

Original Research Article

Molecular docking analysis of *Moringa oleifera* leaf extract with estrogen receptor-beta and effects on histology of brain tissues of pregnant rats exposed to chronic stress

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Sent for review: 15 January 2025

Revised accepted: 22 July 2025

Abstract

Purpose: To evaluate the docking of *Moringa oleifera* Lam. (Moringaceae) leaf extract (MoLE) phytochemicals to estrogen receptor-beta (ER- β) and assess its neuroprotective effect on the hippocampus and amygdala in pregnant rats under chronic unpredictable stress (CUS).

Methods: Thirty healthy virgin female Albino-Wistar rats were randomly assigned to six groups ($n = 5$ per group). Pregnancy was confirmed and treatments were administered orally via gavage from gestational day (GD) 8 to GD 21. Group I (Normal Control) received only water and chow; Group II (CUS Control) was exposed to CUS without treatment; Group III (MoLE Low Dose) received 5 mg/kg/day of aqueous MoLE; Group IV (MoLE High Dose) received 10 mg/kg/day of methanol MoLE; Group V (CUS + MoLE Low Dose) received CUS + 5 mg/kg/day MoLE; and Group VI (CUS + MoLE High Dose) received CUS + 10 mg/kg/day MoLE. At the end of two weeks, histological assessments of the hippocampus and amygdala were conducted to evaluate tissue integrity.

Results: Molecular docking revealed that 1-Propanol, 3,3'-oxy bis and 1,2,3-Trimethyldiaziridine were the most potent ligands for ER- β among the MoLE phytoconstituents. Histological examination showed severe neuronal damage in the hippocampus and amygdala of the CUS Control group compared to Normal Control. MoLE treatment significantly reduced neurodegeneration, especially in the CUS + MoLE High Dose group ($p < 0.05$ vs CUS Control). This neuroprotective effect was dose-dependent and most evident in groups receiving both CUS and MoLE (Groups V and VI).

Conclusion: Orally administered MoLE during mid-to-late pregnancy reduces stress-induced neuronal damage, highlighting its potential as a neuroprotective agent. Further studies should isolate and characterize its active compounds.

Keywords: Estrogen receptor- beta (ER- β), Stress, Phytoconstituents, Hippocampus, Amygdala

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INTRODUCTION

Pregnancy is essential for the continuity of mammals, and people are becoming more aware of the potentially negative consequences of stress on the brains of both the mother and the growing foetus [1]. Hippocampus and amygdala are two key components of the stress system that provide insight into the cellular and molecular processes and mechanisms, as well as the intricate interactions that stress entails. They are connected to several psychological disorders like depression, schizophrenia, obsessive-compulsive disorder, and post-traumatic stress disorder [2]. They integrate sensory information from multiple brain areas, including the thalamus and sensory cortex, and transmit signals to regions involved in coordinating defensive behaviors. The chronic unpredictable stress (CUS) protocol, widely utilized in animal studies, reliably induces physiological changes that mimic those seen in prolonged stress exposure. CUS is characterized by unpredictable, sporadic, and random exposure to various stressors over several weeks [3]. Herbal remedies are frequently used by pregnant women to treat non-life-threatening diseases or to improve their welfare because they are typically seen as a safe and natural alternative to conventional pharmaceuticals. Geographical location, ethnicity, cultural traditions, and social position of the consumer are all factors that affect the prevalence of herbal medicine (HM) use during pregnancy [4].

M. oleifera tree is grown nationwide for its edible and medicinal properties. It belongs to the family *Moringaceae* and the genus *Moringa*. *Moringa oleifera* leaf extract (MoLE), widely used for therapeutic purposes by individuals of reproductive age in Nigeria, contains polyphenolic phytochemicals with diverse physiological effects. Studies have demonstrated estrogenic-like activity using various solvent extracts, methanol, ethanol, and aqueous [5].

In the present study, the methanol extract of *M. oleifera* leaves was used, following standard extraction protocols, to evaluate its neuroprotective potential during pregnancy under chronic stress conditions. A class of ligands known as selective estrogen receptor modulators (SERMs), which can be either natural or synthetic, regulates the activity of estrogen. The estrogen receptor subtype ER- β , which is expressed in a wide range of human cells and tissues, interacts antagonistically to significantly modify the physiological activities of estrogenic drugs [5].

Phytoestrogens are chemicals derived from plants that function estrogenically to varying degrees. These compounds interact with estrogen receptors in a manner similar to SERMs, and this type of action offers favorable pharmacological or nutraceutical advantages [6]. Through careful molecular docking simulations, computer-assisted drug design methodologies have significantly aided in the efficient processing of chemo-informatics and bioinformatics information, accelerating the early phases of drug development [7]. In order to comprehend the mechanisms underlying their estrogenic activity, this work intends to compile data on molecular docking studies of phytochemicals discovered in MoLE that target Estrogen Receptor-beta. Additionally, it seeks to assess the impact of MoLE injection on the tissues of the Dam's brain under circumstances of chronic stress.

EXPERIMENTAL

Plant collection, identification and extraction procedure

Moringa oleifera fresh leaves were collected from a garden in Abakaliki, Ebonyi State, Nigeria, in September 2022. Authentication of the plant was carried out by a botanist at the Herbarium Unit, Department of Biological Science, Alex Ekwueme Federal University Ndufu-Alike (AE-FUNAI), Ebonyi State, Nigeria. Herbarium specimen was deposited with the herbarium number AE-FUNAI UH 504a. Collected leaves were shade-dried for 2 weeks and subsequently ground into a fine powder (particle size < 250 μ m) using a grinding machine (Model MS-233, China). A standardized Soxhlet extraction method was employed using methanol as the solvent. Two hundred grams (200 g) of the powdered leaves were packed into the thimble of the Soxhlet apparatus, containing 500 mL of methanol, for extraction over 48 hours [7]. This was repeated three times and all three extracts were mixed together to obtain the total methanolic extract, which was further processed. The extract was filtered through Whatman filter paper No.1, and the filtrate was concentrated with the aid of vacuum pump and rotatory evaporator at 40 °C used for the experiment. The yield was calculated and the concentrated extract was stored in air-tight container, labeled and refrigerated at 4 °C before use [1].

Phytochemical and Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The leaf extract was screened for the presence of alkaloids, saponins, glycosides, flavonoids,

phenols, tannins, and steroids using established procedures [7]. The GC-MS analysis of leaf extracts was performed using Agilent 6890 gas chromatograph, USA.

Molecular docking analysis

The crystal structures of the estrogen receptor alpha and beta were made available by the Protein Data Bank (www.rcsb.org) under the PDB IDs 1A52, 1L2J, and 3BQD, respectively. The protein and ligand molecules were further converted into the dockable format using Autodock technology. The ligands were docked to several protein targets using Vina [8], and binding affinities were calculated. The molecular interactions between proteins and extraordinary ligands were compared to those of the reference drugs using Discovery Studio 2020.

Design and ethical approval

Thirty (30) mature, inbred, healthy virgin female Albino-Wistar rats were procured from the Animal House, AE-FUNAI, Ebonyi State. They were acclimatized to their feed (Vital feed®, Nigeria) and water *ad libitum* for 2 weeks before commencement of the experiment. The study was approved by the Faculty of Basic Medical Sciences Research Ethics Committee, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State, Nigeria, with code FBMS/EC/AE/1983.

On the first day of gestation, the animals were randomly assigned to one of five groups, each containing five rats.

Group I (Normal Control) received only distilled water and standard laboratory chow *ad libitum* throughout the gestational period.

Group II (CUS Control) was exposed exclusively to the Chronic Unpredictable Stress (CUS) protocol from gestational day (GD) 8 to GD 21.

Group III (Low-Dose MoLE) received daily administration of MoLE at a dosage of 5 mg/kg body weight per day from GD 8 to GD 21.

Group IV (High-Dose MoLE) received daily administration of MoLE at 10 mg/kg body weight per day from GD 8 to GD 21.

Group V (CUS + Low-Dose MoLE) was exposed to the CUS protocol with daily administration of 5 mg/kg body weight of MoLE from GD 8 to GD 21.

Group VI (CUS + High-Dose MoLE) was exposed to CUS protocol with daily

administration of 10 mg/kg of MoLE throughout GD 8 to GD 21.

Protocols

MoLE was freshly reconstituted each day in distilled water and administered via oral gavage in a volume not exceeding 1 mL per 100 g body weight to prevent gastric discomfort. The extract was mixed thoroughly to ensure uniform dispersion and administered using a flexible, blunt-ended oral gavage needle attached to a sterile 2 mL syringe. Oral gavage was performed each morning at a consistent time to maintain dosing regularity and minimize circadian variation. All administrations were done carefully to reduce stress to the animals.

After GD 21, pups were weaned, maintained with free access to tap water and standard chow, and kept within the same experimental grouping as their respective dams. Male and female pups were housed separately after weaning. The gestational period from GD 8 to GD 21 was selected for MoLE administration due to its equivalence to the second and third trimesters of human pregnancy, which corresponds to a critical phase of neurodevelopment in rats. The doses of MoLE (5 and 10 mg/kg/day) were chosen based on previous studies demonstrating their safety and bioactivity in rodent models [1,7].

Initiation of pregnancy

Estrus cycle monitoring was conducted via light microscopy. Female rats exhibiting two consecutive regular four-day estrus cycles were selected for the study. The pro-estrus phase was identified cytologically by the predominance of nucleated epithelial cells in vaginal smears, with few leukocytes and an absence of cornified cells. During this phase, male rats were introduced into the female rat cages at a ratio of 1:2 to facilitate mating. The following morning, the presence of spermatozoa in vaginal smears confirmed successful mating and marked the first day of pregnancy [9]. The stress group of animals was subjected to CUS protocol from gestational day (GD) 8 (which is around the period of onset of organogenesis in rats) to GD 21 (expected day of parturition).

Chronic unpredictable stress (CUS) protocol

From GD 8 to GD 21, animals in the CUS groups were subjected to an unpredictable stress protocol, consisting of the following stressors administered in a random sequence:

- Wet Bedding: 1 L of sawdust bedding mixed with 300 mL of water.
- Cage Tilting: Food and water were positioned at the top of the cage, which was tilted 45°.
- Food Deprivation: Rats were denied food for one overnight period.
- Psychological Stress: Rats were exposed to a caged cat.
- Sleep Deprivation: A 6 cm diameter, 5 cm height pedestal was placed in the cage, and the cage was flooded with 3 cm deep water to prevent sleep.
- Restraint Stress: Rats were placed in a 50 mL plastic tube for 6 hours (three 2-hour sessions with a 30-minute gap).
- Permanent Light: Rats were exposed to continuous light overnight.
- Social Isolation (SI): Rats were housed individually during each stressor application.

Stressors were administered once per day, lasting 6 hours per session, except for the sleep deprivation sessions, which lasted 12 hours. Each stressor was applied twice throughout the protocol, mimicking the unpredictable and uncontrollable nature of human prenatal stress. This CUS protocol has been widely used to investigate maternal and fetal outcomes in preclinical studies [2].

Sample collection

On GD 18, about 2 mL of venous blood was obtained via the orbital route [10], poured into plain tubes, and allowed to clot. Sera were obtained from the clotted sample after centrifuging at 3000 rpm for 10 min and refrigerated until further use to analyze biochemical parameters. The rats were then sacrificed by cervical dislocation at GD 20 and following abdominal incision, the fetuses along with the Placenta were first removed and placed in a new formaldehyde solution after which the rat's brain was also removed and placed in a new formaldehyde solution for 24 hours before being dehydrated using ethanol (70 % for 24 h, 90 % for 1 h and 100 % for 1 h).

Biochemical and histological study

The serum malondialdehyde (MDA) level was measured using the thiobarbituric acid assay

following an earlier reported method [11]. The tissue sample was cleaned in xylene and embedded in paraffin. Coronal sections were cut with a microtome at 5 μ m thickness, mounted on glass slides and stained using the hematoxylin and eosin technique [10]. Examination of slides, morphometric studies, and photomicrography was then done.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with GraphPad Prism software (GraphPad® Software, San Diego, CA, USA). Results are expressed as means $\pm$ standard error of the mean (SEM). The Tukey post hoc test was employed for pairwise comparisons between groups. Statistical significance was set at $p < 0.05$.

RESULTS

Chromatogram of GC-MS analysis identified the presence of forty-one (41) compounds from the methanolic leaf extract, as indicated in Table 1. The main compounds were steroidal fatty acid methyl esters, such as hexadecanoic acid methyl ester, with possible estrogenic bioactivities.

Binding affinity of compounds to ER- α and ER- β

Among the phytochemicals identified from MoLE in this study, 1-Propanol, 3,3'-oxybis-demonstrated notable binding affinity for the active site of estrogen receptor beta (ER- β), with a docking score of -7.3 kcal/mol. This was comparable to the reference drug clomiphene, which exhibited a slightly stronger binding affinity, with a docking score of -8.0 kcal/mol. Another compound screened from MoLE, 1,2,3-Trimethyldiaziridine, also showed a high binding affinity to ER- β , with a docking score of -7.1 kcal/mol.

Clomiphene's interaction with ER- β (as shown in Figure 1 and 2) includes carbon-hydrogen and π -alkyl hydrophobic interactions with ARG A:346, as well as π -alkyl and π -sigma hydrophobic bonds with HIS A:279. Clomiphene also forms π -alkyl interactions with ILE A:355 and PRO A:358. Moreover, an electrostatic π -cation interaction was noted between clomiphene and ASP A:349, supported by neighboring residues including LEU A:392, ILE A:348, TRP A:345, GLN A:393, HIS A:394, and ALA A:357. An unfavorable donor-donor interaction was also detected in this complex.

As illustrated in Figure 3 and Figure 4, 1-Propanol, 3,3'-oxybis- engages in π -alkyl hydrophobic interactions with LYS A:425 and π - π stacking interactions with TRP A:439. Additional residues in the binding pocket include SER A:264, HIS A:428, LEU A:429, LEU A:263, ALA A:432, PRO A:265, ASP A:435, VAL A:438, and ALA A:442. An unfavorable donor-donor interaction was also observed.

Figure 6 presents the 2D interaction profile of 1,2,3-Trimethyldiaziridine within the ER- β binding site. This compound forms π -alkyl hydrophobic interactions with LEU A:339 and LEU A:343, in the presence of several additional amino acid residues, including MET A:340, ILE A:373, MET

A:336, LEU A:476, VAL A:487, TRP A:335, ALA A:302, PHE A:356, LEU A:301, GLU A:305, and ARG A:346.

Serum MDA levels of dams exposed to CUS and MoLE

There was a significant ($p < 0.05$) increase in serum MDA levels in the CUS Control group, as well as in the CUS + Low Dose MoLE and CUS + High Dose MoLE groups, when compared to Normal Control group. Additionally, an increase in MDA levels was observed in the Low Dose MoLE and High Dose MoLE groups compared to Normal Control, although this increase was not statistically significant ($p > 0.05$).

Table 1: Binding affinity of compounds to ER- β

S/no	Name of compound	Binding affinity (kcal/mol)
		ER- β
R	Clomiphene	-8.0
R	Mifepristone	-
1	1-Propanol, 3,3'-oxybis-	-7.3
2	1-Propanamine, 3-propoxy-	-4.5
3	2-Pentene, 2-methyl-	-4.5
4	Pyridine	-3.8
5	2-Pentanone, 5-hydroxy-	-4.5
6	5-Hexen-2-ol, 5-methyl-	-5.3
7	1,3-Propanediamine, N-(1-methylethyl)-	-5.9
8	1,4-Butanediamine, N, N'-diethyl-	-5.4
9	Hexanoic acid, methyl ester	-5.1
10	Cyclotetrasiloxane, octamethyl-	-
11	1, 2, 3-Trimethyldiaziridine	-7.1
12	2-Hexyn-1-ol	-4.5
13	Heptanoic acid, methyl ester	-5.4
14	1-Heptene, 3-methyl-	-5.4
15	1-Fluorononane	-5.5
16	Octanoic acid, methyl ester	-5.4
17	Erythritol	-5.2
18	2-Mercaptopropanoic acid	-3.9
19	Triethylene glycol	-5.0
20	Decanoic acid, methyl ester	-5.8
21	Benzene,2-methoxy-1,3,4-trimethyl	-6.6
22	Ethylene, 1, 2-dichloro-, (Z)-	-3.9
23	10-Undecenoic acid, methyl ester	-5.4
24	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis((trimethylsilyl)oxy)-	-
25	7-Hexadecenal, (Z)-	-5.9
26	1-Octanol, 2-butyl-	-6.7
27	3,8-Dioxatricyclo (5.1.0.0(2,4)) octane, 4-ethenyl-	-6.1
28	Dodecanoic acid, methyl ester	-6.8
29	1-Decanol, 2-hexyl-	-6.2
30	Myristyl stearate	-4.9
31	2-Tridecenal, (E)-	-6.3
32	Cyclotetradecane	-8.2
33	Methyl tetradecanoate	-5.8
34	2-Piperidinone, N-(4-bromo-n-butyl)-	-5.6
35	Tetradecanal	-5.6
36	Ethanol, 2-(octadecyloxy)-	-6.4
37	Hexadecanoic acid, methyl ester	-5.9
38	6-Ethoxy-6-methyl-2-cyclohexenone	-6.2
39	n-Dodecyl methacrylate	-5.8
40	9-Octadecenoic acid (Z)-, methyl ester	-6.9
41	Methyl stearate	-4.4

Conversely, there was a significant ($p < 0.05$) decrease in serum MDA levels in all groups treated with MoLE, specifically the Low Dose MoLE, High Dose MoLE, CUS + Low Dose MoLE, and CUS + High Dose MoLE groups, when compared to the CUS Control group.

Photomicrograph

Photomicrograph of CUS control rats' section of hippocampus shows severe degeneration with severe vacuolation (V) of the Granular Cells (GC) with non-distinct cell outline, which is the consequence of an inflammatory response caused by stress, when compared to the photomicrograph examination in Normal control, which displayed Normal histological architecture of the rat hippocampus.

Photomicrograph of CUS-exposed rats treated with MoLE (5 and 10 mg/kg). The section of the hippocampus shows mild to moderate degeneration, moderate vacuolation (V) with Pyknotic (P) Granular Cells, and mild infiltration of inflammatory Cells (IIC) on the background. Photomicrograph of CUS control group section of Amygdala showed moderate degeneration with moderate Vacuolated Granular Cells (VGC) and moderate focal area of Hemorrhage (H) around the Vacuolated Granular Cells when compared with normal control group section of Amygdala showing numerous normal aggregate of Granular

Cells and other Neuronal Cell, while Photomicrograph of CUS exposed rats treated with MoLE (5 and 10 mg/kg) section of Amygdala showed active aggregate of Granular Cells with mild Pyknotic (P) Cells on the background, when compared with CUS control group.

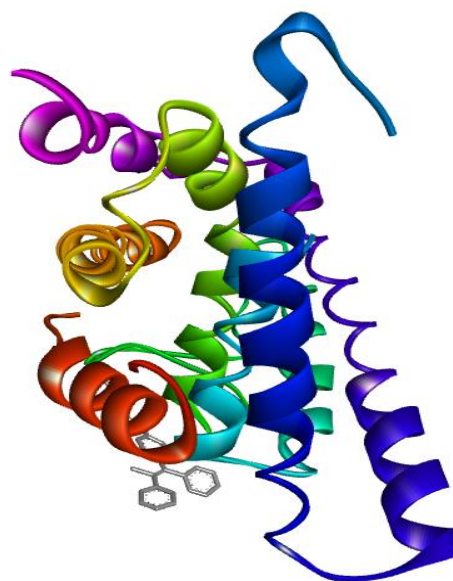


Figure 1: 3D view of the interaction between clomiphen and the binding site of estrogen receptor beta

Table 2: Serum MDA Levels of Dams exposed to CUS and MoLE

Variable	Group I	Group II	Group III	Group IV	Group V	Group VI
MDA (nmol/mL)	1.38±0.05	3.86±0.19*	1.56±0.07 [#]	1.42±0.06 [#]	2.84±0.13**	2.21±0.17**

Values are expressed as Mean ± SEM; * $p < 0.05$ vs Normal Control (Group I); [#] $p < 0.05$ vs chronic stress control (Group II); Group III (Low Dose MoLE), Group IV (High Dose MoLE); Group V (CUS + Low Dose MoLE), Group VI (CUS + High Dose MoLE), all groups were exposed throughout GD 8 – GD 21st day

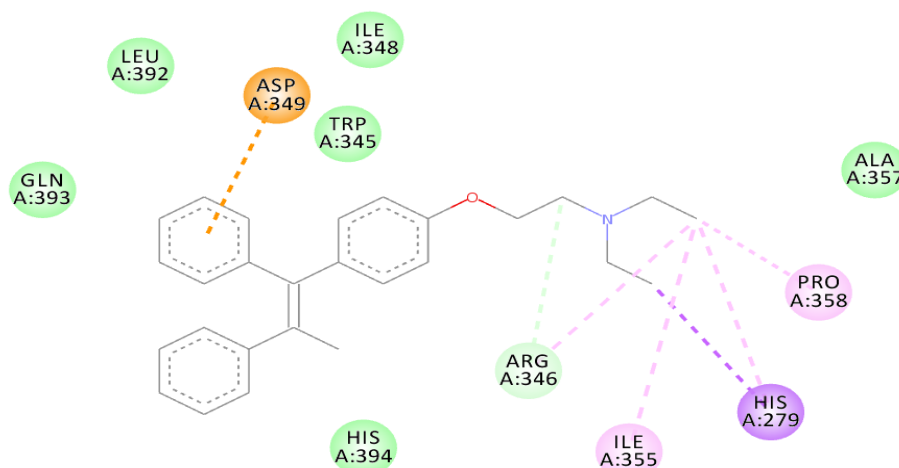


Figure 2: 2D view of the interaction between clomiphen and amino acids in the binding site of estrogen receptor beta

DISCUSSION

When excessive reactive oxygen species (ROS) synthesis outpaces antioxidant defenses under pathophysiological conditions, harmful oxidative stress results because excessive ROS damages cellular components [3]. As a result, there was a significant ($p < 0.05$) rise in MDA levels in CUS control group, Groups II, V, and VI, compared to normal control group. The brain experiences morphological, molecular, and neurochemical changes as a result of acute and long-term stress [12]. Studies on both humans and animals have shown that stress changes the structure of neurons, prevents their growth, and structurally reduces the volume of the hippocampus. Since the beginning of stress research nearly 80 years ago, the neuroendocrine hormones of the hypothalamic-pituitary-adrenal (HPA) axis, particularly glucocorticoids, have drawn a lot of attention as mediators of the myriad effects of stress on the hippocampus and as contributing factors to stress-associated psychopathologies like post-traumatic stress disorder (PTSD) [13]. It has also been demonstrated that persistent and/or stressful stressors can alter the hippocampus' morphology. Human brain imaging investigations have revealed, in particular, and with major therapeutic implications, that PTSD patients had decreased hippocampus volumes, which were connected with deficiencies in verbal memory [12,14]. Photomicrograph of rats treated with MoLE (5 and 10 mg/kg) in groups III and IV section of hippocampus showed moderate Vacuolation (V) with mild area of Hemorrhage, with active Granular Cells.

The amygdala, which increases glucocorticoid release in response to stress, experiences alterations in Long-Term Potentiation (LTP) and cytoarchitecture as a result of stress. According to studies, prolonged exposure to corticosterone can affect the amygdala neurons in several ways that might enhance anxiety, fear, and stress reactions as well as impair learning and memory. They might also aid in the emergence of mental illnesses like PTSD, mood disorders, and anxiety disorders [15]. According to studies, rats exposed to prolonged stress responded more quickly to frightening stimuli in the amygdala. The rats in these studies also had lower dendritic spine levels, which are crucial for memory and learning, in their amygdala neurons [5,12].

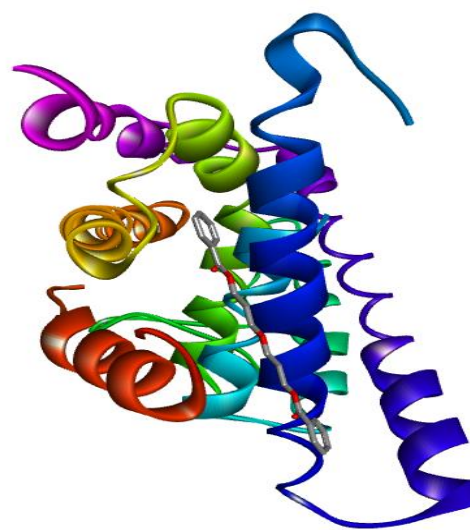


Figure 3: 3D view of the interaction between 1-Propanol, 3, 3'-oxybis- and the binding site of estrogen receptor beta

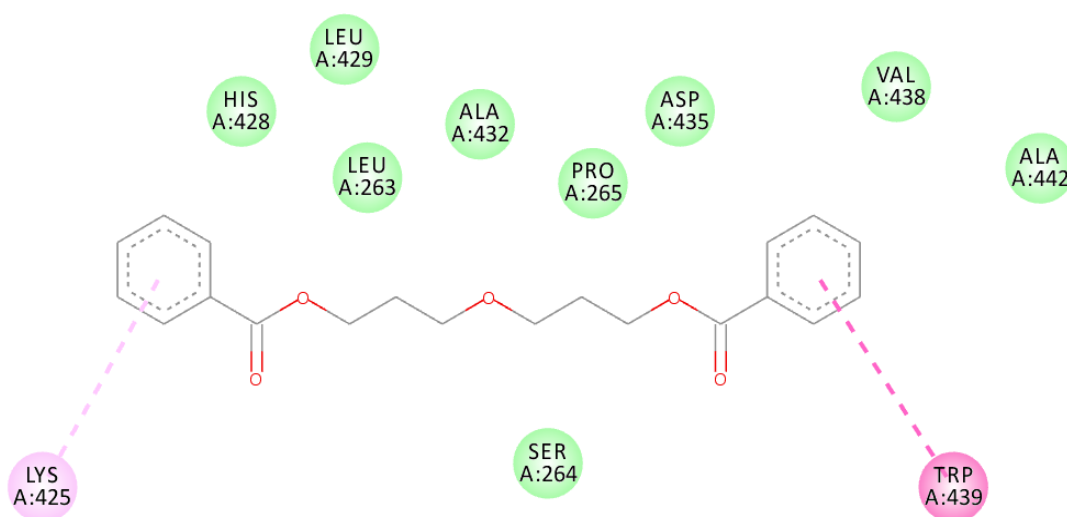


Figure 4: 2D view of the interaction between 1-Propanol, 3, 3'-oxybis- and amino acids in the binding site of estrogen receptor beta

Another study reported that long-term exposure to corticosterone in mice caused atrophy of the amygdala also in addition to these mice having impaired fear conditioning, a learning process that is dependent on the amygdala [16]. A study found that people with PTSD had increased levels of corticosterone in their blood and cerebrospinal fluid. In addition, these people had reduced volume of the amygdala [14].

Phytoestrogens have been shown to have some beneficial effects on the brain, including protecting amygdala neurons from the negative effects of corticosterone. Genistein, a type of phytoestrogen was found to not only protect the amygdala neurons from damage caused by corticosterone in a study, but also enhanced the learning and memory of rats exposed to chronic stress [17]. Another study reported that isoflavones, another type of phytoestrogen, protected amygdala neurons from damage caused by oxidative stress, which is a process that damages cells and tissues, and is thought to be a contributing factor to a number of neurological disorders [18]. This may be due to their strategies, which include maintaining cerebral blood flow, maintaining endogenous antioxidants, and suppressing reactive oxygen species directly to produce direct antioxidant action. Additionally, they include the reduction of excitotoxicity, stimulation of neuroprotective gene products such as B-cell lymphoma 2 (Bcl-2), inhibition of the production of glial cytokines and reactive gliosis, anti-apoptotic activity with preservation of neuronal integrity, and improvement of synaptic plasticity [19].

Cells in the central neurological systems exhibit ER- β in the nucleus, mitochondria, plasma membrane, and endoplasmic reticulum and it plays a role in regulating a variety of functions, including learning, memory, and stress response [5,20]. In this molecular docking analysis, stronger ligand-protein interactions were indicated by lower (more negative) binding affinity values, reflecting more effective binding of the ligand to the active site of the target protein.

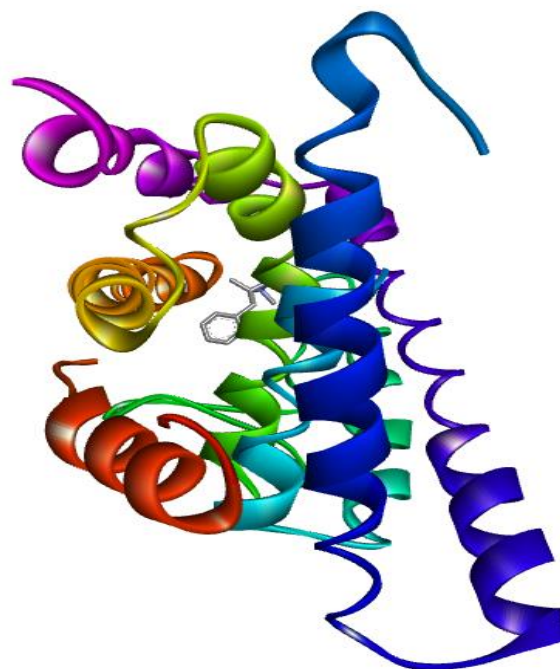


Figure 5: 3D view of the interaction between 1, 2, 3-Trimethyldiaziridine and the binding site of estrogen receptor beta

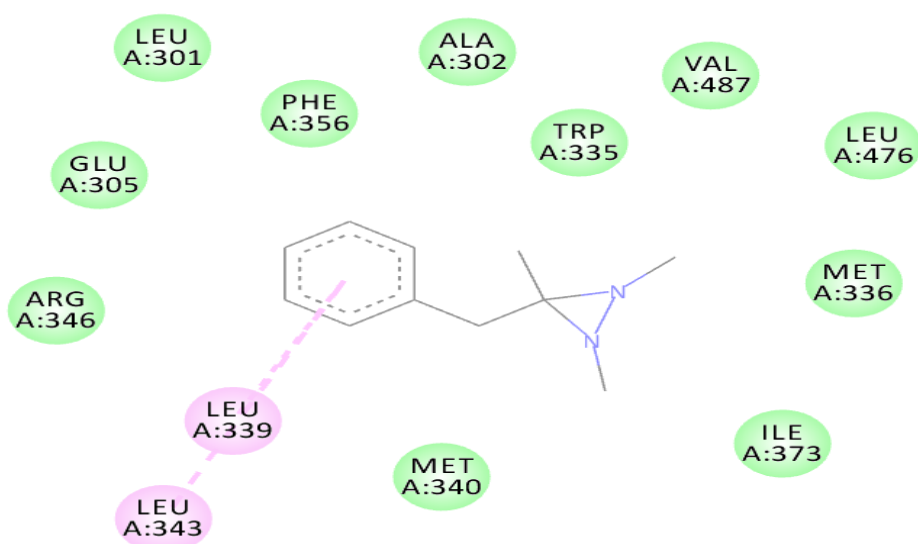


Figure 6: 2D view of the interaction between 1, 2, 3-Trimethyldiaziridine and amino acids in the binding site of estrogen receptor beta

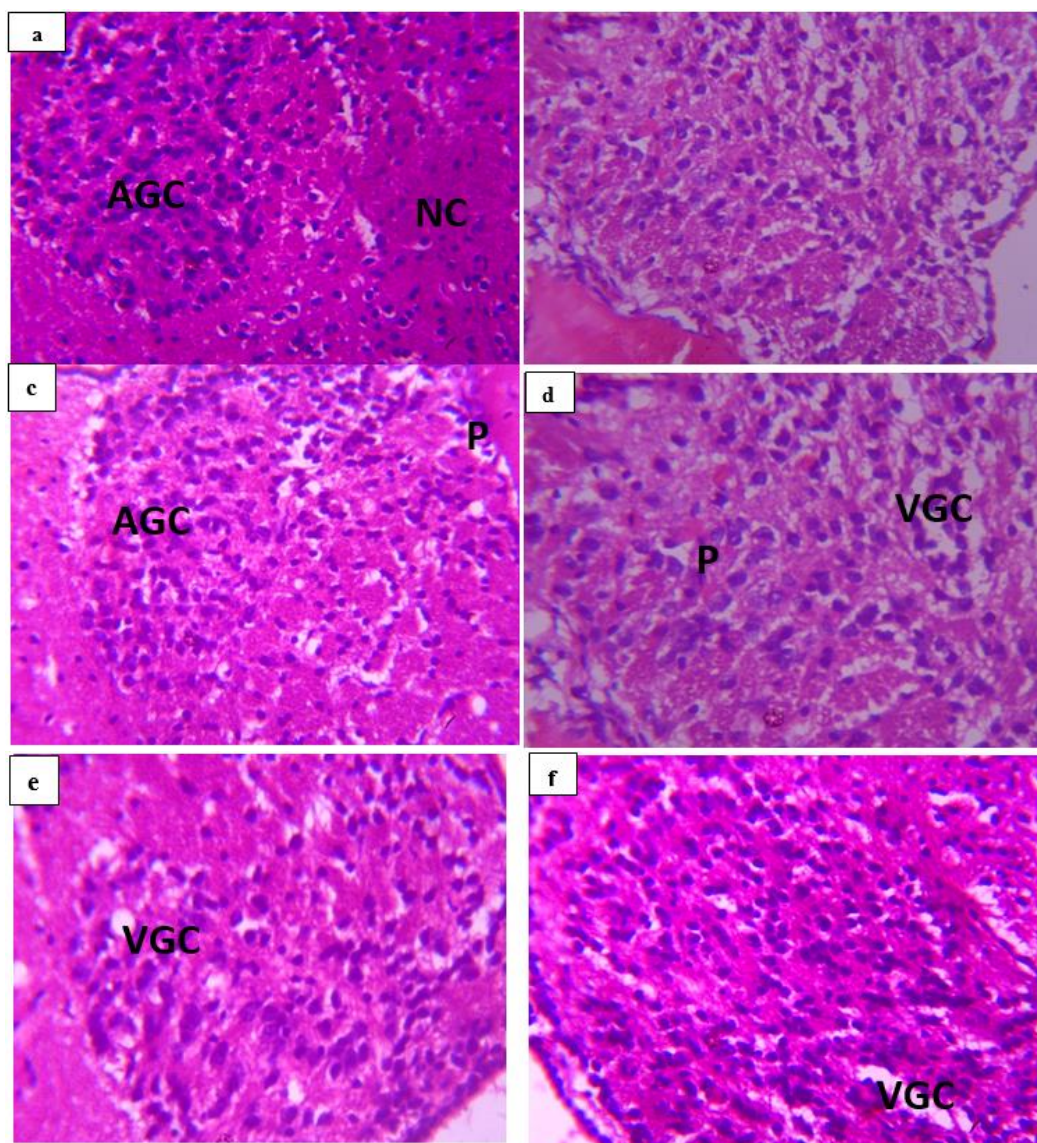


Figure 7: Photomicrograph of sections of the Amygdala (x400; H/E). (a) Normal Control group shows numerous aggregates of granular cells (AGC) and other neuronal cells (NC) that are normal. (b) CUS Control group shows moderate degeneration with moderate vacuolated granular cells (VGC) and moderate focal areas of hemorrhage (H) around the vacuolated granular cells. (c) low-dose MoLE group shows active AGC with mild Pyknotic (P) Cells on the background. (d) The high-dose MoLE group shows mild pyknotic (P) and vacuolated granular cells on the background. (e) CUS protocol + 5 mg/kg body weight/day of MoLE group shows moderate degeneration with moderate VGC. (f) CUS protocol + 10 mg/kg body group shows mild degeneration with VGC

The docking scores for all ligands targeting estrogen receptor alpha (ER- α) ranged from -4.1 to -8.8 kcal/mol, as presented in Table 1. Among the phytochemicals derived from *Moringa oleifera* leaf extract (MoLE), 1-Propanol, 3,3'-oxybis- exhibited a notable binding affinity toward the active site of estrogen receptor beta (ER- β), with a docking score of -7.3 kcal/mol. This binding strength was comparable to that of the reference compound clomiphene, which demonstrated a slightly higher affinity with a docking score of -8.0 kcal/mol. Additionally, 1,2,3-Trimethyldiaziridine, another compound identified from MoLE, showed a considerable

interaction with ER- β , yielding a docking score of -7.1 kcal/mol. The functional characteristics of exogenous or synthetic ligands, such as their agonistic or antagonistic activity, can be elucidated through structural investigations of estrogen receptors and other steroid hormone receptors.

Depending on receptor subtype, cellular environment, and promoter context, some ligands may exhibit both agonistic and antagonistic behavior. This dual activity has led to the classification of such compounds as selective estrogen receptor modulators (SERMs).

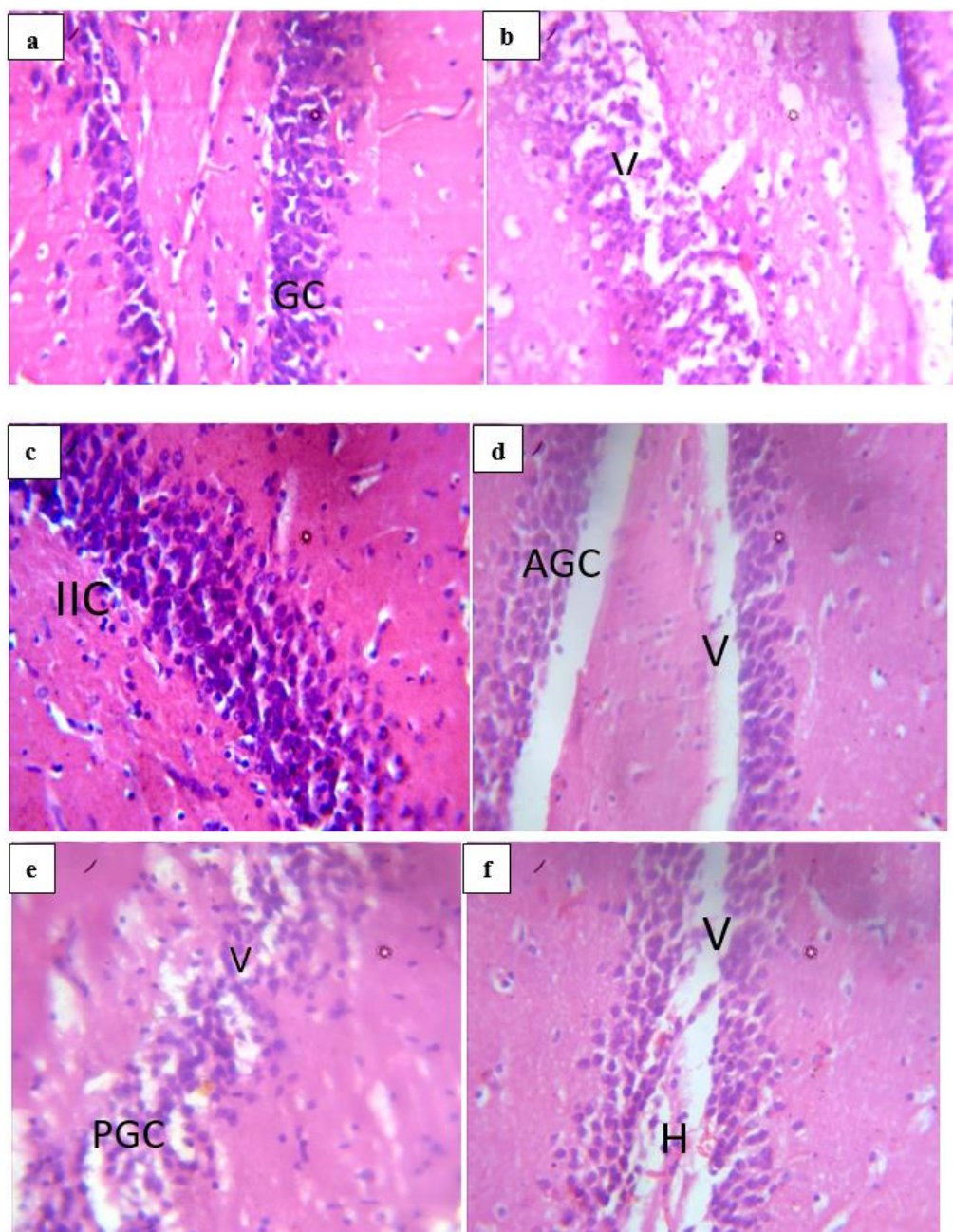


Figure 8: Photomicrograph section of hippocampus (x400) (H/E) (a) Normal Control group shows hippocampus with distinct layers and Granular Cells (GC) outline. (b) The CUS Control group shows severe degeneration with severe vacuolation (V) of the Granular Cells (GC) with non-distinct cell outline. (c) The Low Dose MoLE group mild degeneration with mild infiltration of inflammatory Cells (IIC) on the background. (d) The High Dose MoLE group shows moderate vacuolation (V) with Active Granular Cells (AGCs). (e) The CUS + Low Dose MoLE group shows moderate vacuolation (V) with Pyknotic Granular Cells (PGCs). (f) The CUS protocol + High Dose MoLE group shows moderate vacuolation (V) with a mild area of Hemorrhage (H)

Different non-genomic activities of 17β -estradiol (E2) are sometimes grouped based on how easily they are inhibited by traditional ER antagonists; ICI-type drugs are one such example [5,20]. The involvement of membrane-associated ER- β is suggested by the inhibition of E2-induced activation of membrane ion channels, endothelial nitric oxide synthase, and mitogen-activated protein kinase by ER

antagonists [5]. Due to the potential clinical uses, including treatments for postmenopausal symptoms, osteoporosis, cognitive function, breast and gynecological malignancies, cardiovascular disease, and osteoporosis, anti-estrogens and SERMS have been the subject of extensive investigation.

CONCLUSION

Moringa oleifera leaf extract (MoLE) consumption offers mild to moderate protection to the hippocampus and amygdala from damaging effects of chronic unpredictable stress CUS, which suggests that MoLE treatment may have increased the expression of ER- β or caused activation of ER- β in the hippocampus and amygdala thereby protecting the brain from damage and thus making MoLE a potential treatment for the negative effects of CUS on the brain. These findings suggest that the active phytoconstituents of MoLE may be further investigated for the development of novel therapeutics.

DECLARATIONS

Acknowledgement/Funding

The authors thank the Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, AE-FUNAI, Ebonyi State, for supporting this study.

Ethical approval

The study was approved by Faculty of Basic Medical Sciences Research Ethics Committee, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria. with code FBMS/EC/AE/1983.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Odochi O. Chukwu and Anthony C. U. Ezimah conceived and designed the study, collected and analyzed the data. Odochi O. Chukwu; Cordilia O. Iyare wrote the manuscript. Nnaemeka T. Asogwa; Grace Ottah-Umahi; Albert E. Okorochoa; Sylvester N. Ibekailo; Nwaeze G. Konyefom; Ofovwe O. Ekakitie; Gertrude N. Onyeji; analyzed data and provided technical expertise and reviewed the manuscript.

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