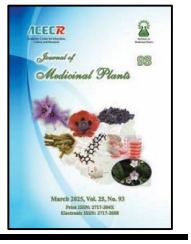
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Research Article

Moringa oleifera Lam. is a potential mitigator of neurodevelopmental defects caused by prenatal stress in Wistar rats

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ABSTRACT

Background: Gestational stress is a key factor in neurodevelopmental impairments in offspring, affecting learning, memory, and emotional regulation. *Moringa oleifera* Lam. leaf extract (MoLE) with its anti-inflammatory and antioxidant properties, may offer neuroprotective benefits. **Objective:** This study evaluated impact of *MoLE* on the neurodevelopment of Wistar rat pups subjected to Chronic Unpredictable Stress (CUS). **Methods:** Twenty-five healthy virgin female Albino-Wistar rats were randomly assigned to five groups of five rats each, which underwent a two-week CUS protocol. Groups were as follows: Group I (received *ad libitum* water and standard rat chow), Group II (received *MoLE* at 5 mg/kg body weight/day), Group III (received *MoLE* at 10 mg/kg body weight/day), Group IV (CUS + *MoLE* at dosage of 5 mg/kg body weight/day), and Group V (CUS + *MoLE* at dosage of 10 mg/kg body weight/day). Offspring were evaluated for histological changes in the hippocampus, prefrontal cortex, and amygdala, while serum malondialdehyde (MDA) levels, and behavioral tests were carried out to assess anxiety-like behaviors and spatial memory. **Results:** Histological analysis revealed *MoLE* supplementation mitigated cellular damage. MDA levels were elevated among pups exposed to *MoLE*-supplemented and CUS, but not in *MoLE*-supplemented groups. Behavioral results for anxiety-like behavior were inconclusive. Spatial memory results showed a non-significant decrease in all groups, requiring further investigation. **Conclusion:** This study provides preliminary evidence for *M. oleifera's* potential neuroprotective effects against gestational stress-induced damage. Further research with more specific behavioral tests is needed to confirm its effects on anxiety-like behavior and spatial memory.

Abbreviations: CUS, Chronicunpredictable stress; MDA, Malondialdehyde; MSG, Monosodium Glutamate; TBARS, Thiobarbituric Acid Reactive Substances

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1. Introduction

Chronic exposure to prenatal stress disrupts various neurodevelopmental processes, resulting in impairments in learning, memory, emotional regulation, and the ability to adapt to new situations [1, 2]. These developmental issues are linked to changes in both structure and function of critical brain areas like the hippocampus, amygdala, and prefrontal cortex [3].

The hippocampus, which plays a central role in memory formation and learning [4], is especially sensitive to prenatal stress. Such stress can hinder neurogenesis, thereby compromising hippocampal function and contributing to memory-related problems [5]. Similarly, the prefrontal cortex essential for high-level cognitive functions including decision-making and problem-solving is notably affected by prenatal stress. Offspring exposed to stress in utero frequently demonstrate reduced cognitive flexibility and struggle to adjust to shifting circumstances [6].

Furthermore, the amygdala, a key brain region for emotional processing, is affected by gestational stress, potentially contributing to anxiety and emotional dysregulation in offspring [7, 8]. An increasing number of studies indicate that oxidative stress defined as a disruption in the balance between reactive free radicals and the body's antioxidant defense mechanisms may play a central role in the negative impact of prenatal stress on brain development [9]. Prolonged stress conditions can raise the concentration of reactive oxygen species (ROS), which contributes to neuronal damage and impairs brain function [10]. Pregnant women often turn to herbal remedies to manage minor health issues or support general wellness, as these products are generally perceived as a natural and safer alternative to conventional pharmaceuticals [11]. The global interest in herbal remedies is growing, driven by a renewed focus on using medicinal plants for health maintenance, their natural origins, affordability, relatively low risk of side effects, and their potential for pharmaceutical discovery [12]. *Moringa oleifera* Lam., a widely recognized medicinal plant found in Africa, Asia, and the Americas, belongs to the *Moringa* genus within the *Moringaceae* family. In Nigeria, *M. oleifera* leaf extract (MoLE) is frequently used by men and women of reproductive age for its therapeutic benefits. The plant is also popularly referred to as the "Mother's best friend," the "Ben oil tree," and the "Drumstick tree" [13]. *M. oleifera* is widely acknowledged for its significant medicinal potential, largely due to its rich content of essential minerals, proteins, vitamins, and β-carotene, which contribute to its strong antioxidant and anti-inflammatory effects. [14]. These properties have led researchers to explore its potential neuroprotective effects. Recent studies in rodents have demonstrated that *M. oleifera* extracts exhibit neuroprotective and mood-enhancing properties, suggesting its potential as a therapeutic agent for neurological disorders [15, 16]. This study was designed to examine whether administering *MoLE* could offer neuroprotective benefits for the developing brains of offspring born to Wistar rats subjected to chronic unpredictable stress during pregnancy. Through this investigation, the goal was to enhance our understanding of *MoLE'*s possible therapeutic role in mitigating the harmful impact of prenatal stress on neurodevelopment.

2. Materials and Methods

2.1. Ethical approval

The study received ethical approval from the Research Ethics Committee of the Faculty of Basic Medical Sciences, Alex Ekwueme Federal University Ndufu-Alike (AE-FUNAI), Ebonyi State, Nigeria, with the approval reference FBMS/EC/AE/1983.

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2.2. Collection of plant, authentication, and extraction procedure

Fresh leaves of *M. oleifera* (MO) were harvested in the early hours of the day from a cultivated plot located in Abakaliki, Ebonyi State, Nigeria. The plant material was taxonomically identified and verified by a botanist at the Herbarium Unit within the Department of Biological Sciences, AE-FUNAI. A voucher specimen was archived in the herbarium with the reference number AE-FUNAI UH 504a. Post-collection, the leaves underwent air-drying under shaded conditions at ambient temperature for approximately two weeks, a measure intended to preserve sensitive phytochemicals from degradation due to sunlight. The desiccated leaves were pulverized into a fine consistency using a mechanical grinder (Miller, model ms-233, China), and then sieved to ensure uniform particle size, enhancing the efficiency and consistency of subsequent extraction steps. For the extraction of bioactive constituents, a conventional Soxhlet apparatus was employed. A total of 200 grams of the prepared leaf powder was packed into the extraction chamber, and methanol (500 ml) was used as the solvent. The extraction was conducted over a continuous 48-hour period and repeated two additional times to maximize yield. All resultant extracts were pooled and subjected to solvent removal using a rotary evaporator operated under reduced pressure at 40°C. The total yield of the methanolic extract was calculated and documented. The final product, a dark green semi-solid paste, was stored in sealed containers at 4°C for use in further experiments.

To complement quercetin-based standardization, the methanolic extract was analyzed for its phytochemical profile using Gas Chromatography-Mass Spectrometry (GC-MS). This technique enabled the identification of a wider array of bioactive constituents. The GC-MS system used included an Agilent 6890 gas chromatograph equipped with an automated on-column injector, a flame ionization detector, and an HP 88 capillary column (dimensions: 100 m × 0.25 mm film thickness). The specific analytical conditions were as follows:

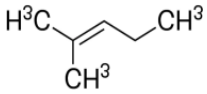
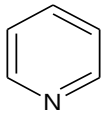
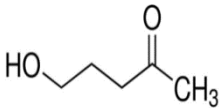
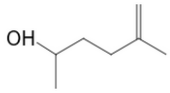
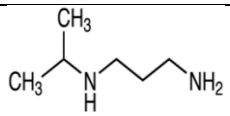
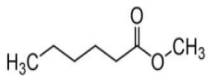
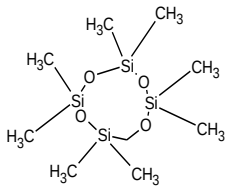
- Ionization voltage: 70 eV
- Oven temperature program: Initial at 180°C and final at 181°C (held for 1 minute)
- Injection volume: 1 µL
- Total run time: 15 minutes

The resulting GC-MS data was analyzed to identify and potentially quantify the various phytochemical constituents present in the MO extract (Table 1) [17]. Prior to chemical profiling, the total methanolic extract of MO leaves underwent a methylation process to facilitate the detection of fatty acid methyl esters. This step involved the chemical transformation of free fatty acids into their corresponding methyl esters via a transesterification reaction. The extract was mixed with methanol containing a strong acid catalyst typically sulfuric acid to drive the esterification process. The reaction was carried out under carefully regulated temperature and time conditions to ensure optimal conversion. Upon completion, the resulting methyl esters were isolated from the reaction mixture and subjected to GC-MS for detailed identification and quantification. This derivatization step was crucial for enhancing the visibility of fatty acid constituents in the MO extract during analytical evaluation.

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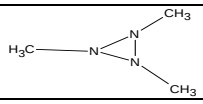
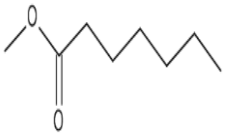
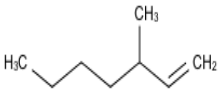
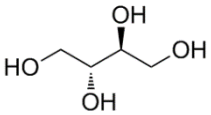
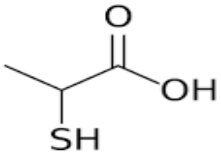
Table 1. GC-MS analysis of MOLE; An exempt from "GC–MS analysis of *M. oleifera* leaf extract and effects of administration on histology of reproductive organs and liver of female rats exposed to chronic unpredictable stress" by Chukwu *et al.*, 2024 [17]

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.

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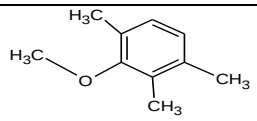
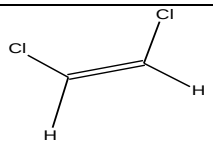
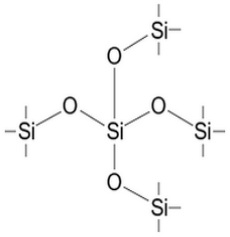
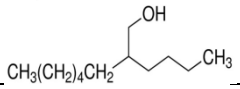
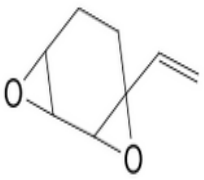
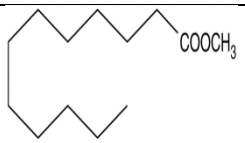
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S/No	Name of Compound	Mol. formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
11	1,2,3-Trimethyldiaziridine	$C_4H_{10}N_2$	86	6.285	0.515		Unidentified
12	2-Hexyn-1-ol	$C_6H_{10}O$	98	6.426	0.212		Flavour and fragrance
13	Heptanoic acid, methyl 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

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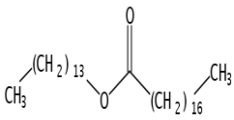
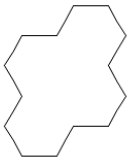
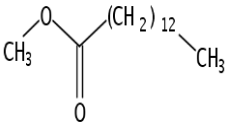
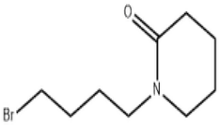
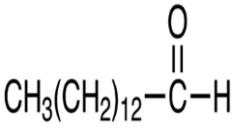
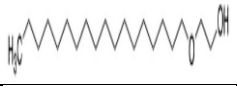
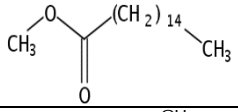
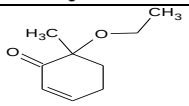
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S/No	Name of Compound	Mol. formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
21	Benzene,2-methoxy-1,3,4-trimethyl	C10H14O	150	10.849	2.298		unidentified
22	Ethylene, 1,2-dichloro-, (Z)-	C2H2Cl2	97	10.894	0.200		22
23	10-Undecenoic acid, methyl 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

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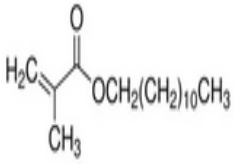
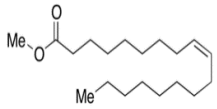
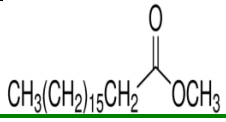
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S/No	Name of Compound	Mol. formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
29	1-Decanol, 2-hexyl-	C16H34O			29	1-Decanol, 2-hexyl-	C16H34O
30	Myristyl stearate	$C_{32}H_{64}O_2$	481	14.623	0.181		Skin conditioning and Deodorant
31	2-Tridecenal, (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

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[DOI: 10.61882/jmp.24.93.79]

Table 1. GC-MS analysis of MOLE; An exempt from "GC–MS analysis of *M. 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 [17] (Continued)

S/No	Name of Compound	Mol. formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
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

2.3. Experimental design

Twenty-five female (25) healthy adult virgin Albino-Wistar rats were sourced from Animal House facility of AE-FUNAI. Before the experiment commenced, the animals underwent a two-week acclimatization period during which they were provided unrestricted access to clean water and a standard diet (Vital Feed®, Nigeria). On confirmation of gestation, designated as Gestational Day (GD) 1, the animals were randomly assigned into five experimental groups, each comprising five rats. The treatment groups were organized as follows:

Group I: Received only standard feed and water throughout gestation without any treatment.

Group II: Administered *MoLE* at of 5 mg/kg body weight per day from GD 8 to GD 21.

Group III: Administered *MoLE* at a dosage 10 mg/kg body weight per day from GD 8 to GD 21.

Group IV (CUS + 5 mg/kg/day of *MoLE*): Subjected to Chronic Unpredictable Stress (CUS) Protocol and treated with 5 mg/kg/day of *MoLE* from GD 8 to GD 21.

Group V (CUS + 10 mg/kg/day of *MoLE*): Subjected to CUS regimen and concurrently given 10 mg/kg/day of *MoLE* from GD 8 to GD 21.

All treatments were administered orally using a gavage method once daily, following the daily exposure to the CUS protocol where applicable. Upon completion of the 21-day gestational period, the offspring were weaned and provided access to water and food *ad libitum*. Post-weaning, the pups remained in the same experimental groups as their respective dams, and were housed in gender-separated cages. The period between gestational days 8 and 21 was deliberately selected, as it marks a critical phase in rat fetal brain development, corresponding to the late second and third trimesters of human gestation. This period is known for heightened vulnerability of the developing brain to environmental and pharmacological influences. The selected dosages of 5 mg/kg and 10 mg/kg of *MoLE* were based on prior findings by Chukwu *et al*. [17], which validated the safety and neuroprotective potential of these concentrations in rodent models. Furthermore, the two dosing levels allowed for evaluation of a potential dose-dependent effect of the extract.

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2.4. Initiation of pregnancy

To identify suitable animals for the study, estrous cycle monitoring was conducted through light microscopy. Female rats that displayed two successive, regular four-day estrous cycles were selected. During the proestrus phase, male rats were introduced into the cages of the females in a 1:2 male-to-female ratio to encourage mating. The following morning was considered GD 1, and successful copulation was confirmed by the detection of sperm cells in vaginal smears collected from the females [19, 20].

2.5. Chronic unpredictable stress protocol

The CUS procedure began on GD 8, which aligns with the early stages of organogenesis in rats, and continued through GD 21, the approximate term for delivery. The protocol incorporated a variety of stress-inducing conditions applied in a random, non-repetitive sequence to prevent habituation. The stressors used included:

i. Wet Bedding – Bedding composed of sawdust was soaked by mixing 1 liter of material with 300 mL of water.

ii. Cage Tilting – The cages were inclined at an angle of up to 45°, positioning food and water at the elevated end.

iii. Food Restriction Overnight – Rats were subjected to limited access to food during one night.

iv. Psychosocial Stress – Animals were exposed to the presence of a confined cat to evoke psychological stress.

v. Sleep Deprivation – A cylindrical platform (6 cm in diameter and 5 cm tall) was positioned on the side of the cage opposite the food and water, with the cage floor covered by 3 cm of water. This arrangement compelled the animal to stay awake by requiring it to balance on the platform to avoid getting wet.

vi. Physical Restraint – Rats were confined in 50 mL plastic tubes with breathing holes for 6 hours per session, divided into three 2-hour intervals separated by 30-minute breaks.

vii. Constant Illumination – Rats were kept under continuous light exposure for one night.

viii. Social Isolation (SI) – Each rat was housed individually, a stressor consistently incorporated into the CUS regimen.

Each stressor was administered once per day, and all were repeated twice throughout the course of the pregnancy. While most stressors lasted 6 hours, sleep deprivation sessions extended to 12 hours. This stress paradigm was designed to simulate the unpredictable and uncontrollable features of prenatal stress encountered by humans, and are widely accepted in animal studies to explore maternal and fetal stress responses [17, 21]. Postnatal development was tracked daily beginning from postnatal day 21, with attention to indicators of pubertal onset such as vaginal opening in females and balano-preputial separation in males. The day on which these events occurred was recorded to determine the timing of puberty.

2.6. Behavioral assessments

Behavioral evaluations were performed in a quiet, spacious room during the daytime, specifically between 8:00 a.m. and 4:00 p.m. To assess anxiety-related behavior, the elevated plus maze was utilized, whereas spatial working memory and locomotor function were measured using the Y-maze test. Observations were manually recorded with the aid of a stopwatch, and all animals within a group were tested on the same day to ensure consistency.

2.7. Elevated plus maze

The elevated plus maze consisted of four arms forming a cross, following the design described by Handley and Mithani [22]. Two opposite arms

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were open (25 × 5 × 5 cm), while the other two were enclosed (25 × 5 × 16 cm). These arms intersected at a central square platform measuring 5 × 5 × 0.5 cm. The closed arms were bordered by 16 cm high walls, whereas the open arms were without side enclosures. After the administration of monosodium glutamate (MSG) or a vehicle control, each mouse was positioned on the central platform, facing a closed arm, and observed for five minutes based on procedures outlined by Montgomery [23]. A full arm entry was noted only when all four paws of the mouse entered an arm. After each session, the maze was sanitized using a 5% ethanol solution. This setup exploits rodents' natural preference for enclosed, dark environments (indicative of approach behavior) and their inherent aversion to open, elevated areas (reflecting avoidance behavior). The proportion of time spent in each type of arm was calculated as (time in open or closed arms / total test time) × 100, and the frequency of entries was determined as (entries into open or closed arms / total number of entries) [23].

2.8. Y-maze

Spontaneous alternation in the Y-maze is a well-established indicator of short-term spatial memory and also provides information on general locomotor activity and repetitive movement patterns [24]. The Y-maze apparatus consisted of three arms arranged at 120° angles, each 41 cm long and 15 cm high, with a 5 cm wide transparent Perspex floor. Mice were introduced individually into one of the arms and allowed to explore the maze freely. An arm entry was counted only when the mouse's tail completely passed into the arm. Entries were manually tracked and designated as A, B, or C. An alternation was defined as three successive entries into different arms (e.g., ACB or CAB). For example, if the sequence was ACB, C, A, B, C, A, CAB, C, A, it would result in 13 total entries and 8 correct alternations. Each test lasted five minutes, and the maze was cleaned with 5% alcohol and air-dried between animals.

2.9. Sample collection and laboratory analysis

Pups were chosen at random from each group for the serum malondialdehyde (MDA) levels, venous blood measuring a volume of 2ml, were obtained from the orbital route of each rat at onset of puberty and transferred into plain tubes, where it was left undisturbed to initiate the clotting process.

2.10. Biochemical analysis

Biochemical evaluations were carried out using serum extracted from blood samples that had been allowed to clot and were then centrifuged at 3000 revolutions per minute for 10 minutes. At the beginning of puberty, blood was collected into plain tubes specifically for biochemical testing. Levels of serum MDA, an indicator of lipid peroxidation, were determined using the thiobarbituric acid reactive substances (TBARS) method, as described by Khoubnasabjafari [25].

2.11. Histological studies

The brain tissues were carefully removed and thoroughly cleansed of any attached tissues and immersed in 10% formaline, for standard histological preparation and subsequent examination for histopathological analysis. Then, 15-micron thick paraffin sections were made, stained with hematoxylin and eosin, and then examined under a microscope for the histopathological studies [26, 20].

2.12. Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA) performed with GraphPad

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Prism software (GraphPad® Software, San Diego, CA, USA). The results are presented as the mean ± standard error of the mean (SEM). Tukey's post hoc test was employed to determine significant differences between groups, with a p-value of less than 0.05 considered statistically significant.

3. Results

3.1. Serum MDA level of offspring exposed to CUS and MoLE

Table 2 indicates that serum MDA levels were significantly elevated ($P < 0.05$) in both Groups IV and V compared to Group I. In contrast, no statistically significant difference was observed in Groups II and III relative to Group I.

3.2. Effects of antenatal CUS and administration of MoLE on offspring at onset of puberty on frequency and duration of close arm entry using elevated plus maze

Table 3 demonstrates that there was no significant difference ($P > 0.05$) in the number of open arm entries among Groups III, IV and V, compared to Group I. Likewise, the duration spent in the open arms (in milliseconds) did not differ significantly between the Groups II, III, IV and V groups when compared with Group I.

3.3. Effects of antenatal CUS exposure and administration of MoLE on offspring locomotor activity and spatial memory at onset of puberty using Y – Maze

Regarding locomotor activity assessed after 5 minutes of exploration in the Y-maze, there was no significant difference in arm entries among the Groups II, III, and V groups compared to Group I. However, Group IV exhibited a significant reduction ($P < 0.05$) in the number of arm entries relative to Group I. As for spatial memory, measured by total alternations during the same exploration period, no significant differences were found among Group II, III, IV, and V when compared to Group I (Table 4).

Table 2. Serum MDA level of offspring from dams exposed to chronic unpredictable stress and *M. oleifera* Leaf extract

Variable	Groups				
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**
MDA (nmol/mL)	1.20 ± 0.01	1.66 ± 0.19	1.58 ± 0.16	3.55 ± 0.26*	2.83 ± 0.10*

Values are expressed as Mean ± SEM; * = $P < 0.05$ versus control

Table 3. Effects of antenatal chronic unpredictable stress and administration of *M. oleifera* Leaf extract on offspring at onset of puberty on frequency and duration of close arm entry using elevated plus maze

Variable	Groups				
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**
Number of entries into Close Arms	0.33 ± 14.36	0.33 ± 1.03	0.67 ± 3.03	2.67 ± 26.60*	1.00 ± 13.09
Time (ms) spent in Close Arms	14.36 ± 14.36	1.030 ± 1.030	3.030 ± 3.030	45.69 ± 26.60*	17.37 ± 13.09

Values are expressed as Mean ± SEM; * = $P < 0.05$ versus control; ms: milliseconds

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Table 4. Effects of antenatal chronic unpredictable stress exposure and administration of *M. oleifera* Leaf extract on offspring locomotor activity and spatial memory at onset of puberty using Y – Maze

Variable	Groups				
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**
Number of ArmEnteries	8.00 ± 0.58	6.667 ± 0.33	5.667 ± 0.33	4.667 ± 0.33*	5.667 ± 0.88
Total Alternations	4.00 ± 1.00	3.00 ± 0.00	2.00 ± 0.58	1.67 ± 0.33	2.00 ± 0.58

Values are expressed as Mean ± SEM; * = P < 0.05 versus control.

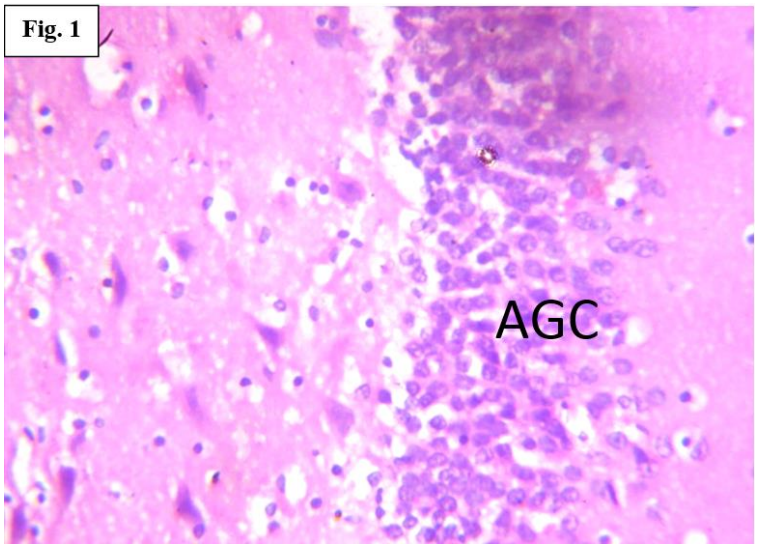


Fig. 1. Photomicrograph of group I section of the Hippocampus (x100/x400) (H/E) shows Hippocampus with distinct layer and active Granular cells (AGC) outline

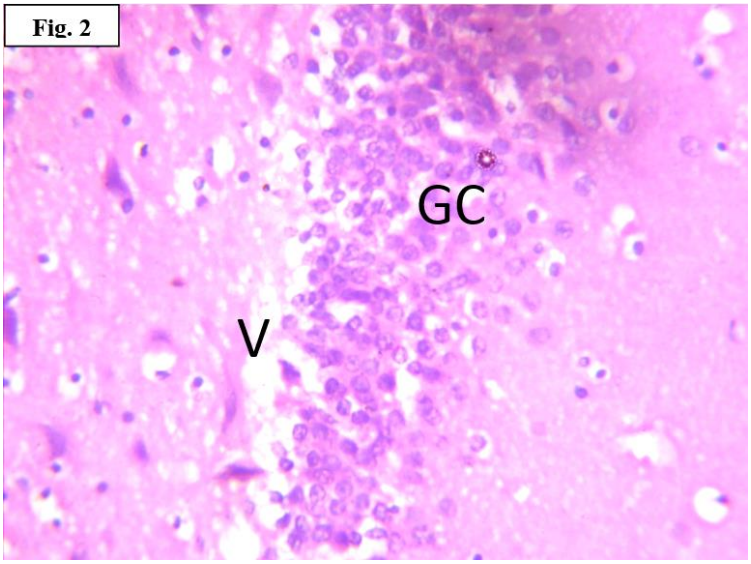


Fig. 2. Photomicrograph of group II section of the Hippocampus (x100/x400) (H/E) shows moderate vacuolation (V) with active Granular cells (GC)

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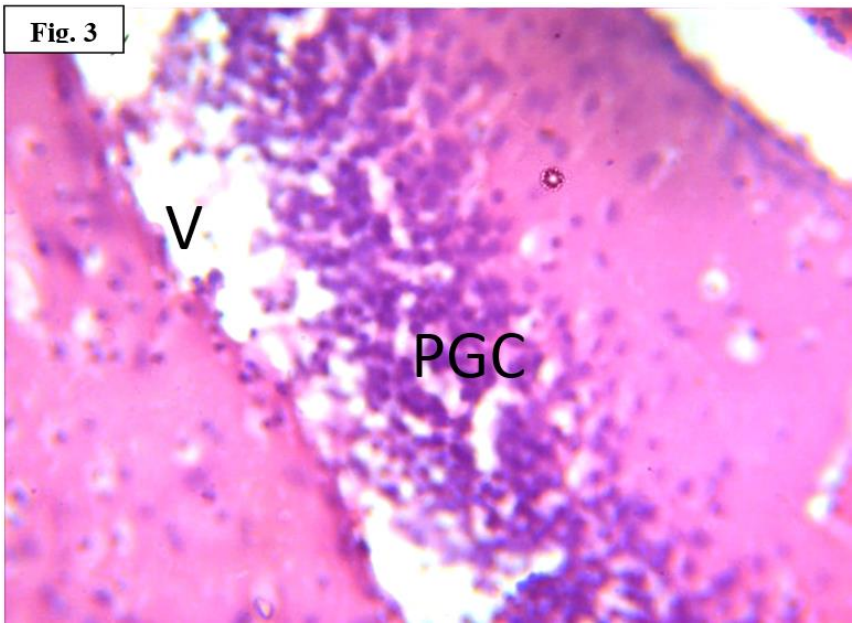


Fig. 3. Photomicrograph of group III section of the Hippocampus (x100/x400) (H/E) shows moderate degeneration with moderate vacuolation (V) and Pyknotic Granular cells (PGC)

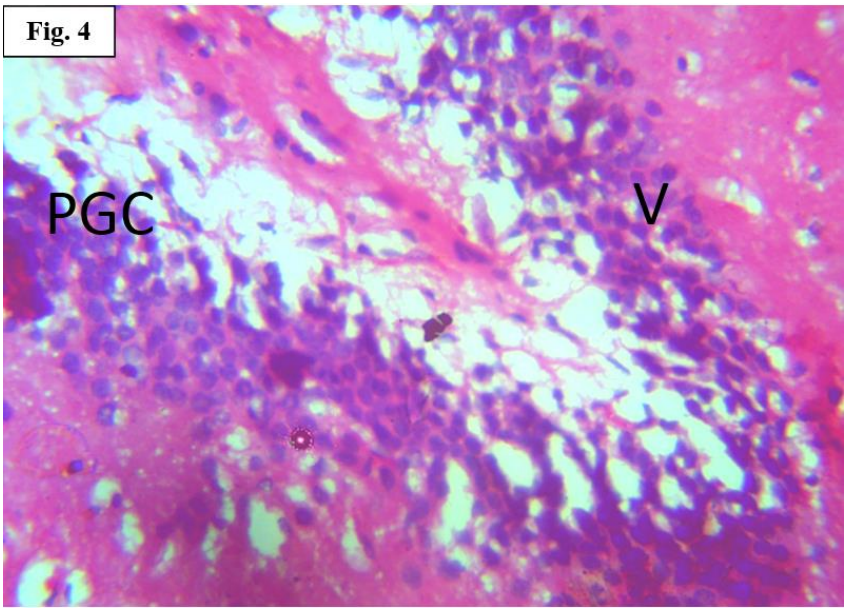


Fig. 4. Photomicrograph of group IV section of the Hippocampus (x100/x400) (H/E) shows severe Degeneration with severe Vacoulation (V) and Pyknotic Granular cells (PGC).

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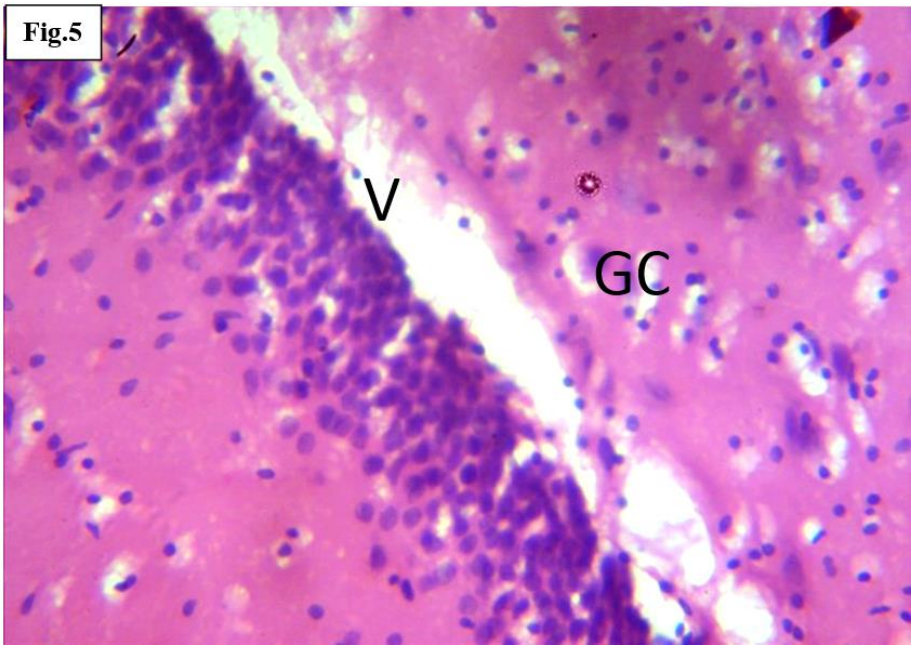


Fig. 5. Photomicrograph of group V section of the Hippocampus (x100/x400) (H/E) shows moderate Degeneration with mild Vacoulation (V) and active Granular cells (GC)

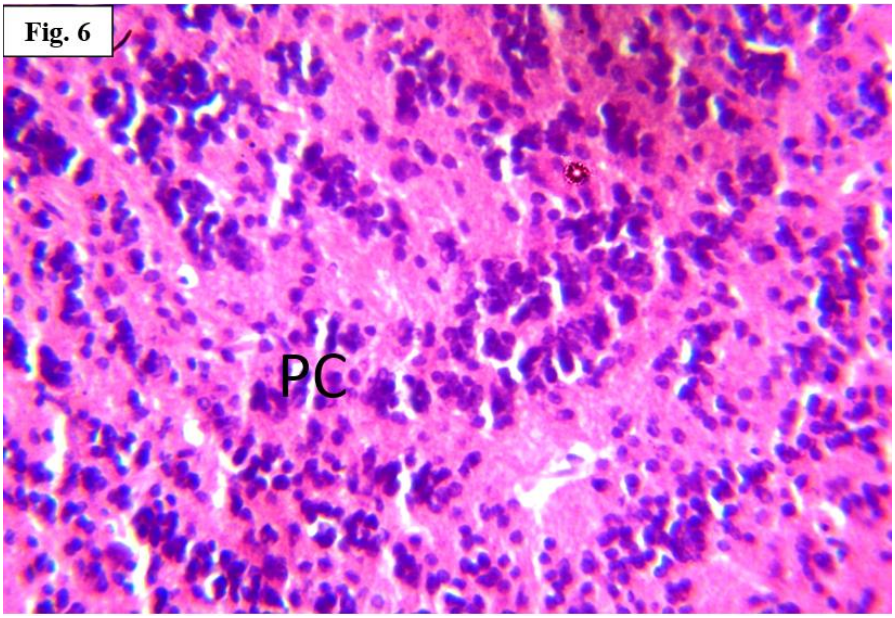


Fig. 6. Photomicrograph of group I section of the Prefrontal Cortex (x100/x400) (H/E) shows Prefrontal Cortex with active and Distinct Pyramidal cells (PC)

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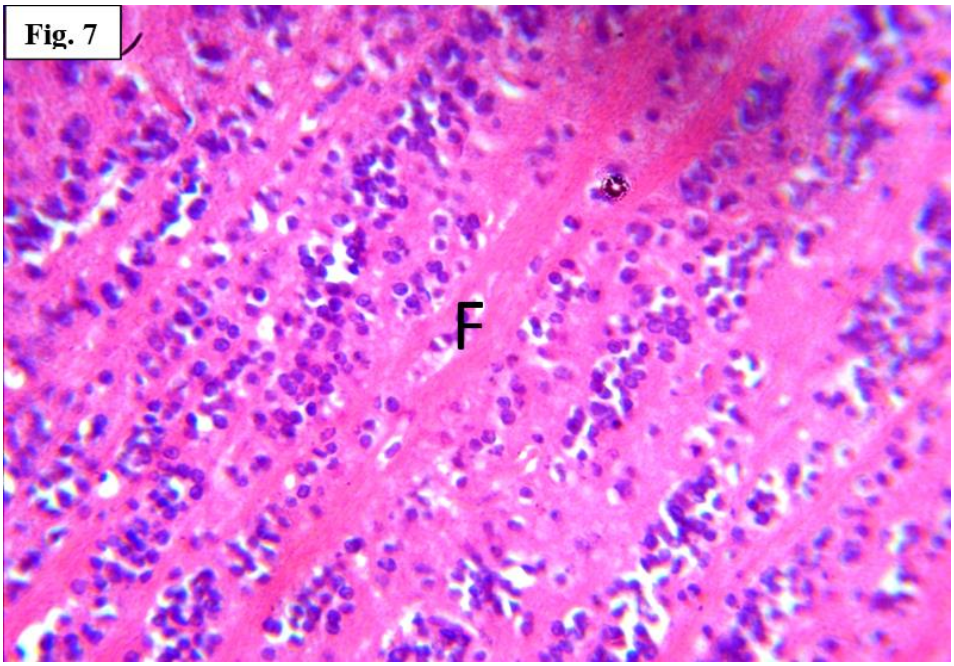


Fig. 7. Photomicrograph of group II section of the Prefrontal Cortex (x100/x400) (H/E) shows mild Degeneration with Moderate Fibrosis (F).

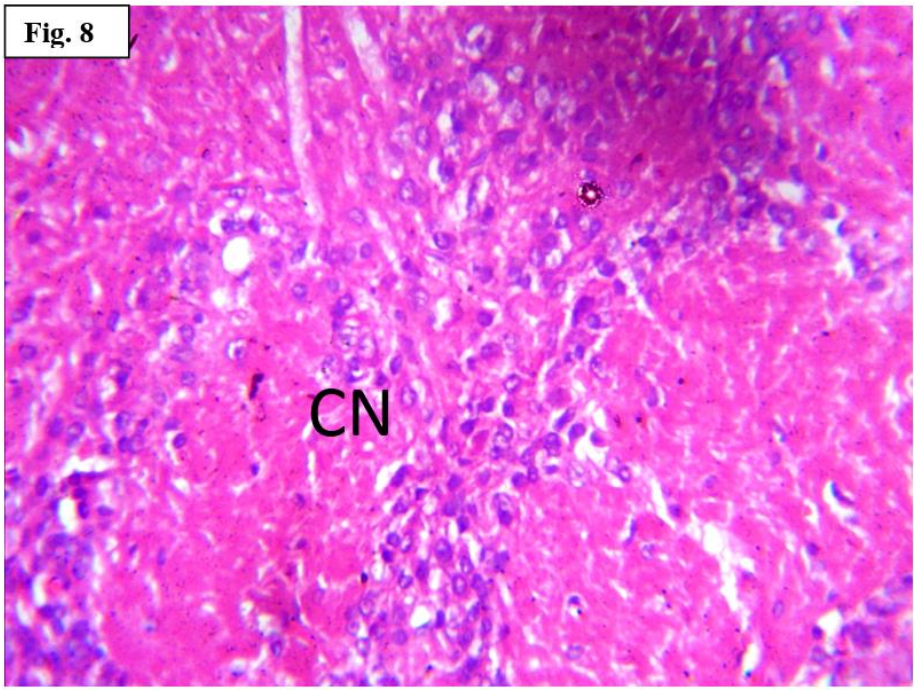


Fig. 8. Photomicrograph of group III section of the Prefrontal Cortex (x100/x400) (H/E) shows moderate focal area of Coagulative Necrosis (CN).

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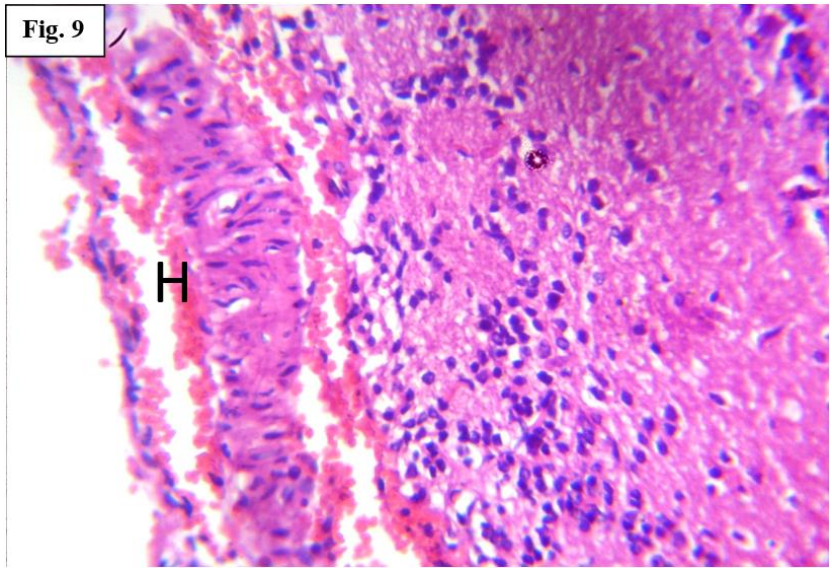


Fig. 9. Photomicrograph of group IV section of the Prefrontal Cortex (x100/x400) (H/E) shows severe Degeneration with severe focal area of Hemorrhage (H).

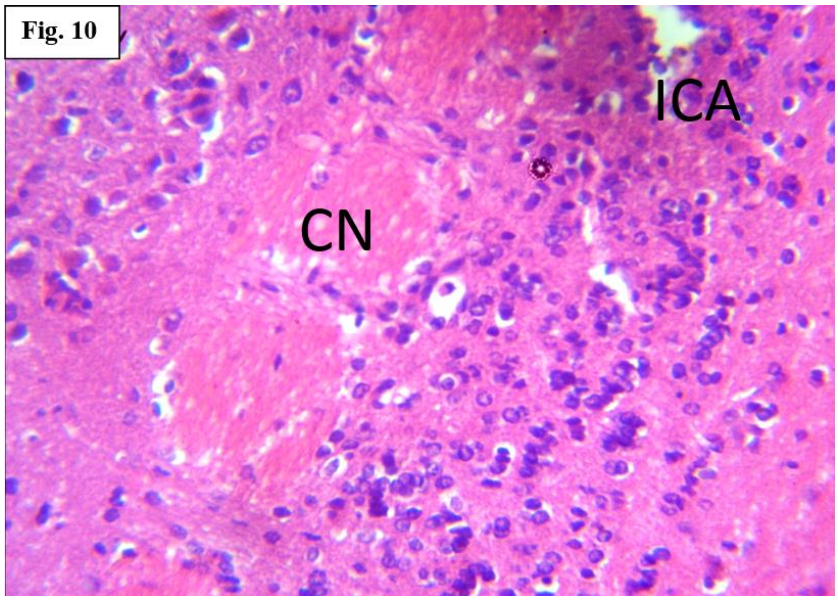


Fig. 10. Photomicrograph of group V section of the Prefrontal Cortex (x100/x400) (H/E) shows moderate Degeneration with focal area of Coagulative Necrosis (CN) and Mild Inflammatory Cells (IC) Aggregate

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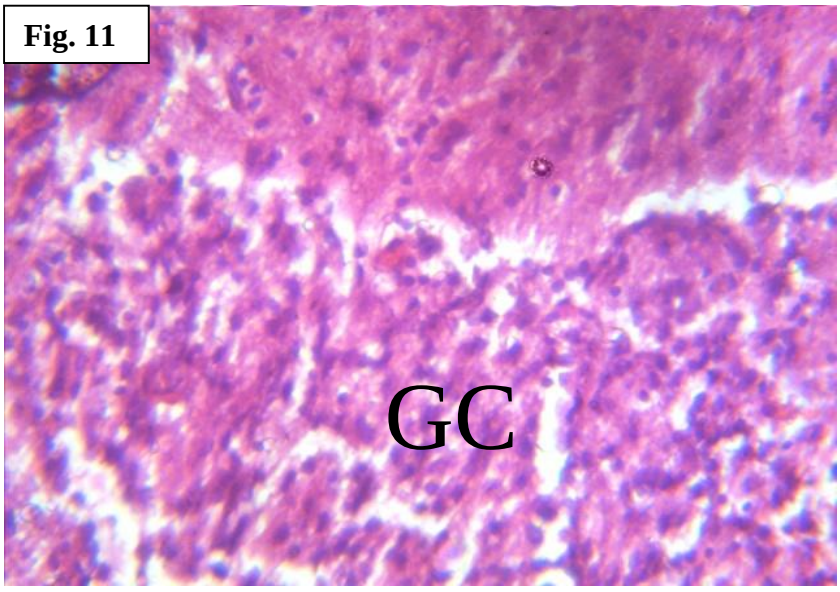


Fig. 11. Microscopic view of a group I amygdala section (H&E stain, magnification x100/x400) reveals abundant clusters of granular cells along with other morphologically normal neuronal cells

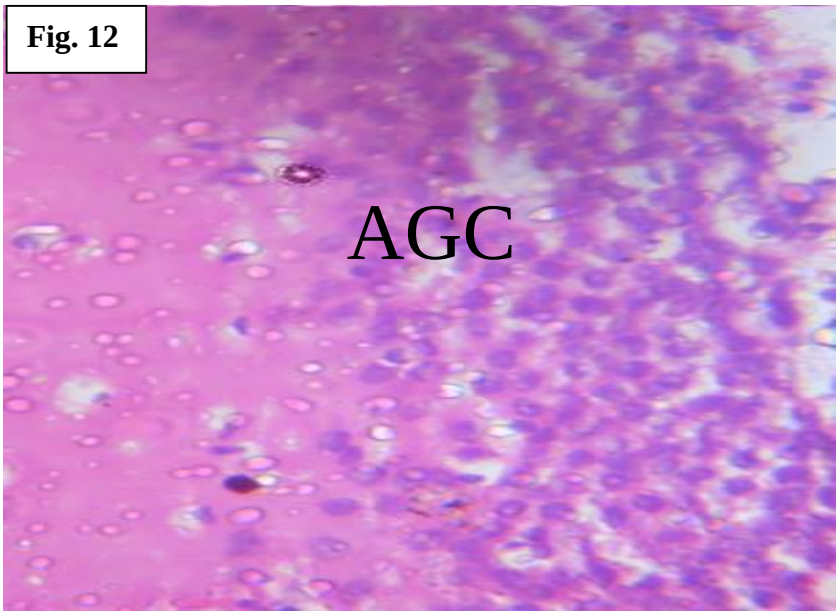


Fig. 12. Microscopic image of a group II amygdala section (H&E stain, magnification x100/x400) shows moderate neuronal degeneration accompanied by an active aggregation of Granular cells (AAGC).

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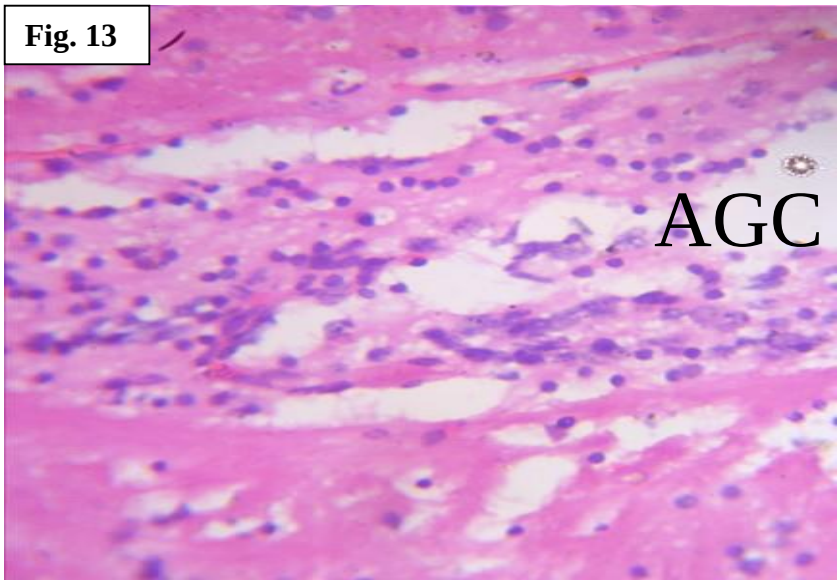


Fig. 13. Microscopic view of a group III amygdala section (H&E stain, magnification x100/x400) displays mild atrophy of Granular cells (AGC)

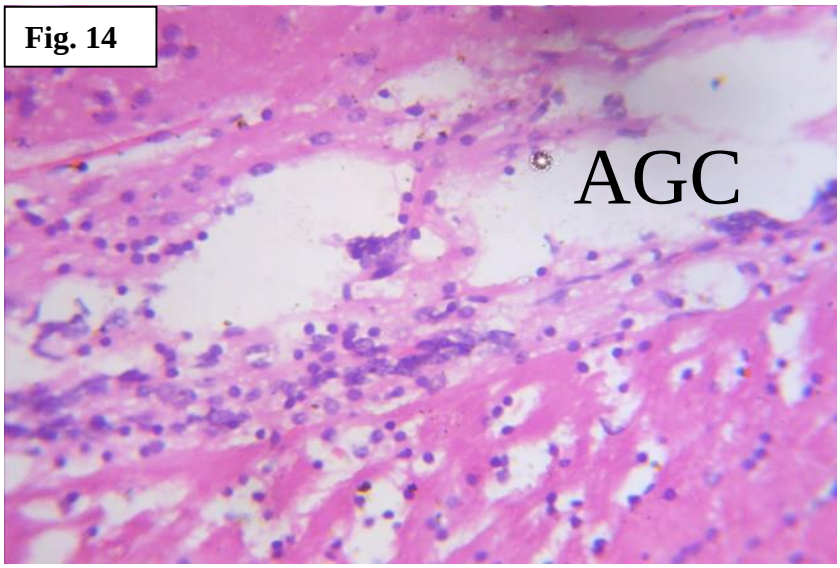


Fig. 14. Microscopic image of a group IV amygdala section (H&E stain, magnification x100/x400) reveals extensive degeneration along with marked atrophy of Granular cells (AGC)

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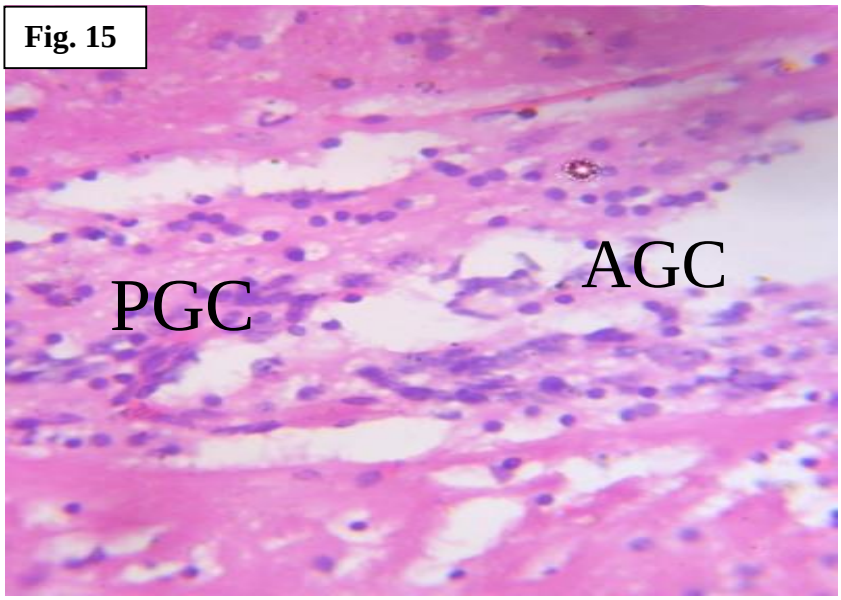


Fig. 15. Microscopic view of a group V amygdala section (H&E stain, magnification x100/x400) shows mildly pyknotic granular cells (PGC) in the background along with atrophic Granular cells (AGC)

4. Discussion

The present study aimed to investigate the potential neuroprotective effects of *M. oleifera* leaf extract against gestational stress-induced neurodevelopmental deficits in Wistar rats. Our findings revealed that CUS significantly elevated serum MDA levels, indicating increased oxidative stress [1] This aligns with previous studies demonstrating that gestational stress can elevate oxidative stress markers [2]. Increased oxidative stress is implicated in various neurodevelopmental disorders and may contribute to neuronal dysfunction [3, 4]. Histological analysis revealed neuronal damage in the hippocampus, amygdala and prefrontal cortex of the treatment groups. While *MoLE* supplementation appeared to mitigate some of this damage, the effects were not significant. Behavioral assessments, including the elevated plus maze and Y-maze tests which is commonly used to assess anxiety-like behavior in rodents [21, 22, 23], did not reveal significant differences between the treatment groups and the control group.

This suggests that *MoLE* supplementation both at low and high doses, despite its known antioxidant properties, failed to significantly mitigate this oxidative stress, and that while *MoLE* may possess antioxidant properties, it may not be sufficient to counteract the oxidative stress induced by CUS during gestation. This could be attributed to several factors, including the timing and duration of supplementation, the dose of the extract, or the severity of the stressor. Histological analysis revealed significant neuronal damage in the hippocampus, amygdala, prefrontal cortex of the treatment group, when compare to the control groups, which is consistent with previous studies as shown in Fig. 1-15 [5, 6, 7, 8]. Interestingly, the CUS + MoLE groups exhibited less severe neuronal damage, suggesting a potential neuroprotective effect of *MoLE*. This aligns with the known antioxidant properties of *M. oleifera*, which could help mitigate oxidative stress-induced neuronal damage [15,16]. However, the lack of significant improvement in behavioral outcomes, such as anxiety-like behavior and spatial memory,

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suggests that the neuroprotective effects of *M. oleifera* may be limited in this context and thus, *MoLE* may not have a significant impact on anxiety-like behavior or spatial memory in this model, which could be due to several factors, including the timing and duration of supplementation, the dose of the extract, or the severity of the stressor [12, 13]. Studies investigating the effects of chronic unpredictable stress (CUS) on pregnant rodents often utilize protocols with unpredictable stressors like cage tilting, social isolation, or changes in light-dark cycles [9]. The mechanism by which *MoLE* may exert its neuroprotective effects is not fully understood. However, it is likely that its antioxidant properties play a crucial role. *MoLE*is rich in various antioxidants, including flavonoids, phenolic acids, and vitamins C and E, which can scavenge free radicals and reduce oxidative stress [27]. Additionally, *MoLE* may also have anti-inflammatory properties, which could contribute to its neuroprotective effects [28]. Our findings partially align with previous studies that have demonstrated the neuroprotective effects of *MoLE* in various animal models. However, it is important to note that the specific effects of *MoLE* may vary depending on the experimental design, the dose and duration of treatment, and the specific neurodegenerative condition being studied.

5. Conclusion

This study provides promising preliminary evidence for the neuroprotective potential of *MoLE* against gestational stress-induced neurodevelopmental deficits. The histological and biochemical findings suggest that *MoLE* might mitigate cellular damage and oxidative stress, potentially contributing to its neuroprotective effects. However, further research is necessary to solidify the behavioral effects, particularly regarding anxiety-like behavior and spatial memory.

Authors' contributions

O.C: Conceptualization, Data curation, laboratory analysis, Methodology, Software, Writing – original draft. **C.I:** Methodology, Resources. **N.K:** Investigation, Project administration. **C. E:** Investigation, Project administration, Supervision, Writing – review & editing. **A. O:** Investigation, Writing – review & editing. **G. O:** Investigation, Writing – review & editing. **N. I:** laboratory analysis, Methodology. **A. E:** Investigation, Project administration, Supervision. All authors read and approved the final manuscript.

Conflict of Interest

The authors confirm that there are no conflicts of interest related to this publication.

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