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Research Article

Toxicological and anti-hyperglycemic studies of five plants used for the treatment of type 2 diabetes in Benin

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Abstract

In Benin as in the African sub-region, the burden of Non-Communicable Diseases (NCDs) and the threats they represent constitute a major public health problem which hinders their economic and social development. NCDs kill 36 million people each year and 80% of deaths occur in developing countries, including Benin. The objective of our work is to evaluate the acute, subacute toxicity and the anti-hyperglycemic activity of five plants listed through an ethno pharmacological survey which is used for the treatment of type 2 diabetes in Benin. The acute toxicity test was conducted on male and female wistar rats at a single dose of 2000 mg/kg. The subacute toxicity test was carried out over a period of 28 days, with 6 batches of 3 rats. Batch 1 received daily 1 ml/100 g of distilled water and batches 2, 3, 4 and 5 respectively the leaf extracts of *Bambusa vulgaris* Schrad. Ex-Wendel; *Parkia biglobosa* (Jacq.) R. Br. Ex G. Don; *Mangifera indica* L.; *Saccharum officinarum* L.; And *Annona muricata* L. at a daily dose of 200mg/kg per day for 28 days. Administration of the single dose of the extract did not cause any deaths. In rats treated with repeated doses of 200mg/kg for 28 days the variation in weight depends on the extracts. The level of transaminases (AST and ALT) did not vary. Plant extracts did not induce any significant change in creatinine level. All five extracts have anti-hyperglycemic activity. Our results allow us to deduce that these plants can be used to prevent and treat diabetes without the risk of hepatic and renal toxicity.

Keywords: Medicinal plants; Type 2 diabetes; Toxicity; efficacy

I INTRODUCTION

Diabetes is a serious chronic disease that occurs when the pancreas does not produce enough insulin, or when the body does not properly use the insulin it produces. Globally, 463 million people have diabetes worldwide, according to the International Diabetes Federation. This describes the phenomenon as a real pandemic, because the progression is considerable. The WHO predicts 700 million people with diabetes by 2045 and the biggest increases will be in countries moving from low to middle income. In Africa the number of diabetics is estimated at 19.4 million and in Benin this number is estimated at 44.6 thousand. One person dies of diabetes every 6 seconds worldwide, more than AIDS, tuberculosis and malaria ^{1,2}. According to the World Health Organization (2WHO, 2002) about 65-80% of the population of developing countries, due to poverty and lack of access to modern medicine, depend mainly on traditional medicinal plants for their Primary health care. Traditional, complementary and alternative medicine are commonly used to treat or prevent chronic diseases and to improve the quality of life of the population. There is some evidence that traditional medicine is promising. Many people use traditional medicine for self-medication, because a widespread misconception makes "natural" synonymous with "safe". People do not know what are the possible side effects of herbal medicines and how and

when they can be used safely ³. The taking of medicinal plants can be done in different types. These can be capsules, tablets, infusions, tinctures, extracts, raw herbs or in different forms including enemas or poultice applications ^{4,5}. Herbal medicines can be toxic if misused and cause damage to organs such as the liver and kidneys. We can cite in particular kidney failure linked to Chinese plants, cardiac damage by poisoning with aconite or lung damage linked to certain mints ^{6,7}. But it is certainly the liver damage that is the most striking ¹. The particular risks that contribute to the hepatotoxicity of medicinal plants are essentially linked to the poor identification of the plant, its storage or its packaging ⁸. Kidney lesions induced by medicinal plants can be reversible or permanent and involve the glomerulus, the tubule and the interstitium, these last two structures being the most frequently involved. Direct vascular damage can sometimes be involved and the resulting renal ischemia can generate secondary tubulo-interstitial lesions ⁹. Due to lack of quality control and lack of consumer information, cases of misuse of herbal preparations have been reported ³. The scientific promotion of traditional medicine should lead in particular to the development of effective and non-toxic herbal medicines. Benin has made great efforts since 2001 for a better organization of the practice of traditional medicine. It is up to researchers to verify the efficacy and safety of these herbal medicines. Little scientific data is available from tests

performed to assess the safety and effectiveness of herbal medicines. The objective of our work is to evaluate the acute, subacute toxicity and the anti-hyperglycemic activity of five plants used for the treatment of Diabetes in Benin.

II MATERIALS AND METHODS

II.1 Vegetal material

It consisted of the leaves of five medicinal plants selected for their use in the treatment of hypertension in some communes of Benin. These were the leaves of *Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum*, And *Annona muricata*. The plants were selected after an ethnopharmacological study considering their frequency of use by traditional healers and the existence of studies already carried out on the plants ¹⁰. They had been identified and authorized in the national herbarium of the University of Abomey Calavi. After harvesting, the samples were dried at laboratory temperature until their plant mass stabilized and then reduced to powder.

II.2 Animal Material

The experimental animals were male and female Wistar rats weighing between 150 and 250 g. All animals had health status of SPF (specific pathogen Exempt). Work on wistar rats were authorized by the national committee of ethics of Benin science academy. Upon receipt, the rats were divided into groups of five (5) and placed in standard cages for a period of acclimatization (2 weeks) before being used in various experiments. During this period the animals had free access to food and water and remained kept at constant temperature ($22 \pm 2^\circ\text{C}$). They were subjected to a light/dark cycle 12 h/12h. The dark phase of the cycle begins at 12 h and different experiences have always been held from 11AM to 6PM due to the nocturnal activity (active phase) of rats.

II.3 Preparation of hydroethanolic extracts

Ethanol and water were used as extraction solvents. For the hydro-ethanolic extraction, a quantity of 50 g of powder was macerated in 500 mL of the two solvents, i.e. 250 mL of distilled water and 250 mL of ethanol. The mixture was subjected to mechanical stirring for 24 hours at room temperature. After filtration, the extracts were lyophilized to obtain the dry crude extracts.

The yield was calculated using the following formula:

$$\text{Yield}(\%) = m/M \times 100$$

m = mass of the prepared extract; M = mass of the powder used.

II.4 Assessment of acute toxicity

The acute oral toxicity test was performed according to the guidelines of the Organization for Economic Co-operation and Development (OECD) ^[11]. The rats were fasted for 4 hours with free access to water. They were randomly divided into 6 groups of three rats and treated orally with a single dose of hydroethanolic extract of the leaves of the five plants (2000 mg/Kg) respectively or distilled water (20 ml/Kg) used as control. After the treatment, the rats were monitored and observed individually every hour for 4 hours and then every day for 14 days. An information sheet was produced for each group of rats in order to collect possible signs of toxicity (mortality, weight, changes in the skin, hair, eyes, somatomotor activity and behavior).

II.5 Assessment of subacute toxicity.

The subacute oral toxicity test was performed according to the 407 guidelines of the Organization for Economic Co-operation and Development (OECD). It was carried out on 18 albino

Wistar rats divided into six equal groups of 3 as follows: group 1, receiving daily distilled water at a rate of 1 ml/100 mg of body weight (control group); batches 2, 3, 4, 5 and 6 receiving daily a solution of the hydro-ethanolic extract of the leaves of the five plants at a rate of 200mg/kg of body weight respectively for each sample. The treatment lasted 28 days. Rats were fed and hydrated freely, then weighed every 14 days. A blood sample was taken before and at the end of the treatment. Several biochemical parameters were assayed in particular; creatinine by the Jaffé colorimetric method. Alanine aminotransferase by the optimized IFCC UV kinetic method, using the GPT-ALT LR GSMitalia kit and aspartate aminotransferase by the optimized IFCC UV kinetic method, using the GOT-AST LR GSMitalia kit.

II.6 Determination of Glucose Concentration in the Blood

The blood of the rats was drawn before treatment at day 0 and day 28 after treatment. Glucose assay is a colorimetric assay following two coupled enzymatic reactions. A closely specific enzymatic reaction (glucose oxidase) oxidizes glucose present in the sample to gluconic acid and hydrogen peroxide. It serves as the substrate for the peroxidase in a coupled reaction resulting in the oxidation of o-dianisidine to a colored product. The intensity of the color is proportional to the glucose concentration. A capsule of enzymes (glucose oxidase-peroxidase) is dissolved in 100 ml of water followed by 1.6 ml of solution of o-dianisidine. The reagent is ready to use and can be kept several days in the refrigerator. We conducted a blank tube containing only distilled water (20 µl), two standard tubes containing 20 µl of glucose (1 g/l). And then we have prepared two tubes each containing 20 µl of supernatant (blood plasma) for each sample. 2 ml of reagent was then added into each tube and all placed in an oven at 37° for 10 minutes. After steaming completed, the absorbance was measured by spectrophotometry at 470 nm, taking as zero the white tube. We kept the average of the two values for duplicate tubes when they are compatible. The glucose concentration is found with the following

$$\text{Calculation: } [G] = D_o/D_{sg} \times C_{sg}$$

With: D_o = absorbance, D_{sg} = Do standard glucose, C_{sg} = concentration standard glucose.

II.7 Statistical analyzes

The data (variation in weight, transaminases, creatinine) collected in the rats before and after treatment with the different plant extracts were entered into Excel software. R software was used for testing. Thus, statistical inference, the normality test (Ryan Joiner test) was used to verify the normality of the data. Levene's test was used to check the homogeneity of the variances. With $P < 0.05$.

III RESULTS

III.1 Yield of extractions

The yields obtained varied from one plant to another. They are between 13,2 and 19,2%. *Bambusa vulgaris* and *Mangifera indica* had the best yields (table 1).

Table 1: Yield of hydroethanolic extraction of each sample

Samples	Yields (%)
<i>Saccharum officinarum</i>	13,2
<i>Annona muricata</i>	17,6
<i>Bambusa vulgaris</i>	18,6
<i>Parkia biglobosa</i>	16,4
<i>Mangifera indica</i>	19,2

III. 2 Toxicological Activity

III.2-1 acute toxicity

✓ Variation of the weight of the rats during the acute toxicity test

From the analysis of the results, we noticed that there was no significant variation in the weight of the rats which received

the extracts of leaves of *Mangifera indica*, *Annona muricata* and *Bambusa vulgaris* and *Saccharum officinarum*. On the other hand, a significant increase was observed in the rats which received the *Parkia biglobosa* extract (P value = $0.03 < 0.05$) and the control rats (P value = 0.015 (Figure 1).

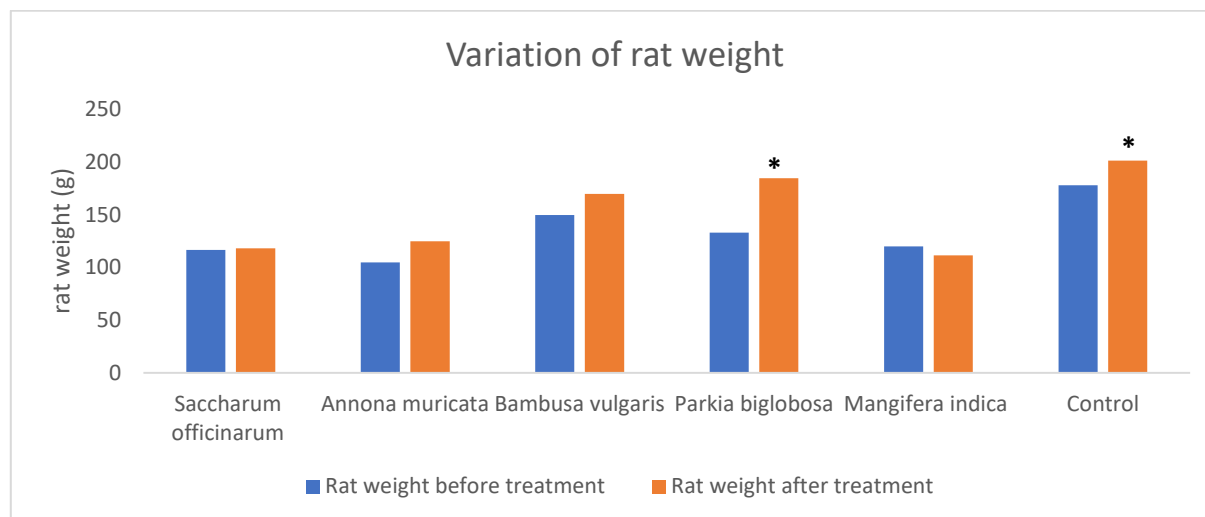


Figure 1: Weight change of rats during acute toxicity test (* $P < 0.05$)

✓ Mortality and other signs of toxicity

No mortality was recorded in the first hours after administration of the single dose of 2000 mg of leaf extracts of the five plants to rats. On the other hand, the treated rats

showed a general weakness compared to the control rats. After 14 days of observation, no death was observed in the treated rats. In addition, no other signs of toxicity such as tremor, reaction to noise, change in coat were observed (Table 2).

Table 2: Effect of single dose of extracts on mortality and other physiological parameters in rats.

group	Hydroethanolic extract	Number of deaths	Tremor	Abnormal motility	Reaction to noise	Coat change
1	<i>Saccharum officinarum</i>	0	No	No	No	No
2	<i>Annona muricata</i>	0	No	No	No	No
3	<i>Mangifera indica</i>	0	No	No	No	No
4	<i>Parkia biglobosa</i>	0	No	No	No	No
5	<i>Bambusa vulgaris</i>	0	No	No	No	No

III.2-2 Subacute toxicity

✓ Evolution of the weight of rats during the subacute toxicity test.

The rats which received the extracts of the leaves of *Annona muricata*, *Parkia biglobosa*, *Mangifera indica* showed no significant variation in their weight on the 14th and 28th day of treatment. On the other hand, in the rats which had received

Saccharum officinarum, a significant reduction (Pvalue = $0.019 < 0.05$) in weight was observed after the 28 days of treatment. In addition a significant increase (Pvalue = $0.013 < 0.05$) was observed in rats that received *Bambusa vulgaris* 14 days after treatment. In the controls, a non-significant increase was observed 14 and 28 days after treatment (figure 2).

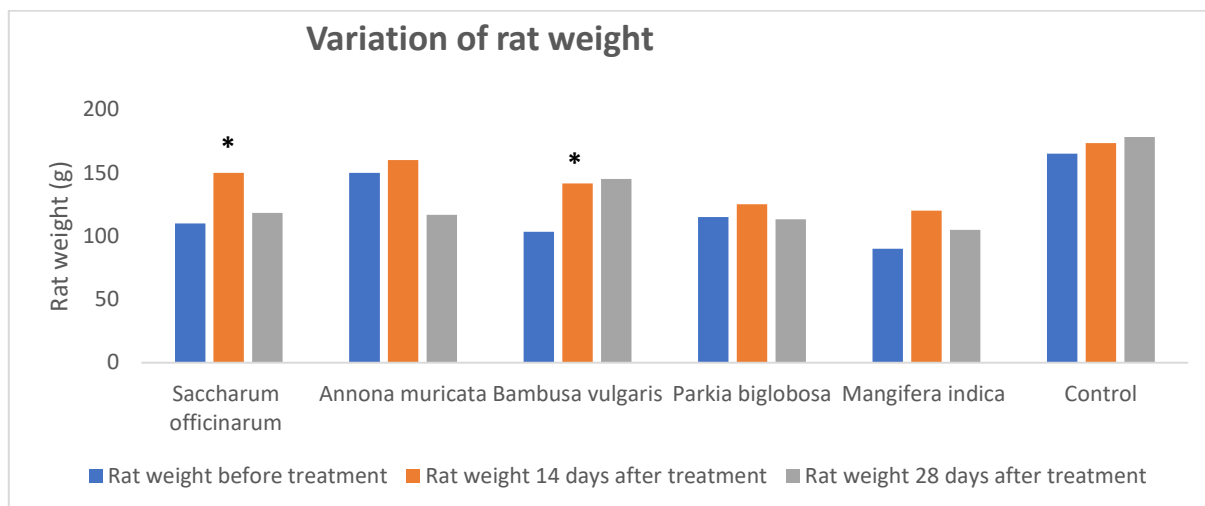


Figure 2: variation of rat weights 14 and 28 days after treatment with extracts (*= P<0,05)

✓ Variation in Alanine aminoTransaminase (ALT) Level

We noted a non-significant decrease in the level of alanine amino transaminase in rats treated with extracts from the leaves of *Saccharum officinarum*, *Bambusa vulgaris*, *Parkia*

biglobosa and *mangifera indica*. But a non-significant increase in ALT levels was observed in rats treated with the hydroethanolic extract of *Annona muricata*. In the control rats we noted a significant decrease (Pvalue=0.03595) in the level of Alanine transaminases (figure 3).

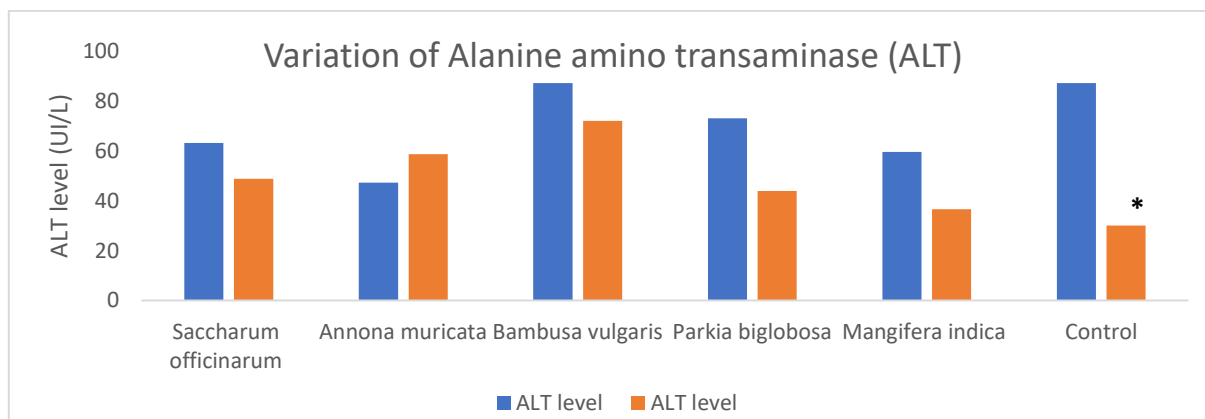


Figure 3: Variation of Alanine transaminase (ALT) in treated rats blood (* P<0,05).

✓ Variation in Aspartate amino Transaminase (AST) level

It appeared from the analysis of the results, that only in rats treated with the hydroethanolic extract of the leaves of

Bambusa vulgaris was observed a significant decrease in the level of AST. The other extracts do not show a significant variation in the ASAT level before and after treatment, as do the control rats (FIG. 4).

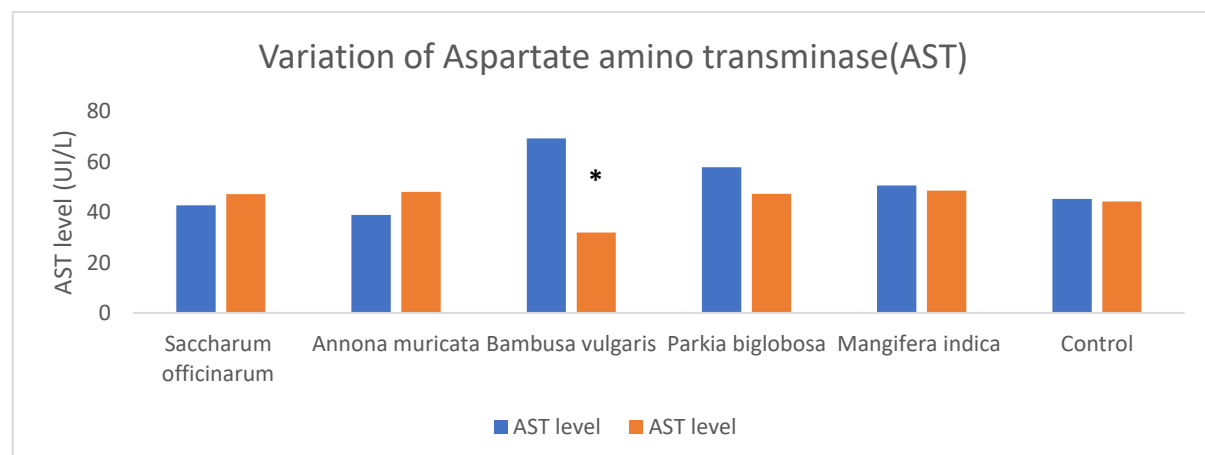


Figure 4 : Variation of Aspartate transaminase (AST) in treated rats blood

✓ Variation in Creatinine level

We noted a non-significant increase in creatinine in rats treated with the hydroethanolic extract of *Saccharum*

officinarum, *Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*. A non-significant decrease was recorded in rats that received *Annona muricata* as well as in control rats (Figure 5).

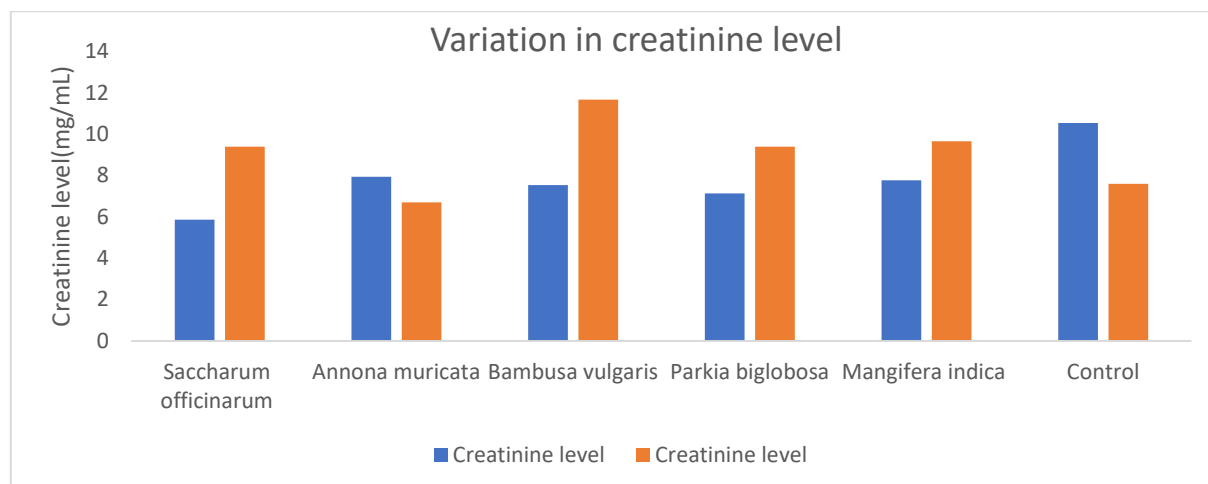


Figure 5: Variation of creatinine level in treated rat blood

III.3 Anti-hyperglycemic activity of plants

To verify the efficacy of the leaf extract of the 5 plants to lower glycemia, we measured the glycemia of the rats before and after 28 days treatment. Analysis of the results showed a non-significant increase in glycemia levels in rats treated with

Bambusa vulgaris and *Parkia biglobosa* extract. In rats treated with hydroethanolic extract of *Saccharum officinarum*, *Annona muricata* and *Mangifera indica*, the glycemia levels remained almost constant. On the other hand, we recorded a significant increase of blood sugar ($P\text{-value} = 0.0148 < 0.05$) in the control rats which received distilled water (figure 6).

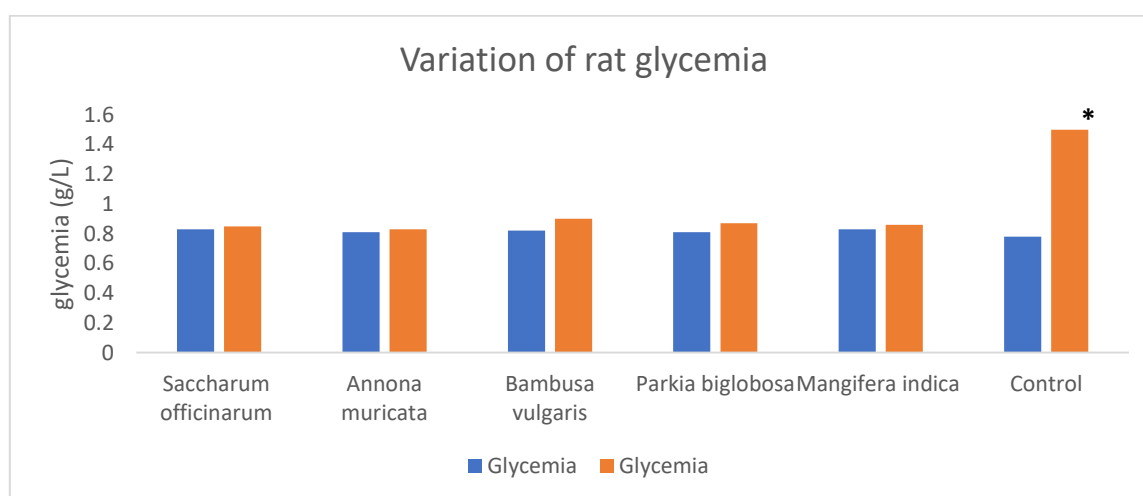


Figure 6: Variation in blood sugar levels in rats (* $P < 0.05$).

VI DISCUSSION

Plant Safety

acute toxicity

Checking the variation of body weight, food intake and general behaviors are important for the evaluation of the toxic effect of a substance because they are the first signs of toxicity^{11; 12}. The body weight of the rats treated under the conditions of acute toxicity at the single dose of 2000 mg/kg of the extract of *Annona muricata*, *Bambusa vulgaris*, *Saccharum officinarum* and *Mangifera indica* did not vary. A significant increase was observed in rats that received *Parkia biglobosa* extract. The weight gain of the rats treated with this plant as of the control rats could be explained by a good tolerance of the organism of

the rats to the leaf extracts of the plant which did not act on the food consumption of the rats during treatment¹³. The absence of signs of toxicity and death of the rats during the fourteen¹⁴ days of observation indicated that the hydroethanolic extracts administered orally up to a maximum dose of 2000mg/kg of our different samples are devoid of acute toxicity in rats under the conditions of our study. The chemical components of our extracts therefore appear to be non-toxic.

Subacute toxicity

The analysis of the results showed that the weight of the rats having received the hydroethanolic extracts of the leaves of *Annona muricata*, *Parkia biglobosa* and *Mangifera indica* and of the controls did not vary significantly during the treatment,

which could mean that these plants were well tolerated by rats. In addition, the weight of the rats treated with the hydroethanolic extract of *Bambusa vulgaris* increased significantly on the 14th day of treatment, which could mean that not only the rats tolerated the plant, it induced appetite stimulation in the rats ¹⁵. However, the rats treated with the extract of the leaves of *Saccharum officinarum* suffered a reduction in their weight on the 28th day of treatment. The duration of the treatment induced a lack of appetite in the rats which led to a reduction in their weight. Toxicity of this plant is suspected. The analyzes carried out showed no significant increase in AST and ALT transaminases in the rats treated with the dose of 200mg/kg of body weight. The sign of liver damage is the leakage of cellular enzyme into the plasma; when the plasma membrane of hepatocytes is damaged, a variety of enzymes normally located in the cytosol are released into the blood, their serum values are a useful marker for the extent and type of hepatocellular damage ¹⁶. ALT is a cytoplasmic enzyme found in very high concentrations in the liver and an increase in serum of this enzyme suggests hepatocellular damage. However, AST is an enzyme that is present in high amounts in the cytoplasm and mitochondria of various tissues, including liver, heart, skeletal muscle, kidney, and brain ^{17,18}. ALTs are more specific for liver damage, but ASTs are somewhat more sensitive ¹⁹. Their stability in rats treated with the plants suggested that our samples at this dose have no hepatotoxic effect. Our results showed no significant increase in creatinine in rats treated with the different extracts of the leaves of the 5 plants. In addition to the liver, the kidney plays an important role in the body's homeostasis, ensuring the filtration of toxic waste products from the bloodstream and their excretion in the urine ²⁰. Serum urea and creatinine are considered the primary markers of nephrotoxicity, although serum urea is often considered a more reliable predictor of kidney function than serum creatinine ²¹. The non-significant increase in creatinine allowed us to suggest that our plants do not induce renal toxicity.

Anti-hyperglycemic activity of plants

The glycaemia of the rats treated with the plant extracts did not vary after the 28 days of treatment, whereas that of the control rats increased significantly. This result demonstrated that our plants could play an anti-hyperglycemic role. Type 2 diabetes is characterized by permanent hyperglycaemia and is caused by insulin resistance created by several factors including oxidative stress ²². Indeed, hyperglycemia induces auto-oxidation of glucose which generates hydroxyl radicals ²³ and leads to the formation of advanced glycation products that influence the transcription of pro-inflammatory genes to further promote oxidative stress ^{24, 25}. In healthy subjects, hyperglycemia has also been associated with oxidative stress ²⁶. Several previous studies have shown that our plants are rich in phenolic acid and have significant anti-radical activity ^{27, 28, 29}. Polyphenols and phenolic acids have anti-hyperglycemic activity which could explain the stability of glycemia in healthy rats ²⁸. In diabetics, by reducing oxidative stress, plant extracts could prevent insulin resistance and thus could treat type 2 diabetes and its complications.

V CONCLUSION

Our results showed that the five plants studied do not induce acute toxicity and do not cause hepatic or renal toxicity when used for a period of 28 days. Plants also have an anti-hyperglycemic effect which could give them antidiabetic activity.

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