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Research Article
Consumption of *Gongronema latifolium* Aqueous Leaf Extract During Lactation May Improve Metabolic Homeostasis in Young Adult Offspring

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Abstract

Background and Objective: Medicinal plants constitute a fundamental component of the traditional healthcare system in rural communities throughout Africa and *Gongronema latifolium* (GL), is widely trusted in Nigeria to have strong nutritional and medicinal values. This study was done to determine the effect of maternal consumption of GL during lactation in young adult offspring. **Materials and Methods:** Twenty four female albino Wistar rats were used for this study and were randomly assigned to four (4) groups. Group I: Control, Group II, 100 mg kg^{-1}, III, 200 mg kg^{-1} and IV: 400 mg kg^{-1} at delivery. The extract was administered orally and daily throughout lactation. **Results:** At postnatal day 42, offspring of extract-treated groups showed a dose-related significant decrease ($p<0.05$) in body weight, food intake, glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and a significant increase in liver weight index, pancreatic weight index, high-density lipoprotein cholesterol (HDL-C) and insulin concentrations of the offspring when compared with control in both sexes. Histological examination showed that GL extract caused histological alterations of the liver structures with various changes in the size of the sinusoids, with mild inflammatory cells without hepatotoxicity and cellular multiplication when compared with control. **Conclusion:** This study suggested that consumption of GL extract by lactating dams may improve metabolic homeostasis in young adult offspring.

Key words: *Gongronema latifolium*, leaf extract, lipid profile, lactation, metabolic homeostasis, offspring

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The potential of herbs and other plant-based formulations have been increasingly recognized in the prevention and treatment of human diseases. The discipline of ethnopharmacology, traditionally employed or observed by man, has in recent years received increased attention and there is presently a widespread interest in medicinal plants used by different cultures[1]. Nigeria has a rich source of medicinal herbs, with high potential for a holistic system of medicines. Medicinal plants constitute a fundamental component of the traditional healthcare system in rural communities throughout Africa and beyond. It is noted that about 80% of people in the developing world rely on traditional medicine for most of their healthcare[2]. Traditional medicines based on herbal remedies have always played a key role in the health system of many countries in which Nigeria is not an exception. This is due to the presence of a wide range of bioactive phytochemicals and secondary metabolites have made plants a promising source of modern synthetic drugs for the management of several diseases[3].

Gongronema latifolium (GL) is a non-wood forest product of West African origin and known as "utazi" in South-Eastern Nigeria GL and commonly called "Arokeke" in the South Western and South-Eastern parts of Nigeria, respectively. GL has long been an integral part of African traditional medicine. It has been used for a variety of medical conditions. An infusion or decoction of the whole plants (the leaves, stems and roots) is used traditionally in the treatment of diabetes, hypertension, cough, intestinal worms, dysentery, dyspepsia, malaria, cough, asthma, viral hepatitis, bilharzia and other microbial infection, abdominal pain after childbirth and womb cleansing[4-6]. Various reports on the pharmacological actions of GL have been reported. Metabolic events such as during gestation and/or the early postnatal development modulate metabolic disease risks in later life giving rise to enhanced susceptibility to develop diseases later in life[7,8]. Among them, feeding conditions likely constitute one of the most influential parameters on the health of the adult. Since the management of these ailments is very expensive when full-blown, the modern trend now in the scientific world is the development of strategies that can prevent the expression of these programmed diseases GL is abundantly present locally and it is also widely consumed locally. This study, therefore, was aimed at determining whether maternal consumption of GL during lactation will improve metabolic homeostasis in young adult offspring.

MATERIALS AND METHODS

Study area: The study area was the animal house of the Department of Physiology and the research work was carried out in May, 2019 through November, 2019.

Plant material: Fresh leaves of *Gongronema latifolium* used for this study were obtained from local farmers in Nsukka, Enugu state in Nigeria. They were identified and authenticated by a taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka where a voucher specimen (PCG/UNN/004) was deposited at the herbarium.

Plant aqueous extract preparation: The method used by Holy *et al.*[9] was adopted for the extraction of the aqueous plant extract. Thousand grams of the finely ground dried plant leaves were immersed into 1000 mL of distilled water. The mixture was allowed to stand for 72 h. It was then filtered using a Whatman No. 1 filter paper. Following filtration; the filtrate was evaporated to dryness over a water bath at 45°C. The pasty filtrate obtained after drying was weighed using an electronic weighing balance. The stock solution of the extract was prepared by dissolving 10 g of the extract in 100 mL of distilled water. The stock solution was appropriately labeled and refrigerated until required for administration via oral gavage.

Animals and experimental procedure: A total of twenty-four female albino Wistar rats weighing 180-210 g were used for this study. The animals were purchased from the Faculty of Basic Medical Sciences' Animal House, University of Nigeria, Enugu Campus. They were weighed, randomly assigned into metallic cages, kept in a room where a 12 h light/dark cycle was maintained and was allowed free access to livestock feed (Vital Feeds, Nigeria Ltd) and tap water *ad libitum* throughout the experiment. The rats were allowed for 2 weeks to acclimatize before the commencement of the study.

Induction of pregnancy: The female Wistar rats were randomly selected and housed two per cage with a mature male rat (2 females with 1 male in a cage), to ensure that all the female rats get pregnant. Vaginal smear was examined under a microscope every morning and a successful mating was ascertained by the presence of sperm cells and denotes day one of pregnancy.

At delivery, the pups were culled to 6-8 pups per dam to prevent over-nutrition and under-nutrition of the pups and were randomly assigned to 4 groups[10]:

Group I : Normal control received drinking water throughout lactation
Group II : Treated with 100 mg (kg b.wt.) of extract as a single dose daily throughout lactation
Group III : Treated with 200 mg (kg b.wt.) of extract as a single dose daily throughout lactation
Group IV : Treated with 400 mg (kg b.wt.) of extract as a single dose daily throughout lactation

Weaning: Weaning occurred at post-natal day (PND) 21. Pups were removed from their mothers and group-housed, with free access to chow and water.

Measurement of body weights, hepatic and pancreatic weight: Offspring body weights were measured using a digital electronic compact balance from weaning to postnatal day (PND) 42 while hepatic and pancreatic weights were measured on PND 42.

Measurement of food intake: Offspring daily food intake was calculated by giving each of the rats a known weight of feed after which the remaining feed was weighed the next day and was subtracted from the amount of feed given to the rat the previous day:

$$\text{Food intake (g)} = \text{Amount given (g)} - \text{Amount remaining (g)}$$

Oral glucose tolerance test (OGTT): The offspring were deprived of food for 12 h and were then given 2 g kg^{-1} b.wt., as a glucose solution by oral. The tail blood samples were collected at 0, 30, 60, 90 and120 min time interval. The blood glucose levels were determined in mg dL^{-1} with the aid of One Touch basic glucometer.

Blood sample collection and serum preparations: At PND 42, blood samples of the offspring per group were collected by cardiac puncture into specimen bottles and allowed to clot and separated by centrifugation. Serum was used for the determination of lipid profile and insulin levels.

Biochemical assays and analysis: The levels of cholesterol and triglyceride were assayed for using enzymatic colorimetric diagnostic kits obtained from Randox laboratories, UK in which the glycerol phosphate oxidase method was employed. HDL-C was determined according to the method of Burnstein *et al.*[11], LDL cholesterol was calculated as:

$$\text{LDL (mg dL}^{-1}) = \frac{\text{Total cholesterol-HDL+Total triglyceride}}{5}$$

While, VLDL - cholesterol was estimated as:

$$\text{VLDL (mg dL}^{-1}) = \frac{\text{Triglyceride}}{5}$$

Insulin assay: Serum insulin was measured in (uIU mL^{-1}) by enzyme-linked immunosorbent assay (ELISA) using an insulin Elisa kit.

Histological examination: The liver and pancreas of the control and treated rats were carefully removed, cleared of connective tissues and fixed in 10% buffered formaldehyde. Sections were obtained and stained with hematoxylin and eosin (H and E) stains. The microscopic slides were labeled appropriately. Photomicrographs were taken at a magnification of 100× using a light microscope.

All procedures used in this study conformed to the guiding principle for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and use of Animals[12] and approved by the Ethics Committee of the University.

Statistical analysis: Results were expressed as Mean±Standard error and statistically evaluated. The Difference between all studied groups was assessed using analysis of variance (ANOVA) followed by a *post hoc* Student's Newman-Keuls test using SPSS software version 21. Values of $p<0.05$ were regarded as being statistically significant.

RESULTS

Effects of treatments on offspring body weight: Oral administration of 100 mg kg^{-1} GL aqueous extract showed the lowest significant decrease ($p<0.05$) in body weight from postnatal day (PND) 14 till the end of the study when compared with normal control and other treated groups. Treatment with 200 mg kg^{-1} GL showed a significant ($p<0.05$) increase over 100 mg kg^{-1} GL group from postnatal day 21 till PND 42 Offspring of rats in 400 mg kg^{-1} group and 200 mg kg^{-1} GL group also showed a significant ($p<0.05$) decrease in body weight when compared with the normal control (Fig. 1).

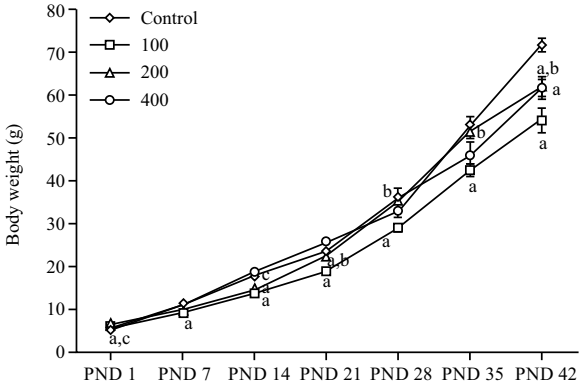

Fig. 1: Trend of body weight of offspring of rats that consumed extract of GL during lactation

Control: Normal control, 100: 100 mg kg^{-1} GL, 200: 200 mg kg^{-1} GL, 400: 400 mg kg^{-1} GL, ^{a}p<0.05 vs. control, ^{b}p<0.05 vs. 100, ^{c}p<0.05 vs. 200

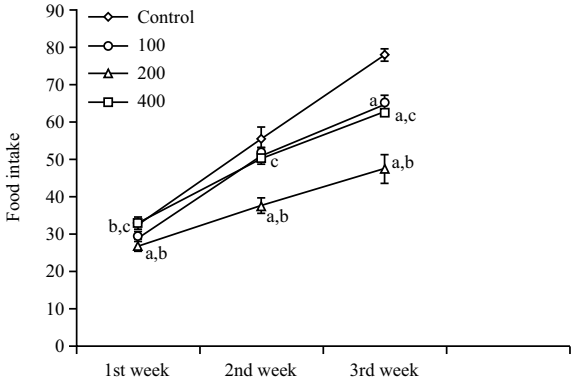

Fig. 2: Trend of food intake of offspring of rats that consumed extract of GL during lactation

Control: Normal control, 100: 100 mg kg^{-1} GL, 200: 200 mg kg^{-1} GL, 400: 400 mg kg^{-1} GL, ^{a}p<0.05 vs. control, ^{b}p<0.05 vs. 100, ^{c}p<0.05 vs. 200

Effects of treatments on offspring food intake: The offspring food intake in the normal control group had an increasing value from the first week of birth till the end of the third week maintaining significant (p<0.05) higher values more than other treated groups. Treatment with 200 mg kg^{-1} GL showed significant p<0.05) lowest values when compared with normal control and 100 mg kg^{-1} GL treated group. The 100 mg kg^{-1} GL and 400 mg kg^{-1} GL groups had no significant difference between each other (Fig. 2).

Effects of treatments on offspring liver weight index: Figure 3a and b showed the offspring liver weight index of normal control and extract treated groups of female and male offspring respectively. Results for the female weight index showed that the weight index of the liver of female offspring

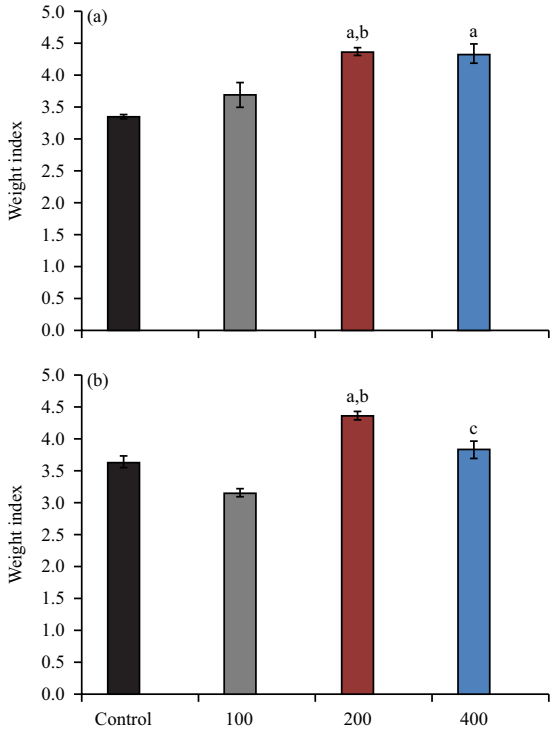

Fig. 3(a-b): Changes in weight index of the liver of (a) Female and (b) Male of rats that consumed extract of GL during lactation

Control: Normal control, 100: 100 mg kg^{-1} GL, 200: 200 mg kg^{-1} GL, 400: 400 mg kg^{-1} GL, ^{a}p<0.05 vs. control, ^{b}p<0.05 vs. 100, ^{c}p<0.05 vs. 200

of rats administered 200 and 400 mg kg^{-1} of the extract increased significantly (p<0.05) when compared with normal control while 200 mg kg^{-1} of the extract increased the liver weight index significantly (p<0.05) when compared with 100 mg kg^{-1} in both sexes.

Effects of treatments on offspring pancreatic weight index: Figure 4a and b showed values for pancreatic weight index in normal control and animals treated with the only extract for female and male offspring respectively, it showed that 100 and 200 mg kg^{-1} GL groups are significantly (p<0.05) higher than the normal control while those treated with 400 mg kg^{-1} extract is significantly (p<0.05) lower than 200 mg kg^{-1} GL group in both female and male offspring.

Effects of treatments on oral glucose tolerance test (OGTT): The obtained results showed that blood glucose decreased significantly (p<0.05) in 400 and 200 mg kg^{-1} GL treated groups after oral glucose solution administration in the 30th min when compared with normal control in the

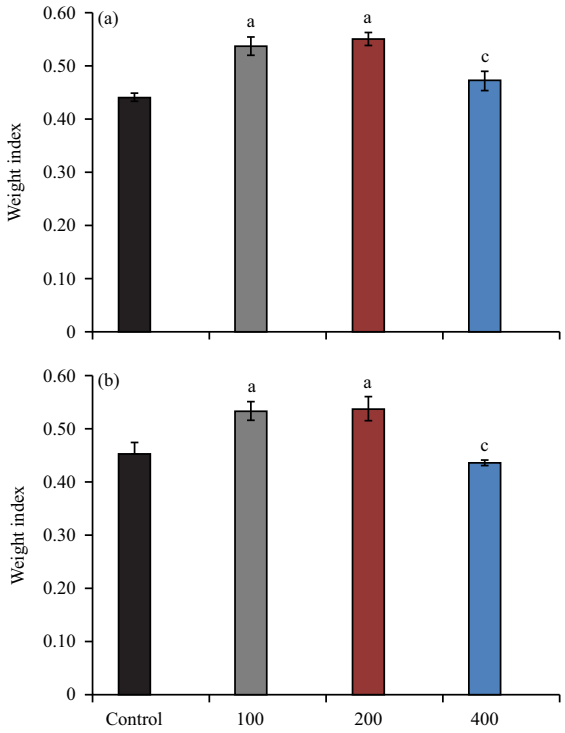

Fig. 4(a-b): Changes in weight index of the pancreas of (a) Female experimental rats of No sucrose group and (b) Male of rats that consumed extract of GL during lactation
Control: Normal control, 100: 100 mg kg^{-1} GL, 200: 200 mg kg^{-1} GL, 400: 400 mg kg^{-1} GL, ^{a}p<0.05 vs. control, ^{c}p<0.05 vs. 200

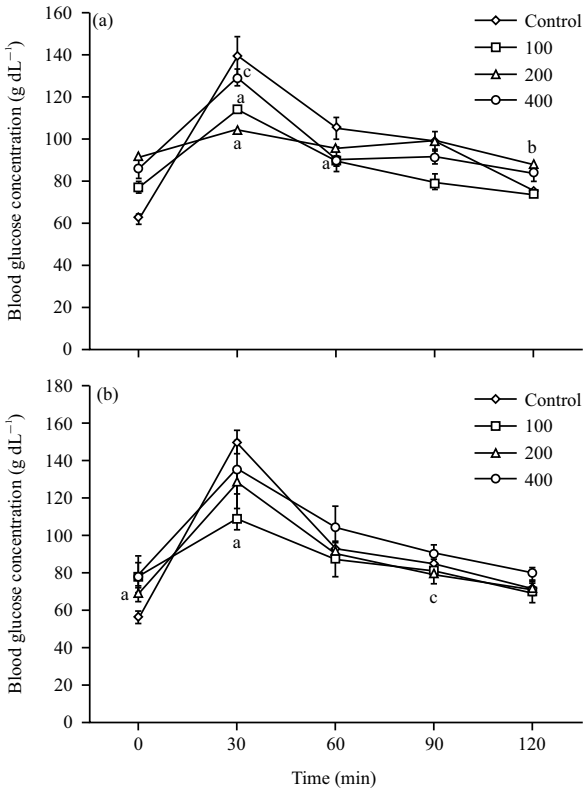

Fig. 5(a-b): Trend of OGTT of (a) Female and (b) Male offspring of rats that consumed extract of GL during lactation
Control: Normal control, 100: 100 mg kg^{-1} GL, 200: 200 mg kg^{-1} GL, 400: 400 mg kg^{-1} GL, ^{a}p<0.05 vs. control, ^{c}p<0.05 vs. 200

female offspring (Fig. 5a) while the blood glucose level of 200 mg kg^{-1} GL group was significantly (p<0.05) higher than the 100 mg kg^{-1} GL group in the 120th min. In Fig. 5b, values for male control and extract groups were shown to have the same pattern. Oral administration of 100 and 200 mg kg^{-1} GL aqueous extract showed the lowest significant decrease (p<0.05) l when compared with normal control while the 400 mg kg^{-1} group was significantly (p<0.05) higher than other extract treated groups.

Effects of treatments on offspring lipid profile: Table 1 show the comparative results for lipid profile levels between the normal control group and the extract-treated group in female offspring. It shows that the TC value for 100, 200 and 400 mg kg^{-1} extract only groups was significantly (p<0.05) lower than the normal control group. Results for TG shows that the 200 mg kg^{-1} the only group had the lowest significant (p<0.05) value when compared with the normal control and other extract treated groups. HDL values were shown to be significantly (p<0.05) higher in the 100 mg kg^{-1} group than another extract only group. For LDL, the result showed that 100 mg kg^{-1} extracts the only group had the lowest significant (p<0.05) value when compared with the normal control and other extract treated groups. For VLDL, the 400 mg kg^{-1} extract the only group had the lowest significant (p<0.05) value.

Table 2 shows the lipid profile values in mg dL^{-1} for male offspring treated with the extract. Results for TC showed that 400 mg kg^{-1} extract only group was significantly (p<0.05) lower than the normal control and other extract treated groups. The 200 mg kg^{-1} extract only group had the lowest significant (p<0.05) value for TC when compared with the normal control group and other extract treated groups. Results for HDL showed that the 200 mg kg^{-1} extract only group had the highest significant (p<0.05) value when compared with the normal control and other extract treated groups. For the LDL, 200 mg kg^{-1} extract only group had the lowest significant (p<0.05) value when compared with the normal control and other extract treated groups. Results for VLDL shows that the 400 mg kg^{-1} extract only group had the lowest significant (p<0.05) value when compared with the normal control.

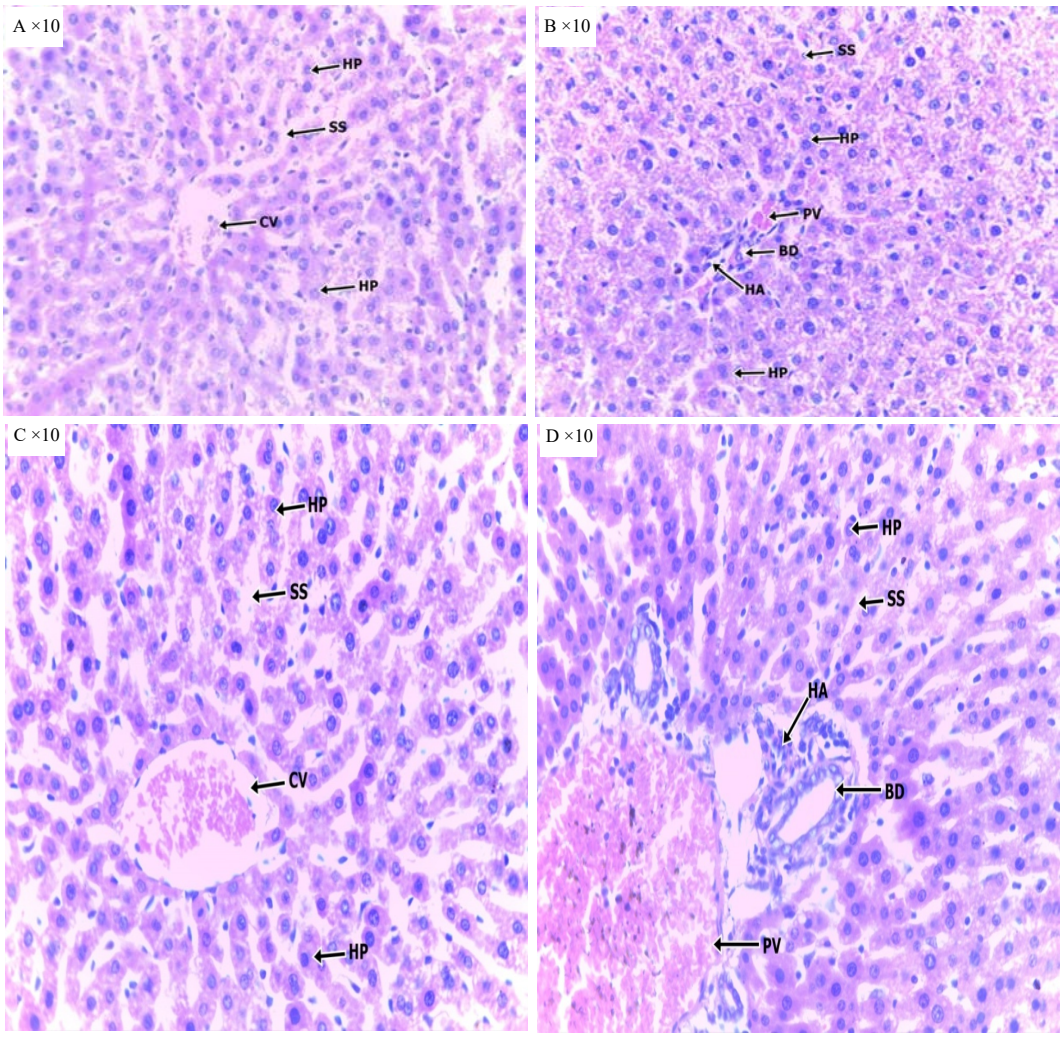

Fig. 6(A-D): Histological sections stained with H and E showed the liver of the experimental groups, (A) Normal treated rats, (B) 100 mg kg^{-1} treated rats, (C) 200 mg kg^{-1} treated rats and (D) 400 mg kg^{-1} treated rats. The histoarchitecture of the liver in Group A and B is normal in which the hepatocytes (HP) arranged around the central vein (CV) with sinusoids. The portal area contains the bile duct, hepatic artery and portal vein with an intact limiting plate hepatocyte. Significant changes with a moderate amount of inflammatory infiltrate with intact limiting plates on the CV, moderate reversible cellular injury was observed in the liver section of Group C while mild inflammatory cells in the sinusoidal spaces (SS), vascular congestion of the CV with no cellular injury was also observed in Group D

Effects of treatments on offspring insulin level: Table 3 shows the effect of graded doses of GL extract on insulin levels in female and male offspring of albino Wistar rats. The female offspring treated with 200 and 400 mg kg^{-1} GL were significantly ($p<0.05$) higher than the normal control and 100 mg kg^{-1} GL group. The male offspring treated with 200 mg kg^{-1} GL was significantly ($p<0.05$) higher than in other groups.

Histological results: Microscopic observation in the liver showed that all doses of GL extract caused histological alterations of the liver structures such as a congested central vein, with various changes in the size of the sinusoids, with mild inflammatory cells, moderate reversible cellular injury without hepatotoxicity (Fig. 6). Similarly, the histological observation in the pancreas, treatment with extract resulted in hyperplasia without the destruction of the islet cells when compared with normal control (Fig. 7).

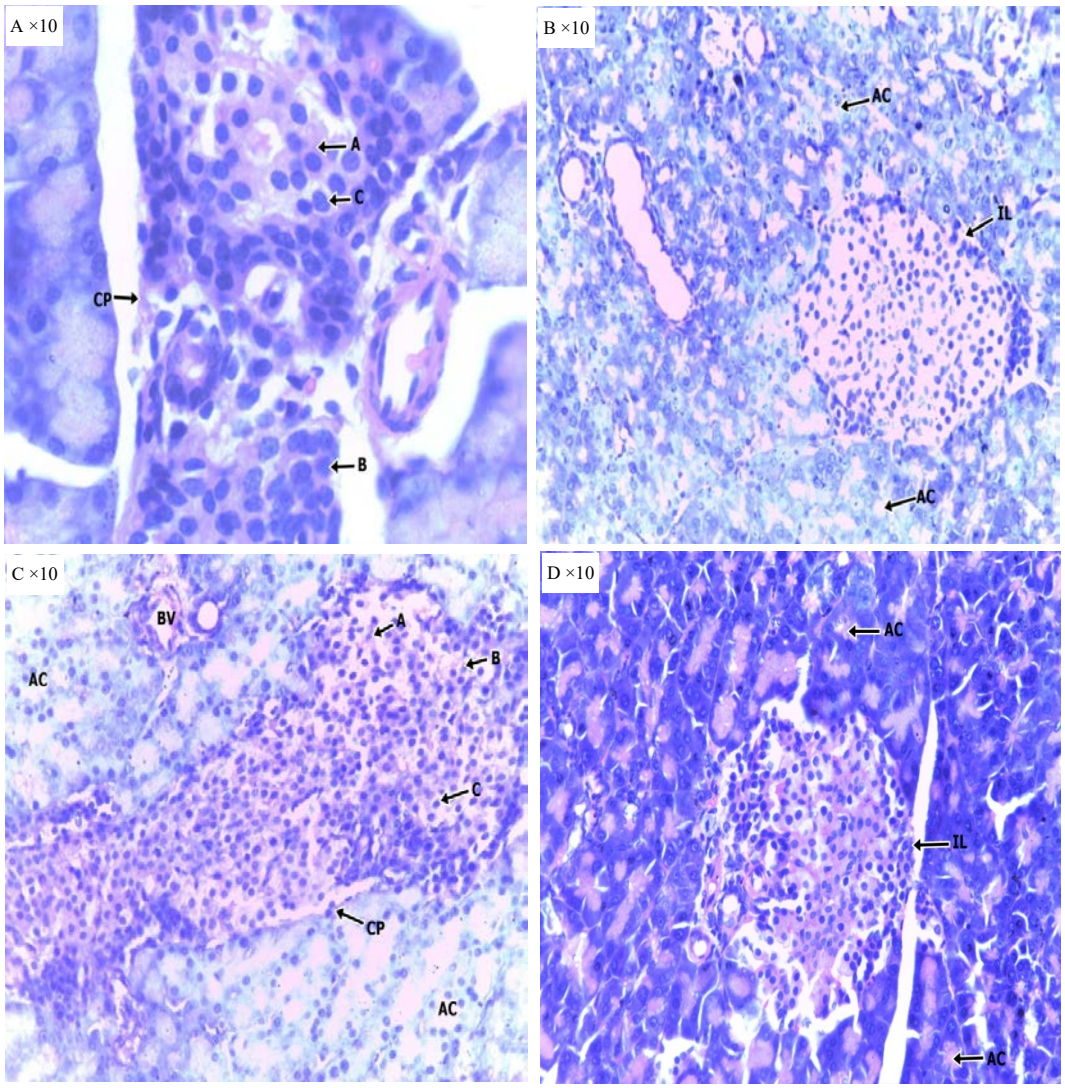

Fig. 7(A-D): Histological sections stained with H and E showed the pancreas of the experimental groups, (A) Normal treated rats, (B) 100 mg kg^{-1} treated rats, (C) 200 mg kg^{-1} treated rats and (D) 400 mg kg^{-1} treated rats. Pancreas histoarchitecture in Group A is normal. Significant changes observed in the pancreas of Group B, C and D showed hyperplasia of the islet cells

Table 1: Effects of treatment with aqueous extract of GL on lipid profile level of female offspring of Wistar rats

	Total cholesterol (mg dL^{-1})	Total triglyceride (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
Normal control	157.300±2.00	142.400±2.22	52.667±4.72	76.100±4.31	28.480±0.44
100 mg kg^{-1} extract	135.733±3.93^{a}	125.000±3.76	66.533±4.65^{a}	31.980±3.49^{a}	26.780±1.75
200 mg kg^{-1} extract	144.033±3.35^{a}	118.333±4.04^{a,b}	53.367±3.38^{b}	49.833±2.34^{a,b}	23.667±1.21^{a,b}
400 mg kg^{-1} extract	138.150±4.38^{a}	141.767±3.05^{c}	52.933±2.44	52.753±0.49^{a,b}	23.340±0.61^{c}

Control: Normal control, 100: 100 mg kg^{-1} GL, 200: 200 mg kg^{-1} GL, 400: 400 mg kg^{-1} GL, ^{a}p<0.05 vs. control, ^{b}p<0.05 vs. 100, ^{c}p<0.05 vs. 200

Table 2: Effects of treatment with aqueous extract of GL on lipid profile level of male offspring of Wistar rats

	Total cholesterol (mg dL^{-1})	Total triglyceride (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
Normal control	160.10±1.33	146.67±1.94	56.63±3.01	78.33±3.12	29.33±0.39
100 mg kg^{-1} extract	160.10±1.33	133.97±5.53^{a}	65.50±2.73	67.90±2.73	26.79±1.11^{a}
200 mg kg^{-1} extract	156.27±1.21	119.37±1.31^{a,b}	71.63±2.28^{a}	55.97±2.44^{a,b}	23.87±0.26^{a,b}
400 mg kg^{-1} extract	142.70±1.24^{a,c}	137.67±1.11^{c}	66.44±2.60^{c}	58.33±1.54^{a}	22.93±0.22^{c}

Control: Normal control, 100: 100 mg kg^{-1}GL, 200: 200 mg kg^{-1} GL, 400: 400 mg kg^{-1} GL, ^{a}p<0.05 vs. control, ^{b}p<0.05 vs. 100, ^{c}p<0.05 vs. 200

Table 3: Effects of graded doses of GL extract on insulin level in female and male offspring of albino Wistar rats

Experimental groups	Insulin (μIU mL^{-1}) female	Insulin (μIU $m L^{-1}$) male
Normal control	55.77±1.56	58.70±0.95
100 mg kg^{-1} extract	58.93±0.81	62.33±1.27
200 mg kg^{-1} extract	68.63±1.26[a]	63.20±1.22[a]
400 mg kg^{-1} extract	66.20±1.42[a]	59.90±1.20

DISCUSSION

In the present study GL extract consumption by the lactating dams caused a dose-related decrease in body weight, food intake, glucose, total cholesterol, triglyceride, LDL-C, VLDL-C and a significant increase in liver weight index, pancreatic weight index, HDL-C and insulin concentrations of the offspring in both sexes. Histological examination showed that GL extract caused alterations of the liver structures with various changes in the size of the sinusoids, with mild inflammatory cells without hepatotoxicity and cellular multiplication.

Early life nutrition is related to offspring's growth and development[13]. Anthropometric indices such as total body weight and weight changes are necessary because weight changes of tissues are useful measures of pathological conditions[14]. About 100 mg/b.wt. of GL decreased body weight more potently when compared with other doses of the extract. This study is consistent with the report of Nwaka *et al.*[15] who reported a decrease in the bodyweight of rats supplemented with the extracts of GL, that could be due to the presence of saponin, which has been reported to reduce body weight and food intake in both normal and high-fat diet rats[16] or because of the unpalatability of GL due to the bitter substances present in the leaves which could have reduced the rat's appetite[17]. Extract caused decreased offspring food intake after weaning suggesting that constituents of the GL extract passed through breast milk during lactation to program reduced offspring' food intake. OGTT measures the ability to metabolize glucose and determines body's response to glucose loading[18]. Results from this study show that OGTT of female and male offspring showed a dose-dependent reduction in extract groups only. Extract treated groups at doses of 100 and 200 mg kg^{-1} produced blood glucose levels significantly lower than 400 mg kg^{-1} thus indicating that 100 and 200 mg kg^{-1} may be the optimum dose at which GL extract exerts its maximum hypoglycemic effect. The reduction in blood glucose level in OGTT suggests that these might probably be due to saponin which has been reported to possess antidiabetic activity[19]. This observed hypoglycemic potency of GL has been reported by Ugochukwu *et al.*[20]. The extract might bind insulin receptors on the plasma membrane or cause the release of insulin from β-cells to initiate a signaling cascade that could promote translocation and fusion of GLUT4 containing vesicles with the plasma membrane to facilitate glucose transport into cells and organs[21].

Dyslipidaemia is a common manifestation in diabetes mellitus and associated with greater risk of atherosclerosis characterized by increased levels of triglycerides, VLDL and LDL, presence of small dense LDL particles and decreased HDL levels[22]. In this study, the administration of the extract showed a dose-related decrease in total cholesterol, triglyceride, LDL-C, VLD and increased HDL concentrations in both male and female offspring. This agrees with previous studies that reported that GL possesses some phytochemicals like flavonoids, saponins and phytosterols that may play some roles in the hypolipidaemic effect elicited by the plant in this study[23,24].

Insulin level increased in both male and female offspring in the GL groups. This suggests that the antidiabetic effect of GL maybe by increasing insulin release from the pancreas and probably acting directly on beta cells indicating an insulin-sensitizing activity. The extract caused cellular multiplication (hyperplasia) as evident by an increased number of cells which can be translated to increased activities and production of the end products such as insulin by the beta cells suggesting the possibility of regeneration as a means for generating new β-cells.

CONCLUSION

The findings from this study show that maternal consumption of aqueous leaf extract of *Gongronema latifolium* during lactation may improve metabolic homeostasis in the young adult offspring by protecting against elevation of blood glucose level, improving against dyslipidemia in both male and female offspring later in life.

SIGNIFICANCE STATEMENT

This study discovers the possible metabolic homeostatic effect of aqueous leaf extract of *Gongronema latifolium* that may be of future therapeutic importance especially in areas where obesity and/or metabolic diseases are endemic. This study will help many researchers to understand the critical role of breast milk in the possible transfer of plant-derived phytochemicals that may affect the metabolic status of the offspring.

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