

Effect of Maternal Consumption of Sucrose and Saponin Extract from *Gongronema latifolium* Leaves During Lactation on Sexual Maturity in Rats Offsprings

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Publication Date: 2026/08/22

Abstract:

➤ Introduction:

Dietary effects of the mother on the metabolism and reproduction of the offspring. *Gongronema latifolium* is a medicinal plant with high saponin and flavonoids content, which has been reported to have hypoglycemic and hypolipidemic activity.

➤ Objectives:

This study aimed to explore the metabolic and reproductive effects of maternal consumption of sucrose and saponin extract of *G. latifolium* during lactation period in the rat pups.

➤ Study Method:

20 female albino Wistar rats were assigned randomly into various groups, control group (water only), sucrose group, sucrose + 100 mg/kg b.w. saponin, sucrose + 200 mg/kg b.w. saponin and sucrose + metformin (500 mg/70 kg b.w.). The treatments were given during lactation for 21 days. Body weight, food consumption, glucose tolerance, lipid profile, insulin, leptin, sex hormones and gonadal histology were evaluated in offspring.

➤ Results:

The maternal saponin intake had significantly reduced the body weight of the offspring compared to controls, but maternal saponin intake had increased the body weight of the offspring relative to the sucrose group, as with metformin. The intake of food was significantly reduced in the saponin groups (weeks 4–6), and 100 mg/kg was the most effective. Blood glucose was significantly reduced, especially at 200mg/kg, indicating a dose dependent hypoglycemic effect. The reduction in triglyceride and VLDL was noted compared to sucrose and there was no change in cholesterol, HDL and LDL levels. There was no effect on insulin levels, but there was a significant increase in leptin in the 100 mg/kg saponin group when compared with sucrose. Although the metabolic benefits were apparent, there were also potential reproductive risks as seen in the ovarian follicular atrophy and mild disruption to the testicles (testes) by histological examination.

➤ **Conclusion:**

G. latifolium saponin extract during lactation had beneficial effects on metabolic homeostasis, possibly by decreasing glucose and triglyceride, but had negative effects on gonadal tissue integrity. The results suggest that both therapeutic and reproductive risks exist and these agents should be used with caution.

Keywords: *Gongronema latifolium*, Saponin, Lactation, Metabolism, Reproductive Health.

How to Cite: Ebube Linda Nwanneka; John Chukwuebuka Abraham; Albert Nkereuwem Eteudo; Ikechi Chukwudi Mbaeri; Michael Arinze Epete (2026) Effect of Maternal Consumption of Sucrose and Saponin Extract from *Gongronema latifolium* Leaves During Lactation on Sexual Maturity in Rats Offsprings. *International Journal of Innovative Science and Research Technology*, 11(8), 1483-1491. <https://doi.org/10.38124/ijisrt/26aug562>

I. INTRODUCTION

Medicinal plants have been long known as one of the oldest and most reliable source of therapeutic agents in the history of humankind. In both culture and continent, they have played a role in disease prevention and treatment as part of traditional medicine and now have a place in modern pharmacology. Their medicinal properties are due to the chemical components they possess, and these components have specific physiological effects on the human body. The bioactive compounds, including flavonoids, tannins, phenolic compounds, and alkaloids, play a pivotal role in drug development and synthesis, and are highly significant in treating various diseases (Fitzgerald et al., 2019; Kumar et al., 2023).

Traditional medicine still has an important role in the delivery of healthcare worldwide. In developing countries, it is estimated that 80% of people use herbal medicine for primary healthcare (Hoenders et al., 2024). In addition to limited availability of modern medical facilities, the observed effectiveness of some herbs in treating ailments like malaria, diabetes, gastrointestinal ailments, hypertension, and so on are also driving this reliance (Chali et al., 2021). Therefore, medicinal plants are more than just cultural artefact, they are integral to public health systems, providing cheap, easily available and culturally appropriate alternatives to chemical drugs.

Of these plants, *Gongronema latifolium* is one major plant that plays significant role in West African ethnomedicine. It is an edible rainforest plant indigenous to Nigeria's S.E. region and is also fairly widespread throughout tropical countries including Guinea-Bissau, Cameroon and Sierra Leone and is locally called "Utazi" by the Igbo, "Arokeke" by Yoruba, and "Utasi" by the Efik and Ibibio (Morebise, 2015; Orumwensodia & Uadia, 2022). The crude extract from the leaves has traditionally been used for treating malaria, diabetes, hypertension, nausea and anorexia, besides its use as a spice and vegetable in local cuisines (Eke et al., 2014; Ogunwande et al., 2007). The beneficial effects have been attributed to its phytochemical constituents, among which saponins are secondary metabolites with a great variety of biological activities.

Saponins, which is from the Latin word "sapo" soap, are well characterized for their soaplike properties and ability to be soluble in water and fat (Timilsena et al., 2023; Faria-Silva et al., 2025). They've been studied extensively for their

pharmaceutical potential and found to be blood lipid lowering, cancer-reduction, blood glucose response reduction and antioxidants. The consumption of high amounts of saponins have also been associated with the inhibition of dental caries, platelet aggregation and protection against acute lead poisoning (Jolly et al., 2024). Given their properties, saponins are of special interest with regard to metabolic disorders, a growing problem in contemporary societies.

Lactational nutrition is a crucial factor in the development of offspring. Breast milk offers perfect nutrition, immune protection and regulation of growth and metabolism (Lokossou et al., 2022). Yet, the dietary habits of mothers can substantially affect the outcomes of babies. Excessive sucrose consumption during lactation has been linked to obesity, insulin resistance, dyslipidaemia, hypertension and early sexual maturation in the offspring (Samuelsson et al., 2013; Bokor et al., 2024; Goran et al., 2019). What has been demonstrated is that consuming sugary diets during pregnancy endangers the cognitive development and metabolic homeostasis of the infants, which impacts their long-term health (Cohen et al., 2018; Hernández-Aragón et al., 2026).

Notwithstanding these observations, research on possible dietary interventions to reduce the negative effects of sucrose exposure during gestation is limited. Although the negative effects of high sugar consumption have been well established, the protective effects of bioactive compounds like saponins are under studied. Considering the pharmacological activity of *Gongronema latifolium* with hypoglycemic, hypolipidemic and antioxidative properties, it is feasible that saponin extracted from the plant can reverse the metabolic and reproductive disorders caused by exposure to sucrose in the offspring (Eke et al., 2014; Ojike et al., 2022).

This study thus fills an important void in the existing literature. The effect of maternal diet on the lactational period on offspring health has been well-studied, however, few studies have investigated the combined effect of sucrose and saponin intake during lactation. This is important to understand when designing nutrition strategies in the mothers to prevent obesity, hormonal imbalance and early puberty in children. The study is especially warranted in the current era of increasing metabolic diseases globally and with the knowledge that diet is a practical and culturally appropriate method for disease prevention.

Thus, the present study was designed to examine the effects of maternal sucrose and saponin extract of *Gongronema latifolium* leaves during lactation on the sexual maturity of rat pups. In particular, the study investigated the timing of puberty, body weight, body mass index (BMI), sex hormones and gonadal histology.

➤ *Based on this Background, the Following Hypotheses were Tested:*

- H₀1: Maternal consumption of sucrose and *Gongronema latifolium* saponin extract during lactation has no significant effect on offspring puberty onset, body weight, and BMI.
- H₀2: Maternal consumption of sucrose and *Gongronema latifolium* saponin extract during lactation has no significant effect on offspring sex hormone levels and gonadal histology.

II. MATERIALS AND METHODS

➤ *Plant Material*

The leaves of *Gongronema latifolium* were collected locally and identified and authenticated by the chief taxonomist of the Plant Science Unit Department of Biotechnology, University of Nigeria, Nsukka (Osuagwu et al., 2013).

➤ *Preparation of Saponin-Rich Extract*

G. latifolium leaves were rinsed in water, dried in shade and dried powders were ground into coarse powder using Thomas-Wiley laboratory mill (Model 4) to give two kg of leaves. The preparation of saponin extract was done according to Harborne (as cited by Fordos et al., 2025) with some modifications. In brief, 1 kg of powdered leaves was extracted for 72 h using 80% methanol with the extraction medium changed every 24 h. The filtrates were combined and concentrated on a rotary evaporator at 40°C and defatted with hexane. The extract obtained was used for its saponin content and it was stored at 4°C until required.

➤ *Biological Studies and Designs*

The twenty matured female albino rats weighing 180-210 g were kept in ventilated metallic cages with 12 hour light/dark cycle at an ambient temperature of 22 °C with free access to standard pelleted feed (Vital Feed Nigeria Ltd.) and water. Animals were preconditioned for 7 days prior to the experiment.

- *The Rats were Randomly Divided into Five Groups (n = 4 for Each Group):*

- ✓ Group 1 (Control) was distilled water alone.
- ✓ Group 2 (Sucrose group): sucrose solution (30% w/v).
- ✓ Group 3: Sucrose solution (30% w/v) + 100 mg/kg b.w. saponin extract.
- ✓ Group 4: sucrose solution (30% w/v) + 200 mg/kg b.w. saponin extract.
- ✓ Group 5 (Standard control): sucrose solution (30% w/v) + metformin (500 mg/70 kg b.w.).

Lactating dams were orally treated for 21 days during the lactation period. On post-natal day (PND) 21, offspring were weaned and kept separately with chow and water ad lib. (Ajayi & Akhigbe, 2020).

➤ *The Measurement of the Body Weight and Length.*

Pup body weights were obtained each week from PND 1 to PND 42 on a digital weighing balance. The length of the body was measured using a calibrated ruler. Body Mass Index (BMI) was defined as:

$$BMI = \frac{\text{Body weight (g)}}{\text{Body length (cm)}^2} \text{ (Stephenson et al., 2022).}$$

➤ *Puberty Assessment*

Puberty was considered to have occurred in female pups when the vagina opened and in males when there was balanopreputial separation (Ajayi & Akhigbe, 2020).

➤ *The Collection of Blood and Organs.*

Samples of blood were obtained at the onset of puberty by cardiac puncture under anaesthetic. The serum was separated and kept at -20°C for hormonal assays. Gonadal tissues (ovaries and testes) were removed for histological examination (Richards & Pangas, 2010).

➤ *Hormonal Assays*

Estrogen level in serum was measured by enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer's instructions (Aguilera-Mijares et al., 2021), as well as the hormones of luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone and testosterone.

➤ *Histological Procedures*

Ovarian and Testicular tissues were fixed in 10% formalin, processed and embedded in paraffin. Sections (5 µm) were stained with hematoxylin and eosin (H&E) and then viewed using a light microscope to evaluate the morphology of the seminiferous tubules, the integrity of the corpora lutea and the number of follicles (Cora et al., 2015).

➤ *Statistical Analysis*

This was analysed using one way ANOVA with Tukey's post hoc test. Means and standard deviation (SD) were used to report results. A P value of <0.05 was considered significant (Cora et al., 2015).

➤ *Ethical Approval*

Ethical clearance with NO MPC/2502/30/002 for this experiment was obtained from the Ethical Committee for Research of the Faculty of Basic Medical Sciences, Ebonyi State University following the guidelines for animal welfare.

III. RESULTS

Table 1 shows females offspring body weight, which was observed to be significantly reduced when the extracts of *Gongronema latifolium* were consumed by mothers. However, the extracts significantly increased the body weight when compared to sucrose group; these effects were similar to that of metformin treatment.

No significant differences were found between offspring given saponin extract and either the control or sucrose groups with regard to hepatic and pancreatic weight (Table 2).

The effect of the maternal consumption of saponin extract during lactation on offspring food intake post-weaning (PND 22–42) is shown in Table 3. During weeks 4–6, a significant decrease ($p < 0.05$) in feed intake was observed in saponin treated groups (100 mg/kg and 200 mg/kg) as compared to the control group. Likewise, during week 6, these groups consumed significantly less food than the sucrose group, with saponin 100 mg/kg showing the lowest amount of food consumed.

The results from Table 4 show that the saponin extract (100 mg/kg and 200 mg/kg) fed to the mothers significantly

reduced the blood glucose level of their offspring compared to the control and sucrose groups. The largest reduction was seen in the saponin 200 mg/kg group.

As per the table 5, the level of cholesterol, triglycerides, HDL, LDL and VLDL were not significantly changed after giving saponin extract to the animals. Triglyceride and VLDL were, however, significantly lowered in comparison to the sucrose group.

No significant difference in insulin level was observed between maternal consumption of saponin extract and control and sucrose groups as shown in Table 6. There was no effect on leptin level relative to the control, however, there was a significant rise in leptin level relative to the sucrose group when saponin 100 mg/kg was used.

Table 1 Mean Values of Neonatal Weight, Length and Vaginal Opening of the Female Offspring

Parameters	Groups	mean±SEM	P-values		
Female offspring neonatal weight (FNW) (g)	1	108.80±8.03			
	2	92.64±3.45	0.174		
	3	74.10±3.0	0.001	0.113	
	4	94.03±2.07	0.563	1.000	0.260
	5	79.75±5.36	0.003	0.409	0.983
Female offspring neonatal length (FNL) (cm)	1	17.27±0.59			
	2	16.19±0.32	0.382		
	3	14.46±0.24	0.001	0.049	
	4	16.40±0.12	0.852	1.000	0.114
	5	14.90±0.50	0.003	0.197	0.987
Vaginal opening (VOP) (days)	1	53.67±5.85			
	2	49.63±1.95	0.932		
	3	42.60±1.63	0.160	0.582	
	4	43.67±0.67	0.423	0.864	1.000
	5	41.50±1.18	0.071	0.352	1.000
Female offspring body mass index (FBMI) (g/cm ²)	1	0.36±0.01			
	2	0.35±0.01	0.996		
	3	0.35±0.01	0.997	1.000	
	4	0.35±0.00	0.988	1.000	1.000
	5	0.36±0.01	1.000	1.000	1.000

Table 2 Results of Mean Values of Selected Female Offspring Hormones and Comparison Negative and Positive Control Groups to Each other and to Other Groups

Groups	Female			
	FSH	LH	Progesterone	Estradiol
	mean±SEM	mean±SEM	mean±SEM	mean±SEM
1	9.66±0.44	4.91±0.20	6.64±0.23	21.51±0.57
2	10.02±0.30 ^{a#}	4.37±0.20 ^{a#}	7.40±0.24 ^{a#}	20.04±1.33 ^{a#}
3	9.48±0.51 ^{a#b#}	4.95±0.13 ^{a#b#}	4.62±0.13 ^{a*b*}	20.71±0.36 ^{a#b#}
4	9.77±0.09 ^{a#b#}	4.84±0.26 ^{a#b#}	4.63±0.21 ^{a*b*}	20.27±0.81 ^{a#b#}
5	9.54±0.42 ^{a#b#}	4.61±0.11 ^{a#b#}	4.93±0.07 ^{a#b*}	20.64±0.45 ^{a#b#}

LH and FSH stood for Luteinizing Hormone and Follicle Stimulating Hormone respectively, a* denoted significant difference when each of groups 2, 3, 4 and 5 were compared to group 1 while b* represented significant difference when each of the groups 3, 4 and 5 were compared

to group 2 at $P < 0.05$ a# denoted no significant difference when each of groups 2, 3, 4 and 5 were compared to group 1 while be represented no significant difference when each of the groups 3, 4 and 5 were compared to group 2 at $P < 0.05$.

Table 3 Showing Mean Values of Neonatal Weight, Length and Balano-Preputial Separation of the Male Offspring

Parameters	Groups	Mean $\pm$ SD	P-values		
Male offspring neonatal weight (MNW) (g)	1	124.92 $\pm$ 9.00			
	2	89.23 $\pm$ 6.97	0.002		
	3	80.78 $\pm$ 3.87	0.000	0.936	
	4	119.00 $\pm$ 5.46	0.984	0.012	0.000
	5	92.13 $\pm$ 2.58	0.002	1.000	0.689
Male offspring neonatal weight (MNL) (cm)	1	17.44 $\pm$ 0.41			
	2	15.68 $\pm$ 0.62	0.006		
	3	14.97 $\pm$ 0.28	0.000	0.650	
	4	17.63 $\pm$ 0.23	0.999	0.001	0.000
	5	15.70 $\pm$ 0.09	0.002	1.000	0.486
Balano-Preputial Separation(days)	1	49.60 $\pm$ 4.07			
	2	48.00 $\pm$ 2.00	0.995		
	3	41.67 $\pm$ 0.92	0.019	0.144	
	4	43.50 $\pm$ 0.34	0.124	0.505	0.976
	5	41.83 $\pm$ 0.70	0.023	0.165	1.000
Male offspring (MBMI) (g/cm ²)	1	0.41 $\pm$ 0.01			
	2	0.36 $\pm$ 0.00	0.130		
	3	0.36 $\pm$ 0.01	0.051	1.000	
	4	0.38 $\pm$ 0.01	0.641	0.872	0.724
	5	0.37 $\pm$ 0.01	0.331	0.987	0.953

Table 4 Showing Mean Values of Selected Male Offspring Hormones and Comparison Negative and Positive Control Group to Each other and to Other Groups

Groups	Male			
	Testosterone	Estradiol	LH	FSH
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
1	7.53 $\pm$ 0.09	24.43 $\pm$ 1.44	5.44 $\pm$ 0.31	13.04 $\pm$ 0.34
2	7.51 $\pm$ 0.15 ^{a#}	29.54 $\pm$ 0.82 ^{a#}	5.80 $\pm$ 0.41 ^{a#}	14.65 $\pm$ 0.18 ^{a#}
3	5.03 $\pm$ 0.04 ^{a*b*}	21.34 $\pm$ 0.24 ^{a#b*}	4.81 $\pm$ 0.06 ^{a#b#}	9.58 $\pm$ 0.23 ^{a*b*}
4	5.28 $\pm$ 0.12 ^{a*b*}	21.23 $\pm$ 0.62 ^{a#b*}	6.40 $\pm$ 2.28 ^{a#b#}	9.56 $\pm$ 0.76 ^{a*b*}
5	4.94 $\pm$ 0.09 ^{a*b*}	20.55 $\pm$ 0.44 ^{a#b*}	4.78 $\pm$ 0.01 ^{a#b#}	9.52 $\pm$ 0.29 ^{a*b*}

LH and FSH stood for Luteinizing Hormone and Follicle Stimulating Hormone respectively. a* denoted significant difference when each of groups 2, 3, 4 and 5 were compared to group 1 while b* represented significant difference when each of the groups 3, 4 and 5 were compared to group 2 at P < 0.05. a# denoted no significant difference when each of groups 2, 3, 4 and 5 were compared to group 1 while b# represented no significant difference when each of the groups 3, 4 and 5 were compared to group 2 at P < 0.05

➤ Ovarian Histology of the Offspring

• Plate 1:

Group 1 (negative control) showing section of offspring ovarian tissue with different stages of follicular development (FD) and the whorl ovarian stroma (WOS) appear normal with mild loss of follicular fluid (X100) (H/E).

• Plate 2:

Group 2 (consumed 30% sucrose only) showing section of ovarian tissue with different stages of follicular development (FD) with moderate follicular fluid atrophy (FFA) and mild fibrotic change (FC). (X100) (H/E).

• Plate 3:

Group 3 (consumed 30% sucrose and 100mg (kg b.w) of saponin extract from *G. latifolium*) showing ovarian tissue with moderate follicular fluid atrophy (FFA), focal area of hemorrhage (H) and infiltration of inflammatory cell (IIC) within the ovarian stroma. (X100) (H/E).

• Plate 4:

Group 4 (consumed 30% sucrose and 200mg (kg b.w) of saponin extract from *G. latifolium*) showing ovarian tissue with decrease in the number of ovarian follicles and moderate infiltration of inflammatory cell (IIC) within the whorl ovarian stroma. (X100) (H/E).

• Plate 5:

Group 5 (consumed 30% sucrose and methformine 500mg/70kg) showing ovarian tissue with necrotic (n) corpus luteum (cl), moderate follicular fluid atrophy (A) and infiltration of inflammatory cell (Iic). (X100) (H/E).

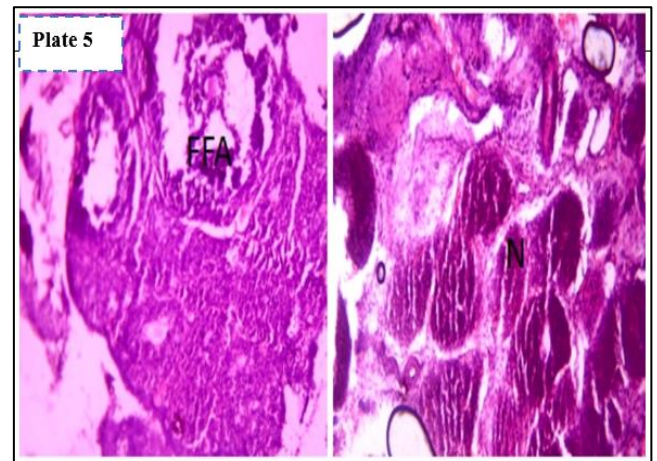
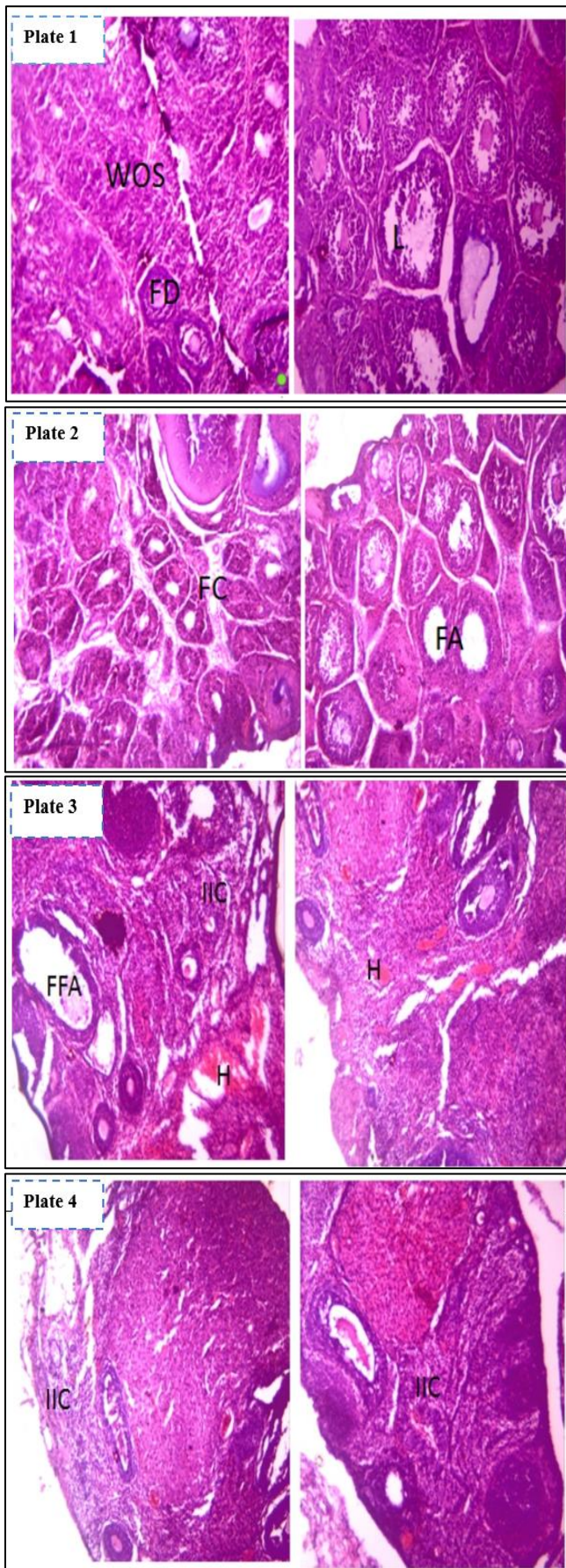


Fig 1 Ovarian Histology of the Offspring

➤ Histology of the Testes

• Plate 6:

Group 1(negative control) testicular section showing active seminiferous tubules that are lined with interstitial cells of the leydig (ILC), sertoli cells (SC) and well enhanced spermatogenesis (WES). (x400) (H and E).

• Plate 7:

Group 2(consumed 30% sucrose only) testicular section showing mild effect on the testicular tissue with mild distortion of seminiferous tubules (D) and mild spermatogenic arrest(SA). (x400) (H and E).

• Plate 8:

Group 3 (consumed 30% sucrose and 100mg (kg b.w) of saponin extract from *G. latifolium*) testicular section showing mild distortion (D) of seminiferous tubules and mild atrophy (A) of seminiferous tubules in some area .(x400) H and E.

• Plate 9:

Group 4 (consumed 30% sucrose and 200mg (kg b.w) of saponin extract from *G. latifolium*) testicular section showing moderate distortion (D) of seminiferous tubules and mild spermatogenic arrest (SA). (x400) H and E.

• Plate 10:

Group 5 (consumed 30% sucrose and methformine 500mg/70kg) testicular section showing sever necrotic (N) seminiferous tubules and mild distortion (D). (x400) H and E.

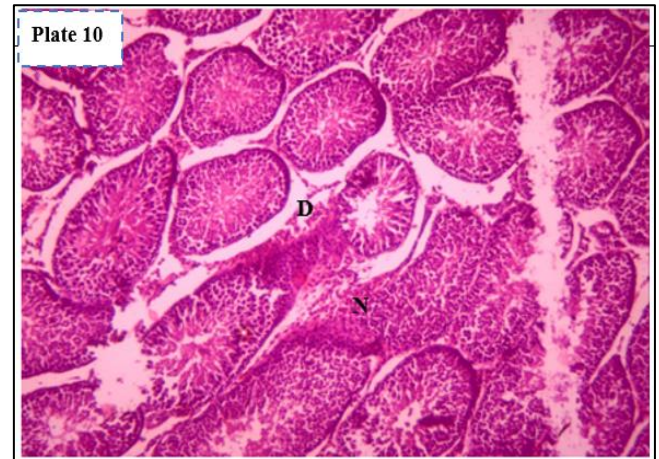
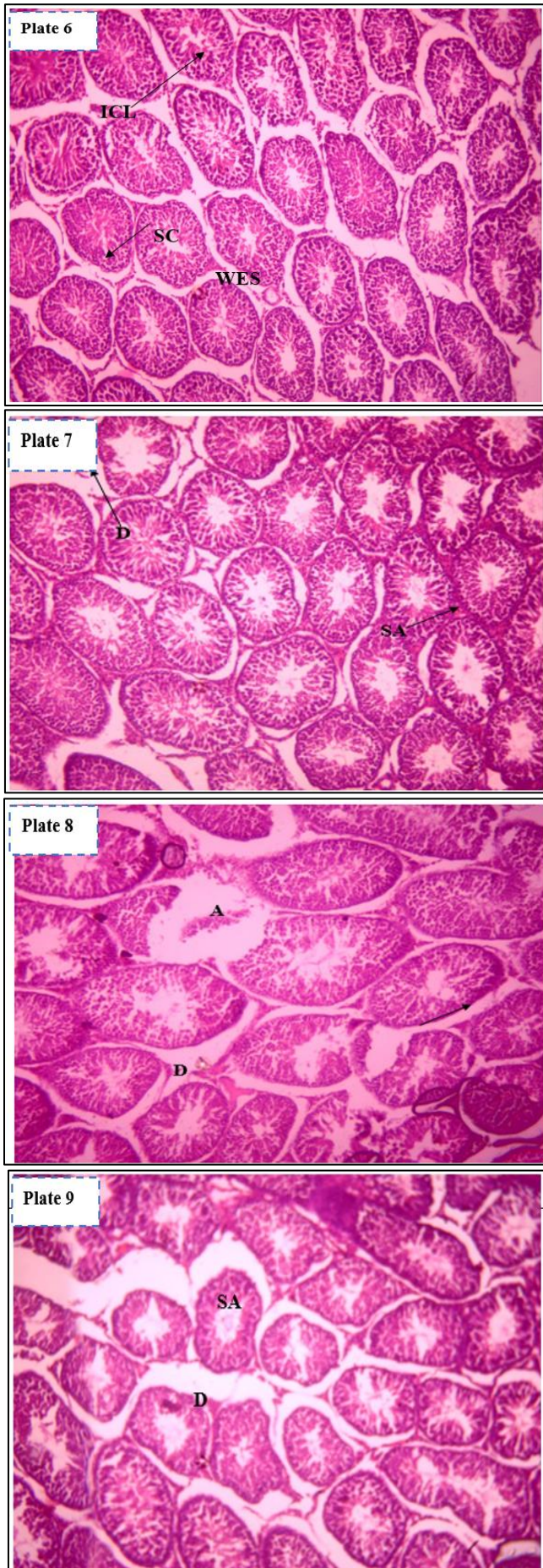


Fig 2 Histology of the Testes

IV. DISCUSSIONS

The effect of the addition of sucrose to the diet of lactating mothers and the application of saponin extract of *Gongronema latifolium* on the growth of their offspring, puberty onset, sex hormones, and gonadal histology was investigated in this study.

Key findings indicated that saponin extract significantly reduced the body weight and body length of the neonates in a dose-dependent manner while having no effect on the onset of puberty in females or BMI. This finding is consistent with previous studies demonstrating *G. latifolium* extracts' ability to decrease food intake and body weight in rodents (Kim et al., 2005), possibly attributed to the bitter phytochemicals' effects on appetite reduction or metabolic impacts of saponin on insulin signaling (Khunti & Davies, 2012). Although saponin does not affect insulin sensitivity or ovulatory function, unlike metformin (Salomaki et al., 2014; Palomba et al., 2010), it does not impact vaginal opening, which demonstrates its differences from metabolic effects.

In females, the hormonal analysis showed that there was a significant increase in the level of progesterone in the saponin treated group of female rats, which is consistent with the findings of Adewumi et al. (2020); Ogbonna et al. (2022), which reported that *G. latifolium* improved the levels of lactogenic hormones. Contrary to this, FSH and LH did not change, indicating that saponin exerts its effects on particular steroidogenic pathways. Testosterone was decreased in male children, but this is not in agreement with previous reports showing induction of hyperglycaemia in diabetic models of mice and rats (Pashapour et al., 2023).

The histological findings revealed ovarian disruption characterized by a follicular atrophy and necrosis with inflammatory infiltration, mostly in the saponin treated groups. These changes are similar to what is observed in the case of impaired folliculogenesis and atresia brought about by the phytochemicals (Sharma et al., 2013; Lilaram et al., 2012). The sections of the testicles showed mild atrophy and spermatogenic arrest, which also indicates that the gonadal effects were adverse.

Several limitations of the study were identified: the use of Wistar rats in this study may not fully reflect the human physiology and the study was restricted to selected reproductive and metabolic indices.

Clinical implications are that maternal nutrition during lactation may affect reproductive development in the offspring, and that this effect may be long-lasting and have implications for fertility and metabolic health.

V. CONCLUSION

Sucrose was fed to mothers during lactation and offspring body weight and length were decreased while BMI and puberal onset were unaffected by feeding sucrose with *Gongronema latifolium* saponin extract. Increased levels of progesterone were observed with the saponin extract, which confirms its lactogenic effects, but histological results showed it had negative effects on ovarian and testicular tissue integrity. These results indicate that *G. latifolium* can be beneficial for lactation but can also be detrimental for gonadal development when fed in conjunction with a high sucrose diet.

ACKNOWLEDGEMENTS

The authors extend their gratitude to all individuals who willingly gave their consent and participated by responding to the questionnaire. Special appreciation is also directed to the traditional leaders of the selected communities for their support and cooperation in mobilizing participants for this study.

➤ Financial Support and Sponsorship

This research was fully funded through self-sponsorship by the authors.

➤ Competing Interests

The authors declare that there are no conflicts of interest associated with this paper.

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