjects than in healthy subjects.

Regarding the anti-hyperglycemic activity of "Pancréas free," it showed a non-significant decrease in blood sugar levels after glucose overload. Our previous study on the combination of "Pancréas free" and "Diabète free" revealed significant anti-hyperglycemic activity [15]. According to the traditional practitioner, these remedies are most effective when combined, which could explain the lack of anti-hyperglycemic activity observed with "Pancréas free" alone. According to the traditional practitioner, "Pancréas free" stimulates pancreatic function, i.e., in the presence of hyperglycemia, "Pancréas free" stimulates the pancreas to secrete insulin in order to regulate blood sugar levels. We observed that "Pancréas free" alone generally led to a non-significant decrease in blood sugar levels from the third hour. A longer observation period could have confirmed or contradicted this property claimed by the traditional practitioner for "Pancréas free." Furthermore, studies conducted on the ginseng contained in "Pancréas free" have shown results that differ from ours. Vuksan et al. on ginseng [25] showed that consuming American ginseng before a meal significantly reduces postprandial blood sugar levels in healthy individuals. Kim et al. on [27] also showed that Korean red ginseng improves insulin sensitivity in patients with type 2 diabetes. Shanmugasundaram et al. [28] showed that administration of gurmar normalized blood sugar levels and regenerated pancreatic beta cells in diabetic rats. The difference observed in this study could be explained by the fact that "Pancréas Free" contains two additional undisclosed plant extracts, which could have interacted and attenuate the anti-hyperglycemic effect of ginseng and gurmar.

Conclusion

This study aimed to evaluate the safety and antihyperglycemic activity of "Pancréas Free," a remedy formulated by a traditional practitioner. In a single and massive administration, no signs of clinical toxicity or lethality were observed up to 2000 mg/kg. Concerning subacute toxicity, repeated administration of the remedy at the tested doses over 28 days did not induce behavioural changes or alterations in haematological and biological parameters used to assess renal and hepatic function in laboratory rats. "Pancréas Free" alone also showed weak anti-hyperglycemic activity without hypoglycemic risk. "Pancréas Free" therefore has a good safety profile. Its combination with "Diabète free" according to the traditional practitioner's protocol could be necessary for a better activity in the management of diabetes.

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Conflict of Interest

None declared.

Acknowledgement

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