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Autoregulatory glycemic activity of the aqueous extract of cashew apple cake (*Anacardium occidentale* L.)

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Abstract
Type II diabetes is a concern for the whole world, given the ever-increasing number of people suffering from this pathology and the limitations of oral anti-diabetics. Agro-resources also possessing undeniable therapeutic effects represent an alternative in the fight against this pathology, hence our interest in the cashew apple. In addition to its nutritional potential, the cashew apple is used as an anti-diabetic. In the present study, the aim will be to evaluate these anti-hyperglycemic properties. In normo-glycemic mice, Aqueous extract of cashew apple, causes an increase in blood sugar. However, for increasing concentrations of cashew apple, ranging from 50 to 250 mg /Kg b.w., the glycemic peaks of these increases decrease from 56.14 to 61.02%. In mice made hyperglycemic, the aqueous extract of cashew apple with the same concentrations, shows a double activity on hyperglycemia induced by glucose at 3g/Kg b.w. For concentrations below 175 mg/Kg b.w., cashew apple has an anti-hyperglycemic effect which varies between 38.27 and 59.57%; on the other hand, above the tolerant dose of 200 mg/Kg b.w., cashew apple causes an 11.19 to 28.22% increase in glucose-induced hyperglycemia. The phytochemical screening of aqueous extract of cashew apple revealed the presence of tannin which could be at the origin of the anti-hyperglycemic effect of this natural essence. The toxicological study has shown that cashew apple, with an LD_{50} much greater than 1000 mg/Kg b.w., is not toxic. These data indicate that the aqueous extract of cashew apple cake may therefore contribute to the control of diabetes, provided that the concentrations of this extract do not exceed the tolerable dose in the presence of another hydrate source of carbon.

Keywords: *Anacardium occidental*, cashew apple, mouse, diabetes and anti-hyperglycemic agent

Introduction
Diabetes, a metabolic disease is characterized by chronic hyperglycemia [1]. In advanced stages, complications such as retinopathy, nephropathy, diabetic neuropathy, and cardiovascular disorders appear. Affecting more than 143 million people [2], it is predicted according to the American Diabetes Association [1] that 9% of the world's adult population will be diabetic by 2025. Having become a public health problem to date, given the increasing number of diabetics and the limitations of oral anti-diabetics, several avenues, including those of agro-resources, are being considered as part of its treatment. In view of its chemical composition, *Aanacardium Occidental*, could be one of these agricultural resources of interest. A tree 8 to 10 meters high, *Aanacardium Occidental* L belongs to the anacardiaceae family, which also includes mango (*Mangifera indica* L.) and pistachio (*Pistacia vera* L.). It has dense and branched foliage, its diameter can reach 12 to 15 meters [3]. The leaves are alternate, simple and leathery. The flowers, numerous, grouped in a terminal panicle [4], are mainly hermaphroditic, although up to 20% male flowers can be found [3]. The fruit, the cashew nut, hangs from a fleshy, juicy, red or yellow stalk when ripe called a cashew apple [5, 6].
In traditional medicine, the cashew apple, rich in vitamin C, acid, carotenoids and polyphenolic compounds, is used as an anti-flu and anti-diabetic. The caustic oil of walnuts, treats sores, warts and corns. Bark decocted, which appears to be the most widely used, is also an adjunct to diabetes, an anti-diarrhea, and an antihypertensive drug. The roasted fruit powder is said to be used against athlete's feet [7]. Although recognized as having therapeutic virtues in traditional medicine, the cashew apple has so far been the subject of few pharmacological studies.
The aim of the present study is to evaluate the anti-hyperglycemic properties of the aqueous extract of the cashew apple in mice.

Materials and Methods

Material

Plant material

The cashew apples harvested from the trees or picked up after they fell in December 2019 come from Korhogo, a town located in the far north of the Ivory Coast. Apples of red or yellow color without any injury were selected. They are then carefully separated from the nuts. Then, they were transported to the laboratory.

Animal material

Mice of the species *Mus musculus* (Muridae) male and female weighing between 20 and 30 g were used for the toxicological and pharmacological tests. Fed with granules supplied by the company IVOGRAIN, they were reared at an average temperature of 28°C with a relative humidity of 70%, at the pet shop of the Pasteur Institute of Côte d'Ivoire, located on the road to Dabou, Km17 Adiopodoumé (Abidjan, Côte d'Ivoire).

Chemicals and physiological fluid

- Glibenclamide arrow: (SANOFI-AVENTS (France).
- D (+) Glucose anhydrous: E. MERCK, Darmstadt (Germany).
- Nacl 0, 9%:

Methods

Extraction

The apples collected are cleaned, washed and then disinfected for 30 min with 100 ppm of active chlorine in tanks. They are then rinsed with water before being squeezed. Pressing and spinning are done manually. The cakes, separated from the juice, were dried in the shade between 25 and 28°C. Dried, these cakes are crushed and reduced to powder. This powder was used in the preparation of the aqueous extract of cashew apple. Fifty grams (50) of crushed cashew apple are mixed on a magnetic stirrer of the AGIMATIC-N type for 24 hours in a liter of distilled water. The resulting solution is filtered through cotton wool and Wattman paper. The same operation is repeated. Distilled water is added to the pellet, then mixed for 2 hours and also filtered. The filtrates are collected in a flask and dried in the study at 60° C. The powder obtained, perfectly soluble in water, is used as the aqueous extract of cashew apple.

Toxicological study

Acute toxicity study by gavage

For the study of the acute toxicity of the aqueous extract of cashew apple, we have 60 *Mus musculus* mice (males and females) at our disposal. Randomly divided into 6 batches of 10 animals each (5 males and 5 females), 5 batches were given increasing doses of CAJ aqueous extract, ranging from 100 to 2.10^3 mg/Kg b.w. orally with an intragastric tube. The control lot was treated with a saline solution of NaCl (0.9%). The number of dead mice was recorded after 30 minutes, two hours or even two weeks after administration. This study was performed 3 times.

Phytochemical screening

A phytochemical screening of the aqueous extract of cashew apple cake was carried out with a view to looking for the major groups of chemical constituents of pharmacological interest: sterols, polyphenols, polyterpenes, flavonoids, tannins, quinone compounds, saponosides and alkaloids [8]. The protocols used are those of Adjoungoua *et al.*, [9], Kablan *et al.*, [10] and Traoré *et al.*, [11].

Pharmacological study

Evaluation of the effect of the aqueous extract of the cashew apple on the glycemia of normo-glycemic mice

We have 25 mice divided into 5 batches, each batch comprising 5 animals. After 12 hours of fasting, the initial blood sugar level of the mice is taken, then they are subjected to the different treatments as indicated below:

- Batch 1: represents that of the control mice receiving 0.5ml of NaCl (0, 9%)
- Batch 2: mice treated with aqueous extract of cashew apple at 50 mg/Kg b.w.
- Batch 3: mice treated with aqueous extract of cashew apple at 140 mg/Kg b.w.
- Batch 4: mice treated with aqueous extract of cashew apple at 200 mg/Kg b.w.
- Batch 5: mice treated with aqueous extract of cashew apple at 250 mg/Kg b.w.

After these different treatments, the animals' blood sugar levels are measured every 30 minutes for 2 hours 30 minutes. The blood glucose level was measured using an ACCU-CHECK Active glucometer on blood taken from the tail of the mouse. The percentage change in blood glucose was calculated.

Evaluation of the hyperglycemic activity of glucose in normo-glycemic mice

We have 25 mice divided into 5 batches, each batch comprising 5 animals. After 12 hours of fasting, the initial blood sugar level of the mice is taken, then they are subjected to the different treatments as indicated below:

- Batch 2: mice treated with glucose at 3 mg/Kg b.w.
- Batch 2: mice treated with glucose at 2 mg/Kg b.w.
- Batch 3: mice treated with glucose at 1 g / Kg b.w.
- Batch 4: mice treated with glucose at 0.5 mg/Kg b.w.
- Batch 5: mice treated with glucose at 0.1 mg/Kg b.w.

After these different treatments, the animals' blood sugar levels are measured every 30 minutes for 2 hours 30 minutes. The blood glucose level was measured using an ACCU-CHECK Active glucometer on blood taken from the tail of the mouse. The percentage change in blood glucose was calculated.

Evaluation of the anti-hyperglycemic activity of the aqueous extract of cashew apple (CAJ) in mice temporarily hyperglycemic (HGPO test)

During this study, 25 male and female *Mus musculus* mice were divided into 5 batches of 5 animals each. These animals, after 12 hours fasting, were pre-treated with aqueous extract of cashew apple or glibenclamide (positive control) and then subjected to temporary hyperglycaemia by oral administration of a glucose solution at 3g/Kg b.w. [12]:

- Batch 1: control mice receiving glucose at 3g /Kg b.w.
- Batch 2: mice treated with aqueous extract of cashew apple at 85 mg/Kg b.w., then glucose at 3g/Kg b.w.
- Batch 3: mice treated with aqueous extract of cashew apple at 175 mg/Kg b.w., then glucose at 3g/Kg b.w.
- Batch 4: mice treated with aqueous extract of cashew apple at 200 mg/Kg b.w., then glucose at 3g/Kg b.w.
- Batch 5: mice treated with aqueous extract of cashew apple at 250 mg/Kg b.w., then glucose at 3g/Kg b.w.

The blood glucose levels of the mice in each batch are measured, just before administration and after treatment, at 30-minute intervals for 2 hours 30 minutes. The percentage change in blood glucose levels is calculated.

Evaluation of the anti-hyperglycemic activity of glibenclamide in mice made temporarily hyperglycaemic (HGPO test)

During this study, 25 male and female *Mus musculus* mice were divided into 5 batches of 5 animals each. These animals, after 12 hours of fasting, are pretreated with glibenclamide at increasing concentrations and then subjected to temporary hyperglycemia by oral administration of a glucose solution at 3g / Kg b.w. [12]:

- Batch 1: mice treated with NaCl (0.9%), then glucose at 3g / Kg b.w.
- Batch 2: mice treated with glibenclamide at 5 mg/Kg b.w., then glucose at 3g /Kg b.w.
- Batch 3: mice treated with glibenclamide at 10 mg/Kg b.w., then glucose at 3g /Kg b.w.
- Batch 4: mice treated with glibenclamide at 20 mg /Kg b.w., then glucose at 3g /Kg b.w.
- Batch 5: mice treated with glibenclamide at 30mg /Kg b.w., then glucose at 3g /Kg b.w.

The blood glucose levels of the mice in each batch are measured, just before administration and after treatment, at 30-minute intervals for 2 hours 30 minutes. The percentage change in blood glucose levels is calculated.

The biological tests were carried out in accordance with the internationally accepted principles of the European Community Directive (Council Directive 86/609/EEC of 24 November 1986).

Statistical analysis

Statistical data expressed as means ± standard error were obtained from the (n = 5) separate experiments. The averages calculated were compared from Student's test (t). When $p \leq 0.05$, the difference is said to be significant. The curves and statistical analysis were performed using Graph Pad Prism 5.1 San Diego, CA, USA.

Results

Acute toxicity study by gavage

The results of the acute gavage toxicity study in mice showed no deaths, with doses of 100, 500, 1000, 1500 and 2000 mg/Kg b.w. of the aqueous extract of cashew apple. All animals were observed over 14 days. The LD_{50} of the aqueous extract of cashew apple *was* greater than 2000 mg/Kg b.w.

Phytochemical screening

The qualitative tests carried out on this extract allowed us to obtain the results shown in Table 1. This Table 1, shows that the extract of the cashew apple contains catechetical tannins, alkaloids, sterols and polyterpenes, polyphenols, flavonoids and saponosides. On the other hand, there is an absence of quinones and gallic tannins in this extract.

Table 1: Chemical composition of the aqueous extract of cashew apple cake

Chemical constituents		Aqueous solution of cashew apple extract
Quinones		-
Tanins	Catechics	+
	Gallic	-
Alkaloids	B	+
	D	+
Sterols and polyterpenes		+
Polyphenols		+
Flavonoids		+
Saponosides		+

Presence (+), absence (-)

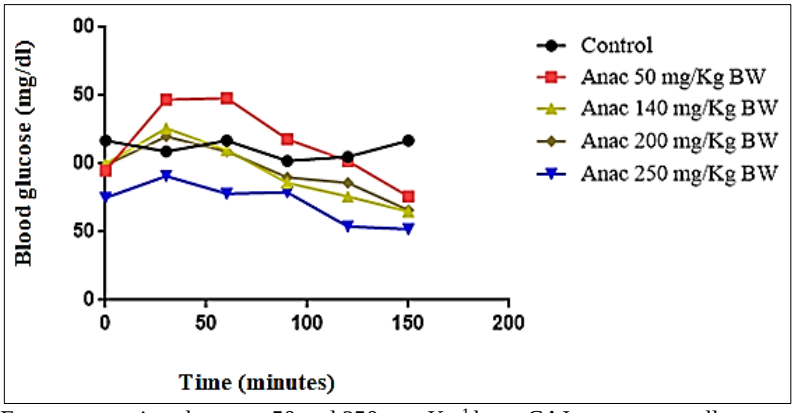
Pharmacological study

Dose-response effect of the aqueous extract of cashew apple cake and glucose on the Glycemia of Normoglycemic mice

Dose-response effect of the aqueous extract of cashew apple cake on the glycemia of normoglycemic mice

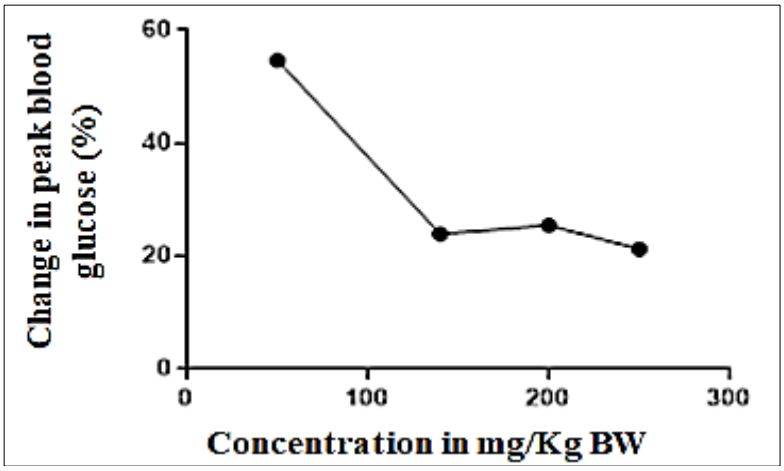
Figure 1 shows the dose-response effect of the aqueous extract of cashew apple cake on the blood sugar levels of normoglycemic mice. The oral administration of the aqueous extract of cashew apple cakes at concentrations between 50 and 250 mg. Kg^{-1} b.w. causes a small and less rapid increase in blood glucose, in normo-glycemic mice. This increase is 148 mg/dl and 109 mg/dl, respectively for cashew apple cake concentrations of 50 and 200 mg/Kg b.w., after 60 minutes. The maximum blood glucose level of 148 mg/dl is obtained with a concentration of the aqueous extract of cashew apple cake equal to 50 mg/Kg b.w. Compared to this, a significant reduction in the glucose level of 81.54% ($p \leq 0.01$) is recorded for cashew apple cake concentration corresponding to 200 mg/Kg b.w., after 60 minutes of treatment. These reductions in induced hyperglycemia increase with concentration. In addition, beyond ninety minutes, significant hypoglycaemia ($P < 0.05$-0.01) are recorded. These hypoglycaemias are respectively -35% and -30.66% ($P < 0.05$-0.01) for cashew apple cake concentrations of 140 mg/Kg and 250 mg/Kg b.w.

To better appreciate the anti-hyperglycemic effect of the aqueous extract of cashew apple cake, the glycemic peaks corresponding to the variation in blood glucose, 30 minutes after treatment, were shown as a function of the concentration of the extract of the cashew apple, in Figure 2**.** Figure 2 shows that oral administration of cashew apple cake in mice causes a decrease in the blood sugar peak. This decrease is significant and 56.14% ($p < 0.05$) for a concentration of aqueous extract of cashew apple of 140 $mg.Kg^{-1}$ b.w. It is rather 53.37% ($p < 0.05$) for a concentration of aqueous extract of cashew apple cake equal to 200 $mg.Kg^{-1}$b.w.



For concentrations between 50 and 250 mg. Kg^{-1} b.w., CAJ, causes a small and less rapid increase in blood glucose, which decreases with increasing concentration.

Fig 1: Dose-response effect of aqueous extract of cashew apple cake on blood glucose levels in normoglycemic mice



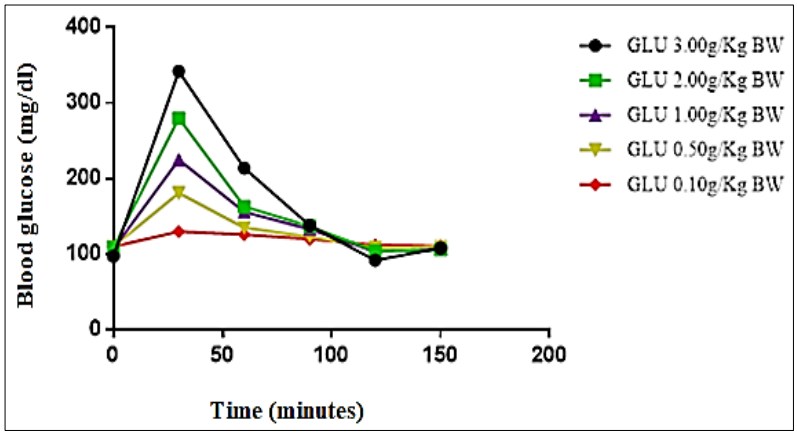
The glycemic peak recorded with CAJ, for concentrations between 50 and 250 mg. Kg^{-1} b.w., decreases as its concentration increases.

Fig 2: Variation of the glycemic peak in the presence of increasing concentration of the aqueous extract of cashew apple cake (CAJ)

Dose-response effect of glucose on glycemia in normoglycemic mice

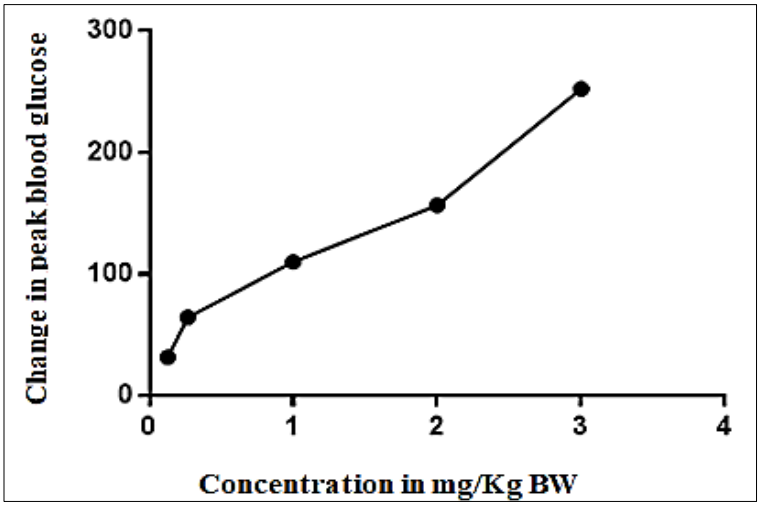
Figure 3 shows the dose-response effect of glucose on the blood glucose levels of normo-glycemic mice. The oral administration of glucose in concentrations ranging from 0.1 to 3.0 g/Kg b.w., causes a rapid and high increase in blood sugar in normal-glycemic mice. This increase is 130 mg/dl for a glucose concentration of 0.1 g/Kg b.w. It is maximum and 342 mg/dl, for a concentration of 3 g/Kg b.w., of glucose. At this concentration, reactive hypoglycemia is also recorded around 120 minutes.

Figure 4: This figure shows that oral administration of glucose in increasing dose, in mice, causes a dose-dependent increase in the glycemic peak. At 0.10 g/Kg b.w., the glycemic peak is 31.81% (P<0.05). This glycemic peak is 64.54% (P<0.05), for a glucose concentration equal to 0.5 g/Kg b.w. The maximum glycemic peak of 252.52% (P<0.01) is reached with a glucose concentration equal to 3.0 g/Kg b.w.



Glucose for concentrations ranging from 0.1 to 3.0 g/Kg b.w., causes a rapid and high dose-dependent increase in blood glucose

Fig 3: Dose-response effect of glucose on blood glucose in normoglycemic mice



Glucose for concentrations ranging from 0.1 to 3.0 g/Kg b.w., causes a dose-dependent increase in peak blood glucose

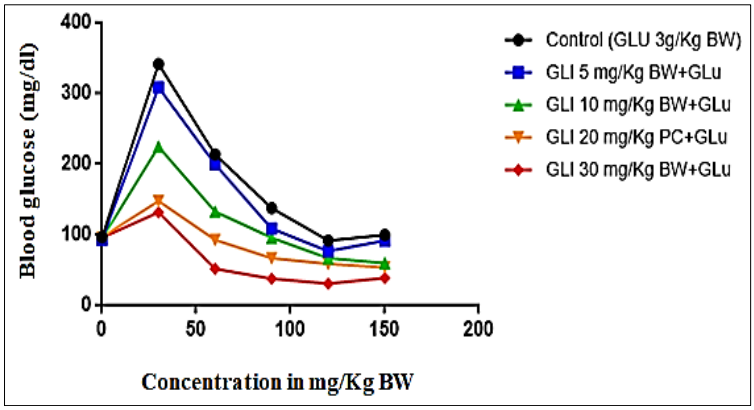
Fig 4: Change in peak glycaemia in mice, depending on of the glucose concentration administered in g/kg b.w.

Dose-response effect of the aqueous extract of cashew apple or of Glibenclamide on the glycemia of mice made hyper-glycemic by oral administration of glucose (HGPO test).

Dose-response effect of Glibenclamide on the glycaemia of mice rendered hyperglycemic by oral administration of glucose (HGPO test)

Figure 5, shows the dose response effect of Glibenclamide on hyperglycemia induced by oral glucose administration in mice.

Glibenclamide decreases in a dose-dependent manner the hyperglycemia induced by oral administration of glucose at 3g/Kg b.w., in mice. For a Glibenclamide concentration of 5 mg/kg b.w., a blood glucose level of 200 mg/dl is recorded, corresponding to a 5.56% reduction in hyperglycemia induced by glucose, after 60 minutes of treatment. In the presence of a Glibenclamide concentration of 20 mg/Kg b.w., the blood sugar level is 93 mg/dl, after 60 minutes of treatment. Compared to that of the control of 97 mg/dl, this value represents a significant hypoglycemia of -4.21 ± 1.5 (P<0.01), after 60 minutes of treatment. With 30 mg/ Kg b.w., the antihyperglycemic effect of Glibenclamide is maximum, it results in hypoglycemia beyond 30 minutes.



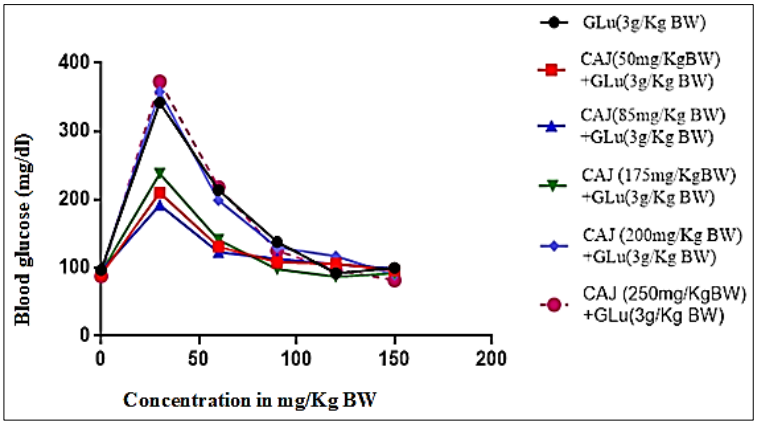
Glibenclamide decreases in a dose-dependent manner the hyperglycemia induced by the oral administration of glucose at 3g/Kg BW.

Fig 5: Dose-response effect of glibenclamide on blood glucose in hyper-glycemic mice

Dose-response effect of the aqueous extract of cashew apple on the blood glucose levels of hyper-glycemic mice (HGPO test)

Figure 6, shows the dose response effect of the aqueous extract of cashew apple cake on hyperglycemia induced by oral administration of glucose, in mice, at 3 g/kg b.w. It shows a double effect of the aqueous extract of cashew apple cake on the glycemia of mice made hyperglycemic by oral administration of glucose.

Concentrations of CAJ ranging from 50 to 175 mg/Kg b.w., decrease hyperglycemia caused by glucose to 3 g/Kg b.w., in mice. Whereas those of the cashew apple included between 200 and 250 mg/Kg PC, cause a not significant increase of this hyper-glycemia. For a cashew apple cake concentration of 85 mg/Kg b.w., a glucose level of 123 mg/dl is recorded after 60 minutes of treatment. However, at a concentration of 250 mg/Kg b.w., the glucose level is 218 mg/dl compared to the reference value of 214 mg/dl. These values reflect a significant decrease in blood glucose of 75.56% (p<0.01) for cashew apple cake concentration of 85 mg/Kg b.w. A non-significant increase in glucose level of 22.47%, is instead obtained with a CAJ concentration of 250 mg/Kg b.w.



CAJ has a dual effect on the blood glucose of mice made hyperglycemic by oral administration of glucose at 3 g/Kg PC. CAJ from 50 to 175 mg/Kg PC, decrease the induced hyperglycemia; on the other hand between 200 and 250 mg/Kg PC, CAJ enhances the induced hyperglycemia

Fig 6: Dose-response effect of the aqueous extract on blood glucose in hyper-glycemic mice

Discussion

The objective of this study is to evaluate the anti-hyperglycemic properties of the aqueous extract of cashew apple cake in mice.

The oral administration of the aqueous extract of cashew apple cakes, increases the glycemia in normo-glycemic mice, as well as glucose. This increase can be explained by the presence in this extract of reducing sugars (fructose, glucose), as shown by numerous studies [5, 13]. However, in the presence of an increasing dose of aqueous extract of cashew apple cake, between 50 and 250mg/Kg b.w., the decrease in glycemic peaks recorded, suggests that in addition to the presence of reducing sugars in this natural extract, there would be substances with antagonistic effects, otherwise anti-hyperglycemic. Associated with the low and less rapid increases in blood glucose, the hypoglycemia observed beyond the ninety minutes, also seem to testify to this. The thesis of a reactive hypoglycemia cannot be considered in this case, because it implies a rapid and high increase in blood glucose, hyperinsulinism due to a high presence of carbohydrate [14]. This natural substance would therefore have a self-regulating glycemic activity. Fruits and vegetables with anti-hyperglycemic effects exist, such as *Raphia gentiliana* De Wild [15], garlic (*Allium sativum*) and onion (*Allium cepa*) [16].

In order to confirm these results, tests of hyperglycemia induced by oral administration of glucose at 3.0 g/Kg b.w., in the presence and absence of glibenclamide or cashew apple cake, were performed. The oral administration of glucose at 3.0 g/Kg b.w., causes hyperglycemia, in mice. The glycemic peak is reached 30 minutes after glucose administration. These results are similar to those of N'Doua *et al.*, [17] and Masunda *et al.*, [15] obtained in rats and mice respectively. They are also reminiscent of those of Adam *et al.*, [18] carried out with rabbits, in which the glycemic peak is obtained 90 minutes after glucose administration. In the presence of glibenclamide at concentrations between 5 and 30 mg/Kg b.w., the glycemic peak induced by glucose at 3.0g /Kg b.w., decreases in a dose-dependent manner. On the other hand, with cashew apple cake, a dual activity is observed. Cashew apple, for concentrations ranging from 50 to 175 mg/Kg b.w., decreases the hyperglycemia induced by glucose, to be without notable anti-hyperglycemic effect at 200 mg/Kg b.w. Beyond this tolerant concentration, the extract of this natural substance, causes an increase of this hyperglycemia, although not significant according to the statistical analysis.

These results support the idea of coexistence of groups of molecules with opposite effects in the aqueous extract of cashew apple cake. They are also partly reminiscent of the results obtained with *Mormordica charantia*, following hyperglycemia induced by alloxane monohydrate in mice [2].

According to the authors of this work, the antihyperglycemic activity of *Mormordica charantia* decreases above 200mg/Kg b.w., the most active antihyperglycemic dose. This most active dose is higher than those of cashew apple cake and *colocynthis vulgaris* [18], which are respectively 85 mg/Kg b.w. and 100 mg/Kg b.w. Like *Mormordica charantia* [2], the anti-hyperglycemic effect of CAJ does not follow a linear dose-response relationship, commonly encountered in pharmacology.

The anti-hyperglycemic effects of the aqueous extract of cashew apple cakes could be explained by the presence in this natural substance of tannins of pharmacological groups recognized as anti-hyperglycemic [17]. This extract also contains other molecules of pharmacological interest (flavonoids and sterol polyphenols and polyterpenes, alkaloids, saponosides) that can also justify these effects. According to the OCDE 423 protocols [19] and the toxicological data of Clarke and Clarke (1977) [20], this plant substance, like other food plants [2], is not toxic because its LD_{50} is well above 1000 mg/Kg b.w.

Conclusion

The non-toxic aqueous extract of cashew apple cakes contains, in addition to carbohydrates (glucose, fructose etc.), anti-hyperglycemic substances. The presence of these anti-hyperglycemic substances in this natural essence gives it a self-regulating property of glycemia.

The catechic tannins present in this vegetable substance could be at the origin of its anti-hyperglycemic effect. The use in this form of the cashew apple cake in the fight against diabetes, can be considered, provided that its tolerant dose is not exceeded in the presence of another source of carbohydrate whose glycemic load it could optimize. To identify and elucidate the mechanism of action, the molecular group responsible for the said effects, fractionation tests are necessary

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

DM: protocols, ethno-botanical survey, experiments, analyses, interpretations and writing.

NAM., S.D.: Provision of products for the experiments, and of equipment for the extraction of the aqueous extract of cashew apple cakes

CA: Contribution to pharmacological analysis and interpretation

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