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Research Article

Pregnancy Outcomes of Wistar Rats Exposed to Gestational Stress and *Moringa oleifera* Leaf Extract

O.O. Chukwu[1,2,3*], A.C.U. Ezimah[2], A.E. Okorocha[2], C.O. Iyare[1], and N.G. Konyefom[2]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, David Umahi Federal University of Health Sciences, Ebonyi State, Nigeria.[2]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike. Abakaliki, Ebonyi, State, Nigeria.[3]Physiology Unit, College of Health Sciences, Evangel University, Akaeze, Nigeria.

Keywords:
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*** Address for Correspondence: Email:** chukwuoo@dufuhs.edu.ng

Tel: +2348128756074

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ABSTRACT

Background: Stress during pregnancy significantly impacts offspring early physiological programming.MoLE is believed to have both anti-stress and antioxidant properties. This study aimed to determine the effects of antenatal administration of *Moringa oleifera* leaf extract (MoLE; 5 or 10 mg/kg body weight/day via oral gavage from gestational day 8 to 21) under chronic unpredictable stress (CUS) conditions on pregnancy outcomes in Wistar rats.

Methods: Thirty healthy virgin female rats were randomly placed into 6 groups of 5 rats per group and exposed to CUS protocol comprised of variety of stressors applied in unpredictable manner, for two weeks, as follows: Group I (distilled water via oral gavage + normal rat chow *ad libitum*), Group II (CUS only), Group III (5mg/Kg body weight/day of MoLE), Group IV (10mg/Kg body weight/day of MoLE), Group V (CUS + 5mg/Kg body weight/day of MoLE), Group VI (CUS + 10mg/Kg body weight/day of MoLE).

Results: Pregnant rats exposed to CUS showed increased level of Cortisol (42.65±3.12 ng/mL), decreased Bodyweight gain at second and third trimester, shorter Gestational Length (20.50±0.29days), decreased Litter Size (3.00±0.58), Stillbirth (22%), Neonatal Mortality (66%), decreased pups birth weight (3.22±0.15g), birth length (4.26±0.11cm), BMI at birth (0.18±0.00g/cm²), when compared to Normal Control and MoLE exposed Groups. MoLE administration, particularly at 10 mg/kg, significantly attenuated these adverse effects.

Conclusion: MoLE demonstrates both anti-stress and antioxidant properties that protect against gestational stress-induced adverse pregnancy outcomes. The 10 mg/kg dose showed optimal protective effects. More research is necessary to comprehend underlying molecular pathways.

O. O. Chukwu **ORCID:**https://orcid.org/0000-0002-3269-1200

1. Introduction

Pregnancy is a critical stage in an animal's life cycle, and there is increased public awareness of the potential negative effects of stress exposure during pregnancy-on-pregnancy outcomes (de Kloet *et al.,* 2005). The chronic unpredictable stress (CUS) protocol, which is frequently used to explore the effects of stress exposure in a variety of animal models, has consistently been shown to modify the physiological milieu characteristic of the chronic stress-response. CUS is distinguished by unpredictable, sporadic, and random exposure to a variety of stressors over a period spanning several weeks (Lautarescu *et al.* 2020). Unpredictable stress better mimics the real-life human prenatal stress experience, which is characterized by random, uncontrollable, and diverse stressors, unlike predictable stress models that fail to capture the chronicity and unpredictability inherent in human psychosocial stressors during pregnancy. Maternal stress during pregnancy has been shown in studies on both animal models and human patients to cause birth defects (Sandman *et al.,* 1994; Fowden *et al*., 2022), including stillbirth and infant mortality in the offspring. Major socioeconomic difficulties in developing and low-income countries include birth defects, neonatal mortality, and stillbirths.

Cortisol, a hormone produced by the "hypothalamic-pituitary-adrenal" (HPA) axis, is essential for the body's response to physical and psychological stress as well as for maintaining homeostasis (McEwen and Akil, 2020). As a result, it is not surprising that cortisol production and availability are altered in response to numerous undesirable situations, such as increased psychosocial stress and psychopathology (Sapolsky, 2000). These problems can occur before or during pregnancy and have been linked to an increased risk of developmental abnormalities in children (Sandman *et al.,* 1994). The study of maternal glucocorticoids (GCs), specifically cortisol in humans, during pregnancy is an important topic in understanding pregnancy outcomes. This is because maternal GCs are a frequently utilized biomarker in a variety of unfavorable prenatal circumstances linked to an increased risk of developmental problems in offspring. Furthermore, maternal GCs have the ability to influence fetal GC exposure via different routes, underlining their importance in this context. Herbal products are frequently viewed by pregnant women as a feasible and safe alternative to conventional drugs (Frawley *et al.,* 2015). They frequently utilize these products to improve their overall health or to address minor diseases. The use of Herbal Medicine (HM) during pregnancy varies in prevalence depending on factors such as the consumer's geographical location, ethnicity, cultural traditions, and social standing (Duguá, 2010).

All portions of the *Moringa oleifera* tree are edible and are grown for their nutritional and medicinal benefits throughout the nation. It is a member of the genus *Moringa* and family *Moringaceae*. Both males and females in Nigeria of reproductive age regularly consume *Moringa oleifera* leaf extract (MoLE), primarily for therapeutic purposes. The polyphenolic phytochemicals in *M. oleifera* have various chemical and physiological characteristics, and the application of its methanol, ethanol, and aqueous extracts has been shown to have typical estrogenic-like effects in vivo and in vitro studies (Patisaul and Jefferson, 2010). Despite the widespread use of *M. oleifera* by pregnant women in Nigeria and other developing countries, no study has systematically examined the effects of MoLE administration under chronic unpredictable stress conditions during gestation on pregnancy outcomes. While the individual effects of MoLE and stress on pregnancy have been studied separately, the interaction between antenatal MoLE supplementation and maternal stress remains unexplored. The current study aims to assess the impact of prenatal administration of MoLE under chronic stress circumstances on pregnancy outcomes.

2. Materials and Methods

2.1 Animal Procurement and Housing

Thirty mature, inbred, virgin female Albino-Wistar rats (weighing 180-220g) and fifteen male rats were obtained from the Animal House at Alex Ekwueme Federal University, Ndufu-Alike (AE-FUNAI). Animals were housed in standard plastic cages (45×30×20 cm) under controlled environmental conditions (12-hour light/dark cycle). All animals had *ad libitum* access to standard rat chow (Vital feed®, Nigeria) and distilled water. Animals were acclimatized for two weeks prior to the study.

2.2 Plant Collection, Identification and Extraction Procedure

Fresh leaves of *Moringa oleifera* were collected early in the morning from a garden in Abakaliki, Ebonyi State, and authenticated by a botanist from the Herbarium Unit of the Department of Biology, Alex Ekwueme Federal University Ndufu-Alike (AE-FUNAI), Ebonyi State. The leaves were washed and allowed to air dry for seven days at room temperature. The leaves were processed in an electric blender (model ms-233, China) into a coarse powder. *M. Oleifera* was extracted with methanol using the recommended procedure (Chukwu *et al.,* 2024;

Chukwu *et al.,*2025). The extract was collected, dried at 40^{o}C, to obtain a pasty dark green extract for the experiment. The yield was calculated, and the concentrated extract was stored in an airtight container, labelled and refrigerated at 4^{o}C before use (Chukwu *et al.,*2025).

2.3 Phytochemical Analysis via Gas Chromatography-Mass Spectrometry (GC-MS)

The phytochemical profile of the extract was characterized by gas chromatography–mass spectrometry (GC–MS) as previously described by Chukwu *et al*. (2024). Briefly, analysis was performed using an Agilent GC–MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm). The oven temperature was programmed from 60°C (2 min hold) to 280°C at 10°C/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Major compounds identified are known for antioxidant and anti-inflammatory activity (Chukwu *et al*. 2024) (Table 1)

2.4 Ethical Approval

All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals and approved by the Faculty of Basic Medical Sciences Research Ethics Committee, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria (Approval Code: FBMS/EC/AE/1983).

2.5 Initiation of Pregnancy

Estrous cycles were monitored daily between 8:00-9:00 AM using vaginal smear analysis. Smears were collected using a sterile saline-moistened cotton swab, air-dried on glass slides, stained with Giemsa stain, and examined under light microscopy (×400 magnification). The estrous cycle phases were determined based on cell morphology: proestrus (predominantly nucleated epithelial cells), estrus (predominantly cornified cells), metestrus (equal proportions of leukocytes and cornified cells), and diestrus (predominantly leukocytes). Animals exhibiting two consecutive regular 4-day cycles were selected for the study. During proestrus, female rats were paired with male rats at a 2:1 ratio for mating. The following morning (gestational day 1), successful mating was confirmed by the presence of spermatozoa in vaginal smears (Ajayi and Akhigbe, 2020; Chukwu *et al.,*2025).

2.6 Experimental Design

A total of thirty mature, inbred, and seemingly healthy virgin female Albino-Wistar rats were obtained from the Animal House at AE-FUNAI. The animals were allowed to adapt to their diet (Vital feed®, Nigeria) and access to water without restriction for a period of two weeks prior to the start of the study. On the first day of gestation, the animals were assigned to six groups, with each group consisting of five (n=5) randomly selected rats. The grouping was as follows:

- **Group I (Control):** received distilled water via oral gavage (1 mL/kg/day) and normal rat chow *ad libitum* throughout pregnancy.
- **Group II:** was exposed to CUS protocol only from GD 8 – GD 21st day + distilled water via oral gavage.
- **Group III (Low Dose MoLE):** was administered 5mg/Kg body weight/day of MoLE throughout GD 8 – GD 21st day.
- **Group IV (High Dose MoLE):** was administered only 10mg/Kg body weight/day MoLE throughout GD 8 – GD 21st day.
- **Group V (CUS + Low Dose MoLE):** was exposed to CUS protocol + 5mg/Kg body weight/day of MoLE throughout GD 8 – GD 21st day.
- **Group VI (CUS + High Dose MoLE):** was exposed to CUS protocol + 10mg/Kg body weight/day of MoLE throughout GD 8 – GD 21st day.

Doses (5 and 10 mg/kg) were selected based on previous studies demonstrating dose-dependent antioxidant effects of MoLE in rodents. Groups III and IV received MoLE alone to establish baseline effects, while Groups V and VI received the same doses under stress conditions to evaluate potential protective effects. This parallel dosing design allows direct comparison of MoLE effects with and without stress. All administration was via oral gavage between 9:00-11:00 AM daily after exposure to the CUS protocol.

2.7 Chronic Unpredictable Stress 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 (Sequeira-Cordero *et al*., 2019).The CUS protocol employs multiple diverse stressors to prevent habituation and ensure psychological and physiological stress responses that better model real-world human stress experiences. 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 (Grigoryan *et al.,* 2022; Sequeira-Cordero *et al*., 2019).

2.8 Maternal Observation

In order to examine factors like death rates, general physical condition, and food consumption, the dams were weighed and monitored daily for the course of their gestation period. Partial abortion was determined based on the appearance of blood drops and/or a sudden fall in the weight of the pregnant dams, and the percentage of partial abortions was calculated within each group. The pups retained the groups of the dams. Weights and lengths of pups were recorded at birth and weaning, after which the BMI was calculated from the values obtained.

2.9 Sample Collection and Hormonal Profile Analysis

Venous blood was collected through the orbital route on GD 18 (Salami and Raji, 2015) and transferred into plain tubes, where it was left undisturbed to initiate the clotting process. The biochemical parameters were analyzed using sera recovered from the clotted sample following centrifugation at 3000 rpm for 10 minutes (Chukwu *et al.,* 2025). The enzyme linked immunosorbent assay (ELISA) was employed to test the serum cortisol levels, following the manufacturer's kit instructions.

2.10 Newborn Pups Birthweight, BirthLength and Body Mass Index (BMI)

The weights and lengths of the pups were measured upon birth and at weaning, and their body mass index (BMI) was subsequently computed using the appropriate formula.

$BMI = Weight\ (g) / Length\ (cm)^2$

2.11 Stillbirth

Newborn offspring were subjected to meticulous examination following the process of giving birth, with deceased offspring being classified as stillbirths. The calculation method employed to determine the percentage of stillbirths is as follows:

The percentage of the stillbirths = Number of stillbirths / Number of total births in group × 100.

2.12 Neonatal Mortality Rate

Neonatal mortality was noted as the occurrence of death among newborn pups, excluding still-born pups, within the period of three weeks after parturition. Calculation of Neonatal mortality rate was performed using the following method:

Percentage of Neonatal mortality = Number of neonatal mortality / Number of total births in group × 100

2.13 Birth Defects

Birth defects encompass a range of anatomical or physiological abnormalities that manifest at the moment of birth, originating from developmental disruptions occurring either prior to or during the birthing process. The newborn pup had a meticulous examination to identify any congenital abnormalities, and subsequently, Calculation of the percentage of birth defects was performed using the following method:

Percentage of birth defects = Number of birth defects / Number of total births in group × 100

2.14 Data 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 considered" significant at $p<0.05$. Means ± standard error of means (SEM) was used to express the results.

3. Results

3.1 Phytochemical Analysis via GC-MS

GC-MS phytochemical summary GC-MS analysis of the methanolic MoLE used in this study identified 41 compounds as reported previously by Chukwu., et al. (Chukwu *et al.,* 2024) (Table 1). Major constituents (by % total ion chromatogram) included hexadecanoic acid, methyl ester (methyl palmitate; 26.18% TIC), methyl stearate (12.11% TIC), dodecanoic acid methyl ester (6.01% TIC), and erythritol (6.84% TIC) among others. Several fatty-acid methyl esters and steroidal derivatives of potential endocrine relevance were present (Chukwu *et al.,* 2024)

3.2 Serum Cortisol Level of Dams.

Serum cortisol levels were significantly elevated in Group II compared to Group I (42.65±3.12 vs 24.25±0.22 ng/mL, $p<0.001$). MoLE administration significantly reduced cortisol levels: Group III (27.13±0.91), Group IV (27.33±0.56), and Group VI (30.44±1.05) showed significantly lower cortisol levels compared to Group II. Group V (38.73±1.98) also showed reduced cortisol compared to Group II, though the difference was not statistically significant (Table 2).

3.3 Average Weight of Feed intake of Dams.

Average daily food intake ranged from 48.00±1.83g (Group I) to 73.00±2.32g (Group III) across trimesters. In the first trimester, Group III showed significantly increased feed intake compared to Group I (68.86±4.57 vs 48.00±1.83g). During the third trimester, Group II and III showed significant increases compared to Group I (71.43±0.97 and 73.00±2.32 vs 60.29±0.25g, respectively), while Group VI showed significant decrease (48.86±1.06g) (Table 3).

3.4 Bodyweight of Dams exposed to CUS and MoLE at the End of each Trimester

Results presented on table 4 shows that, at the end of first trimester, an increase in bodyweight was recorded across all the groups with Group VI recording the highest bodyweight and Group I recording the Lowest. At the end of second trimester, an increase in bodyweight was recorded across all the groups with Group VI recording the highest bodyweight and Group I recording the lowest. At day 20, an increase in bodyweight was recorded across all the groups with Group VI recording the highest bodyweight and Group II recording the lowest.

3.5 Dams Mortality and Partial Abortion following Exposure to CUS and MoLE from Gestational Day 8 to 21

Results presented on table 5; shows that partial abortion was seen among dams in Group II, IV, V, VI with the same percentage seen across the groups. Dam's mortality was only recorded in Group II and V, as there was no mortality across the other groups.

3.6 Gestational Length of Dams exposed to CUS and MoLE

Results presented on table 6 shows significant decrease in gestational length in Group II and V as compared to Group I (20.50±0.29 vs 22.00±0.00 days). Non-significant decrease in gestational length was also recorded across the other groups as compared to Group I.

3.7 Litter Size from Dams exposed to CUS and MoLE during Pregnancy

Table 7 shows that there was a significant decrease in litter size in Group II as compared to Group I (3.00±0.58 vs 10.00±1.53), as well as a non-significant decrease in litter size in Group III, IV, V, and VI, compared to Group I.

3.8 Body Parameters of offspring from Dams exposed to CUS and MoLE at Birth.

Birth weight was significantly decreased in Group II (3.22±0.15g) and Group V (4.24±0.21g) compared to Group I (5.40±0.34g). Groups III (6.12±0.31g), IV (4.80±0.15g), and VI (5.26±0.19g) showed improved birth weights compared to Group II. Birth length was significantly decreased in Group II (4.26±0.11cm) compared to Group I (5.18±0.16cm). All treatment groups showed improved lengths compared to Group II. A significant decrease in BMI was recorded in Group V compared to Groups I and II (Table 8).

3.9 Stillbirth and Birth defect among Pups of Dams exposed to CUS and MoLE

Stillbirth was observed only in Group II (22%, 2/9 pups). No birth defects were observed across all groups (Table 9).

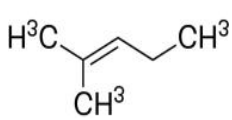
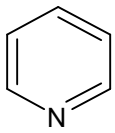
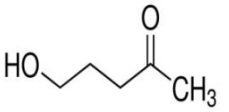
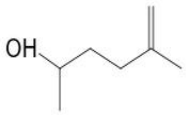
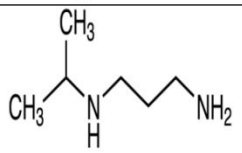
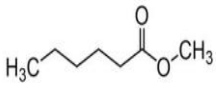
3.10 Neonatal Mortality Rate of Pups from Dams from Postnatal Day 1 to 21

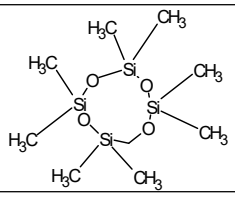
Neonatal mortality was highest in Group II (66% at week 1; 100% by week 2). Group V showed the next highest mortality (29% at week 1; 20% at week 2). Group I had the lowest mortality (3% at week 1; 3.5% at week 2). No mortality was recorded in any group during week 3 (Table 10)

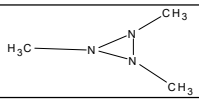
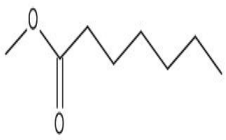
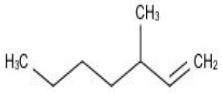
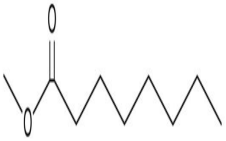
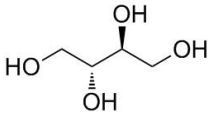
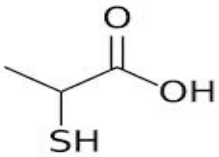
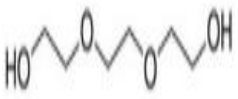
3.11 Body Parameters of offspring at Weaning of Dams

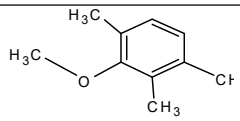
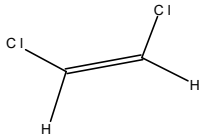
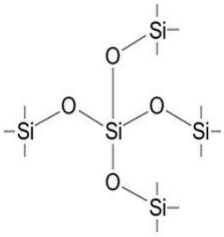
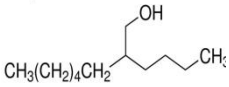
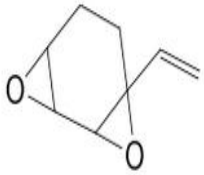
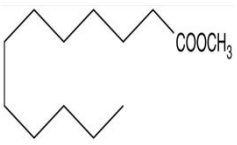
At weaning, significant increases in body weight were recorded in Groups IV (33.36±1.42g), V (35.34±0.73g), and VI (33.50±1.02g) compared to Group I (28.34±0.78g). Significant increases in length were recorded in Groups V (10.90±0.19cm) and VI (11.00±0.16cm) compared to Group I (10.14±0.10cm). A significant increase in BMI was observed in Group IV (0.29±0.01 g/cm²) compared to Group I (0.27±0.00 g/cm²). All treatment groups showed significantly delayed fur appearance compared to Group I (Table 11).

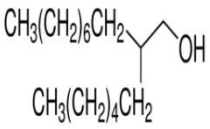
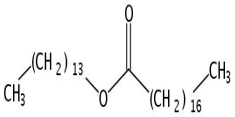
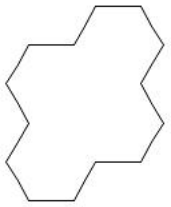
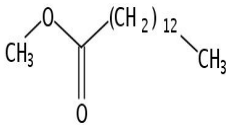
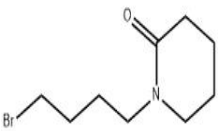
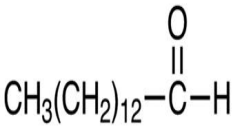
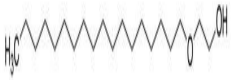
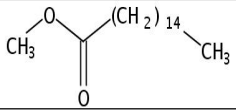
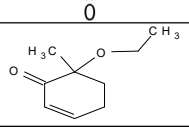
Table 1: GC-MS analysis of MOLE; An exempt from "GC–MS analysis of *Moringa oleifera* leaf extract and effects of administration on histology of reproductive organs and liver of female rats exposed to chronic unpredictable stress" by Chukwu*et al.*, 2024 (11).

S/No	Name of Compound	Mol. formular	Mol. Wt(g)	RT (min)	% TIC	Structure	Activity
1	1-Propanol, 3,3'-oxybis-	$C_6H_{14}O_3$	134.	3.045	1.521		Humectants (Food additive/Moisturizer)
2	1-Propanamine, 3-propoxy-	$C_5H_{12}NO$	117	3.327	0.803		Textile resins,Drugs, Pesticides
3	2-Pentene, 2-methyl-	C_6H_{12}	84	3.778	0.593		Photochemical and ozonolysis studies
4	Pyridine	C_5H_5N	79	4.285	4.763		Drugs, Vitamins, Food flavorings, Pesticides,
5	2-Pentanone, 5-hydroxy-	$C_5H_{10}O_2$	102	4.820	0.630		Anti-malarial drugs, Vitamin B1
6	5-Hexen-2-ol, 5-methyl-	$C_7H_{14}O$	114	5.102	0.887		Natural substances and Extractives
7	1,3-Propanediamine, N-(1-methylethyl)-	$C_6H_{16}N_2$	116	5.440	0.546		Useful research chemical compound
8	1,4-Butanediamine, N,N'-diethyl-	$C_8H_{20}N_2$	144	5.553	0.183		Unidentified
9	Hexanoic acid, methyl ester	$C_7H_{14}O_2$	130	5.722	0.843		Flavouring agents
10	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296	6.031	0.468		Pharmaceuticals, Polymers, Hair/Skin care products,

							Antiperspirants and Deodorants, Lubricants, Sealants, Adhesives, Waxes and Coating.

S/No	Name of Compound	Mol. formular	Mol. Wt(g)	RT (min)	% TIC	Structure	Activity
11	1,2,3-Trimethyldiaziridine	$C_4H_{10}N_2$	86	6.285	0.515		Unidentified
12	2-Hexyn-1-ol	$C_6H_{10}O$	98	6.426	0.212		Flavour and fragrance
13	Heptanoic acid, methyl ester	$C_8H_{16}O_2$	144	6.595	1.204		Human Metabolite, Flavouring agents, Fragrance,
14	1-Heptene, 3-methyl-	C_8H_{16}	112	6.905	0.608		Hydrocarbon
15	1-Fluorononane	$C_9H_{19}F$	146	7.158	0.459		Unidentified
16	Octanoic acid, methyl ester	$C_9H_{18}O_2$	158	7.440	2.299		Metabolite
17	Erythritol	$C_4H_{10}O_4$	122	7.834	6.842		Food additive and Sugar substitutes
18	2-Mercaptopropanoic acid	$C_3H_6O_2S$	106	8.313	2.240		Flavour and Fragrance agents
19	Triethylene glycol	$C_6H_{14}O_4$	150	9.243	1.079		Pesticides, Fragrance, Humectant, Disinfectant, Plasticizer for vinyl polymers
20	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	9.440	1.750		Biodiesel surrogate

S/No	Name of Compound	Mol. formular	Mol. Wt(g)	RT (min)	% TIC	Structure	Activity
21	Benzene,2-methoxy-1,3,4-trimethyl	$C_{10}H_{14}O$	150	10.849	2.298		unidentified
22	Ethylene, 1,2-dichloro-, (Z)-	$C_2H_2Cl_2$	97	10.894	0.200		Pharmacology, Refrigerant, Degreaser, Adhesives, Lacquers, oils, and Resins
23	10-Undecenoic acid, methyl ester	$C_{12}H_{22}O_2$	198	11.102	0.275		Flavouring agents
24	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis((trimethylsilyl)oxy)-	$C_{12}H_{36}O_4Si_5$	385	11.581	0.360		Paints, Coatings, and Cosmetics, including some Personal care products
25	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238	12.539	0.016		Derivative of essential oils with potential antibacterial activities.
26	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186	12.623	0.015		Human metabolite, Humectant
27	3,8-Dioxatricyclo(5.1.0.0(2,4))octane, 4-ethenyl-	$C_8H_{10}O_2$	138	12.905	0.003		Undefined
28	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	13.356	6.008		Therapeutic uses, Flavouring agents

S/No	Name of Compound	Mol. formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
29	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242	14.342	0.497		Fungicidal properties, Inhibitor (Candida glabrata), suggesting useful for treating Skin cancer
30	Myristyl stearate	$C_{32}H_{64}O_2$	481	14.623	0.181		Skin conditioning and Deodorant
31	2-Tridecenal, (E)-	$C_{13}H_{24}O$	196	14.877	0.219		Flavour and Fragrance agents
32	Cyclotetradecane	$C_{14}H_{28}$	196	15.468	1.427		Plant metabolite and a human metabolite
33	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	15.891	4.899		Plant metabolite, Flavouring agent and a Fragrance.
34	2-Piperidinone, N-(4-bromo-n-butyl)-	$C_9H_{16}BrNO$	234	16.426	1.766		Antimicrobial
35	Tetradecanal	$C_{14}H_{28}O$	212	16.736	1.853		Human metabolite, Flavouring agent and Fragrance
36	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314	16.877	1.420		Surfactant
37	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	17.384	26.182		Metabolite
38	6-Ethoxy-6-methyl-2-cyclohexenone	$C_9H_{14}O_2$	154	17.778	3.234		Flavouring agent

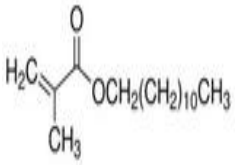
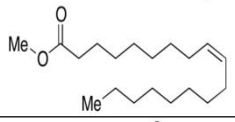
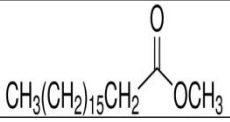
39	n-Dodecyl methacrylate	$C_{16}H_{30}O_2$	254	18.004	3.814		Drug (Clinical trials), Metabolites, Fragrance, Pesticides etc.
40	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	18.567	4.770		Flavouring agents and Fragrance
41	Methyl stearate	$C_{19}H_{38}O_2$	298	18.736	12.113		Metabolites and Flavouring agents

Table 2: Serum Cortisol Levels of Dams exposed to Chronic Unpredictable Stress and *Moringaoleifera* Leaf Extract

Variable	Groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Cortisol (ng/ml)	24.25±0.22	42.65±3.12*	27.13±0.91#	27.33±0.56#	38.73±1.98*	30.44±1.05#

Values are expressed as Mean ± SEM; * = p<0.05 versus control; # = p<0.05 versus stress control.

Table 3: Average Weight (g) of Food intake of Dams exposed to Chronic Unpredictable Stress and *Moringaoleifera* Leaf Extract (MoLE) during Pregnancy

Variable	Groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Food intake @ 1st trimester	48.00±1.83	58.86±1.86	68.86±4.57*	51.43±7.44	61.43±1.13	49.29±2.71
Food intake @2nd trimester	57.16±1.34	58.86±1.97	72.43±5.08*#	54.00±1.46	55.86±0.71	51.86±1.10
Food intake @ 3rd trimester	60.29±0.25	71.43±0.97*	73.00±2.32*	58.86±1.32#	58.29±0.71#	48.86±1.06*#

Values are expressed as Mean ± SEM; * = p<0.05 versus control; # = p<0.05 versus stress control.

Table 4: Bodyweight (g) of Dams exposed to Chronic Unpredictable Stress and *Moringaoleifera* Leaf Extract (MoLE) during pregnancy at the End of each Trimester

Variable	Groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Body Weight(g) @ Day 1	197.37±3.41	193.90±13.42	191.83±2.19	187.83±6.39	204.00±11.96	225.23±5.76
Body Weight(g) @ Day 7	209.47±0.55	217.37±16.63	206.17±2.35	220.47±5.93	223.10±17.34	241.33±6.65
Body Weight(g) @ Day 14	222.13±4.57	222.37±20.73	229.37±4.35	234.73±9.70	238.63±20.76	251.13±3.53
Body Weight(g) @ Day 20	242.30±11.55	220.37±16.99	236.67±5.33	265.70±17.64	258.83±22.59	259.63±2.45

Values are expressed as Mean ± SEM; * = p<0.05 versus control; # = p<0.05 versus stress control.

Table 5: Dams Mortality and Partial Abortion following Exposure to CUS and MoLEFrom Gestational Day 8 To 21

Variable	Groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Partial Abortion	0/5 (0%)	2/5 (40%)	0/5 (0%)	2/5 (40%)	2/5 (40%)	2/5 (40%)
Dams Mortality	0 (0%)	1 (20%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)

(*) Percentage Partial Abortion; */* is the number of Partial Abortion divided by number of total Dams in group. x100. (*) Percentage Dams Mortality; */* is the number of Dams Mortality divided by number of total Dams in group x100.

Table 6: Gestational Length of Dams exposed to Chronic Unpredictable Stress and *MoringaOleifera* Leaf Extract

Variable	Groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Gestational Length (Days)	22.00±0.00	20.50±0.29*	21.75±0.25	21.50±0.29	20.50±0.29*	21.25±0.25

*Values are expressed as Mean ± SEM; * = p<0.05 versus control; # = p<0.05 versus stress control.*

Table 7: Litter Size from Dams exposed to Chronic Unpredictable Stress (CUS) and *MoringaOleifera* Leaf Extract (MoLE) during pregnancy

Variable	Groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Litter Size	10.00±1.53	3.00±0.58*	8.33±1.20	5.67±1.20	4.67±0.67	6.00±1.53

*Values are expressed as Mean ± SEM; * = p<0.05 versus control; # = p<0.05 versus stress control.*

Table 8: Body Parameters of offspring at Birth from Dams exposed to Chronic Unpredictable Stress (CUS) and *MoringaOleifera* Leaf Extract (MoLE).

Groups	Variable		
	Body Weight (g)	Length (cm)	BMI (g/cm^2)
Group I	5.40±0.34	5.18±0.16	0.20±0.01
Group II	3.22±0.15*	4.26±0.11*	0.18±0.00
Group III	6.12±0.31$^{\#}$	5.58±0.19$^{\#}$	0.20±0.01
Group IV	4.80±0.15$^{\#}$	5.42±0.19$^{\#}$	0.17±0.01
Group V	4.24±0.21*	5.80±0.12$^{\#}$	0.13±0.00*$^{\#}$
Group VI	5.26±0.19$^{\#}$	5.40±0.19$^{\#}$	0.18±0.01

Values are expressed as Mean ± SEM; * = $p<0.05$ versus control; # = $p<0.05$ versus stress control.

Table 9: Stillbirth and Birthdefect of Pups from Dams exposed to Chronic Unpredictable Stress and *MoringaOleifera* Leaf Extract (MoLE) during pregnancy from Postnatal Day 1 to 21

Variable	Groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Stillbirth	0/30 (0%)	2/9 (22%)	0/25 (0%)	0/17 (0%)	0/14 (0%)	0/18 (0%)
Birthdefect	0/30 (0%)	0/9 (0%)	0/25 (0%)	0/17 (0%)	0/14 (0%)	0/18 (0%)

(*) Percentage stillbirth; */* is the number of stillbirth divided by number of total births in group x 100.
(*) Percentage birthdefect; */* is the number of birthdefect divided by number of total births in group x 100.

Table 10: Neonatal Mortality Rate of offspring from Dams exposed to Chronic Unpredictable Stress and *MoringaOleifera* Leaf Extract (MoLE) during pregnancy from Postnatal Day 1 to 21

Variable	Groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
1st Week	1/30 (3%)	6/9 (66%)	2/25 (8%)	3/17 (18%)	4/14 (29%)	3/18 (17%)
2nd Week	1/29 (3.5%)	3/3 (100%)	1/23 (4%)	2/14 (15%)	2/10 (20%)	0/15 (0%)
3rd Week	0/28 (0%)	0/0 (0%)	0/22 (0%)	0/12 (0%)	0/8 (0%)	0/15 (0%)

(*) Percentage newborn mortality rate; */* is the number of neonatal mortality divided by number of total births in group x100.

Table 11: Body Parameters of offspring at Weaning from Dams exposed to CUS and *MoLE*

Groups	Variable			
	Body Weight (g)	Length (cm)	BMI (g/cm^2)	Appearance of Fur (Days)
Group I	28.34±0.78	10.14±0.10	0.27±0.00	8.50±0.29
Group II	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00*
Group III	29.38±0.70	10.52±0.16	0.27±0.01	8.75±0.25
Group IV	33.36±1.42*	10.78±0.14	0.29±0.01*	9.75±0.25*
Group V	35.34±0.73*	10.90±0.19*	0.28±0.01	10.50±0.29*
Group VI	33.50±1.02*	11.00±0.16*	0.27±0.00	10.25±0.25*

Values are expressed as Mean ± SEM; * = $p<0.05$ versus control; # = $p<0.05$ versus stress control.

4. Discussion

The investigation of maternal glucocorticoids during pregnancy is important in terms of pregnancy outcomes because they are a common biomarker in a variety of severe prenatal circumstances that are 'associated with an increased risk of developmental abnormalities' in offspring (Chukwu *et al.,* 2025b). Furthermore, maternal glucocorticoids can impact fetal glucocorticoid exposure via a range of mechanisms. Cortisol levels are frequently observed to be higher during pregnancy. Many embryonic organs, including the liver, lungs, and brain, rely on this phenomenon for appropriate growth and maturation (Challis *et al.,* 2001). However, it is important to emphasize that excessive cortisol levels can have detrimental consequences on the development of the fetus (Chukwu *et al.,* 2025b). Results of this present study showed that CUS, a powerful stressor that can cause increased levels of cortisol, that aids the body in coping with stress, increased cortisol levels in dams exposed to CUS only. However, this study also showed that MoLE was able to significantly lower cortisol levels in dams exposed to CUS. This is consistent with earlier studies that showed *M. oleifera* leaf powder was able to lower serum cortisol levels in rabbits exposed to chronic heat stress (Hamed and El-Sayed, 2019) and that MoLE was able to protect rats from liver damage brought on by thioacetamide (Mousa *et al.,* 2019). It also suggests that MoLE may have anti-stress properties that may have helped to reduce stress and anxiety, as well as that its antioxidant properties may have helped to protect cells from damage caused by stress. The observed anti-stress effects of MoLE may be attributed to its rich polyphenolic content, particularly quercetin, kaempferol, and chlorogenic acid, which were identified in our GC-MS analysis. These compounds are well-documented antioxidants that can neutralize reactive oxygen species (ROS) generated during chronic stress, protect cellular structures, and modulate HPA axis activity.

In the present study, a wide range in pregnant dams' average feed intake throughout gestation was observed. Since pregnancy is stressful for dams and can result in increased appetite in the first trimester, it is possible that the dams increased their feed intake in response to the stress of pregnancy in order to meet the increased “nutritional needs” of the fetus. Average daily food intake ranged from 48.00±1.83g (Group I) to 73.00±2.32g (Group III) across trimesters. The results show that the average food intake of dams increased in the first trimester in all groups as compared to Group I. The altered liver metabolism, elevated gluconeogenesis, and changed levels of the hormones that control appetite in the hypothalamus during stress owing to the increased GC levels may be the causes of the average food intake seen in dams of groups exposed to CUS and administered MoLE in the second and third trimester when compared to Group I. This is consistent with prior studies that showed MoLE reduced feed intake and weight in rats exposed to CUS (Vegiopoulos and Herzig, 2007). Although it is noted that pregnant women generally have an increase in body weight because to "fetal growth", the huge variation in weight gain between the dams could be brought about by variable feed intake, cortisol levels, and the quantity of fetuses conceived.

The shorter gestational length and higher incidence of preterm birth seen in Group II and V, in this study may be due to an "increased level of glucocorticoids". To maintain pregnancy, a higher level of estrogen is required, which raises the plasma concentration of cortisol. Consequently, slightly enhanced amounts of estrogen may be seen during pregnancy. These levels are further raised when animals are exposed to stress during the pregnancy, which affects the timing of birth. The increased level of estrogen alters the cortisol catabolism in the liver, and cortisol levels spiked suddenly as a result, causing preterm birth. In utero exposure to “synthetic glucocorticoids” has been linked in several clinical investigations to preterm births. Additionally, unfavorable childhood experiences and elevated placental CRH levels are linked to preterm birth (Sandman *et al.,* 2006; Christiaens *et al.,* 2015).

Globally, stillbirth is a significant contributor to infant death; nevertheless, the exact cause of stillbirth is still under investigation. According to studies conducted on humans, women who experienced psychological stress throughout their pregnancies had a higher rate of stillbirths. A few of the "mechanisms for stress-induced stillbirth" include the increased release of catecholamines, which limits the substrates given to the baby, the increased level of glucocorticoid secretion, which changes "fetal cardiovascular and metabolic" function, and the suppression of hunger (Hobel *et al*., 2008). The CUS group alone experienced stillbirth in the current study, which may be related to the CUS protocol exposure over the duration of gestation, which exposed the fetus to greater glucocorticoid levels during the gestation period and resulting in stillbirth. Maternal hypertension was one of the causes of the 2.6 million stillbirths documented by the WHO in 2015, which resulted in more than 7,178 deaths daily (MacDorman and Gregory, 2015; WHO, 2026). Stillbirth is, in fact, inversely connected to maternal healthcare. An increased risk of stillbirth might result from a number of conditions. These include a high level of glucocorticoids, which can block 'glucose uptake' by the muscle, cause muscular atrophy, and

promote protein degradation and amino acid export while suppressing protein synthesis. Additionally, an increased amount of glucocorticoids can change lung maturation and have an impact on lung shape (Heszele and Price, 2004; Tschanz *et al*., 2002). In the current study, it was observed that none of the groups under consideration had any incidences of birth abnormalities. Given the significant socioeconomic ramifications linked to the prevalence of birth abnormalities in developing countries, this discovery is notable. Nevertheless, a number of studies have connected maternal prenatal stress to its conceivable causative involvement. For instance, studies have revealed that animals who experienced maternal stress during pregnancy were more likely to give birth to offspring who had birth defects (Chukwu *et al*., 2025a; Chukwu *et al*., 2025b). It has been discovered that maternal stress exposure during pregnancy is connected to the occurrence of newborn mortality. As a result, infant mortality was noted in all groups. Notably, when compared to the other groups, Group II had the greatest proportion of newborn death rate. The rate after that was substantially greater for Group V whereas Group I had lowest risk of neonatal death. This pattern may be explained by increased glucocorticoids levels during embryogenesis, which ultimately cause abnormalities in organogenesis, a crucial developmental step. Glucocorticoids is essential for fetal organogenesis, particularly for the development of the liver, brain, and dendritic and axonal terminals (Ward, 1994). Although fetal organ growth and maturation require slightly elevated levels of glucocorticoids, an abrupt rise in these hormones can be harmful to the fetus' postnatal survival. On the other hand, abnormal organ development could result from persistently elevated glucocorticoids levels. The results of earlier research, which found that violence against women and other forms of pregnancy stress raised the risk of baby and child mortality (Butchart and Villaveces, 2003; Class *et al*., 2013), support the findings of the current study.

Notably, MoLE administration in the absence of stress (Groups III and IV) did not adversely affect pregnancy outcomes, and in several parameters (birth weight, litter size) showed trends toward improvement compared to controls. Under stress conditions (Groups V and VI), MoLE demonstrated significant protective effects, with Group VI (10 mg/kg) showing superior outcomes, particularly in cortisol reduction and neonatal survival. Our findings suggest that the 10 mg/kg dose of MoLE provided optimal protection against gestational stress, as evidenced by lower cortisol levels, reduced partial abortion rates, and improved neonatal survival compared to the 5 mg/kg dose (Group V).

Among the groups, a significant decrease in bodyweight was recorded in Groups II and V compared to Group I. There was a significant decrease in body length of Group II compared to Group I. A significant reduction in body length was recorded in Groups III, IV, V and VI compared to Group II. A significant decrease in BMI of Group V compared to Groups I and II was also recorded; this may also be brought on by high glucocorticoids levels, ultimately leading to a reduction in protein synthesis and an increase in muscle breakdown during development (Tiao *et al*., 1996). Glucocorticoid receptor (GR) NR3C1 is involved in cell proliferation and differentiation. Newborn birth weight may have an impact on the level of methylation it experiences, and as a result, gene activity (Mulligan *et al.,* 2012). Several clinical studies have discovered a link between low birth weight and elevated 11-HSD gene methylation (Marsit *et al.,* 2012). Stress related to maternal immobility has been linked to lower birth weights. The metabolism of the liver and skeletal muscles is altered by maternal stress, which impacts postnatal development and growth. Previous studies have demonstrated that high glucocorticoids exposure during the neonatal period can program the liver and skeletal muscle metabolism toward a worse metabolic phenotype in later life because of increased 'GR expression and glucocorticoids sensitivity' in the visceral fat and liver (Seckl and Meaney, 2004). Stress during pregnancy has been linked to a decrease in maternal food intake as well as an increase in corticotropin-releasing hormone (CRH) levels, which have been linked to the occurrence of low birth weight. Intrauterine growth retardation, which might be linked to a decrease in nutrition delivery from the mother to the fetus, is one possible effect of this condition. This decrease has been connected to higher glucocorticoid levels in pregnant women. Glucocorticoid administration causes a decrease in expression of placental 'glucose transporter' GLUT1, which in turn results in a decrease in the transfer of glucose to the fetus (Matsas *et al.,* 2023).

While Wistar rats provide a well-validated mammalian model for studying prenatal stress effects, direct extrapolation to humans requires caution. Rats have a shorter gestation period (21 days) and are litter-bearing, which may influence stress responses differently than singleton human pregnancies. Additionally, the placenta of rodents differs in structure (hemotrichorial vs. human hemomonochorial) and in the regulation of glucocorticoid transfer via 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). However, the shared HPA axis function, conserved glucocorticoid receptor mechanisms, and analogous effects on fetal

programming validate this model for understanding fundamental biological processes relevant to human pregnancy outcomes.

The CUS protocol employed in this study incorporates multiple physical and psychological stressors, including social isolation, predator exposure, sleep deprivation, and environmental disturbances, which parallel key elements of human psychosocial stress such as social disruption, anxiety, environmental unpredictability, and chronic sleep disruption. This multifaceted approach better models the complex, unpredictable nature of real-world human stress during pregnancy than single-stressor models (Grigoryan *et al.,* 2022; Sequeira-Cordero *et al*., 2019).

5. Conclusion

This study demonstrates that gestational stress significantly compromises fetal development through elevated glucocorticoid synthesis and placental transfer, which stimulate gluconeogenesis, impair peripheral glucose uptake, reduce protein synthesis, cause muscle atrophy, and hinder calcium absorption critical for bone formation. These metabolic disruptions lead to reduced maternal feed intake, weight gain, and adverse outcomes including low birth weight, stillbirth, and neonatal mortality. Importantly, *Moringa oleifera* leaf extract, particularly at 10 mg/kg, exhibits significant protective effects against these stress-induced adverse outcomes. MoLE administration effectively reduced serum cortisol, improved maternal weight gain, enhanced neonatal survival, and normalized birth parameters in stressed dams, likely mediated through antioxidant properties of its polyphenolic constituents (quercetin, kaempferol, chlorogenic acid) and HPA axis modulation. These findings suggest MoLE as a potential therapeutic intervention to mitigate gestational stress effects, though clinical translation requires caution due to species differences, necessitating well-controlled human studies before recommendations. Further research is needed to elucidate underlying molecular pathways and characterize gestational CUS adverse effects.

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7. Authors' Contributions

O.O.C., A.C.U.E, C.O.I, A.E.O, N.G.K., conceived and planned and carried out the experiments, data collection, and analysis. O.O.C. and C.O.I. wrote the first draft of the manuscript, and all authors provided critical feedback and helped shape the research, analysis and manuscript.

8. Competing Interests

The authors declare that there is no conflict of interest.

9. References

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