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Evaluation of the antihyperglycemic activity of mature and immature *Blighia sapida* arils (Koenig, 1782) on Wistar rats

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ABSTRACT

The objective of this study was to evaluate the antihyperglycemic activity of *Blighia sapida* arils (mature and immature) on Wistar rats. Forty-two (42) rats were divided into seven batches. They were made hyperglycemic by sugar water administration at concentration of 2500 mg/L. Rats in batches 1, 2 and 3 were administered 200, 400 and 800 mg/Kg of body weight of mature arils of *Blighia sapida* respectively, while those in batches 4, 5 and 6 received 200, 400 and 800 mg/kg of body weight of immature arils respectively. As for the control batch, it received distilled water. Immature arils of *Blighia sapida* lower blood sugar levels more than mature arils. The decrease of glycemia can be justified by the presence of hypoglycin A which is higher in immature arils than mature arils. The use of hypoglycin A as an antidiabetic agent should be considered. Further, studies are needed to evaluate the impact of blocking β-oxidation on insulin secretion. It would also be important to determine therapeutic doses, which should be low given the known toxic effects of hypoglycin A.

Keywords: antihyperglycemic activity, *Blighia sapida* arils, rats

INTRODUCTION

According to the latest data published by the Lancet, more than 800 millions of adults are diabetics. This number of diabetics is four time higher than the number of diabetics in 1990 **[1]**. This number of diabetics represents 11.1% of people in this group of age **[2]**. The number of diabetics could exceed 853 million (13 % of adults) by 2050 **[3]**. Diabetes (type 1 diabetes and type 2 diabetes) are major public health problem that affects all countries **[4]**, but there are disparities in their prevalence depending on the regions of the world. In fact, contrary to popular belief, diabetes are not only affect people of developed countries because low- and middle-income countries, particularly Africa countries **[5, 6]**, Asia countries **[7]** and South and Central America countries **[8]** are seeing their number of diabetics rise rapidly. Today, four out of five people with diabetes live in low- and middle-income countries **[1]**. According to the estimation, 24 million people had diabetes in Africa in 2021, and this number is expected to rise to 55 million by 2045, an increase of 129% **[9]**. This growth, which affect negatively African economy **[10]**, can be explained by a rapid urbanization, the adoption of sedentary lifestyles and the transition to diets rich in high sugars and fats **[11]**.

To fight against this pandemic in Africa, World Health Organization (WHO) has adopted a strategic plan that aims to revolutionize the prevention, early diagnosis and management of diabetes by emphasizing primary health care and integrating diabetes care into existing health services. This strategy has mixed results since half of the population with diabetes in Africa is not diagnosed **[12]** and accessibility to modern treatments are limited **[13]**. All this means that in Africa, premature deaths due to diabetes (defined as deaths occurring before the age of 70) amount to 58%, the global average defined by the WHO being 48% **[12]**. Local solutions should be sought through local food plants which contain molecules that could have beneficial effects against hyperglycemia. Hypoglycin A, a water-soluble molecule which is more abundant in the aril (part of the fruit consumed) of immature *Blighia sapida* than in the mature aril, would

contribute to reduce hyperglycemia associated with diabetes. Indeed, this molecule blocks ß-oxidation by inducing a hypoglycemic coma **[14]**. Then, lipids could not be a source of energy, and then the exclusive use of glucose leads to a hypoglycemic coma which can even lead to death **[14]**. Initially, arils of *Blighia sapida* are not used in traditional medicine, but the fact that they cause hypoglycemia could lead to believe that this molecule would be effective against hyperglycemia and therefore they can constitute a solution to fight against diabetes in Africa countries where the plant *Blighia sapida* is endemic **[15]**. To verify this, a study was conducted. The objective of this work was to contribute to the prevention and treatment of diabetes by evaluating the antihyperglycemic activity of hypoglycin A contained in the aril of *Blighia sapida*.

MATERIALS AND METHODS

Harvesting and Extraction

Immature and mature capsules of *Blighia sapida* were harvested from a field in Korhogo department. Placed separately in plastic bags, they were transported to the Biology Laboratory of Peleforo GON COULIBALY University. At the laboratory, arils were removed from the capsules using a knife, rinsed with distilled water to remove any dirt or impurities and put in transparent plastic bags. After that, each kind of arils (mature or immature) were stored in a freezer until their used. For each group of arils (immature and mature), 500 g were weighed, placed in 1 liter of distilled water and the whole was ground using a blender. The ground material from each group of arils (immature and mature) was filtered through a fine sieve. The filtrate from each group of arils (immature and mature) was placed in different porcelain plates to be dried in an oven at 50°C during 48 hours. After the drying period, the plates were removed from the oven and placed at room temperature to be cool. The dry mass at the bottom of the plates of each group of arils was scraped and put into a blender to be powdered. The powder

obtained from each group of arils was put into plastic jars. The jars of each group of arils were identified, sealed tightly and stored in a cupboard. **Figure 1** shows the methodology used to obtain *Blighia sapida* arils powders.

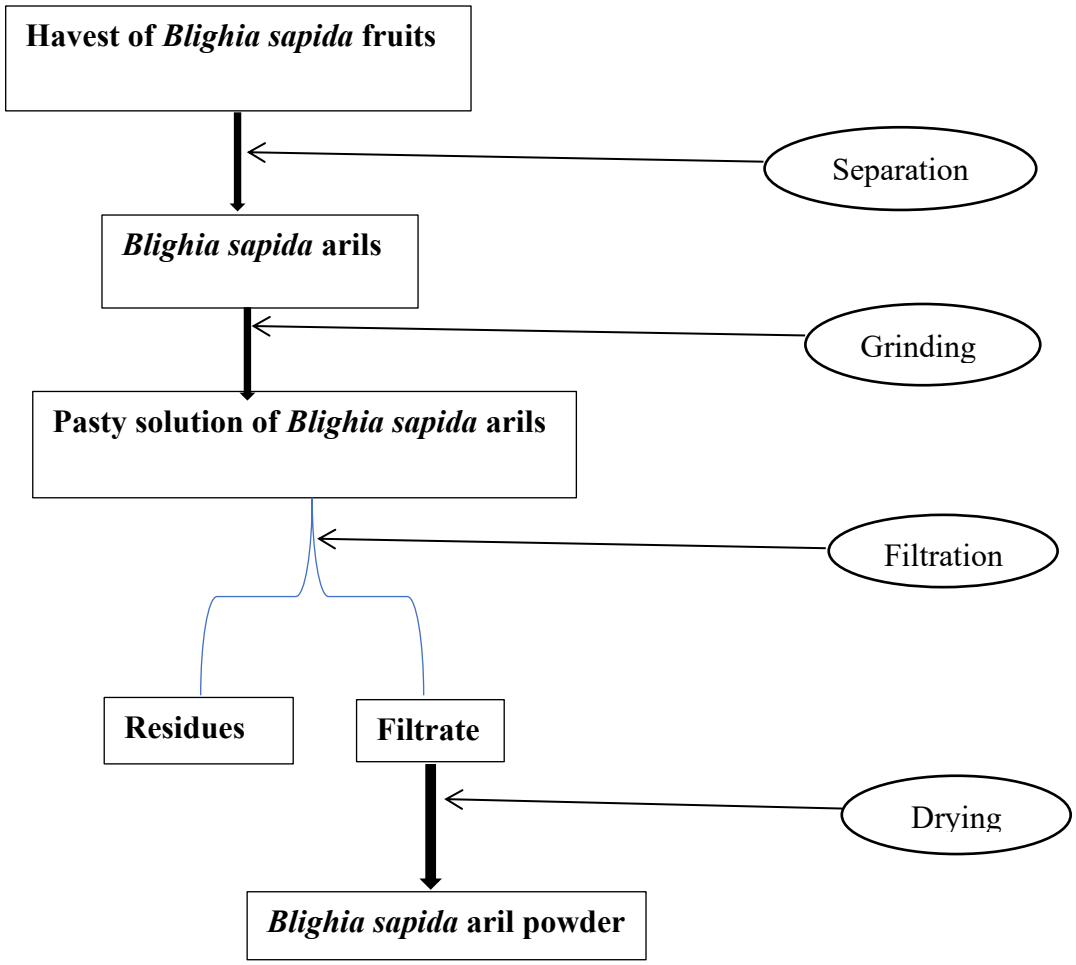

Figure 1: Methodology used to obtain *Blighia sapida* arils powders

Rats raising

Albino Wistar rats were used. They were raised in breeding cages at the animal facility of the Department of Biological Sciences of Peleforo GON COULIBALY University in Korhogo.

During the breeding period, they were fed with a food prepared made by a society call Ivograin who is specialized in animal food formulation. This food is made up of crude protein matter (15%), crude fat matter (3.5%), cellulose matter (12%), mineral matter (9%), calcium (1%), phosphorus (0.9%), sodium (0.3%), vitamin A (15000 UI/kg), vitamin D3 (3000 UI/kg) and vitamin E (10 mg/kg).

Experimentation

42 Wistar rats weighing between $127 \pm 12\ g$ were used. They were divided into seven groups (six per group including 3 males and 3 females). To avoid reproduction, male rats of each group formed were separated from females during the experimental period.

Rats of each group were first made hyperglycemic by sugar water administration at concentration of 2500 mg/liter for one week.

After being made hyperglycemic, they underwent specific treatments for 21 days. The rats in the control group received distilled water by gavage. The rats in groups 1, 2 and 3 received 200, 400 and 800 mg/kg bw of mature arils by gavage, respectively. Those in batches 4, 5 and 6 received 200, 400 and 800 mg/kg bw of immature arils respectively. During the specific treatments, rats of each group still received sugar water at a concentration of 2500 mg/liter. During each gavage, the total volume of each preparation administered was 1 mL and the gavage process was made every day during the duration of specific treatments (21 days). Blood sugar levels of each rat was taken at the beginning of the experimentation (day 0) and at day 7, day 14 and at day 28).

Animals were observed throughout the duration of the experimentation in order to note every abnormality. Any death observed was recorded. Water content in feces was quantified using an oven. During the experimentation, they received the same diet which was consumed during the

breeding period. The environment of the experiment was maintained at temperature of 25±2°C with dark and light cycle of 12h/12 h.

Blood glucose measurement on each rat process

The saphenous vein was identified on the left hind paw of each rat. After that, a sterile blade was used to shave the hair around the saphenous vein. The shaved area was carefully disinfected with 90% alcohol. To ensure the Accu-cheik glucometer was working properly, a blank strip was inserted into it, and the glucometer's signal requesting a drop of blood confirmed that it was ready for use. Using a fine sterile needle, the saphenous vein was pricked, and the drop of blood from the vein was placed on the strip inserted into the glucometer. The value displayed by the glucometer was the blood glucose level of the punctured rat. The pricked area was disinfected with 90° alcohol and rat was monitored to ensure that it recovered normally.

Statistical analysis

The results were analyzed using Statistica software version 7.1. Before comparing the means, the Fisher test was used to compare the variances of the groups in order to verify the assumption of homogeneity of variances. In case of homogeneity, the Student T test was used to compare the glycemic means of the groups two by two. Otherwise, the modified Student T (Welch T test) was used to compare the glycemic means of the groups two by two.

RESULTS

Blood glucose measurement at the beginning of the experiment (day 0)

The average weights obtained in the different groups of rats were not significantly different ($p \geq 0.05$) from each other. When the different blood sugar was measured to each rat of each group, we noticed that there was no significant variation ($p \geq 0.05$) from each of groups **(Figure 2)**.

After one week of sugar water administration, the blood glucose levels of rats in the groups receiving sugar water were not significantly different ($p \geq 0.05$) from each other, but they were elevated ($p \leq 0.05$) compared to the negative control **(Figure 3)**.

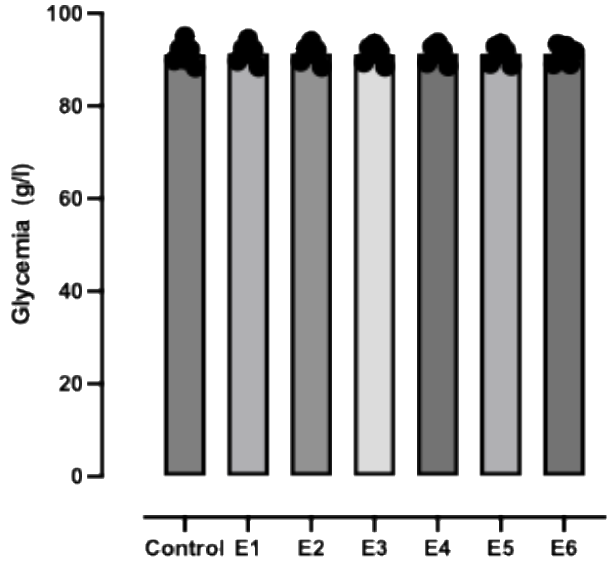

Figure 2 which showed the mean glycemia of each group of rats at the beginning of the experimentation

Control: control group; E1: Trial 1; E2: Trial 2; E3: Trial 3; E4: Trial 4; E5: Trial 5. There is no significant difference of glycemia between the groups ($p \geq 0.05$)

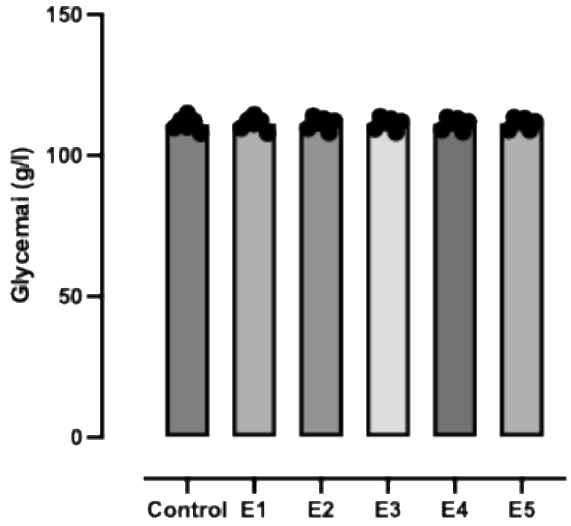

Figure 3 which showed the mean glycemia measured of each group of rats after the used of sugar water during one week

Control: control group; E1: Trial 1; E2: Trial 2; E3: Trial 3; E4: Trial 4; E5: Trial 5. There is no significant difference of glycemia between the groups ($p \geq 0.05$)

Blood glucose measurement after treatment on days 14 and 28

Regardless of the kind of arils used (mature or immature), for the same dose administered, more the dose was highest, more blood glucose measured was low **(Figure 4 and Figure 5)**.

At the same dose, blood glucose was more lowered ($p \leq 0.05$) in rats that received immature aril solutions than in those that received mature aril solutions **(Figure 6)**.

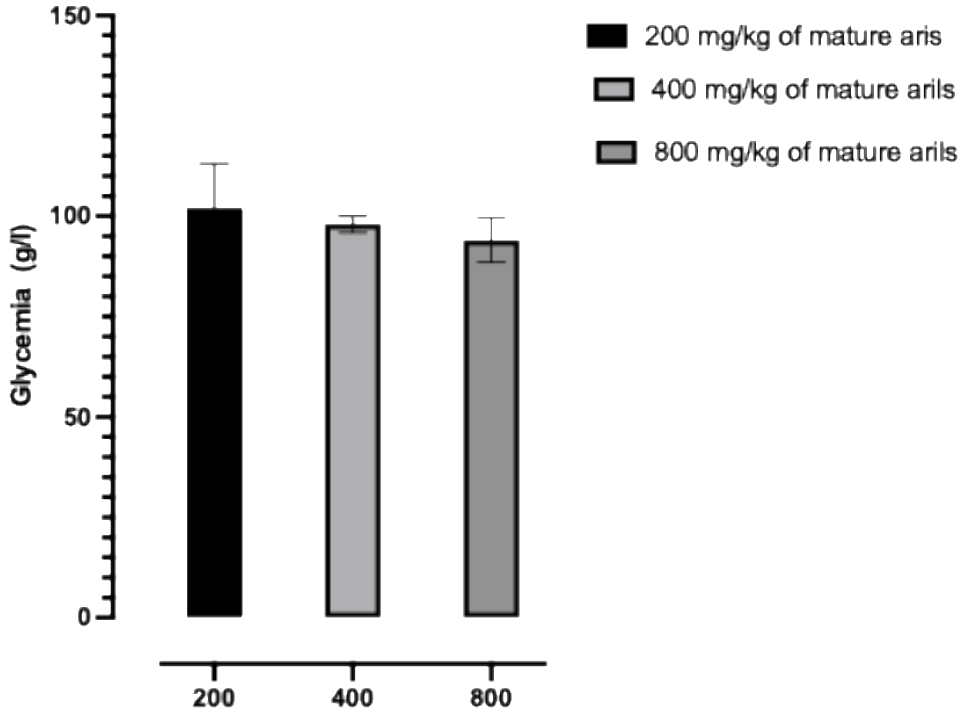

Figure 4 show the variation of glycemia depending to the dose of mature arils administered

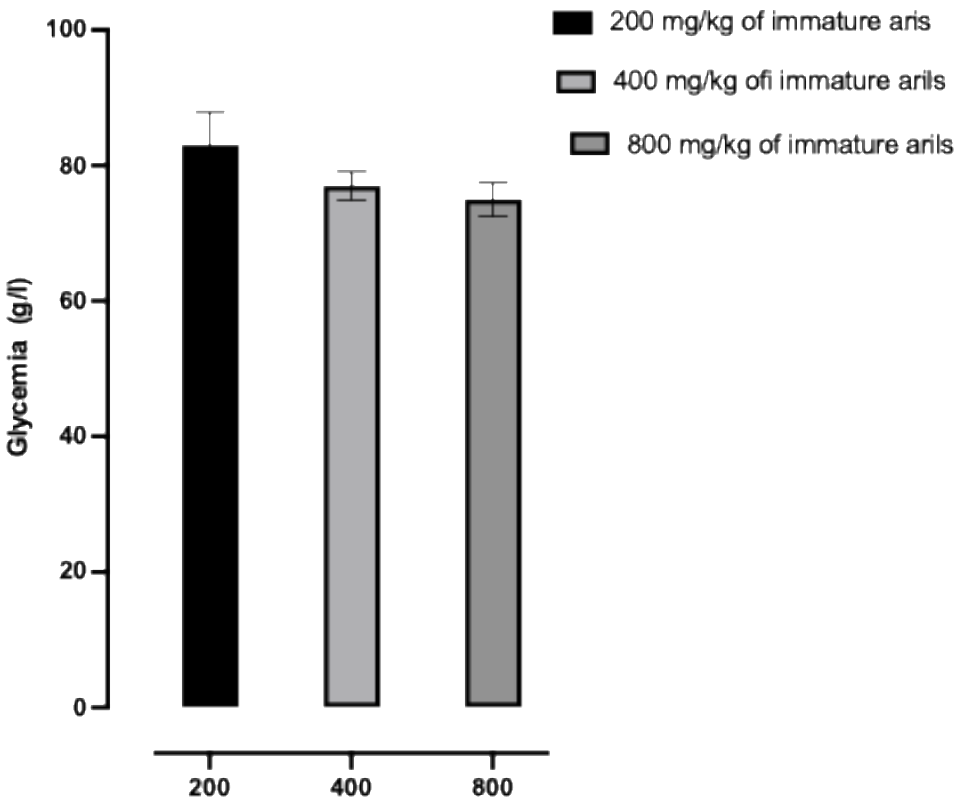

Figure 5 show the variation of glycemia depending to the dose of immature arils administered

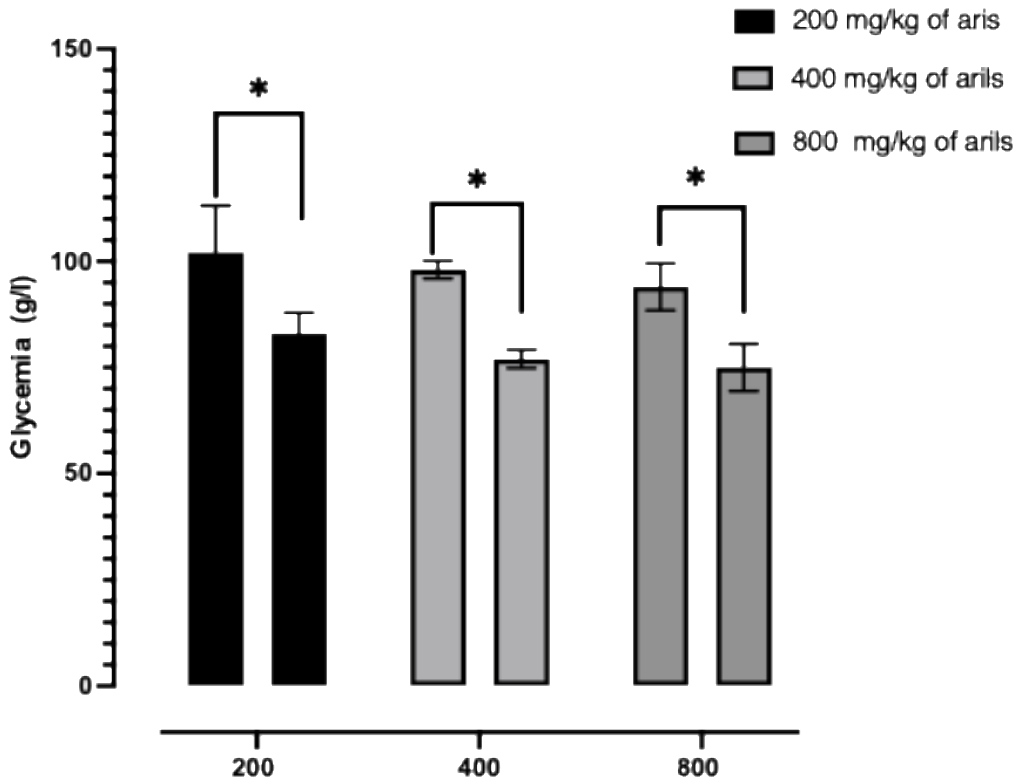

Figure 6 show the variation of glycemia depending to the dose of arils (mature or immature) administered

At the same dose immature arils induced significant diminution of glycemia than mature arils

DISCUSSION

The fact that the average weights obtained in the different groups of rats were not significantly different ($p \geq 0.05$) suggests that homogeneity between the different batches of rats constituted was respected. Thus, if a difference in weight is observed from one batch to another during the experiment, this would be related to the kind of treatment used.

Also, since no significant difference was observed in blood glucose levels between the groups, this means that the groups of rats are in identical blood glucose conditions. Therefore, any variation in blood glucose levels from one group to another during the experiment would be due to the treatment received.

The level of blood glucose in the experiment batch of rats compare to the control batch after one week of the use of sugar in drinking water suggests that glycemia was induced and because there was no significant different on blood glucose level from each experiment batch of rats after the sugar used during one week of use is due to the fact that all the animals are in the same condition.

The fact that blood glucose levels decrease when the dose of aril extract which is administered increase can be explained by the increase in hypoglycin A content, which blocks beta-oxidation, thereby increase the utilization of plasma glucose. Thus, more the amount of hypoglycin A is higher, more plasma glucose is used and more the blood glucose level become lower.

Since solutions of immature arils contain higher concentrations of hypoglycin A than solutions of mature arils, this explains the decrease in blood glucose levels for the same dose administered when immature aril extract is given compared to when mature aril extract is given.

CONCLUSION

We can deduce that immature arils are more hypoglycemic than mature arils and that the decrease in blood glucose is proportional to the amount of hypoglycin A administered. The

use of hypoglycin A as an antidiabetic agent is under consideration. However, what impact would have blocked ß-oxidation on insulin production?

Conflicts of Interest

The authors declare that they have no competing interests.

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