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Anti-Lipidemic and Anti-Glycemic Potential of Lactational Exposure to Flavonoid from *Hibiscus sabdariffa* in Offspring of Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author NYW conceptualized the study and wrote original draft of the manuscript. Author NNR wrote, reviewed and edited the manuscript, Author UCC did formal analysis and data validation, Author PA searched for resources and helped in Project administration, Author EIE investigated the study. Author JUE and AJK performed the methodology. Author AAO did data curation. Author OCS did statistical analysis. All the authors read and approved the final manuscript.

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ABSTRACT

Background and Aim: An individual's growth, organ development, physical makeup, and health are significantly influenced by their nutrition in the early stages of development. *Hibiscus sabdariffa* have been associated with beneficial effects on human health. The anti-lipidemic and anti-glycemic potential of lactational exposure to flavonoid from *Hibiscus sabdariffa* from birth to postnatal day 42 was investigated in Wistar rats.
Methods: Sixteen in-bred pregnant female Wistar rats were randomly assigned to four groups of four rats each. Group 1 was used as normal control. Groups 2, 3 and 4 oral doses of 10, 20 and 50 mg of flavonoid respectively for 21 days. The body weight and length of the dams were measured daily. At postnatal day 21, the dams were assigned into four groups according to their parent rat. Their water and food intake were also measured daily and recorded. At postnatal day 42, blood sample was collected by occulo-puncture for serum lipid profile analysis and glucose tolerance test was done using Accucheck strip.
Results: There was a significant decrease in fluid and food intake, body weight and body length at the doses tested. Also, there was a significant decrease in the lipid profile at different doses. The oral glucose tolerance test revealed the ability of flavonoid to regulate glucose homeostasis in the offspring.
Conclusion: Consumption of flavonoid from *H. sabdariffa* may ameliorate diabetes status and also reduce the risk factors associated with cardiovascular diseases.
Significance: Results from this study can be used as a preventive guide to cardiovascular diseases.

Keywords: Flavonoid; lactational; hibiscus sabdariffa; lipid profile; glucose homeostasis.

1. INTRODUCTION

Fast growth and development, dietary changes, and an increasingly poor lifestyle are the main causes of metabolic syndrome, which is now a global problem that affects people of all socioeconomic levels, races, nationalities, and ethnicities [1]. The causes of the increase in mortality and disease are lipid disorders, high glucose levels while fasting, hypertension, central obesity, and hyperglycemia [2, 3]. An individual's nutrition has a major impact on his or her growth, organ development, appearance, and health during the initial stages of childhood. Diagnostic and epidemiological investigations, as well as a plethora of experiments involving experimental animals, have indicated that kids exposed to specific diets throughout gestation and early infancy are susceptible to a substantial lifetime risk for overweight and metabolic disorders [4]. Studies have demonstrated a correlation between a mother's dietary habits during her pregnancy and lactation and the subsequent development of obesity along with additional metabolic disorders in her offspring [5, 6].

There is a rise in the usage of medicinal plants due to their low environmental impact and potency in treating certain medical conditions [7, 8]. Research has demonstrated that certain plants, like *Hibiscus sabdariffa*, possess characteristics related to metabolic homeostasis and hypoglycemia. Additionally, the use of medicinal herbs and dietary supplements in the treatment of metabolic syndrome is gaining popularity [9, 10]. Nevertheless, not much information about the effects of *Hibiscus sabdariffa's* biologically active elements has been published, regardless of numerous investigations into the plant's crude extract's medicinal properties. Thus, the purpose of this study was to ascertain the anti-lipidemic and anti-glycemic effect of flavonoid from *H. sabdariffa* on young offspring whose mothers were exposed to it during lactation. (Reference not needed

because it's a statement for the aim of the study).

2. MATERIALS AND METHODS

Plant materials: Dried calyces of *Hibiscus sabdariffa* was purchased from New market in Enugu State, Nigeria. A sample of the plant was identified and authenticated at the herbarium section of the department of plant science and biotechnology, University of Nigeria, Nsukka. A 2000 g of the calyxes' powder were extracted by maceration in 5L of methanol for 48h with intermittent agitations. The powder was exhaustively extracted with repeated washings with fresh portions of methanol. The methanol extract was recovered after evaporation of the filtrate in vacuo at 40°C using a rotary evaporator. The concentrated extracts were transferred into sterile beakers with aluminum foil and stored in a refrigerator [11].

Preparation of flavonoid extract: The flavonoid extract was prepared following the methods of [12]. with slight modifications: A 100 g of the crude extract was dissolved in 500 ml of 10 % H_2SO_4 in a conical flask and was hydrolyzed by heating in a water bath for 30 mins at 100 °C. The mixture was then placed on ice for 15mins, so as to allow the precipitations of the flavonoids aglycones. The cooled solution was filtered and the filtrate (flavonoid aglycone mixture) was dissolved in warm 95 % ethanol. The resulting solution was again filtered into a volumetric flask. The filtrate was concentrated to dryness using a rotary evaporator. Test for flavonoid using Zinc-HCL reduction test was performed to confirm the preparation. To the extract, a few drops of concentrated HCL was added, formation of magenta color after few minutes indicated the presence of flavonoids.

Experimental animals and design: Sixteen (16) female Wistar rats weighing 100 g to 130 g with two consecutive regular 4-day estrous cycle obtained from the animal house of the Faculty of Basic Medical Science, University of Nigeria, Enugu Campus was used for this design. The animals were housed in wire mesh cages and were allowed to acclimatize for 7 days during which they were provided with hybrid feed and clean tap water. The rats were grouped into 4 groups of 4 rats each. Group 1 served as the control and were fed with rat chow orally for 42 days while Groups 2, 3 and 4 received 10, 20 and 50 mg/kg body weight of flavonoid respectively for 21 days [13].

Induction of pregnancy in rats: In this work, we employed rats with two concurrent normal four-day estrous cycles, tracking the estrous cycle daily by examining the vaginal smears under light microscopy at predetermined intervals. To facilitate mating in the ratio of one male rat to two female rats, male rats with demonstrated fertility were added to the cages during pro-estrous. The day sperm were visible in the rats' vaginal smear was marked as the first day of pregnancy [14].

Measurement of food and fluid intake in the offspring: The young pups were separated from their mothers on the 21st postnatal day and placed in a group home with unrestricted access to food and water. Every day, the amount of food and water given out was weighed and documented. Before the next meal was served, the leftovers were collected, measured, and documented. Then, the amount that was initially given was deducted and recorded [15].

Measurement of BMI of offspring: The length of pups was measured using a tape and a digital electronic weighing scale was used to determine the weight from birth, day 7, 14, 21, 28, 35 and 42. The body weight and body length (naso-anal length) were then used to determine the Body Mass Index (BMI) [15].

$$BMI = \text{body weight (g)} / \text{Length}^2 (\text{cm}^2)$$

Determination of lipid profile: Each rat was given an eye puncture on postnatal day 42, at which point blood samples were taken and labeled. After that, the samples were spun in a centrifuge to extract serum samples. The random test kit was used to measure the lipid profile in accordance with the manufacturer's instructions. After gathering and combining serum samples with different reagents, they were placed in a water bath to ensure thorough mixing. Following standardization, the samples were put in a Wincom Spectrophotometer (model 721, China), where the optical density was read off [16]. The following formula was used to determine the total cholesterol and triglycerides:

Total cholesterol and triglycerides=

$$\frac{O.D\ value \times Conc.standard}{O.D\ sample}$$

Determination of oral glucose tolerance test: This method was carried out according to reported literatures. The rats were fasted overnight prior to the test. Blood was obtained

from a tail cut and was assessed for baseline glucose levels using an Acucheck glucometer. The rats then received 3 mg/kg body weight of glucose solution in distilled water delivered by oral gavage. At 30, 60, 90, and 120 minutes after the administration of glucose, dried blood and tissue were quickly removed from the tail wound and blood was collected again to measure the glucose concentration [17].

Statistical analysis: All data were tabulated and statistically analyzed. Results were presented as Mean ± Standard error of mean. The results were analyzed using one-way analysis of variance (ANOVA) followed by Student Newman-Keul's post-hoc test using SPSS software version 20. Values of $p<0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

There was a significant decrease in the mean food and fluid of the offspring during lactation. There was also a significant decrease in body weight and body mass index of the offspring from birth to postnatal day 42. This is in agreement with vitro and in vivo studies which showed that *Hibiscus sabdariffa* extract inhibited the activity of α-amylase, blocking sugars and starch absorption, which may assist in weight loss [18-20]. This also suggest that flavonoid from *H. sabdariffa* can guard against obesity that can occur in adulthood and thus is of great benefit.

Table 1. Effect of maternal administration of flavonoid from *Hibiscus sabdariffa* on offspring's mean weekly food intake from PND 21-pnd 42

GROUPS	Food intake at PND 28	Food intake at PND 35	Food intake at PND 42
Control	29.08±4.31	72.25±2.11	56.50±2.19
Group 2 (10 mg)	39.24±2.47*	42.04±4.56*	52.91±8.40*
Group 3 (20 mg)	21.80±3.08*	27.47±2.18*	38.27±6.77*
Group 4 (50 mg)	32.72±7.79*	49.46±5.15*	59.93±4.77*

*Each value represents the Mean ± SEM (Standard error of mean). *means significant difference (p<0.05)*

Table 2. Effect of maternal administration of flavonoid from *Hibiscus sabdariffa* on offspring's mean weekly fluid intake from PND 21 to PND 42

GROUPS	Fluid Intake at PND 21	fluid intake at PND 35	Fluid Intake AT PND 42
Control	132.00±2.26	131.00±6.07	121.43±3.13
Group 2 (10 mg)	75.14±5.29*	78.29±6.75*	72.90±6.48*
Group 3 (20 mg)	49.57±4.87*	55.57±7.55*	59.16±3.78*
Group 4 (50 mg)	56.67±4.03*	82.71±7.88*	91.86±11.42*

*Each value represents the Mean ± SEM (Standard error of mean). *means significant difference (p<0.05)*

Table 3. Effect of maternal administration of flavonoid from *Hibiscus sabdariffa* on offspring's mean serum lipid profile on PND 42

GROUPS	Mean Triglyceride	Mean Cholesterol	Mean HDL	Mean LDL
Control	50.92±13.92	118.02±12.43	65.35±11.53	48.46±21.99
Group 2 (10 mg)	74.97±5.09*	160.10±7.68	79.44±7.43*	71.81±5.81*
Group 3 (20 mg)	146.22±14.00*	137.93±7.27	44.10±4.75*	36.57±24.69*
Group 4 (30 mg)	52.44±28.83*	107.95±26.28	197.75±19.12*	-67.22±51.17*

*Each value represents the Mean ± SEM (Standard error of mean). *means significant difference (p<0.05)*

Table 4. Effect of maternal administration of flavonoid from *Hibiscus sabdariffa* on offspring's mean weekly BMI from birth to PND 42

Groups	PND 0	PND 7	PND 14	PND 21	PND 28	PND 35	PND 42
Control	0.21±0.01	0.29±0.01	0.38±0.1	0.42±0.01	0.51±0.00	0.54±0.01	0.65±0.01
Group 2(10mg)	0.25±0.14*	0.27±0.01*	0.32±0.01*	0.33±0.01*	0.35±0.01*	0.46±0.26*	0.56±0.012*
Group 3(20mg)	0.27±0.00*	0.21±0.01*	0.28±0.00*	0.30±0.01*	0.34±0.14*	0.42±0.01*	0.52±0.11*
Group 4(50mg)	0.24±0.01*	0.21±0.00*	0.32±0.01*	0.31±0.01*	0.36±0.01*	0.44±0.14*	0.50±0.01*

*Each value represents the Mean ± SEM (Standard error of mean). *means significant difference (p<0.05)*

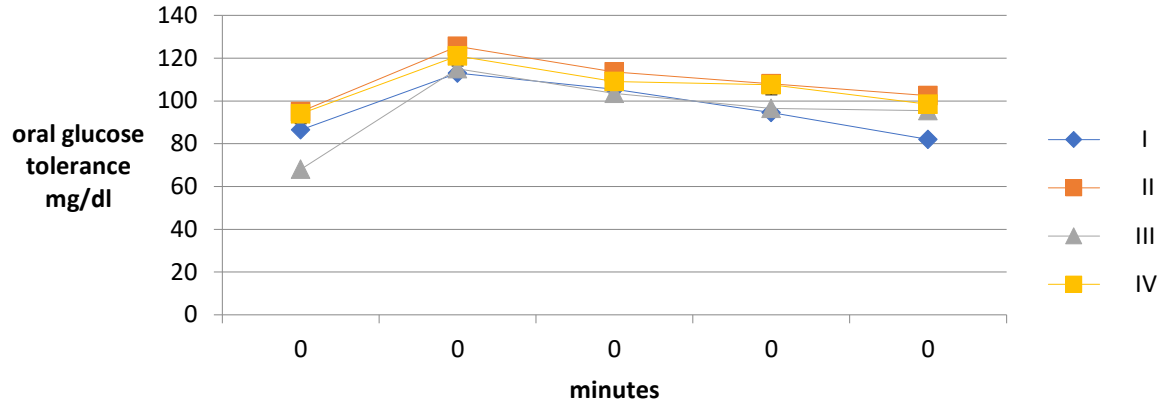

Fig. 1. Trend of OGTT of male offspring of rats that consumed flavonoid from *Hibiscus sabdariffa*

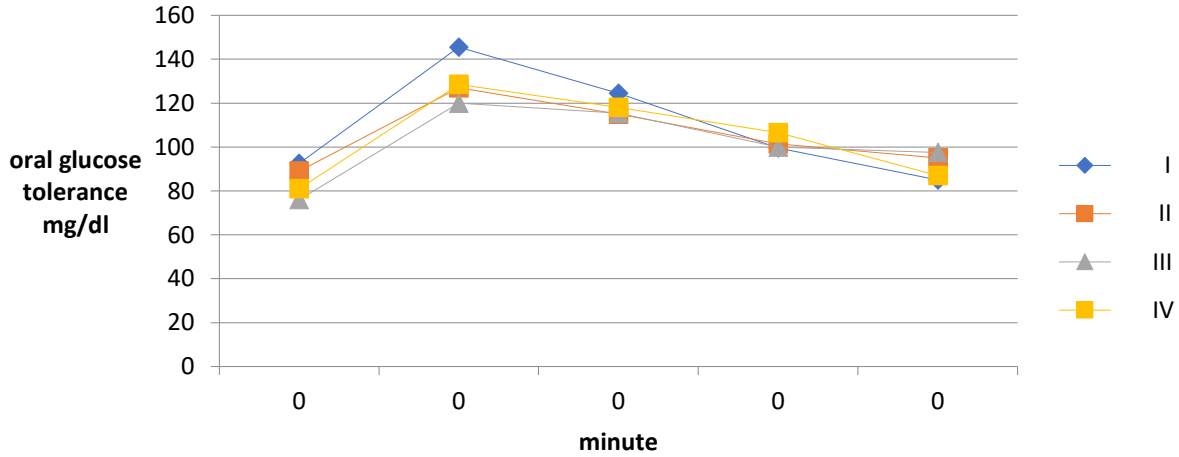

Fig. 2. Trend of OGTT of female offspring of rats that consumed flavonoid from *Hibiscus sabdariffa*

The lipid lowering activity observed from the result is in agreement with studies done by [21-22], that showed that extracts of *H. sabdariffa* have a lipid lowering activity which could prevent against diseases like hyperlipidemia and cardiovascular diseases through the inhibition of the triacylglycerol synthesis or other hypolipidemic effects through the antioxidant activity against LDL-c oxidation and hepatic liver clearance. The extracts (water and ethanolic extracts of dried calyces or leaves) were able to decrease low-density lipoprotein cholesterol (LDL-c), triglycerides (TAG), total cholesterol (TC) and lipid peroxidation in vivo. A few of them even reported that the extract was also able to reduce very-low density lipoprotein cholesterol (VLDL-c) [10][23] along with an increase in serum level of high-density lipoprotein cholesterol (HDL-c) levels [23][22].

This result is also similar with a study on the protective effect of a polyphenol extract of *HS* in a type II diabetic rat model (high fat diet model). At a dose of 200 mg/kg, the extract demonstrated anti-insulin resistance properties as it reduced hyperglycemia and hyperinsulinemia. It decreased serum triacylglycerol, cholesterol and the ratio of low-density lipoprotein/high-density protein (LDL/HDL), as well as reduced the plasma advanced glycation end products (AGE) formation and lipid peroxidation. This suggests that effective control of cholesterol levels in blood by maternal consumption of flavonoid during lactation may help in preventing and hastening the decrease in blood pressure as it has been shown that flavonoids have been implicated in the control of dyslipidemia by inhibiting LDL oxidation.

The result of oral glucose tolerance test confirms that flavonoid consumption was able to regulate glucose homeostasis in the offspring. This could be as a result of the ability of flavonoids to stimulate glucose uptake in peripheral tissues, regulate the activity and or expression of the rate limiting enzymes in the carbohydrate metabolism pathway and act per se as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms of insulin signaling, to ameliorate the diabetes status. Also, a number of studies hypothesized that some flavonoids may increase GLUT-4 expression activity leading to restore insulin sensitivity and might be a viable therapeutic avenue for treating diabetes [24-25].

4. CONCLUSION

The result of this study revealed that lactational exposure to flavonoid from *Hibiscus sabdariffa* was able to regulate glucose homeostasis and lipid profile in the offspring. Thus, consumption of flavonoid from *H. sabdariffa* may ameliorate diabetes status and also reduce the risk factors associated with cardiovascular diseases. It therefore ascertains that the lipid lowering activity of *HS* is as a result of this phytochemical.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance for this study was obtained from Research and Ethics Committee, University of Nigeria, Enugu.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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