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Original Research Article

Impact of Gestational Stress and Administration of *Moringa oleifera* Leaveson Post-Natal Reproductive Development of Male Offspring of Wistar Rats

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ABSTRACT

Maternal health and perinatal environment especially during pregnancy greatly impact offspring postnatal reproductive development. Pregnant women frequently use herbal products to improve their health as safe alternatives to conventional drugs. *Moringaoleifera*Leaf Extract (MoLE) may have antioxidant and anti-stress properties. This study investigated the impact of prenatal MoLE exposure in chronic stress environment on the postnatal reproductive development of male offspring of Wistar rats. Twenty-five (25) healthy virgin female Wistar rats were randomly divided into 5 groups, and exposed to MoLE and chronic unpredictable stress (CUS) protocol for 2 weeks, as follows: Group I (control), Group II (5 mg/kg body weight/day of MoLE), Group III (10 mg/kg body weight/day of MoLE), Group IV (CUS + 5 mg/kg body weight/day of MoLE), Group V (CUS + 10 mg/kg body weight/day of MoLE). Pups were monitored for puberty onset, and their body weight, length, body mass index (BMI), and sex hormone levels were measured at puberty onset. Reproductive organs were weighed and examined histologically. MoLE and CUS exposure were associated with significant increases in postnatal body weight, length, BMI, and reproductive organ weights, along with histological changes and delayed puberty onset in offspring. Findings suggest that prenatal MoLE exposure in a chronic stress environment can lead to accelerated postnatal growth and delayed puberty in offspring.

Keywords: Development, Pregnancy, Puberty, Reproduction, Stress.

Introduction

The development of the offspring is significantly influenced by perinatal environment and maternal health, particularly during pregnancy. During the early stages of fetal brain development (gestation days 14–21) in both experimental animals and humans, alterations to one or both of these factors, such as exposure to stressful events, can result in profound and enduring postnatal effects on progeny reproduction,[1] as well as lifelong modifications to physiology, behaviour, morphology, and neuroendocrine development,[2] and this process is referred to as prenatal programming of neuroendocrine system that controls reproduction.[3] The chronic unpredictable stress (CUS) model is a common experimental paradigm used to investigate the effects of stress exposure in a range of animal models since it has been repeatedly shown to generate alterations in physiological environment typical of chronic stress-response. The main characteristic of CUS is erratic, irregular, and random exposure to a range of stimuli over several weeks.[4]

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Herbal products are frequently used by pregnant women to treat non-life-threatening ailments or to enhance their overall wellbeing because they are typically seen as a safe, natural substitute for conventional medications.[5]

Herbal remedies are also becoming more popular around the world as a result of the resurgence of interest in the use of medicinal plants to maintain human health, as well as their origin, lower cost, minimal side effects, and bioprospecting of medications.[6]

Moringaoleifera, a member of the *Moringa* genus and family, *Moringaceae,* is a well-known medicinal plant with multiple traditional uses, and is native to Asia, America and Africa. *Moringaoleifera*leaf extract (MoLE) which is commonly consumed by both sexes of reproductive age in Nigeria for its purported medicinal benefits. It has been nicknamed the "Mother's best friend", "Ben oil tree", and the "Drumstick tree".[7] The high quantity of essential minerals, proteins, vitamins, and *β*-carotene found in *M. oleifera* has led to its recognition as having tremendous medical value.[8] This study investigated how antenatal MoLE administration under circumstances of chronic stress affected the male offspring's postnatal growth and reproductive development.

Materials and Methods

Collection of Plant, Authentication and Extraction Procedure

Moringaoleifera fresh leaves were collected from a garden in Abakaliki, Ebonyi State, Nigeria, in September, 2022. Authentication of the plant was carried out by a botanist, at the Herbarium Unit, Department of Biological Science, AlexEkwueme Federal University Ndufu-Alike (AE-FUNAI) Ebonyi State, Nigeria.Herbarium specimen was deposited with the herbarium number AE-FUNAI UH 504a. Collected leaves were shade-dried for 2 weeks and subsequently ground into a fine powder (particle size < 250 micrometers) using a grinding

machine (Miller: model ms-233, China).[4,9] A standardized Soxhlet extraction method was employed using methanol as the solvent. Two hundred grams of the powdered leaves were extracted three times for 48 hours each. The combined filtrates were concentrated using a rotary evaporator at 40°C to obtain a pasty dark green extract, which was stored at 4°C in an airtight labelled container to ensure potency, the yield was calculated.[4]

Ethical Approval

This study adhered to the ARRIVE guidelines and was granted ethical approval by the Faculty of Basic Medical Sciences Research Ethics Committee at AE-FUNAI (code: FBMS/EC/AE/1983).

Experimental Design

Twenty-five healthy, mature virgin female Wistar rats were obtained from the Animal House, AE-FUNAI, and acclimatized to standard laboratory conditions for two weeks prior to the study. The rats were randomly divided into 5 groups (n = 5) on the first day of pregnancy. The experimental groups were:
Group I: received only standard feed and water *ad libitum* throughout pregnancy, thus served as the control.
Group II: received MoLE (5 mg/kg body weight/day) from gestational day (GD) 8 to 21.
Group III: received MoLE (10 mg/kg body weight/day) from GD 8 to 21.
Group IV: was exposed to the CUS protocol and MoLE (5 mg/kg body weight/day) from GD 8 to 21.
Group V: was exposed to the CUS protocol and MoLE (10 mg/kg body weight/day) from GD 8 to 21.
MoLE was administered orally once daily via oral gavage following exposure to the CUS regimen. The offspring were weaned to standard feed and water *ad libitum* at 21 days and maintained in their respective groups**.** The male and female pups were housed separately after weaning. The gestational period from GD 8 to 21, was selected as it corresponds to a critical period of fetal brain development in rats, analogous to the second and third trimesters in humans, when the brain is highly susceptible to environmental influences. The doses of MoLE (5 and 10 mg/kg/day) were chosen based on previous study by Chukwu*et al*.,[4] demonstrating the safety and efficacy of *M. oleifera* leaf extracts at similar doses in animal models. Additionally, these doses were selected to evaluate a dose-dependent response to MoLE treatment Chukwu*et al*.[4]

Initiation of Pregnancy

Light microscopy made it feasible to observe the estrus cycles, and the animals selected for the study were those that displayed two consecutive regular 4-day estrus cycles. Healthy, mature male Wistar rats were paired with the female rats at 1:2 ratio in their cages during pro-estrus phase to encourage mating. The next morning, the female rats' vaginal smears revealed the presence of spermatozoa which indicate that mating was successful and then marked the first day of pregnancy.[10, 11]

Chronic Unpredictable Stress (CUS) Protocol

The stress group of animals were subjected to CUS protocol from GD 8 (which is around the period of onset of organogenesis in rats) to GD 21 (expected day of parturition). The CUS protocol, a complex stress paradigm, was employed to expose animals to a variety of stressors in an unpredictable manner. The stressors included: wet bedding, cage tilting, restricted food access, exposure to a predator, sleep deprivation, restraint, and prolonged illumination.
Wet Bedding: was created by combining 300 millilitres of water with 1 litre of sawdust.
Cage Tilting:The cage was tilted at a 45-degree angle, with feed and water placed at the higher end.
Difficulty Accessing Food: animals were subjected to a single night of difficulty in obtaining food.
Psychological Stress: A cat in a cage was introduced to the rats as a psychological stressor.
Sleep Deprivation: was induced by placing a cylindrical wooden pedestal of 6 cm in diameter and 5 cm in height opposite the feed and water compartment on the cage floor which was filled with 3 cm of tap water, preventing the animal from sleeping but allowing it to remain standing on the pedestal.
Restraint Stress: involved housing the rats in a small plastic tube for six hours, divided into three two-hour sessions with 30-minute intervals.
A single night of continuous illumination.
Social Isolation (SI): was a constant stressor due to the animals' isolation during the application of each stressor.
These stressors were administered sequentially, ensuring unpredictability throughout pregnancy. Except for sleep deprivation sessions that lasted 12 hours, all other stressors lasted for 6 hours per day. The animals in the CUS groups were exposed to each stressor twice during the study. The CUS protocol was employed to mimic the unpredictable and uncontrollable nature of human prenatal stress. This model has been widely used in preclinical studies to investigate the effects of stress on maternal and fetal outcomes.[4, 12]

Balano-preputial Separation (BPS) in Offspring

Given the typical onset of puberty shortly after weaning, offspring were monitored daily for BPS beginning on day 21. The age in days when puberty was first observed was recorded.

Body Parameters

The body weights and lengths of offspring were recorded at puberty onset, and BMI was calculated using the formula:

$$BMI = \frac{\text{Weight (g)}}{\text{Length (cm)}^2} \quad \ldots\ldots\ldots (1)$$

Biochemical Study

At puberty onset, male offspring were randomly selected from each group and subjected to blood sampling. Two milliliters of blood were drawn from the retro-orbital sinus of each selected offspring into plain tubes for hormonal analysis. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were determined using enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's instructions.

Histopathological Study

The animals were fasted for 24 hours before being humanely sacrificed. The epididymis and testes were excised and weighed, after abdominal incision. The tissues were carefully cleaned and preserved in Bouin's fixative. Following dehydration with ethanol and clearing with xylene, the tissues were embedded in paraffin wax. Thin sections (5 µm) were cut using a microtome, stained with hematoxylin and eosin (H&E), and mounted in Canada balsam for histological examination.

Sperm Analysis

To evaluate sperm parameters, the cauda epididymides were excised and dissected in 1.5 mL of phosphate-buffered saline (PBS) at 37°C to release stored sperm. Sperm suspensions were incubated at 37°C and analyzed using a light microscope at a magnification of 400x, according to World Health Organization criteria (5th edition), with modifications.[13, 14]

Sperm Motility Assessment

Percentage of motile spermatozoa was determined on a pre-warmed slide. Approximately 10 microliters of sperm suspension were analyzed under an Olympus CX 41 light microscope at 400x magnification. The degree of motility was evaluated in 5 randomly selected fields on each slide, categorizing sperm as actively motile (AM), sluggishly motile (SM), or non-motile (NM).[13, 14]

Sperm Morphology Assessment

Sperm samples were examined for aberrant sperm traits, including pinhead, no-hook, curved mid-piece, bent mid-piece, tail-less, coiled

tail, looped tail, and bent tail. The percentage of abnormal sperm was calculated based on the ratio of total number of abnormal sperm to total number of sperm observed.[13, 14]

Sperm Count Assessment

The sperm count was assessed by diluting sperm suspension with 10% formalin fixative and counting spermatozoa in a Neubauer chamber. Counting discrimination criteria were determined based on the location of the sperm heads within the chamber squares. Sperm with aberrant flagellum and head morphology were excluded.[13, 14]

Statistical Analysis

Data were analyzed using GraphPad® Prism Software (San Diego, CA, USA). Data comparison was performed with one-way ANOVA and the Turkey post hoc test. The results were consideredsignificant at $p < 0.05$. Results were expressed as means ± standard error of means (SEM).

Results and Discussion

Offspring Age and Body Parameters at Puberty Onset

Groups III, IV, and V demonstrated significant increases in body weights compared to Group I (Table 1). In contrast to Group I, Group II exhibited a non-significant weight gain. Groups II, III, and IV demonstrated a statistically significant increase in length compared to Group I. When compared to Group I, Group V showed no significant increase in length. The body mass index (BMI) of Groups IV, and V was significantly higher than that of Group I. Groups II and III showed no significant increase in BMI compared to Group I. Groups II, III, IV, and V reached puberty significantly later than Group I.

Table 1: Offspring Age and Body Parameters at Puberty Onset

Group	Variable			
	Body Weight (g)	**Length (cm)**	**BMI (g/cm^2)**	**Age (Days)**
Group I (Control)	84.25 ± 3.07	16.33 ± 0.17	0.32 ± 0.02	39.60 ± 0.25
Group II (MoLE 5 mg/kg)	94.02 ± 2.79	17.33 ± 0.17 [a]	0.31 ± 0.00	44.00 ± 0.32 [a]
Group III (MoLE 10 mg/kg)	99.26 ± 3.58 [a]	17.33 ± 0.17 [a]	0.33 ± 0.01	44.80 ± 0.37 [a]
Group IV (CUS + MoLE 5 mg/kg)	133.10 ± 1.83 [a]	18.47 ± 0.03 [a]	0.39 ± 0.01 [a]	59.80 ± 0.37 [a]
Group V (CUS + MoLE 10 mg/kg)	107.28 ± 1.91 [a]	17.00 ± 0.29	0.37 ± 0.01 [a]	57.40 ± 0.25 [a]

Values are expressed as Mean ± SEM; [a] = $P < 0.05$ versus control.

Table 2: Absolute weight of Testes and Epididymis from offspring at Onset of Puberty

Variable	Group				
	Group I (Control)	**Group II (MoLE 5 mg/kg)**	**Group III (MoLE 10 mg/kg)**	**Group IV (CUS + MoLE 5 mg/kg)**	**Group V (CUS + MoLE 10 mg/kg)**
Testes (g)	0.57 ± 0.03	0.63 ± 0.02	0.66 ± 0.04	1.03 ± 0.03 [a]	0.77 ± 0.02 [a]
Epididymis (g)	0.033 ± 0.006	0.043 ± 0.003	0.047 ± 0.008	0.050 ± 0.006	0.056 ± 0.003

Values are expressed as Mean ± SEM; [a] = $P < 0.05$ versus control.

Table 3: Relative Weight (mg) of Testes and Epididymis of Offspring at Onset of Puberty

Variable	Group				
	Group I (Control)	**Group II (MoLE 5 mg/kg)**	**Group III (MoLE 10 mg/kg)**	**Group IV (CUS + MoLE 5 mg/kg)**	**Group V (CUS + MoLE 10 mg/kg)**
Testes weight (mg)	6.77 ± 0.12	6.73 ± 0.07	6.63 ± 0.12	7.73 ± 0.19 [a]	7.13 ± 0.09
Epididymis weight (mg)	0.39 ± 0.07	0.46 ± 0.05	0.47 ± 0.08	0.43 ± 0.03	0.46 ± 0.01

Values are expressed as Mean ± SEM; [a] = $P<0.05$ versus control

Table 4: Serum LH, FSH, and Testosterone Level of Offspring

Variable	Group				
	Group I (Control)	**Group II (MoLE 5 mg/kg)**	**Group III (MoLE 10 mg/kg)**	**Group IV (CUS + MoLE 5 mg/kg)**	**Group V (CUS + MoLE 10 mg/kg)**
LH (IU/L)	5.96 ± 0.41	7.47 ± 0.23	8.28 ± 0.24 [a]	8.68 ± 0.65 [a]	7.38 ± 0.13
FSH (IU/L)	3.11 ± 0.12	3.91 ± 0.28 [a]	4.38 ± 0.14 [a]	4.02 ± 0.09 [a]	3.69 ± 0.06
Testosterone (ng/mL)	9.16 ± 0.18	8.37 ± 0.36	7.92 ± 0.06	6.70 ± 0.47 [a]	7.17 ± 0.12 [a]

Values are expressed as Mean ± SEM; [a] = $P < 0.05$ versus control.

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Table 5: Sperm Motility and Sperm Count of Offspring at Onset of Puberty

Variable	Group				
	Group I (Control)	Group II (MoLE 5 mg/kg)	Group III (MoLE 10 mg/kg)	Group IV (CUS + MoLE 5 mg/kg)	Group V (CUS + MoLE 10 mg/kg)
% AM (Active Motility)	45.00 ± 7.64	35.00 ± 7.64	30.00 ± 2.89	8.33 ± 1.67 [a]	21.67 ± 1.67
% SM (Sluggish Motility)	16.67 ± 1.67	18.33 ± 1.67	25.00 ± 2.89	26.67 ± 1.67 [a]	23.33 ± 1.67
% NM (Non-Motile)	30.00 ± 5.77	46.67 ± 6.01	43.33 ± 3.33	75.00 ± 2.89 [a]	55.00 ± 2.89 [a]
Count ($x10^6$/L)	38.33 ± 3.28	34.33 ± 2.33	35.33 ± 3.28	29.67 ± 2.03	32.00 ± 2.31

Values are expressed as Mean ± SEM; [a] = $P < 0.05$ versus control.

Table 6: Sperm Morphology of Offspring at Onset of Puberty

Variable	Group				
	Group I (Control)	Group II (MoLE 5 mg/kg)	Group III (MoLE 10 mg/kg)	Group IV (CUS + MoLE 5 mg/kg)	Group V (CUS + MoLE 10 mg/kg)
Pin Head (PH)	1.33 ± 0.33	2.33 ± 0.33	1.66 ± 0.33	2.00 ± 0.58	2.68 ± 0.33
Round Head (RH)	1.33 ± 0.33	1.33 ± 0.33	1.67 ± 0.33	2.33 ± 0.33	1.33 ± 0.33
Headless Tail (HT)	133 ± 0.33	1.67 ± 0.33	2.33 ± 0.33	6.00 ± 0.58 [a]	4.00 ± 0.58 [a]
Looped Tail (LT)	0.33 ± 0.33	0.67 ± 0.33	1.00 ± 0.00	0.67 ± 0.33	0.67 ± 0.33
Bent Mid Piece (BMP)	1.00 ± 0.58	2.00 ± 0.00	3.00 ± 0.58	2.00 ± 0.58	2.00 ± 0.58
Coiled Mid Piece (CMP)	0.67 ± 0.33	1.67 ± 0.33	1.33 ± 0.33	1.67 ± 0.33	0.67 ± 0.33

Values are expressed as Mean ± SEM; [a] = $P < 0.05$ versus control.

Despite the observed increases in body weight, length, and BMI, gestational stress resulted in a delayed onset of puberty, suggesting a potential growth-promoting effect without concomitant reproductive maturation. This discrepancy between somatic growth and reproductive development indicates a disruption in the hormonal pathways necessary for puberty, as previously reported.[11,15]

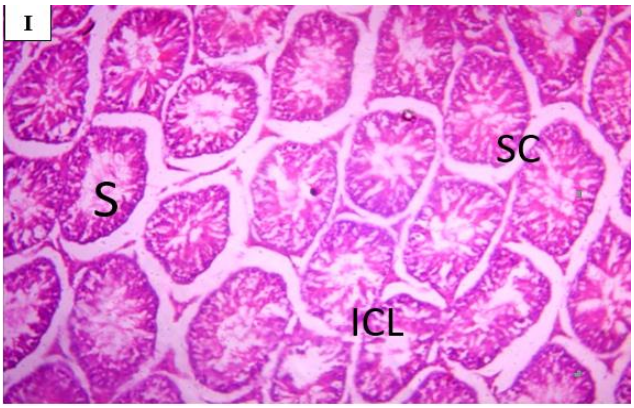

Figure 1: Photomicrograph of Group I section of the Testes (X400) (H/E) shows normal testicular architecture with active seminiferous tubules that are lined with Interstitial Cells of Leydig (ICL), Sertoli Cell (SC) and Spermatogenesis (S)

Potential underlying mechanisms may include hypothalamic-pituitary-gonadal axis dysregulation, oxidative stress, and inflammation.[9,16]MoLE supplementation appeared to mitigate some of these negative effects, particularly at higher doses, suggesting its potential to restore hormonal balance and promote normal pubertal development. These findings underscore the importance of managing maternal stress during pregnancy to optimize child development and reproductive health outcomes. Early interventions, such as nutritional supplementation with

compounds like *Moringaoleifera*, may be beneficial in mitigating the long-term consequences of prenatal stress due to their antioxidant, anti-inflammatory, and hormonal regulatory properties.[7]

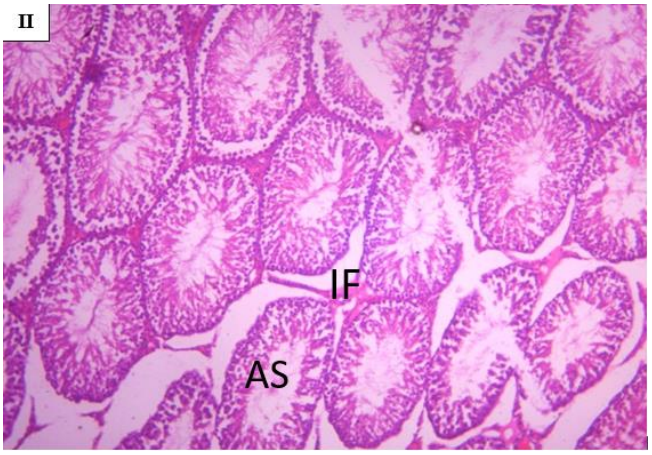

Figure 2: Photomicrograph of Group II section of Testes (X400) (H/E) shows mild Arrest of Spermatogenesis (AS) with mild Interstitial Fibrosis (IF)

Absolute weight (g) and Relative weight (mg) of Testes and Epididymis at Puberty Onset

Table 2 presents the absolute weights of the testes and epididymis, while Table 3 shows the relative weights. Compared to Group I, the absolute weight of the testes increased significantly in Groups IV and V, but not in Groups II and III. When comparing Groups II, III, IV, and

V to Group I, there was a non-significant increase in the absolute organ weight of the epididymis. As presented in Table 3, there was a significant increase in the relative weight of the testes in Group V compared to Group I. Groups II and IV showed no significant increase in the relative weight of the testes, while Group III exhibited no

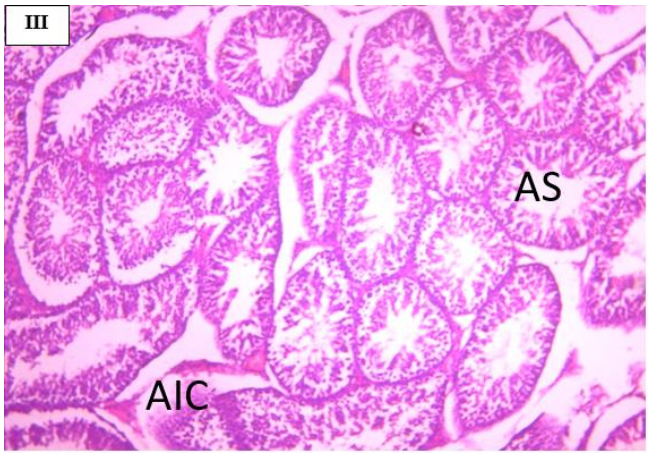

Figure 3: Photomicrograph of Group III section of the Testes (X400) (H/E) shows mild Arrest of Spermatogenesis (AS) with focal Aggregate of Inflammatory Cell (AIC)

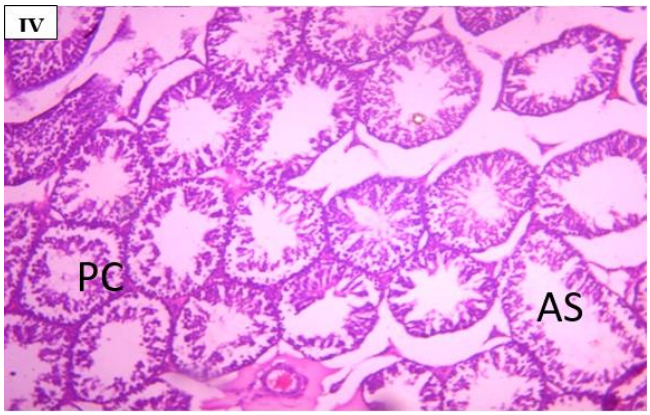

Figure 4: Photomicrograph of Group IV section of Testes (X400) (H/E) shows damage to testicular tissue with severe Arrest of Spermatogenesis (AS) and Pyknotic Testicular Cell (PC)

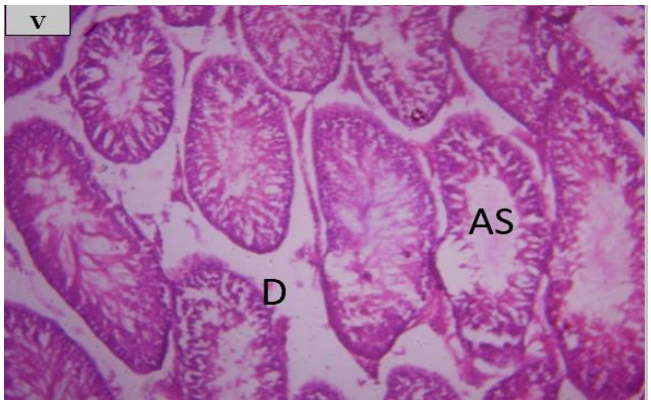

Figure 5: Photomicrograph of Group V section of Testes (X400) (H/E) shows moderate Distortion (D) of the Seminiferous Tubules and mild Arrest of Spermatogenesis (AS)

significant decrease. The relative weight of the epididymis did not change significantly in Groups II, III, IV or V compared to Group I.

Gestational stress significantly compromised testicular and epididymal development, as evidenced by increased absolute and relative testicular weights, histological abnormalities including arrest of spermatogenesis, interstitial fibrosis, and inflammatory cell infiltration, and reduced sperm parameters. Underlying mechanisms likely involve oxidative stress, inflammation, and hormonal dysregulation, which can disrupt testicular and epididymal function.[17]Whileepididymal absolute weight remained relatively unchanged, histological alterations indicated functional impairment (Figures 8-10). *Moringaoleifera* supplementation demonstrated a protective effect on testicular and epididymal tissues, with varying degrees of amelioration depending on the dose, potentially through its antioxidant, anti-inflammatory, and hormonal regulatory properties.[8]These findings highlight the vulnerability of the male reproductive system to prenatal stressors and underscore the need for interventions to protect testicular and epididymal health

.

Serum LH, FSH, Testosterone Level of Offspring at Puberty Onset

The serum levels of FSH, LH, and testosterone in the various groups are summarized in Table 4. When comparing Groups II and V to Group I, there was no significant increase in serum LH levels, while Groups III and IV exhibited a significant elevation in LH levels. Compared to Group I, Group V demonstrated a non-significant increase in serum FSH levels, whereas Groups II, III, and IV exhibited significant increases. Serum testosterone levels decreased significantly in Groups IV and V but there was no significant changes in the testosterone level in Groups II and III compared to Group I.

Gestational stress induced hormonal imbalances characterized by elevated LH and FSH levels, indicative of compensatory pituitary response to impaired testicular function, and decreased testosterone levels due to compromised Leydig cell function.[3,18] This hormonal dysregulation likely mediated by oxidative stress and inflammation, contributed to testicular and epididymal alterations.[19] MoLEinfluence on normalizing hormone levels suggests its potential role in regulating the hypothalamic-pituitary-gonadal axis, consistent with with its reported hormonal regulatory effects.[7,8] These findings underscore the importance of maintaining hormonal balance during pregnancy for optimal male reproductive development.

Sperm Motility, Sperm Count and Sperm Morphology of Offspring at Puberty Onset

Table 5 presents the percentage of active motility (AM), sluggish motility (SM), non-motile (NM) sperm cells, and sperm count in the various groups. Group IV exhibited a significant decrease in the percentage of AM sperm cells compared to Group I. Groups II, III, and V demonstrated non-significant decreases in AM compared to Group I. Group IV showed a significant increase in the percentage of SM sperm cells compared to Group I. Groups II, III, and V exhibited non-significant increases in SM compared to Group I. Groups IV and V demonstrated significant increases in the percentage of NM sperm cells compared to Group I. Groups II and III showed non-significant increases in NM compared to Group I. There was a decrease in sperm count in Groups II, III, IV and V, but this was not significant compared to Group I. The number of pinhead-shaped sperm cells increased in Groups II, III, IV, and V, but not significantly compared to Group I. There were no significant differences in the number of roundhead-shaped sperm cells amomg Groups II, III, IV, and V compared to Group I.Groups IV and V demonstrated a significant increase in the number of headless-tailed sperm cells compared to Group I. on the other hand, the increase in headless-tailed sperm cells in Groups II and III were not significant compared to Group I. In Groups II, III, IV, and V no significant increase was observed in looped-tailed sperm cells compared to Group I. Similarly, Groups II, III, IV, and V exhibited no significant increases in bent mid-piece sperm cells, and coiled-piece sperm cells compared to Group I (Table 6).

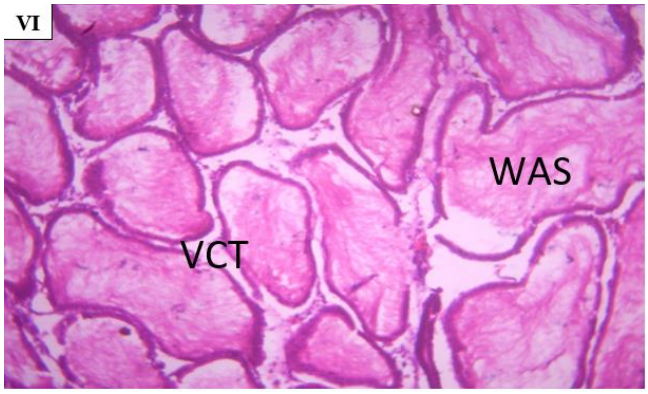

Figure 6: Photomicrograph of Group I section of the Epididymis (X100) (H/E) shows Normal Epididymis with Well Accumulated Spermatozoa (WAS) within the lumen and the Vascular Connective Tissue (VCT)

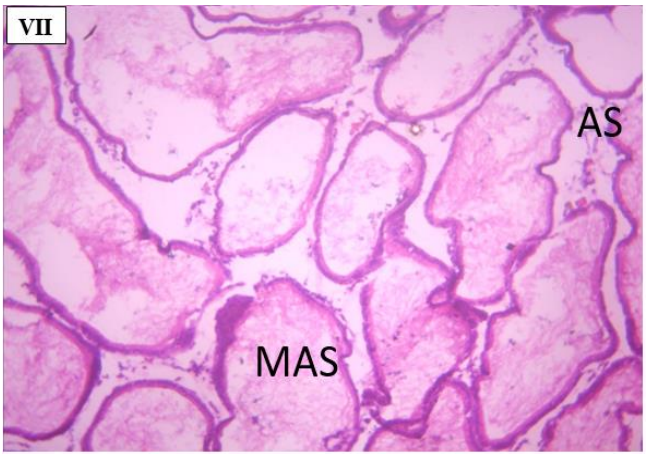

Figure 7: Photomicrograph of Group II section of the Epididymis (X100) (H/E) shows moderate effect on epididymal tissue with Mild Accumulation of Spermatozoa (MAS) within the lumen with mild arrest of spermatozoa in both section

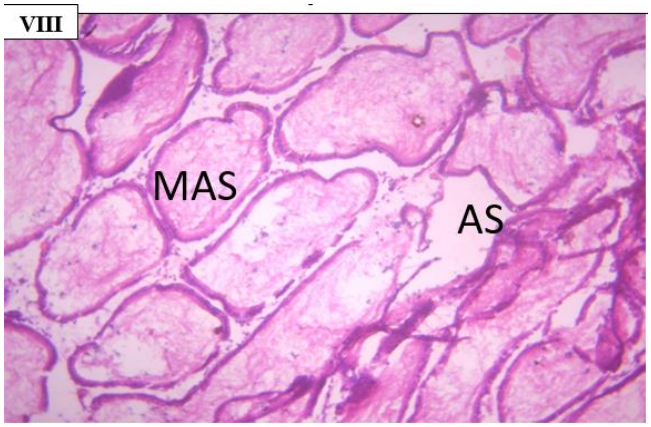

Figure 8: Photomicrograph of Group III section of the Epididymis (X100) (H/E) shows mild effects on Epididymal Tissue with moderate Accumulation of Spermatozoa (MAS) within the Lumen with, mild Arrest of Spermatozoa (AS)

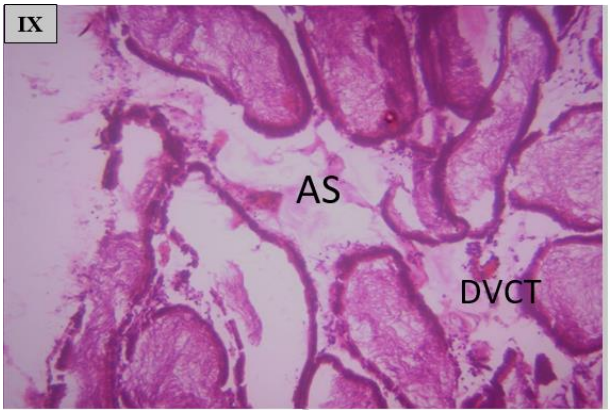

Figure 9: Photomicrograph of Group IV section of the Epididymis (X100) (H/E) shows severe effects on Epididemal Tissue with severe Arrest of Spermatozoa (AS) and Distortion of Vascular Connective Tissue (DVCT)

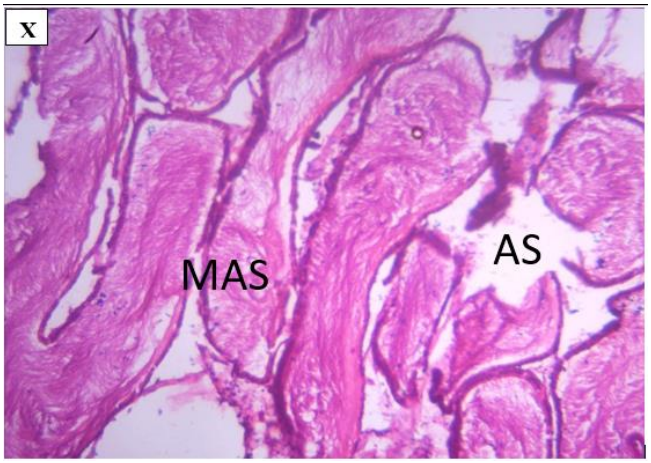

Figure 10: Photomicrograph of Group V section of the Epididymis (X100) (H/E) shows moderate degeneration with Moderate Accumulation of Spermatozoa (MAS) and mild Arrest of Spermatozoa (AS)

Studies have shown that gestational stress significantly impairs sperm quality, which is characterized by reduced motility, increased abnormal morphology, and decreased sperm count, highlighting the potential risk of infertility and reduced reproductive success in offsprings.[20,21] The underlying mechanisms include oxidative stress, DNA damage, and impaired spermatogenesis.[22]MoLEsupplementation showed some beneficial effects on sperm parameters, although inconsistent, suggesting its potential to mitigate the adverse effects of gestational stress on male fertility through its antioxidant and anti-inflammatory properties.[7, 8]

Figures 1-10 present photomicrographs of the testes and epididymis of representative rats in each group, illustrating the effects of the treatments on the morphology of these organs. Group I exhibited normal testicular architecture, including active seminiferous tubules, Leydig cells, and Sertoli cells (Figure 1). Group II showed minor interstitial fibrosis and arrested spermatogenesis (Figure 2). Group III displayed mild spermatogenesis arrest with focal inflammatory cell aggregates (Figure 3). Group IV exhibited severe testicular tissue damage, including arrested spermatogenesis and pyknotic testicular cells (Figure 4). Group V showed moderate distortion of seminiferous tubules and mild spermatogenesis arrest (Figure 5). Group I demonstrated normal epididymal architecture with well-accumulated spermatozoa and vascular connective tissue (Figure 6). Group II exhibited moderate epididymal effects, including mild spermatozoa accumulation and arrest (Figure 7). Group III displayed mild epididymal effects with moderate spermatozoa accumulation and mild arrest (Figure 8). Group

IV showed severe epididymal effects, including severe spermatozoa arrest and distortion of vascular connective tissue (Figure 9). Group V exhibited moderate degeneration, with moderate spermatozoa accumulation and mild arrest (Figure 10). Gestational stress led to dose-dependent alterations in testicular architecture, including arrest of spermatogenesis, interstitial fibrosis, and inflammatory cell infiltration (Figure 3 - 5). The epididymis exhibited varying degrees of damage, with reduced sperm accumulation and morphological abnormalities (Figure 8 - 9). These findings corroborate the biochemical and sperm parameters, emphasizing the detrimental impacts of gestational stress on male reproductive function.

Conclusion

This study revealed the detrimental impacts of gestational stress on male offsprings' reproductive development, characterized by delays in pubertal onset, compromised testicular and epididymal function, hormonal imbalances, and impaired sperm quality. These findings underscore the critical role of a healthy prenatal environment in shaping long-term reproductive health. *Moringaoleifera* supplementation demonstrated potential as a mitigating factor for some of these adverse effects, suggesting its role in protecting against oxidative stress, inflammation, and hormonal dysregulation. In order to elucidate the precise mechanisms of action and optimize its application as a therapeutic intervention, further investigation is warranted. The findings of this study emphasize the need for comprehensive strategies to manage maternal stress during pregnancy and to protect male reproductive health. Early interventions, including nutritional supplementation and stress management is also recommended.

Conflict of Interest

The authors declare no conflict of interests.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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