Effect of alpha-lipoic acid on the depressive-like behavior in oophorectomized rats: Neuroprotective actions of apelin and microRNA 99a

Eman A. Allam[a], Hend S. Zakaria[b], Walaa N. Roushdy[c*], Basant A. Bakr[d], Eman M. Omar[a]
[a]Department of Medical Physiology, Faculty of Medicine, Alexandria University, Alexandria, Egypt.
[b]Department of Clinical Pharmacology, Faculty of Medicine, Alexandria University, Alexandria, Egypt.
[c]Department of Medical Biochemistry, Faculty of Medicine, Alexandria University, Alexandria, Egypt.
[d]Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt.

Abstract

Background: Postmenopausal sharp decline in estrogen levels leads to significant mood and cognitive changes. Although estrogen replacement therapy can alleviate these symptoms, it is not suitable for all women. Alpha-lipoic acid (ALA) is a naturally occurring compound with reported antidepressant effects. Apelin, an adipokine, exerts neuroprotective actions, while microRNA-99a (miRNA-99a) has emerged as a depression-related regulator.

Objectives: assessing ALA's potential antidepressant effects in oophorectomized female rats, focusing on its effect on apelin and miRNA-99a expression in the brain.

Materials and methods: Thirty-two female rats were divided into four groups (control, oophorectomized, estradiol-treated, and ALA-treated). Depressive-like behavior was assessed using behavioral tests. Neurotransmitters, Nrf2, apelin, inflammatory and oxidative stress markers were measured by ELISA and colorimetric assays. MiRNA-99a expression was quantified using real-time PCR.

Results: In oophorectomized group, IL-6 and NF-κB increased significantly, while other biochemical and molecular markers decreased compared with controls ($p<0.001$). ALA and estradiol significantly improved behavioral outcomes, biochemical and molecular parameters, with ALA producing more significant effects. IL-6 decreased to 45.0±5.29 and 57.25±4.89 pg/mg, and NF-κB to 7.7±1.1 and 5.38±0.92 ng/mg, respectively ($p<0.001$). Dopamine and serotonin increased with ALA (15.0±1.77 and 15.13±1.46 ng/mg) and estradiol (18.5±1.2 and 19.63±1.51 ng/mg) ($p<0.001$). ALA induced higher Nrf2 (172.5±9.24 vs 121.5±6.55 pg/mg), apelin (242.1±11.7 vs 204.8±15.3 pg/mg), CAT (5.8±0.83 vs 3.56±0.5 U/g), GPx (32.88±4.52 vs 23.13±2.1 U/g), and miRNA-99a (0.68±0.04 vs 0.50±0.07 fold change) (all $p<0.001$).

Conclusion: These results refer to the promising role of ALA in postmenopausal depression that is augmented by its linking with apelin and miRNA-99a expression.

Keywords: Alpha-lipoic acid (ALA); Apelin; MicroRNA 99a (MiRNA-99a); Oophorectomized rats; Postmenopausal depression; Neuroprotection.

DOI: 10.21608/svuijm.2025.430962.2302

***Correspondence**: walaa.roushdy@alexmed.edu.eg

Received: 15 October, 2025.

Revised: 14 November, 2025.

Accepted: 20 November, 2025.

Published: 25 November, 2025

Cite this article as Eman A. Allam, Hend S. Zakaria, Walaa N. Roushdy, Basant A. Bakr, Eman M. Omar (2025). Effect of alpha-lipoic acid on the depressive-like behavior in oophorectomized rats: Neuroprotective actions of apelin and microRNA 99a. *SVU-International Journal of Medical Sciences*. Vol.8, Issue 2, pp: 823-845.

Introduction

The physiological hormonal fluctuations of estrogen in women play a vital role in shaping their mood, behavior, and cognitive functions throughout their lifespan. The most substantial changes in mood and cognition are caused by the dramatic drop in estrogen levels, which happens during the critical stages, such as the anestrus cycle, menopausal shift, post-menopause period, and menopause induced by surgery **(Bastos et al., 2015)**.

Preclinical research has shown that aging and bilateral ovariectomy increase the susceptibility of female rats to stressful conditions more than control animals, resulting in emotional impairments as anxiety. The pathogenesis of mood changes and depressive like behavior accompanying menopausal transition is multifactorial. Estrogen decline is the cornerstone in this condition as this decline leads to a state of chronic neuroinflammation and oxidative stress **(Liang et al., 2024)**. Also, estrorogen decline is claimed to affect neurotransmitters and neurotrophic factors as brain derived neurotrophic factor (BDNF) in both prefrontal cortex and hippocampus which is closely related to menopausal cognitive and depressive changes **(Guo et al., 2018; Bohm et al., 2020)**. In addition, disturbance in the hypothalamo-pituitary-adrenal axis is another important key player in the development of menopausal associated depressive changes **(Gordon et al., 2016)**.

There is compelling evidence indicating that estrogen replacement therapy can effectively reverse the mood, behavior, cognitive and depressive symptoms accompanying menopausal transition **(Tantipongpiradet et al., 2019)**. However, hormone replacement therapy is not the best choice for all women in menopause. Consequently, extensive research has been conducted to explore alternative approaches that can effectively alleviate the symptoms associated with menopause **(Da Rocha et al., 2021)**.

A naturally occurring compound commonly present in mitochondria, alpha-lipoic acid (ALA), is essential for the functions of several enzymes **(Salehi et al., 2019)**. ALA is synthesized endogenously from fatty acids and cysteine in small amounts. Consequently, obtaining exogenous sources of ALA is critical for achieving positive results **(Mendoza-Núñez et al., 2019)**. ALA is plentiful in a wide range of dietary sources, including animal-derived foods such as bovine kidney, heart, and liver, as well as plant-based options like tomatoes, broccoli, spinach, peas, brussel, rice bran and potatoes **(Triggiani, 2020)**. Structurally, ALA contains two thiol groups capable of oxidation and reduction, making it a redox couple. Both oxidized and reduced forms exhibit antioxidant effects **(Ferreira et al., 2009)**. While ALA has been investigated in different models of depression, the exact underlying mechanism is still not elucidated.

Adipocytokines were found to be effective modulators in cognitive, mood, anxiety and depressive changes. However, the specific mechanism is still unclear **(Fu et al., 2023)**. Apelin, an adipokine with its receptor APJ, plays an important pivotal role in numerous physiological functions and exhibits valuable impacts on glucose metabolism and insulin resistance **(Fernández-Galilea et al., 2011)**. It was found that apelin/APJ system has neuroprotective effects through its ability to promote angiogenesis and its role to suppress inflammatory responses and oxidative stresses. In addition, it has the ability to modulate autophagy and mitigate apoptosis. So, increasing its brain level constitutes an important theraputic target for treatment of many neurological illnesses **(Tian et al., 2023)**.

Research on depression-related MicroRNAs (miRNAs) have garnered much attention nowadays. MiRNAs are a group of short, non-coding RNAs, typically range in length between 17 and 25 nucleotides. These molecules have the ability to bind partially complementary sites in the 3′ untranslated region of their target

messenger RNAs (mRNAs), thereby suppressing their translation or leading to their degradation **(Bartel, 2004)**. It has been propsed that miRNAs engaged in numerous crucial biological processes linked to the nervous system, such as neuronal proliferation, neurogenesis, and synaptic plasticity **(Blandford et al., 2018)**. One member of this family is microRNA 99a (miRNA-99a) that has been found to be significantly lower in the hypothalamus during postmenopausal depression in studies involving animal models **(Yang et al., 2019)**.

Our study aimed to evaluate the potential antidepressant impact of ALA in oophorectomized female rats focusing on its effect on apelin level and miRNA-99a expression.

Materials and methods

Animals and husbandry

Thirty-two healthy adult female albino rats, weighing approximately between 180 and 200 grams and aged 8 weeks, were housed in standard environmental conditions in the animal house of Medical Physiology Department, Alexandria Faculty of Medicine, Egypt. The rats were fed with a standard conventional pellet diet and water. To minimize any potential psychological stress due to their new environment, the rats were given one week to acclimate.

The study protocol has been approved by the Alexandria Faculty of Medicine's Research Ethics Committee (Ethical registration no: 0305632, IRB No: 00012098, FWA No: 00018699). All experimental procedures were conducted following the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines **(Percie et al., 2020)**.

Drugs and reagents

Alpha-lipoic acid and Estradiol were obtained from Sigma-Aldrich Co., St. Louis, MO, USA. (Website: www.sigmaaldrich.com) and prepared as a suspension using gum acacia.

Induction of the model (surgical procedures)

The experimental rats were subjected to surgical procedure either sham operation or oophorectomy. Before surgery, all rats were fasted for the whole night. Aseptic surgical methods were used to perform the animal dissections. The rats were anaesthetized using intraperitoneal injection containing combination of ketamine (50 mg) and xylazine (5 mg/kg). Bilateral ophorectomy was performed following Parhizkar et al procedure **(Parhizkar et al., 2008)**. To reduce any possible surgical complications, the rats were subjected to stabilization period post-operatively. After surgery, the rats were given meloxicam (0.2 mg/kg, subcutaneous) for three days. They stayed beneath a warm mattress during the recovery from anesthesia. The surgical wounds were examined daily during the postoperative period, and rifampicin was used topically for seven days **(Rodríguez-Landa, 2022)**.

Study design and supplementation (Fig.1).

Following surgery, the rats were divided into the following groups: Normal control group (n=8), this group served as negative control group. Animals received 1 ml of gum acacia 2% orally once daily after sham operation throughout the study period (8 weeks). Oophorectomized group (n=8), this group served as positive control group, animals received 1 ml of gum acacia 2% orally once daily after oophorectomy throughout the study period (8 weeks). Estradiol group (n=8), animals received 17-b Estradiol (0.5 mg/kg/day, oral gavage) suspended in 1 ml of gum acacia 2% once daily after oophorectomy for 8 weeks **(El Habachi et al., 2014)**. ALA group (n=8), animals received ALA (200 mg/kg/day, oral gavage) suspended in 1 ml of gum acacia 2% once daily after oophorectomy for 8 weeks **(Yusha'u et al., 2021; Dias et al., 2016).**

Gum acacia 2% was used as a suspending vehicle because it is widely applied in experimental pharmacology for oral delivery of lipid-soluble compounds, providing uniform dispersion and good

palatability for rats. Although gum acacia possesses mild antioxidant properties, it was administered uniformly to all experimental groups, including controls, to eliminate any potential confounding effect attributable to the vehicle itself.

A metallic gastric tube (gavage) was used to administer the treatment orally in a minimum volume of 0.3 ml to ensure delivery of the dose and a maximum volume of 0.8 ml to avoid dosage vomiting. All treatments began the day after surgery and continued for 8 weeks (the end of the study) to allow adequate time for the establishment of post-ovariectomy neurobehavioral and histopathological changes, and for the evaluation of ALA's long-term protective effects **(El Habachi et al., 2014; Yusha'u et al., 2021).** For all experimental rats, body weight was measured before the experiment and then weekly during the experimental period to give the proper dose for each animal based on their weights and to study the impact of oophorectomy and treatment on the animals' body weights **(Yusha'u et al., 2021)**.

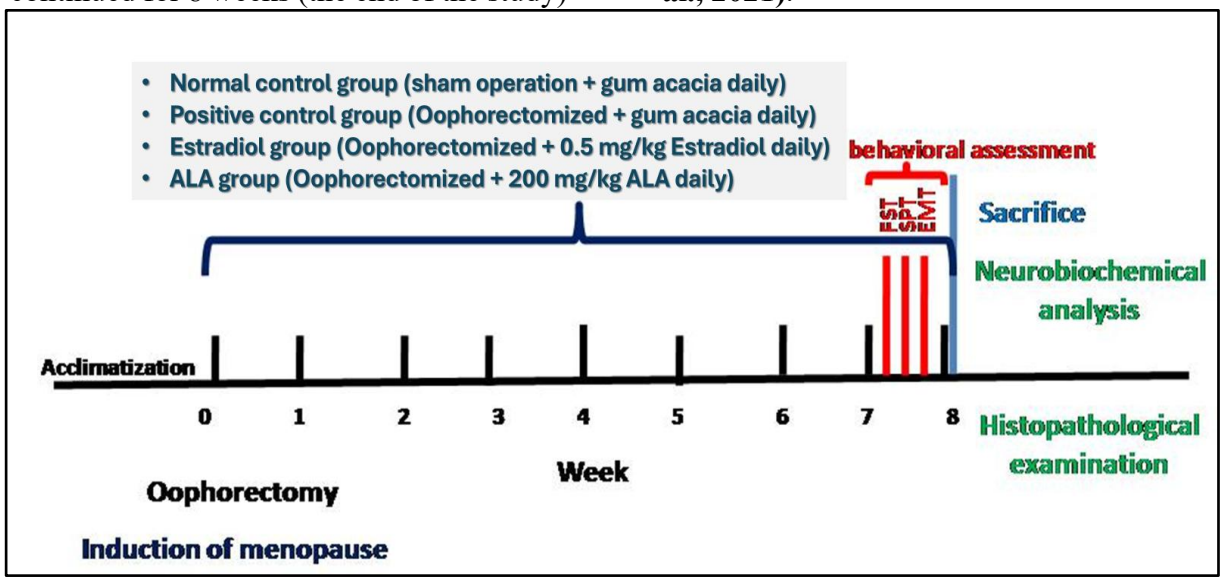

Fig. 1. Flow chart of the experimental design of the study

Menopause was induced by oophorectomy; rats were divided into four groups (n=8 each); they were treated for eight weeks. Following, rats were subjected to neurobehavioral assessment in the last week of the study, and then they were sacrificed for biochemical and histopathological assessment of the brain.

ALA: Alpha lipoic acid ; **FST:** Forced Swim Test ; **SPT:** Sucrose Preference Test ; **EMT:** Elevated Maze Test

Behavioral tests

Behavioral assessments were conducted in a dedicated room spanning from 8:00 am to 1:00 pm by a skilled investigator blinded to the experimental groups in the last week of our study. Before the behavioral tests, rats were placed in the testing room for an hour for habituation. The behavioral tests were performed in the following order: Forced swim test (days 49 and 50), Sucrose preference test (days 51,52 and 53), and Elevated maze test (days 54 and 55) **(Zvozilova et al., 2024).**

1. **Forced Swim Test (FST)**:

Rats were individually placed in a water-filled container at 24±1°C for two consecutive days. On the first day, animals were allowed to swim for 15 minutes (training session). On the following day (test day), rats were tested for 5 minutes, during which the behavior of each rat was recorded as follows: immobility time (the period of rats exhibiting no active movements, only minimal movements needed to keep their head above water surface); climbing time (the duration of forceful forepaw movements against the walls of the container); and swimming time (which corresponds to active forepaw

movements that propel the body around the container, exceeding the minimal effort needed to maintain the head above surface of the water). The water was replaced after each trial to maintain temperature and cleanliness **(Slattery and Cryan, 2012).**

2. Sucrose Preference Test (SPT):

The test was conducted in the rats' home cages over three consecutive days to assess anhedonia in rodents, which is a hallmark of depression symptoms. Two bottles were placed in each rat cage; one containing 250 ml of sucrose solution (1–2% w/v) and the other containing plain drinking water. The positions of the bottles were alternated daily to prevent side preference. The intake of sucrose solution and water was recorded daily, and sucrose preference (%) was calculated using the equation (Sucrose intake×100 / Total fluid intake) **(Kandratavicius et al., 2015).**

3. Elevated Maze Test (EMT):

The test was performed to assess anxiety through passive observation. The maze consisted of two open arms (51 cm × 10 cm) and two closed arms (51 cm × 10 cm × 41 cm) extending from a central platform (10 cm × 10 cm) elevated 55 cm above the ground. Each rat was placed at the center of the maze facing an open arm, and its behavior was observed for 5 minutes, during which the number of entries and the time spent in open and closed arms were measured **(Pellow et al., 1985).**

Study termination and sample preparation

After completing the behavioral assessments, the rats were anaesthetized, decapitated and the brain tissue was immediately excised. Half of the brain was allocated for biochemical analysis, while the remaining half was prepared for histopathological examination. For biochemical analysis, the hippocampus and prefrontal cortex were swiftly dissected and residual blood was removed by washing with pre-cooling 0.01M PBS buffer (pH =7.4). The dissected parts of the brains were stored at -80°C until they were subjected to homogenization in the suitable buffer. The frozen brain tissues were homogenized in cold PBS containing protease inhibitor, in a ratio of 100 mg tissue/ml. The samples were centrifuged for 15 minutes at 12000 xg and the supernatant fluid was collected in aliquots **(Yang et al., 2017).** For histological examination, the specimens of hippocampal and prefrontal cortex were preserved in 10% buffered formol-saline and subsequently processed to get paraffin blocks. These blocks were sectioned to a thickness of 3-5 mm to get coronal sections. Then, Hematoxylin and Eosin (H&E) dyes were used to stain the sections for micooscopic examination under a light microscope **(Bancroft and Gamble, 2008).**

Biochemical assessment

1. Determination of Neurotransmitters; dopamine and serotonin levels in brain tissue:

Commercially available rat ELISA kits from CUSABIO TECHNOLOGY LLC, Houston, USA. (Website: www.cusabio.com) were used to measure the levels of dopamine (Catalog No. E08660r) and serotonin (Catalog No. E08364r) in the brain tissue homogenates following the instructions of the manufacturer. The samples were tested twice on a microplate at 450 nm. Following the normalization of the ELISA findings to the total proteins of brain tissue, which were determined using Lowry's technique **(Lowry et al., 1951)**, the protein levels of dopamine and serotonin were expressed in ng/mg tissue protein.

2.Measurement of Inflammatory Markers; Interleukin 6 (IL-6) and Nuclear factor-kappa B (NF-kB) in brain tissue:

Quantitative measurements of IL-6 and NF-kb in the brain tissue homogenates were performed using commercially available rat ELISA kits following the manufacturer's instructions. Rat IL-6 ELISA Kit was obtained from CUSABIO TECHNOLOGY LLC, Houston, USA. (Catalog NO. E04640r), and rat NF-kB ELISA kit (Catalog No. MBS453975) from MyBioSource, San Diego, California, USA.

(Website: www.MyBioSource.com). The samples were tested twice and the absorbance was read at 450 nm. The levels of IL-6 and NF-kB were expressed in pg/mg and ng/mg tissue protein respectively.

3. Evaluation of Oxidative Stress Markers:

The catalase (CAT) and glutathione perioxidase (GPx) activities in the brain tissue were measured colorimetrically according to the assay kit protocol (Catalog No. CA2517 and GP2524 respectively) from Biodiagnostics Co., Giza, Egypt (Website: www.bio-diagnostic.com).

Catalase assay relied on its reaction with a known amount of hydrogen peroxide (H_2O_2). Catalase inhibitor was used to stop the reaction after one minute. In the presence of peroxidase, 4-aminophenazone (AAP) and 3,5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) reacted with the remaining H_2O_2 to form a chromophore whose color intensity was inversely proportional to the quantity of catalase in the sample. The absorbance of the reaction solution was read at 510 nm **(Aebi, 1984)**.

The activity of GPx was measured indirectly by monitoring the conversion of NADPH to $NADP^+$. Tissue homogenate was added to a solution containing NADPH, glutathione and glutathione reductase. The reaction starts by adding H_2O_2 which is reduced by GPx using reduced glutathione (GSH), producing oxidized glutathione (GSSG). Then, GSH is regenerated from GSSG by glutathione reductase, consuming NADPH. The GPx activity in the sample is directly proportional to the rate at which absorbance decreases at 340 nm **(Paglia and Valentine, 1967)**.

Both CAT and GPx results were expressed as U/g tissue after being adjusted to tissue weight.

4.Enzyme-linked immunoassay (ELISA) of nuclear factor erythroid 2-related factor2 (NrF2) and apelin in brain tissue:

Using commercially available rat ELISA kits, the levels of NrF2 (Catalog No. F33630, Life Span BioSciences Inc., Newark, USA. Website: www.lsbio.com) and apelin (Catalog No. ER0651, Fine Test, Wuhan, China. Website: www.fn-test.com) were measured in brain tissue homogenates. The samples were tested twice on a microplate at 450 nm, and the levels of NrF2 and apelin were expressed in pg/mg tissue protein.

5.Relative expression of miRNA-99a in brain tissue by Quantitative Real-time Polymerase Chain Reaction (RT-qPCR):

RNAlater™ Stabilization Solution (Catalog NO. AM7023) was used to preserve parts of the obtained brain tissues in -80 ºC to avoid RNA breakdown.

Total RNA extraction from the brain tissue samples was carried out using Qiagen® miRNeasy Mini kit (Catalog No. 217004, Qiagen, Germany. Website: www.qiagen.com) that combines phenol/guanidine-based sample lysis with silica membrane-based total RNA purification.

The NanoDrop 2000/2000c Spectrophotometer (from Thermo Scientific, USA) was used to measure the concentration and purity of RNA at 260, 280, and 230 nm. A260/A280 and A260/A230 ratios of 1.8–2 and 2–2.2 respectively indicated highly pure RNA **(Sriram et al., 2021)**. The RNA extract was then stored at -80°C until use.

Complementary DNA (cDNA) was synthesized by the use of miRCURY locked nucleic acid (miRCURY LNA) miRNA PCR Starter Kit from Qiagen, Germany (Catalog No. 339320). The reaction volume was 10 µl (2 µl 5x miRCURYSYBR Green RT Reaction Buffer, 1 µl 10x miRCURY RT Enzyme Mix, 5µl RNase-free water and 2 µL of each RNA "10 ng/ µl" sample). Then, thermocycling was carried out as follows: reverse transcription step at 42°C for 60 min, inactivation step at 95°C for 5 min, then cooling at 4°C for 5 min.

Relative expression of miRNA-99a was determined by quantitative real-time PCR using miRCURY LNA miRNA PCR Starter Kit from Qiagen, Germany (Catalog No.

339320), including 2 LNA PCR assays of primers for miR-99a (YP00205149) as well as U6 (YP00205149) as an endogenous control (reference gene). Two separate PCR reactions were carried out for each sample of cDNA using miR-99a gene primers in the first reaction and U6 gene primers in the second reaction, each reaction contained 5 µl 2x miRCURY SYBR® Green master mix, 0.5 µl of ROX reference dye, 1 µl gene primer, 2.5 µl nuclease-free water and 1 µl cDNA (20 ng/µl). Then, the Real-time cycler conditions for Applied Biosystems were carried out as follows: initial heat activation at 95°C for 2 min, then 40 cycles of denaturation at 95°C for 10 seconds, and combined annealing/extension at 56°C for 60 seconds. In the end, a melting curve analysis was done at 60–95°C. Negative controls were included in all runs to evaluate the back ground signal and avoid misinterpretation. Using the comparative cycle threshold (CT) formula ($2^{-\Delta\Delta CT}$), relative expression of miRNA-99a was calculated and expressed as fold change **(Rao et al., 2013)**.

Statistical analysis (Kotz et al.,2006).

Before analysis, the Shapiro-Wilk test was used to evaluate data distribution and verify normality. The mean, standard deviation (SD), and range were used to summarize the quantitative data. Statistical significance was established at the 5% level. The F-test (ANOVA) was used for comparing more than two groups, and Tukey's post hoc test was utilized for pairwise comparisons. When quantitative variables did not meet the normality assumption, group comparisons were done using the Kruskal-Wallis test, with Dunn's multiple comparisons test applied for pairwise analyses. Additionally, the Pearson correlation coefficient was calculated to evaluate relationships between two quantitative variables that are normally distributed. Data analysis was performed by version 20.0 of IBM SPSS Statistics software (Armonk, NY: IBM Corp).

Ethical approval code

The study protocol has been approved by the Alexandria Faculty of Medicine's Research Ethics Committee (Ethical registration no: 0305632, IRB No: 00012098, FWA No: 00018699). All experimental procedures were conducted following the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Results

Behavioral tests results

1. Forced swim test:

In FST, the amount of time spent immbile was considered as a key measure of the depression. The oophorectomized rats exhibited a significant notable rise in this time (52.12±11.91) when compared to the sham operated rats. However, both estradiol and ALA treatments were successful in reducing the immobility time (31.50±8.64) and (32.12±8.46) respectively, with no significant difference observed between them **(Table.1)**.

2. Sucrose preference test:

In SPT, a decrease in sucrose preference % indicates reduced interest in pleasurable activities, one of the prominent symptoms of depression. A statistically significant diminish in sucrose preference % was seen in the oophorectomized group compared to the normal control group. Treated groups with estradiol and ALA revealed a statistically significant increase in the percentage of sucrose preference when compared to the oophorectomized rats. However, there was no significant disparity between the two treatments concerning this test **(Table.1)**.

3. Elevated maze test:

In EMT, the duration of the time allocated by the rats in the closed arms is a primary indicator used to assess depressive like behavior. The oophorectomized group exhibited a statistically significant decline in the time spent in open areas, coupled with a significant rise in time spent in closed areas, versus the normal control group. Both estradiol and ALA treated groups displayed statistically significant increased time spent in open areas and decreased time spent in

closed areas compared to the oophorectomized group. However, no significant difference was present between the two treatment groups concerning this test **(Table 1)**.

Table 1. Effect of 8 weeks treatment with estradiol & ALA on different behavioral tests in oophorectomized rats

Variables	Normal control group	Oophorectomized group	Estradiol group	ALA group	Test of Sig.
1- Forced swimming test (FST) (Mean ± SD)					
a) Swimming time (seconds)	200.0 ± 15.91	178.6[a] ± 11.38	193.4 ± 8.19	194.6[b] ±9.93	F= 4.887* P=0.007*
b) Climbing time (seconds)	70.13 ± 13.18	69.25 ± 8.88	75.13 ± 4.88	73.25 ± 6.30	F= 0.756 P=0.528
c) Immobility time (seconds)	29.88 ± 5.77	52.12[a] ± 11.91	31.50[b] ± 8.64	32.12[b] ±8.46	F= 11.029* P<0.001*
2. Sucrose preference (SPT) (Mean ± SD)					
(%)	75.38 ± 10.74	44.38[a] ± 14.68	72.38[b] ± 7.17	64.88[b] ±8.97	F= 13.492* P<0.001*
3.Elevated maze test (EMT) (Mean ± SD)					
a) Number of entries					
Open arm	2.63 ± 0.52	0.38[a] ± 0.52	1.63[ab] ± 0.52	1.25[a] ± 0.46	H=22.892* P<0.001*
Closed arm	1.25 ± 0.46	2.38[a] ± 0.52	1.38[b] ± 0.52	1.63[b] ± 0.52	H=13.454* P=0.004*
b) Time spent (seconds)					
Open areas	98.50 ± 23.22	20.0[a] ± 10.35	77.50[b] ± 16.69	71.88[ab] ± 20.86	F=26.157* P<0.001*
Closed areas	208.5 ± 24.23	276.9[a] ± 10.67	220.0[b]±18.52	232.5[b] ± 23.60	F=17.895* P<0.001*

8 replicas in each group **SD:** Standard deviation

F: **F for One way ANOVA test**, Pairwise comparison between each two groups used **Post Hoc Test (Tukey)**

H: H forKruskal Wallis test, Pairwise comparison between each two groups used **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p value for comparison between the studied groups

***:** Statistically significant at ($p \leq 0.05$)

a: Significant vs. normal control group
b: Significant vs. oophorectomized group
c: Significant vs. estradiol group

Neurobiochemical analysis results

1. Impact of estradiol and lipoic acid on the dopamine and serotonin concentration in the brain: (Table 2)

Both dopamine and serotonin are critical neurotransmitters that are essential for modulation of mood, emotions, and cognitive functions. Interestly, the levels of dopamine and serotonin exhibited a statistically significant decrease in oophorectomized rats compared to the normal control group ($p<0.001$). The estradiol and lipoic acid treated groups demonstrated a statistically significant elevation in dopamine and serotonin concentrations in comparison to the oophorectomized group ($p<0.001$) **(Fig 2a)**. Furthermore, dopamine and serotonin displayed negative correlations with immobility time in FST ($r= -0.641$, $p <0.001$ and $r= -0.553$, $p=0.001$, respectively) **(Fig 2b, 2c)**, and time allocated in the closed arm in the EMT ($r= -0.723$, $p<0.001$ and $r= -0.715$, $p<0.001$, respectively) **(Fig 2d, 2e)**. Moreover, dopamine and serotonin exhibited positive correlations with the sucrose preference % in SPT ($r= 0.621$, $p<0.001$, and $r= 0.657$, $p<0.001$, respectively) **(Fig 2f, 2g)**.

2.Effect of estradiol and lipoic acid on inflammatory markers (IL-6 and NF-kB): (Table 2)

The menopause transition prompts an innate inflammatory response. The oophorectomized rats exhibited a significant elevation in the concentrations of IL-6 and NF-kB, when compared to the normal control rats. Both estradiol and lipoic acid had a significant positive effects in reducing inflammation, as showed by the significant decrease in IL-6 and NF-kB concentrations with both treatments in comparison to the oophorectomized group **(Fig 3a)**.

3. Effect of estradiol and lipoic acid on inflammatory markers (IL-6 and NF-kB): (Table 2)

The menopause transition prompts an innate inflammatory response. The oophorectomized rats exhibited a significant elevation in the concentrations of IL-6 and NF-kB, when compared to the normal control rats. Both estradiol and lipoic acid had a significant positive effects in reducing inflammation, as showed by the significant decrease in IL-6 and NF-kB concentrations with both treatments in comparison to the oophorectomized group **(Fig 3a)**.

4. Effect of estradiol and lipoic acid on oxidative stress markers (CAT and GPx): (Table 2)

The insufficiency of estrogen, which is an established antioxidant in the body, results in oxidative stress in different tissues. The oophorectomized rats showed a significant decrease in the activity of CAT and GPx in comparison to the normal control rats. Both estradiol and lipoic acid treated groups showed a statistically significant elevation in the CAT and GPx activity when set side to side to the oophorectomized group. The antioxidant effects of ALA is more evident than estradiol as discernible from the significant variance between the two groups **(Fig.3b)**.

5. Effect of estradiol and lipoic acid on NrF2 and apelin levels: (Table 2)

A statistically significant decline was observed in the NrF2 levels (68.75±6.63 pg/mg) in oophrectomized rats in comparison to the normal control rats (211.9±8.41 pg/mg). Both estradiol and ALA treated groups revealed a statistically significant elevation in the NrF2 levels as compared to the oophorectomized group (121.5±6.55 pg/mg, 172.5±9.24 pg/mg respectively). The ALA was more significant inducer of NrF2 than estradiol in oophorectomized rats ($p<0.001$) **(Fig 4a)**.

Noteably, NrF2 showed a negative correlation with IL-6 and NF-KB ($r= -$

0.889, p<0.001 and r= -0.712, p<0.001 respectively) **(Fig 4b, 4c)**. Moreover, a positive correlation was found between NrF2 and CAT as well as GPx activity (r=0.907, p<0.001 and r=0.941, p<0.001, respectively) **(Fig 4d, 4e)**.

Table 2. Effect of 8 weeks treatment with estradiol & ALA on different biochemical and molecular markers in oophorectomized rats

Variables	Normal group	Oophorectomized group	Estradiol group	ALA group	Test of Sig.
Dopamine (ng/mg)					
Min. – Max.	17.0 – 23.0	9.0 – 12.0	17.0 – 20.0	12.0 – 17.0	F=48.509*
Mean ± SD.	20.25 ± 2.49	10.50[a] ± 1.20	18.50[b] ± 1.20	15.0[abc] ± 1.77	P<0.001*
Serotonin (ng/mg)					
Min. – Max.	22.0 – 28.0	8.0 – 11.0	17.0 – 21.0	13.0 – 17.0	F=109.214*
Mean ± SD.	24.88 ± 2.53	9.75[a] ± 1.16	19.63[ab] ± 1.51	15.13[abc] ± 1.46	P<0.001*
NrF2 (pg/mg)					
Min. – Max.	200.0 – 222.0	79.0 – 95.0	110.0 – 130.0	159.0 – 185.0	F=401.090*
Mean ± SD.	211.9 ± 8.41	86.75[a] ± 6.63	121.5[ab] ± 6.55	172.5[abc] ± 9.24	P<0.001*
Apelin (pg/mg)					
Min. – Max.	320.0 – 360.0	118.0 – 198.0	185.0 – 230.0	225.0 – 265.0	F=143.058*
Mean ± SD.	337.5 ± 11.74	172.9[a] ± 25.09	204.8[ab] ± 15.28	242.1[abc] ± 11.70	P<0.001*
IL6 (pg/mg)					
Min. – Max.	38.0 – 46.0	68.0 – 80.0	52.0 – 65.0	39.0 – 54.0	F=74.270*
Mean ± SD.	42.0 ± 3.07	72.63[a] ± 4.72	57.25[ab] ± 4.89	45.0[bc] ± 5.29	P<0.001*
NF-kB (ng/mg)					
Min. – Max.	2.90 – 6.30	12.46 – 14.70	4.30 – 6.80	5.50 – 8.60	F=122.935*
Mean ± SD.	4.56 ± 1.31	13.58[a] ± 0.73	5.38[b] ± 0.92	7.70[abc] ± 1.11	P<0.001*
CAT (U/g)					
Min. – Max.	5.40 – 6.90	1.80 – 3.10	2.90 – 4.30	5.10 – 6.90	F=68.096*
Mean ± SD.	5.94 ± 0.57	2.35[a] ± 0.42	3.56[ab] ± 0.50	5.80[bc] ± 0.83	P<0.001*
GPx (U/g)					
Min. – Max.	39.0 – 55.0	13.0 – 22.0	21.0 – 26.0	27.0 – 40.0	F=73.607*
Mean ± SD.	46.88 ± 6.03	17.0[a] ± 3.55	23.13[ab] ± 2.10	32.88[abc] ± 4.52	P<0.001*
miRNA-99a (Fold change)					
Min. – Max.	0.87 – 1.20	0.13 – 0.31	0.41 – 0.59	0.61 – 0.74	F=142.662*
Mean ± SD.	1.02 ± 0.11	0.25[a] ± 0.06	0.50[ab] ± 0.07	0.68[abc] ± 0.04	P<0.001*

8 replicas in each group **SD:** Standard deviation

F: **F for One way ANOVA test**, Pairwise comparison between each two groups used **Post Hoc Test (Tukey)**

p: p value for comparison between the studied groups

***:** Statistically significant at ($p \leq 0.05$)

a: Significant vs. normal control group

b: Significant vs. oophorectomized group

c: Significant vs. estradiol group

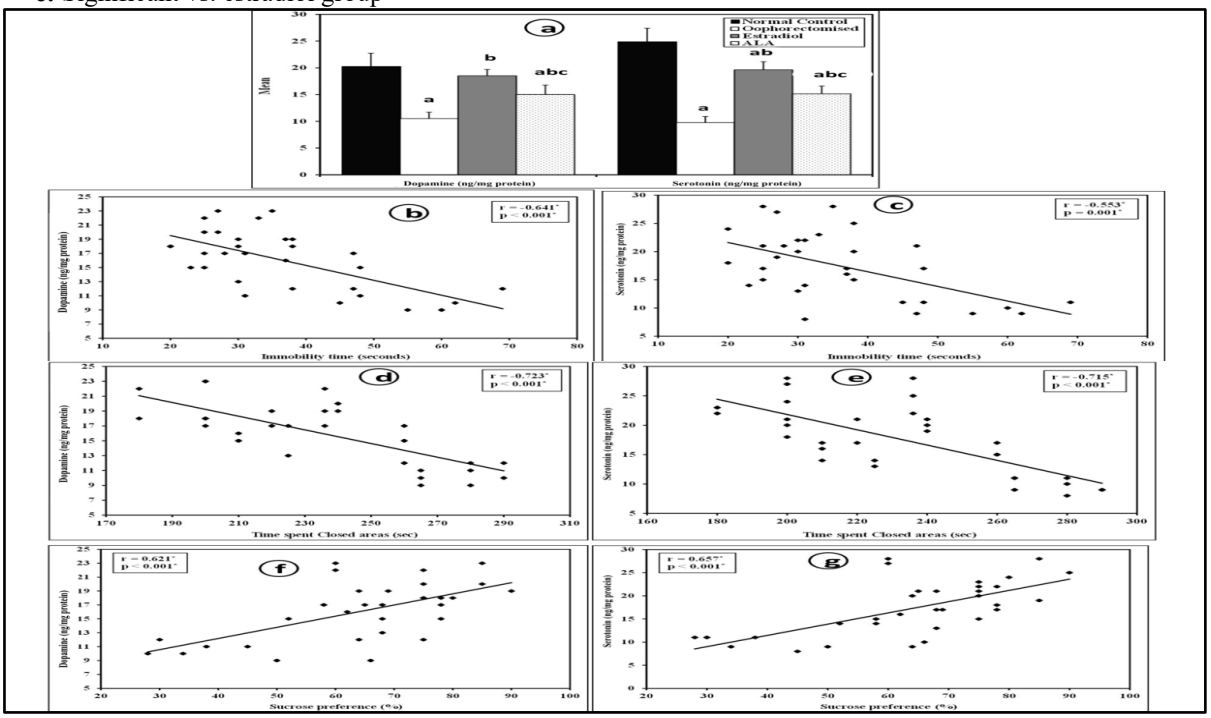

Fig 2. Effect of 8 weeks treatment with estradiol and ALA on brain neurotransmitters (dopamine and serotonin) and their correlations with behavioral assessments

a) Mean levels of dopamine and serotonin (ng/mg protein) in the different studied groups. **Results are represented as mean±SD** ; **Statistically significant at ($p \leq 0.05$)**
a: Significant vs. normal control group, **b:** Significant vs. oophorectomized group, **c:** Significant vs.
estradiol group.

b) Significant negative correlation between dopamine level and immobility time in FST (r= -0.641, p<0.001)

c) Significant negative correlation between serotonin level and immobility time in FST (r= -0.553, p=0.001)

d) Significant negative correlation between dopamine level and time spent in closed areas in EMT (r= -0.723, p<0.001)

e) Significant negative correlation between serotonin level and time spent in closed areas in EMT (r= -0.715, p<0.001)

f) Significant positive correlation between dopamine level and sucrose preference % in SPT (r=0.621, p<0.001)

g) Significant positive correlation between serotonin level and sucrose preference % in SPT (r=0.675, p<0.001)

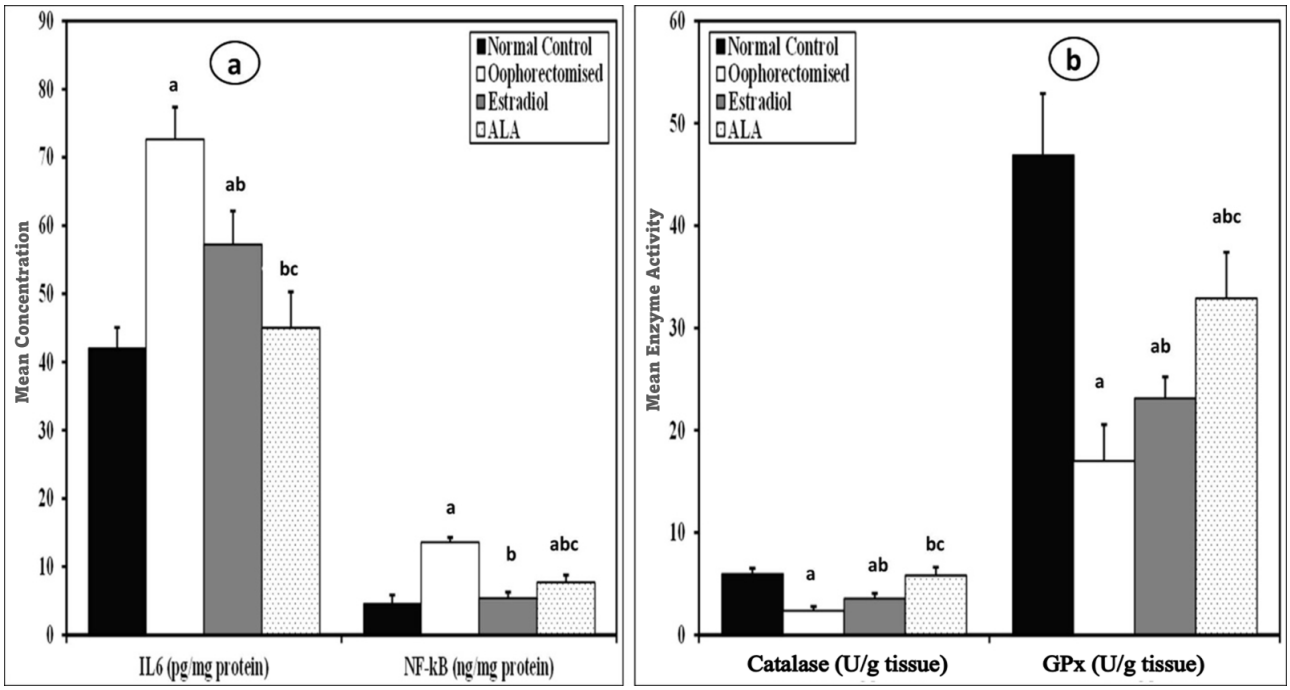

Fig 3. Effects of estradiol and alpha-lipoic acid (ALA) treatment on inflammatory and oxidative stress markers in the different studied groups.

a) Mean levels of IL6 (pg/ mg protein) and NF-kB (ng/mg protein).
b) Mean activities of catalase "CAT" and glutathione peroxidase "GPx" (U/g tissue).

Results are represented as mean±SD ; Statistically significant at ($p \leq 0.05$)

a: Significant vs. normal control group, **b:** Significant vs. oophorectomized group, **c:** Significant vs. estradiol group.

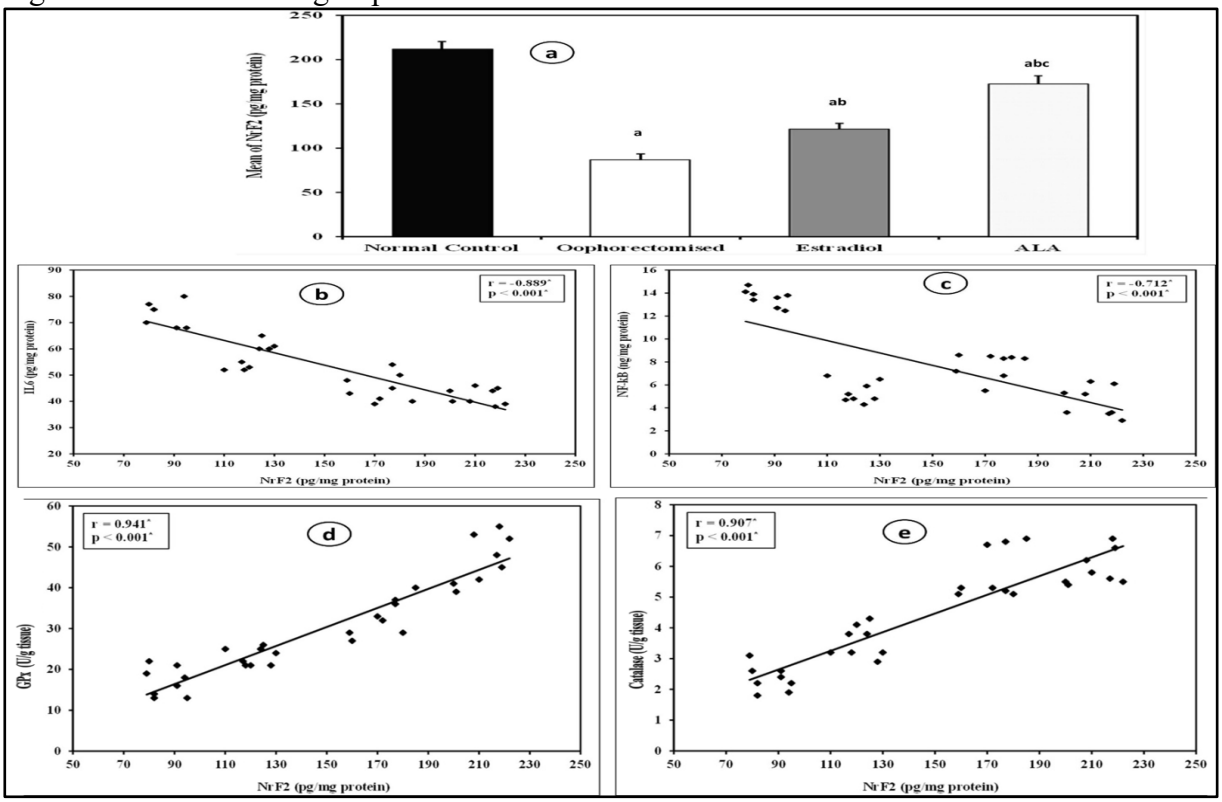

Fig. 4. Effects of estradiol and alpha-lipoic acid (ALA) treatment on NrF2 level and its correlation with inflammatory and oxidative stress markers.

a) Mean level of NrF2 (pg/mg) in the different studied groups.

Results are represented as mean±SD ; **Statistically significant at ($p \leq 0.05$)**

a: Significant vs. normal control group, **b:** Significant vs. oophorectomized group, **c:** Significant vs. estradiol group.

b) Significant negative correlation between NrF2 and IL6 levels ($r= -0.889$, $p<0.001$).

c) Significant negative correlation between NrF2 and NF-kB levels ($r= -0.712$, $p<0.001$).

d) Significant positive correlation between NrF2 and GPX levels ($r=0.941$, $p<0.001$).

e) Significant positive correlation between NrF2 and CAT levels ($r=0.907$, $p<0.001$).

There was a statistically significant decrease in the levels of apelin (172.9±25.09 pg/mg) in rats underwent oopphrectomy versus sham operated rats (337.5±11.74 pg/mg). Both estradiol and lipoic acid treated groups revealed a statistically significant increase in the apelin levels as compared to the oophrectomized group (204.8±15.28 pg/mg, 242.1±11.70 pg/mg respectively). The ALA showed more significant induction of apelin than estradiol in oophorectomized rats ($p<0.001$) **(Fig 5a)**.

To evaluate the role of apelin in oophorectomy induced depression, we assessed the correlations between brain levels of apelin and various parameters in all rats participating in the experiments **(Table 3)**. Regarding behavioral tests, apelin levels were negatively correlated with the immobility time in FST and the time spent in closed areas in EMT and positively correlated with the sucrose preference % in SPT. For biochemical parameters, apelin levels showed positive correlations with the levels of dopamine, serotonin, NrF2, CAT and Gpx and miRNA-99a expression. In addition, apelin was negatively correlated with IL-6 and NF-kB levels. All these correlations were statistically significant ($p<0.001$).

1. **Impacts of estradiol and lipoic acid on miRNA-99a expression: (Table.2)**

The expression of miRNA-99a demonstrated a statistically significant decrease in oophorectomized rats (0.25±0.06 fold change) compared to sham operated rats (1.02±0.11 fold change). The estradiol and ALA treated groups both revealed a statistically significant enhancement in miRNA-99a expression versus the oophorectomized group (0.50±0.07 and 0.68±0.04 fold change respectively). Notably, ALA exhibited a more pronounced induction of miRNA-99a than estradiol in oophorectomized rats ($p<0.001$) **(Fig.5b)**.

To elucidate the role of miRNA-99a in depression induced by oophorectomy, correlations were conducted between brain levels of miRNA-99a and various parameters in all rats participating in the experiments **(Table 3)**. As regards behavioral test, there were negative correlations between miRNA-99a with immobility time in FST and time spent in closed areas in EMT and a positive correlation between miRNA-99a and sucrose preference % in SPT. Regarding the biochemical parameters, miRNA-99a was positively correlated with dopamine, serotonin, NrF2, apelin **(Fig 5c)**, CAT and Gpx levels. In addition, miRNA-99a was negatively correlated with IL-6 and NF-kB levels. All these correlations were statistically significant ($p<0.001$).

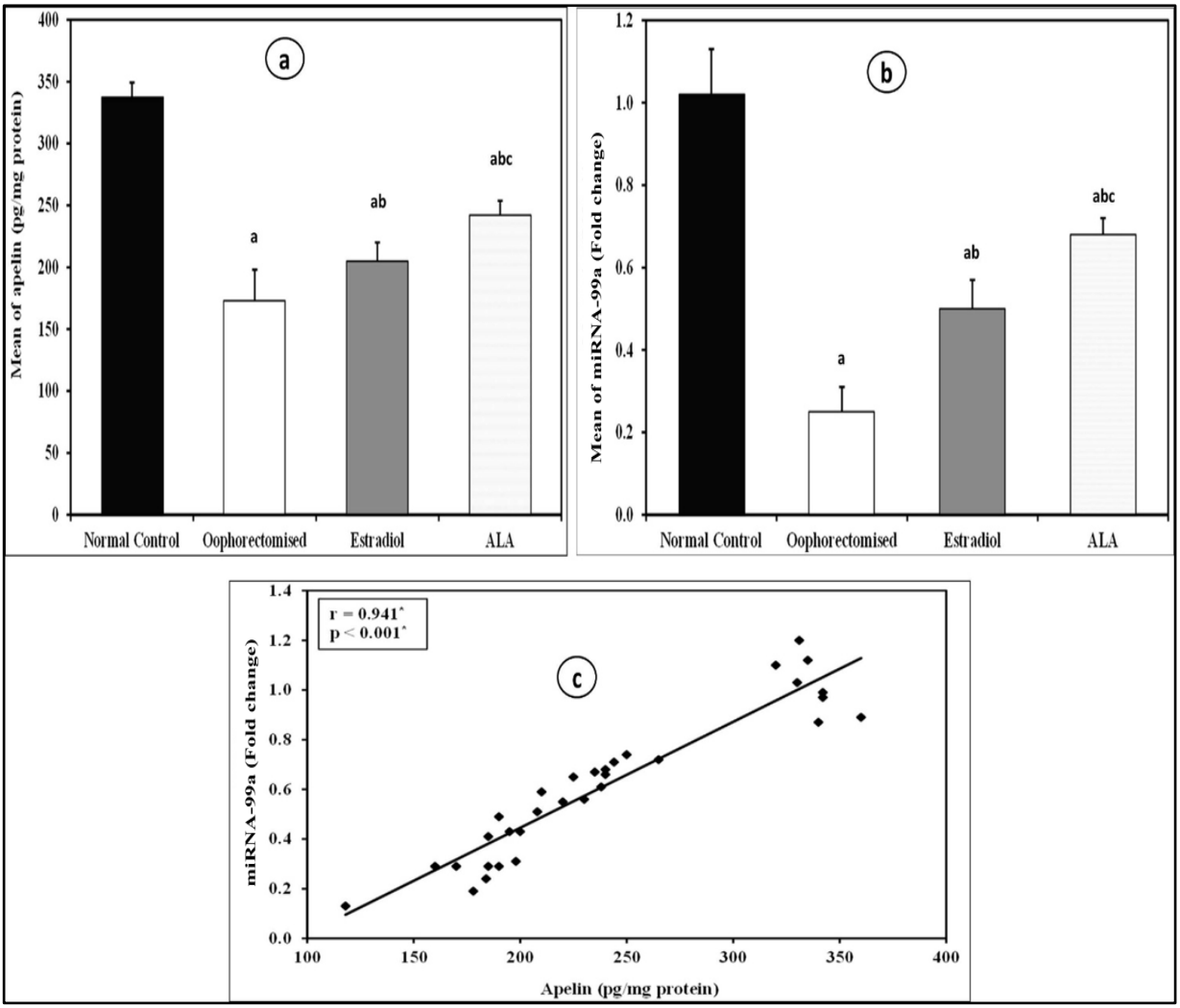

Fig. 5. Effects of estradiol and alpha-lipoic acid (ALA) treatment on levels of apelin and miRNA-99a expression & Correlation between apelin and miRNA-99a expression.

a) Mean level of apelin (pg/mg) in the different studied groups.

b) Mean relative expression of miRNA-99a (fold change) in the different studied groups.

Results are represented as mean±SD ; Statistically significant at ($p \leq 0.05$)

a: Significant vs. normal control group, **b:** Significant vs. oophorectomized group, **c:** Significant vs. estradiol

group.

c) Significant positive correlation between apelin and miRNA-99a expression ($r=0.941$, $p<0.001$).

Table 3. Correlation analysis of brain Apelin and miRNA-99a levels with different parameters in the study group

Parameter	Apelin (pg/mg)		MiRNA-99a (Fold change)	
	r value	p value	r value	p value
Immobility time (sec)	-0.514*	0.003*	-0.564*	0.001*
Sucrose preference (%)	0.550*	0.001*	0.652*	<0.001*
Time spent Closed areas (sec)	-0.561*	0.001*	-0.585*	<0.001*
Dopamine (ng/mg)	0.696*	<0.001*	0.673*	<0.001*
Serotonin (ng/mg)	0.786*	<0.001*	0.793*	<0.001*
NrF2 (pg/mg)	0.929*	<0.001*	0.953*	<0.001*
IL-6 (pg/mg)	-0.755*	<0.001*	-0.819*	<0.001*
NF-kB (ng/mg)	-0.668*	<0.001*	-0.735*	<0.001*
CAT (U/g)	0.770*	<0.001*	0.828*	<0.001*
GPx (U/g)	0.905*	<0.001*	0.904*	<0.001*
MiRNA-99a (Fold change)	0.941*	<0.001*		

r: Pearson coefficient

*: Statistically significant at ($p \leq 0.005$)

Histopathological examination

1. Hippocampus observations

In the normal control group, the three layers: molecular (ML), granule (GL), and polymorphic (PL) were displayed normally. The granular layer consisted of densely packed, small granule cells with their nuclei rounded and vesicular. Also, in both ML and PL, glial cells are numerous, along with pyramidal cells with normal structural integrity. Several indications of hippocampal tissue degeneration were observed in the oophorectomized group. All hippocampal layers exhibited neuronal cytoplasmic rarefaction and spongiform change, along with disrupted cellular architecture, which may impair the modulation of neurotransmitters involved in cognitive processes. Moreover, gliosis was also noticeable. Treatment with estradiol significantly enhanced hippocampal structure, resulting in well-preserved layers with no signs of neuronal degeneration or structural disintegration. There was a noticeable dilation and congestion of blood vessels in the polymorphic layer. Additionally, ALA exhibits the most typical outcomes, as seen by the granular layer's thickness. The distribution of small blood vessels matched that of the control group **(Fig 6)**.

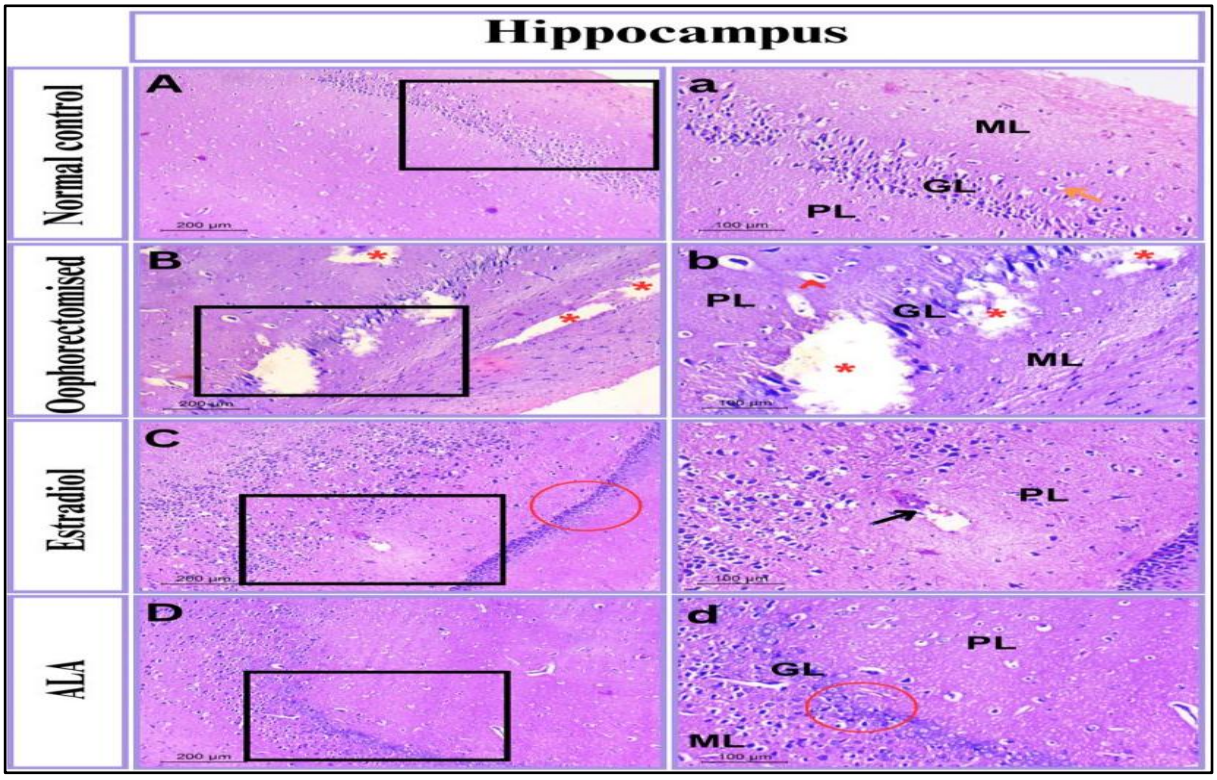

Fig. 6. Representative Hematoxylin and Eosin (H&E) stained sections of the hippocampus from female rat brains.

The left panels (magnification ×100) show the overall hippocampal architecture, and the right panels (magnification ×200) represent higher-magnification views of the insets.

Pointing tools: arrows, arrowheads, circles, and asterisks are used to highlight histopathological features as described below:

(A-a): Normal control group showing normal layers: granular layer (GL), polymorphic (PL) and molecular (ML), and glial cells (orange arrow).

(B-b): Oophorectomized group showing tissue degeneration (red asterisks) at all hippocampal layers (GL, PL, ML), and gliosis (red head arrow).

(C-c): Estradiol treated group showing a condensed granular layer (red circle), normal polymorphic layer (PL), and congested dilated blood vessel (black arrow).

(D-d): ALA-treated group showing normal hippocampal architecture with intact GL, PL, and ML layers, in addition to the granular layer thickness illustrated by the red circle.

2. Prefrontal cortex observations

The prefrontal cortex neurons in the normal control group appear to have normal nuclei and receive blood via numerous blood vessels, reflecting the typical histological organization of this brain region. In the oophorectomized group, several cerebral infarcts and degenerated areas with clogged blood vessels were observed. Both estradiole and ALA treated groups showed marked improvement and the disappearance of these degenerative changes. While the estradiol-treated group displayed vascular dilatation with partially obstructed vessels, ALA treatment produced more significant restoration of normal histoarchitecture **(Fig 7)**.

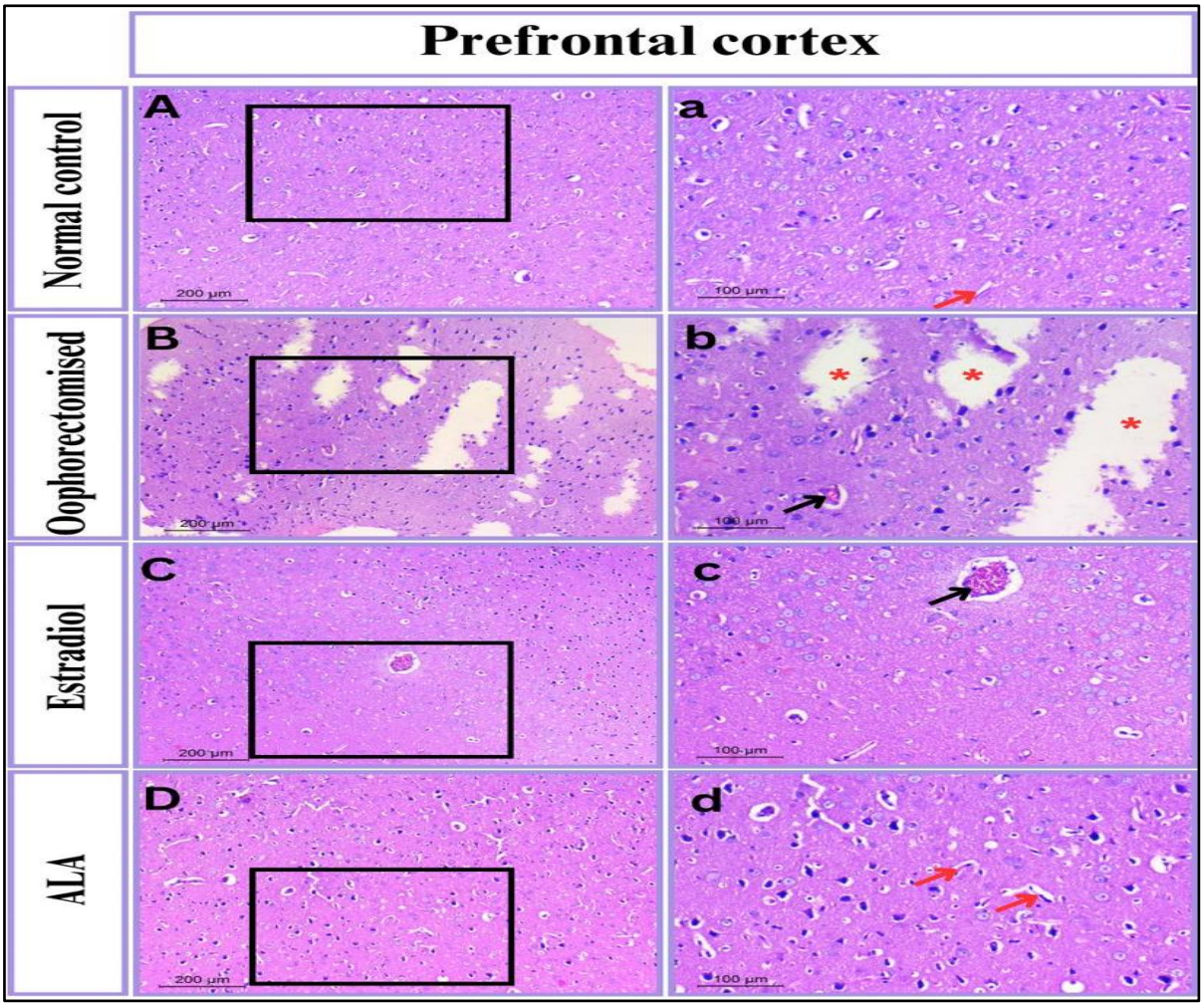

Fig. 7. Representative Hematoxylin and Eosin (H&E) stained sections of the prefrontal cortex from female rat brains.

The left panels (magnification ×100) show the overall cortical architecture, and the right panels (magnification ×200) represent higher-magnification views of the insets.

Pointing tools: arrows and asterisks are used to highlight key histopathological features as described below:

(A-a): Normal control group showing typical cortical histology with intact neurons and normal blood vessels (red arrow).

(B-b): Oophorectomized group showing cerebral infracts and neuronal degeneration (red asterisks) and congested dilated blood vessel (black arrow).

(C-c): Estradiol treated group showing restored cortical structure with marked vascular dilatation (black arrow).

(D-d): ALA treated group showing normal cortical architecture and well-defined vascularization (red arrows)

Discussion

It is well known that depression and mood changes are of the tragic complications in menopausal women. The present study builds upon prior research, which suggested that lipoic acid may possess neuroprotective properties capable of ameliorating or mitigating symptoms associated with depression **(Dias et al., 2016)**. The primary aim of this study is to investigate the potential benefits of alpha lipoic acid over hormonal replacement therapy and to elucidate some of the underlying mechanistic pathways contributing to this observed effect.

In the present work, neurobehavioral tests were performed to observe the behavioral changes in oophrocetomized rats, which are

similar to menopausal women. The oophrocetomized rats showed a reduction in sucrose consumption as determined by the SPT, reflecting a diminished interest in pleasurable activities. Concurrently, an augmentation in immobility time during the FST was observed, indicating heightened despair or hopelessness. Morever, an increase in time spent on closed arm on EMT was observed. These alterations in behavior may be construed as indicative of depressive symptoms or mood disruptions encountered during the menopausal phase. Additionally, the behavioral changes were associated with decreased levels of neurotransmitters dopamine and serotonin in tissue homogenates of hippocampus and prefrontal cortex. Our results also revealed significant correlations between both neurotransmitters and important parameters obtained from the neurobehavioral assessment**.** Dopamine is associated with feelings of reward and motivation, while serotonin is involved in mood stabilization, anxiety regulation, and depression management **(Seo et al., 2008)**.

In our study, evidence indicated that both estradiol and lipoic acid could improve the behavioral symptoms. The amelioration of behavioral outcomes within both treated groups was concomitant with increased levels of neurotransmitters, dopamine and serotonin. Akotkar and his colleague reported that different doses of ALA increased dopamine and serotonin and were partly responsible for improvement in depression associated with rats exposed to mild, chronic, unpredictable stress **(Akotkar et al., 2023)**. While both treatments have shown promise as antidepressants, it is important to explore the underlying mechanisms to ascertain their optimal use in different conditions. Whereas estradiol primarily mediates its effects via estrogen receptor–dependent genomic and non-genomic pathways influencing neurotransmission and synaptic plasticity **(Bastos et al., 2015),** ALA exerts its actions mainly through antioxidant, anti-inflammatory, and mitochondrial-stabilizing pathways, independent of estrogen receptor activation **(Salehi et al., 2019)**.

Estradiol and lipoic acid have been studied for their potential impacts on the NrF2 pathway, a pathway that is essential for cellular antioxidant and cytoprotective responses. In our study, both treatments exhibited an upregulation of NrF2 levels. ALA showed a significant favourable impact on the expression of NrF2 compared to estradiol. The elevation of NrF2 level by ALA leads to increased production of antioxidant enzymes as catalase (CAT) and glutathione peroxidase (GPx), thereby enhancing the cellular antioxidant defense within the body. Our study revealed a positive correlation between antioxidant enzymes (CAT and GPx) and NrF2 expression. In accordance with our results, Xia et al. (2019) found that ALA had a role to mitigate traumatic brain damage by inhibition of oxidative stress and apoptosis through modulation of NrF2 signalling pathway **(Xia et al., 2019)**. In addition, induced NrF2 activation has been linked to cellular cytoprotection, which can help to alleviate tissue damage and inflammation in different pathological conditions **(Ngo and Duennwald, 2022**). Our results showed negative correlation between inflammatory markers and NrF2. The anti-inflammatory and antioxidant effects of ALA through NrF2 were reported in different brain disorders as ischemic stroke **(Lv et al., 2017)**, and chemotherapy induced cognitive impairment **(Lal et al., 2023)**.

Concerning apelin, an adipokine with many neuroprotective actions, our results revelaed that the endogenous brain apelin levels were significantly decreased in brain tissues of oophorectomized rats in comparison to normal rats. In accordance with our results, Yan et al., (2018) revealed that the 21-day mild chronic unpredictable stress significantly decreased apelin mRNA hypothalamic expression **(Yan et al., 2018)**. The anxiolytic and antidepressant actions of apelin were evident in many studies through different mechanisms **(Hu et al., 2022;**

Aminyavari et al., 2019). The mechanism through which apelin exerts its neuroprotective effects may involve its role as an anti-inflammatory adipokine. This is in accordance with Zhang et al. (2019) who reported that repeated apelin-13 intracerebroventricular injection inhibited stress-induced microglial overactivation and cytokine production in response to lipopolysaccharides. This suggests that apelin-13 has antidepressant effects, which may be mediated by blocking the NF-κB-mediated inflammatory response **(Zhang et al., 2019)**. Also Luo et al. (2019) found that apelin was able to mitigate the cognitive deficit in Alzheimer's Disease Model induced by Streptozotocin, and this was through reduction of the inflammatory cytokines brain levels **(Luo et al., 2019)**. This anti-inflammatory effect of apelin goes hand in hand with our results that revealed negative correlation between apelin with both inflammatory markers (IL-6 and NF-kB). Another possible protective effect of apelin that was evident in our study is the antioxidant effect that was shown from the positive correlation between apelin with NrF2 and both antioxidant eznymes (CAT and GPx). This is in accordance with the results obtained by Than et al. (2014) that revealed the ability of apelin to mitigate oxidative stress in human adipocytes **(Than et al., 2014)**. The aforementioned protective effects of apelin in many neurological disorders put it as a promising therapeutic target in our study to promote antidepressant effect. Both ALA and the hormonal replacement therapy in the form of estradiol in oophroctomized rats increased levels of brain apelin. However, in our study, the ALA had a more positive effects than estradiol. Our results are in accordance with Huerta AE et al who found that oral ALA supplementation was effective to change the levels of many mediators and adipokines and one of them was apelin that increased by ALA supplementation **(Huerta et al., 2015)**. Also another in vitro sudy found that lipoic acid was effective to modulate adipocyte apelin expression in rats fed with high fat diet **(Fernández-Galilea et al., 2011)**. To our knowledge, our study is the first to show the effect of ALA on apelin in brain tissue and to correlate it with the antidepressant effect of apelin which was evident from the correlations between apelin and neurotransmitters (dopamine and serotonin) and also the correlations between apelin and parameters obtained from the neurobehavioral assessment.

Furthermore, we explored the expression of microRNA-99a in the brain tissue of different studied groups. MiRNA-99a is a small RNA molecule involved in regulating gene expression and has associations to numerous cellular processes, cell proliferation, apoptosis, and tumor suppression. Our study revealed significantly lower miRNA-99a expression levels in oophorectomized rats compared to normal rats. This observation aligns with a clinical study indicating a negative correlation between progesterone and estrogen levels and miRNA-99a expression **(Xie and Cao, 2019)**. Furthermore, Yang et al. (2019) found a dramatic miRNA-99a downregulation in the hypothalamus of peri/postmenopausal depression model mice **(Yang et al., 2019)**. The mechanism of neuroprotective effect of miRNA-99a is still unknown, however our results revealed positive correlations between this miRNA and NrF2, CAT and GPx which gives clue for its antioxidant effect. In addition, negative correlations were found between miRNA-99a and both inflammatory markers (IL-6 and NF-kB) giving an idea about its anti-inflammatory action. This is in accordance with other studies showing the neuroprotective effects of miRNA-99a in rat models of spinal cord injury **(Wang et al., 2022)**, and cerebral ischemia - reperfusion injury **(Tao et al., 2015)**.

In addition, Yang et al. (2019) suggested that miRNA-99a might be a possible therapeutic target for perimenopausal and postmenopausal depression since it may be responsible for manipulating synaptic plasticity **(Yang et al., 2019)**.

Consequently and based on all previous data, we hypothesized that miRNA-99a might be a therapeutic target for menopause associated depression. Interestingly, our study showed that both the estradiol and ALA were effective to increase miRNA-99a, with ALA having superior effect when compared to estradiol. While there may not be direct research linking lipoic acid and miRNA-99a, it's worth noting that lipoic acid's antioxidant properties could indirectly impact miRNA-99a expression.

Conclusion

Our results revealed a similar effect of hormonal replacement therapy and ALA in improving depressive-like manifestations in oophorectomized rats. Apelin and miRNA-99a appear to be important mediators of ALA neuroprotective effects. ALA may represent a promising adjuvant treatment for cognitive and depressive manifestaions of menopause; however, further research is needed. However, shifting from preclinical to clinical studies must be taken in cosidedation.

The present study has certain limitations, including the use of an animal model that may not fully represent human physiology, the absence of a dose–response assessment, and the lack of mechanistic inhibition or blocking experiments to confirm pathway specificity. Therefore, future studies should address these aspects and validate these findings in clinical settings.

References

- **Aebi H. (1984).** Catalase in vitro. Methods Enzymol, 105;121-126.
- **Akotkar L, Aswar U, Patil R, Kumar D, Aswar M, Pandey J, et al. (2023).** Antidepressant Effect of Alpha Lipoic Acid in Rats Exposed to Chronic Unpredictable Mild Stress: Putative Role of Neurotransmitters and 5HT3 Receptor. Future Pharmacology, 3(2):407-425.
- **Aminyavari S, Zahmatkesh M, Khodagholi F, Sanati M. (2019).** Anxiolytic impact of Apelin-13 in a rat model of Alzheimer's disease: Involvement of glucocorticoid receptor and FKBP5. Peptides, 118:170102.
- **Bancroft JD, Gamble M. (2008).** Theory and Practice of Histological Techniques (6th ed.). Elsevier Health Sciences.
- **Bartel DP. (2004).** MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 116(2):281-297.
- **Bastos CP, Pereira LM, Ferreira-Vieira TH, Drumond LE, Massensini AR, Moraes MF, et al. (2015).** Object recognition memory deficit and depressive-like behavior caused by chronic ovariectomy can be transitorily recovered by the acute activation of hippocampal estrogen receptors. Psychoneuroendocrinology, 57:14-25.
- **Blandford SN, Galloway DA, Moore CS. (2018).** The roles of extracellular vesicle micro RNAs in the central nervous system. Glia, 66(11):2267-2278.
- **Bohm LN, Goldberg A.R, Mariani M, Frankfurt M, Thornton J. (2020).** Reducing luteinizing hormone levels after ovariectomy improves spatial memory: Possible role of brain-derived neurotrophic factor. Hormones and Behavior, 118:104590.
- **Da Rocha RVO, Martins MIM, Antunes FTT, Martins MG, Klein AB, Corrêa DS, et al. (2021).** Behavioral, Oxidative, and Biochemical Effects of Omega-3 on an Ovariectomized Rat Model of Menopause. Journal of menopausal medicine, 27(3):132-140.
- **Dias KC, Patrocínio CF, Barroso PL, Rodrigues RB, Nascimento PA, Medeiros IS, et al. (2016).** Alpha-Lipoic Acid Effects on Reserpine-Induced Depression Like Behavior in Mice. JSM Anxiety Depress, 1:1010-1016.
- **El Habachi NM, Maklad HM, Sharara GM, Allam EA, Fawzy EM. (2014).** A comparative study between the effect of 17-β estradiol and antioxidants combination on some menopausal changes in oophorectomised rats. Middle

East Fertility Society Journal, 19(4):303-313.

- **Fernández-Galilea M, Pérez-Matute P, Prieto-Hontoria P, Martínez JA, Moreno-Aliaga MJ. (2011).** Effects of lipoic acid on apelin in 3T3-L1 adipocytes and in high-fat fed rats. Journal of physiology and biochemistry, 67:479-486.
- **Ferreira PMP, Militão GCG, Freitas RM. (2009).** Lipoic acid effects on lipid peroxidation level, superoxide dismutase activity and monoamines concentration in rat hippocampus. Neuroscience letters, 464:131-134.
- **Fu X, Wang Y, Zhao F, Cui R, Xie W, Liu Q, Yang W. (2023).** Shared biological mechanisms of depression and obesity: focus on adipokines and lipokines. Aging (Albany NY), 15(12):5917-5950.
- **Gordon J.L, Eisenlohr-Moul T.A, Rubinow D.R, Schrubbe L, Girdler S.S. (2016)**. Naturally Occurring Changes in Estradiol Concentrations in the Menopause Transition Predict Morning Cortisol and Negative Mood in Perimenopausal Depression. Clinical psychological science, 4:919-935.
- **Guo L, Ren L, Zhang C. (2018).** Relationship between depression and inflammatory factors and brain-derived neurotrophic factor in patients with perimenopause syndrome. Experimental and therapeutic medicine, 15:4436-4440.
- **Hu S, He L, Chen B, You Y. (2022)**. Apelin-13 attenuates depressive-like behaviors induced by chronic unpredictable mild stress via activating AMPK/PGC-1α/FNDC5/BDNF pathway. Peptides, 156:170847.
- **Huerta AE, Prieto-Hontoria PL, Sáinz N, Martínez JA, Moreno-Aliaga MJ. (2015).** Supplementation with α-Lipoic Acid Alone or in Combination with Eicosapentaenoic Acid Modulates the Inflammatory Status of Healthy Overweight or Obese Women Consuming an Energy-Restricted Diet. The Journal of nutrition, 46(4):889S-896S.
- **Kandratavicius L, Balista PA, Wolf DC, Abrao J, Evora PR, Rodrigues AJ, et al. (2015).** Effects of nitric oxide-related compounds in the acute ketamine animal model of schizophrenia. BMC neuroscience, 16:9.
- **Kotz S, Balakrishnan N, Read CB, Vidakovic B. (2006).** Encyclopedia of statistical sciences. 2nd ed. Hoboken, N.J: Wiley-Interscience.
- **Lal R, Dharavath RN, Chopra K. (2023).** Alpha-Lipoic Acid Ameliorates Doxorubicin-Induced Cognitive Impairments by Modulating Neuroinflammation and Oxidative Stress via NRF-2/HO-1 Signaling Pathway in the Rat Hippocampus. Neurochemical research, 48(8):2476-2489.
- **Liang G, Kow ASF, Yusof R, Tham CL, Ho YC, Lee MT. (2024).** Menopause-Associated Depression: Impact of Oxidative Stress and Neuroinflammation on the Central Nervous System-A Review. Biomedicines, 12(1):184.
- **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951).** Protein measurement with the Folin phenol reagent. The Journal of biological chemistry, 193(1):265-275.
- **Luo H, Xiang Y, Qu X, Liu H, Liu C, Li G, Han L, Qin X. (2019).** Apelin-13 Suppresses Neuroinflammation Against Cognitive Deficit in a Streptozotocin-Induced Rat Model of Alzheimer's Disease Through Activation of BDNF-TrkB Signaling Pathway. Frontiers in pharmacology, 10:395.
- **Lv C, Maharjan S, Wang Q, Sun Y, Han X, Wang S, et al. (2017).** α-Lipoic acid promotes neurological recovery after ischemic stroke by activating the NrF2/HO-1 pathway to attenuate oxidative damage. Cellular physiology and biochemistry, 43(3):1273-1287.
- **Mendoza-Núñez VM, García-Martínez BI, Rosado-Pérez J, Santiago-Osorio E, Pedraza-Chaverri**

J, Hernández-Abad VJ. (2019). The Effect of 600 mg Alpha-lipoic Acid Supplementation on Oxidative Stress, Inflammation, and RAGE in Older Adults with Type 2 Diabetes Mellitus. Oxidative medicine and cellular longevity, 2019:3276958.

- **Ngo V, Duennwald ML. (2022).** Nrf2 and Oxidative Stress: A General Overview of Mechanisms and Implications in Human Disease. Antioxidants, 11(12):2345.
- **Paglia DE, Valentine WN. (1967).** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of laboratory and clinical medicine, 70: 158-169.
- **Parhizkar S, Ibrahim R, Latiff AL. (2008).** Incision choice in laparatomy: a comparison of two incision techniques in ovariectomy of rats. World Applied Sciences Journal, 4:537-540.
- **Pellow S, Chopin P, File SE, Briley M. (1985).** Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of neuroscience methods, 14:149-167.
- **Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M. (2020).** The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS biology,18(7):e3000410.
- **Rao X, Huang X, Zhou Z, Lin X. (2013).** An improvement of the 2ˆ(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostatistics, bioinformatics and biomathematics, 3(3):71-85.
- **Rodríguez-Landa JF. (2022).** Considerations of Timing Post-ovariectomy in Mice and Rats in Studying Anxiety- and Depression-Like Behaviors Associated With Surgical Menopause in Women. Front Behav Neurosci, 16:829274.
- **Salehi B, Berkay Yılmaz Y, Antika G, Boyunegmez Tumer T, Fawzi Mahomoodally M, Lobine D, et al. (2019).** Insights on the Use of α-Lipoic Acid for Therapeutic Purposes. Biomolecules, 9(8):356.
- **Seo D, Patrick CJ, Kennealy PJ. (2008).** Role of Serotonin and Dopamine System Interactions in the Neurobiology of Impulsive Aggression and its Comorbidity with other Clinical Disorders. Aggression and violent behavior, 13(5):383-395.
- **Slattery DA and Cryan JF. (2012).** Using the rat forced swim test to assess antidepressant-like activity in rodents. Nature protocols, 7(6):1009-1014.
- **Sriram H, Khanka T, Kedia S, Tyagi P, Ghogale S, Deshpande N, et al. (2021).** Improved protocol for plasma microRNA extraction and comparison of commercial kits. Biochemia medica, 31(3):030705.
- **Tantipongpiradet A, Monthakantirat O, Vipatpakpaiboon O, Khampukdee C, Umehara K, Noguchi H, et al. (2019).** Effects of Puerarin on the Ovariectomy-Induced Depressive-Like Behavior in ICR Mice and Its Possible Mechanism of Action. Molecules, 24(24):4569.
- **Tao Z, Zhao H, Wang R, Liu P, Yan F, Zhang C, et al. (2015).** Neuroprotective effect of microRNA-99a against focal cerebral ischemia-reperfusion injury in mice. Journal of the neurological sciences, 355(1-2):113-119.
- **Than A, Zhang X, Leow MK, Poh CL, Chong SK, Chen P. (2014).** Apelin attenuates oxidative stress in human adipocytes. The Journal of biological chemistry, 289(6):3763-3774.
- **Tian Y, Wang R, Liu L, Zhang W, Liu H, Jiang L, Jiang Y. (2023).** The regulatory effects of the apelin/APJ system on depression: A prospective therapeutic target. Neuropeptides, 102:102382.
- **Triggiani L. (2020).** Potential therapeutic effects of alpha lipoic acid in memory disorders. Progress in Nutrition, 22(1):12-19.
- **Wang R, Liu Y, Jing L. (2022).** MiRNA-99a alleviates inflammation and

oxidative stress in lipopolysaccharide-stimulated PC-12 cells and rats post spinal cord injury. Bioengineered, 13(4):9520-9531.

- **Xia D, Zhai X, Wang H, Chen Z, Fu C, Zhu M. (2019).** Alpha lipoic acid inhibits oxidative stress-induced apoptosis by modulating of NrF2 signalling pathway after traumatic brain injury. Journal of cellular and molecular medicine, 23(6):4088-4096.
- **Xie J, Cao Y. (2019).** Expression of TGF-β1 and miR-99a in serum of patients with early spontaneous abortion and correlation with hormone levels during pregnancy. Experimental and therapeutic medicine, 17:4593-4597.
- **Yan Z, Jiao H, Ding X, Ma Q, Li X, Pan Q, et al. (2018).** Xiaoyaosan Improves Depressive-Like Behaviors in Mice through Regulating Apelin-APJ System in Hypothalamus. Molecules, 23(5):1073.
- **Yang J, Zhang L, Cao LL, Qi J, Li P, Wang XP, et al. (2019).** MicroRNA-99a is a potential target for regulating hypothalamic synaptic plasticity in the peri/postmenopausal depression model. Cells, 8(9):1081.
- **Yang, X, Song, S, Xu, Y. (2017).** Resveratrol ameliorates chronic unpredictable mild stress-induced depression-like behavior: involvement of the HPA axis, inflammatory markers, BDNF, and Wnt/β-catenin pathway in rats. Neuropsychiatric Disease and Treatment, 13:2727-2736.
- **Yusha'u Y, Muhammad UA, Mustapha S, Umar AH, Imam MI, Umar B, et al. (2021).** Alpha-lipoic acid attenuates depressive symptoms in mice exposed to chronic unpredictable mild stress. Journal of African Association of Physiological Sciences, 9(1):58-68.
- **Zhang ZX, Li E, Yan JP, Fu W, Shen P, Tian SW, et al. (2019).** Apelin attenuates depressive-like behavior and neuroinflammation in rats co-treated with chronic stress and lipopolysaccharide. Neuropeptides, 77:101959.
- **Zvozilova A, Bukatova S, Koprdova R, Mach M. (2024).** Evaluation of New Approaches to Depression Treatment Using an Animal Model of Pharmacoresistant Depression. International Journal of Molecular Sciences, 25(10):5265.