Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

Open Access

Anti-apoptotic effect of menaquinone-7 protects the brain of ovariectomized rats

Sherif W. Mansour[1,2], Soad Abdallah Selim[1], Sarah Ahmed Salama[1], Samia Hussein[3,4*] and Eman Reda Abozaid[1]

Abstract

Background Mood and memory deterioration occurs after ovariectomy (OVX) with various degrees and sometimes requires medical intervention. Menaquinone-7 (MK-7) is a potent isoform of vitamin K_2 and has many effects on the bone and cardiovascular system. However, the effect of MK-7 on the brain and its mechanisms of action are still unclear. This study was performed to investigate the effect of MK-7 on mood and memory disorders following ovariectomy. Thirty-two female albino rats were divided into four groups ($n=8$). Group I (control group) included sham-operated rats with sunflower oil intake. Group II (K_2) included sham-operated rats with an intake of MK-7 dissolved in sunflower oil. Group III (K_2 OVX) included ovariectomized rats with an intake of MK-7 dissolved in sunflower oil. Group IV(OVX) included ovariectomized rats with sunflower oil intake. Working memory, anxiety, depression, and sociability behaviors were investigated in all groups. Gene expression of BAX, BCL2, and p53 was measured in the hippocampus of all groups by real-time PCR. Besides, BAX/BCL2 ratio was calculated.

Results Working memory, anxiety, depression, and sociability behaviors in the OVX rats showed a significant change compared to the sham-operated. However, the intake of MK-7 after the OVX resulted in significant improvement. Regarding hydrogen peroxide and MDA activity, they were significantly higher in the OVX group compared to the sham-operated groups, while in the K_2OVX group, their activity showed a significant decrease in comparison with the OVX group. However, catalase and total antioxidant capacity were significantly lower in the OVX group compared to the sham-operated group, while in the $\mathbf{k}_2$OVX group, their activity showed a significant increase in comparison with the OVX group. The OVX group showed a significant elevation in the BAX, BAX/BCl2 ratio, and P53, but BCL2 was significantly reduced. However, the intake of MK-7 caused a significant improvement.

Conclusions Our study showed that the OVX group showed significant physiological, biochemical, and molecular changes, which can be prevented by MK-7 intake.

Keywords Ovariectomy, MK-7, Memory, Anxiety, Apoptotic genes

1 Background

Menopause is the normal cessation of menstruation for 12 months due to the depletion of ovarian follicles and a subsequent reduction in ovarian production of estradiol and progesterone, with increased follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Burger et al. [1]; Hogervorst and Bandelow [2]). Menopause is always followed by complications including hot flushes (Reed et al. [3]), vulvovaginal atrophy, vaginal dryness, urinary tract infections (Koothirezhi [4]), a decrease in bone mineral density (Yoshida et al. [5]), and cardiovascular

*Correspondence:
Samia Hussein
samiahussein82@hotmail.com
[1] Department of Physiology, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt
[2] Head of Physiology & Biochemistry Department, Mutah School of Medicine, Mutah University, Mutah 61710, Jordan
[3] Medical Biochemistry & Molecular Biology Department, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt
[4] Department of Basic Medical Sciences, Ibn Sina University for Medical Sciences, Amman 11104, Jordan

problems (Miller et al. [6]). Brain function is also greatly affected following menopause. Sleep disturbance (Baker et al. [7]), anxiety-like behavior, depression (Puga-Olguín et al. [8]), and memory changes (Egashira et al. [9]) are common complaints.

The hormonal changes after bilateral oophorectomy are severe and sudden. Consequently, the cognitive consequences become more common and severe following surgical menopause (Sarrel et al. [10]). The severe reduction in estradiol levels with an increase in FSH levels is a direct cause of mood disturbances (Freeman et al. [11]). During menopause, women are at risk of depression attacks 3 folds more than during fertile life. Even years before menopause, severe depressive periods may occur (Bromberger and Epperson [12]). According to a previous study, even women with no previous complaints of depression could be at risk for depressive attacks during menopause (Faubion et al. [13]). Moreover, there is a common complaint of poor memory. The most affected are verbal and spatial memory, with difficulty in remembering names and even telling information. In severe cases, cognitive impairment is in the form of difficulty in trouble organizing, planning, and concentrating (Faubion et al. [13]; Karishma et al. [14]). In rats, ovariectomy is followed by a defect in short- and long-term memory, and the appearance of anxiety-like behavior (Djiogue et al. [15]). The hippocampus is responsible for the formation and recall of long-term memories about people, places, objects, and events (Zemla and Basu [16]).

MK-7 is a lipid-soluble vitamin and acts as a co-factor for γ-glutamyl carboxylation of glutamic acid to γ-carboxy glutamic acid**.**Natto, hard cheese, ground beef, and egg yolk are rich sources of MK-7(Schwalfenberg [17]). MK-7 could increase bone mineral density (Zhang et al. [18]), regulate insulin secretion from the pancreas (Lee et al. [19]), and improve glucose metabolism (Ferron et al. [20]). The effect of MK-7 on the brain has been recently investigated in a few studies. MK-7 has been reported to have antioxidant action, reduce total brain water, and protect astrocytes in neural developmental diseases (Hadipour et al. [21]; Farhadi Moghadam and Fereidoni [22]). Menopause is a part of the aging process that occurs through several mechanisms, including disturbed cell metabolism and apoptosis (Rufini et al. [23]). Menopause is considered a neurological transition period associated with changes in brain structure, particularly the connectivity and metabolic profile (Mosconi et al. [24]). The neuroprotective effect of MK-7 has been a matter of interest recently. However, its effect on the menopausal brain and possible underlying mechanisms are still unclear.

2 Materials and methods

2.1 Animals

Thirty-two fertile, healthy adult (average 3 months) female albino rats (weight:150–200 gm) were obtained. Rats were housed in groups (n=4 groups) in standard cages (n= 8 rats) under a 12:12 h light–dark cycle, kept at a comfortable temperature (20 to 24 °C) with free access to food standard chow, and tap water. Rats were acclimatized to the testing room environment in the Animal Behavior Laboratory of the Physiology Department (for 1 week) (Gancheva and Zhelyazkova-Savova [25]).

Then, the animals were randomly divided into four groups (n = 8 rats in each group)(n = 8).

Group (I) control sham-operated with sunflower oil (Sigma-Aldrich, USA., MFCD00132403) intake by oral gavage once daily for 5 days per week for 10 weeks.

Group (II) K_2sham-operated with MK-7 (Sigma-Aldrich, USA., 900074-1MG) intake of 35 mg/kg dissolved in sunflower oil by oral gavage once daily for 5 days per week for 10 weeks (Gancheva and Zhelyazkova-Savova [25]).

Group (III) K_2 *OVX* ovariectomized with MK-7 intake 35 mg/kg dissolved in sunflower oil by oral gavage once daily for 5 days per week for 10 weeks.

Group (IV) OVX ovariectomized with sunflower oil intake by oral gavage once daily for 5 days per week for 10 weeks.

The study was maintained for ten weeks after the ovariectomy operation. In the last two weeks before the end of the study, behavioral tests were performed. In the last four days of the study, vaginal cytology was performed for the two sham-operated groups. Scarification and blood sampling were performed during the meta estrous phase. Figure 1 shows the experimental study design.

2.2 Vaginal cytology

Vaginal cytology was used to determine the phases of the estrous cycle. It is noninvasive and relatively inexpensive. Also, it is accurate and reliable. The tail was elevated to visualize the vagina. The vaginal cells were flushed by gently introducing 100 µL of phosphate-buffered saline (PBS). The liquid was slowly released into the vagina and drawn back. The process was repeated about 4 to 5 times in the same sterile latex bulb. The pipette and sterile latex bulb should be placed at the entrance of the vaginal canal without penetrating the vaginal orifice. A few drops of cell suspension were put on a glass slide, air-dried, and stained with 0.1% crystal violet stain. The slide was examined under a light microscope immediately at 200 X magnification (Auta and Hassan [26]). A modest number of big, non-granular, cornified epithelial cells and a rise in the number of leucocytes are two features of the metestrus phase (Ajayi and Akhigbe [27]).

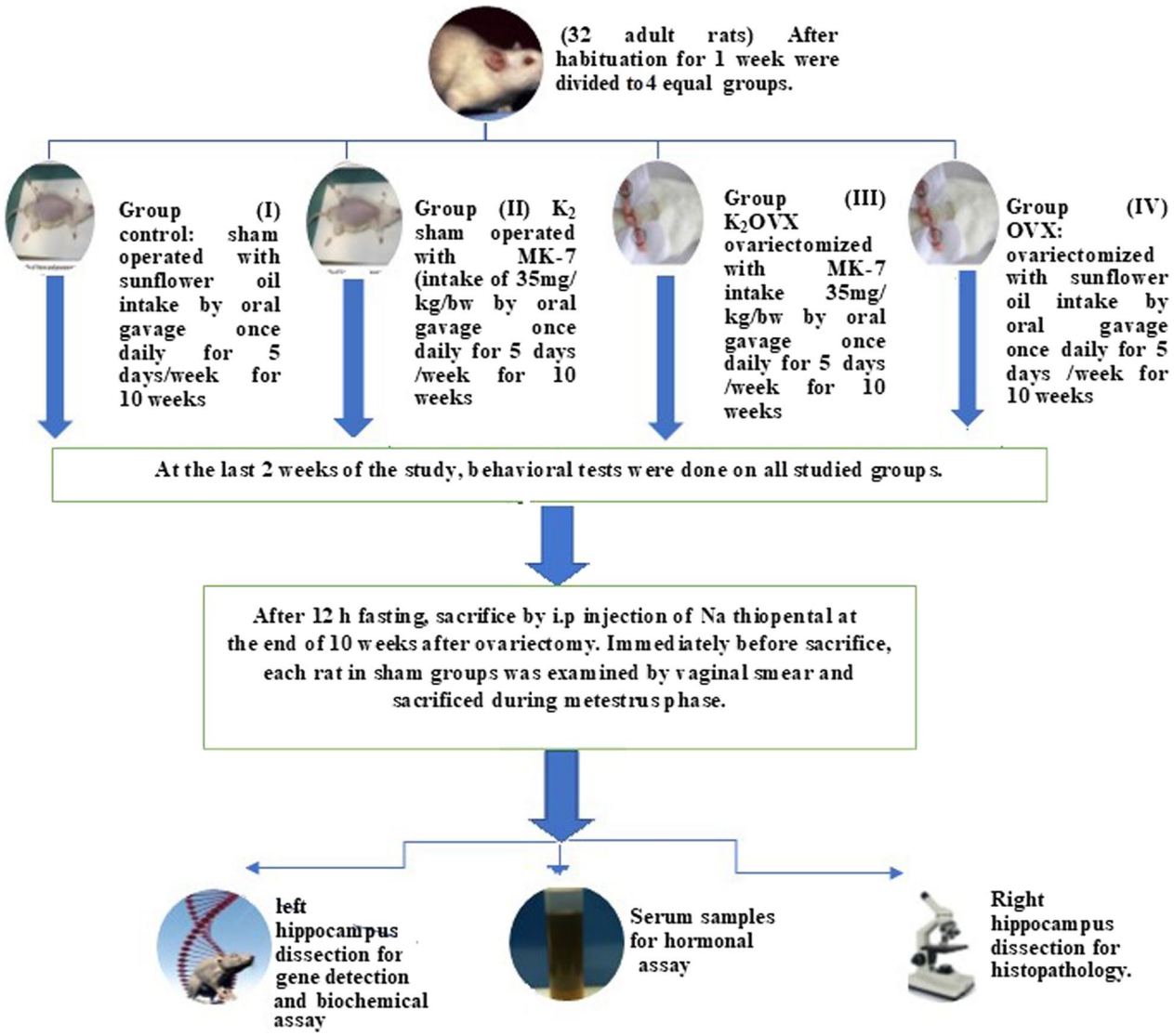

Fig. 1 Schematic presentation of the study

2.3 Ovariectomy

The animals fasted overnight. They were anesthetized with 2% sodium pentobarbital by intraperitoneal injection (0.2 mL/100 g). A central midline incision of 0.5 cm was made with a scalpel after shaving and sterilizing the area with 75% ethyl alcohol. The connective tissue was then separated until it reached the peritoneal wall. The ovaries were palpated and removed bilaterally. Then the peritoneum and the muscle layer were sutured separately with absorbable sutures. While the skin was sutured with nylon non-absorbable suture, which spontaneously detached after 2–3 days. In sham-operated groups, the same procedures were done except that the ovaries were just palpated and not removed (Ajayi and Akhigbe [27]).

2.4 Behavioral tests

2.4.1 Open field test (OF)

The OF test is a behavioral test for testing anxiety-like behavior in rats. The open field maze consists of a black woody square (1 m × 1 m) surrounded by black wooden walls (50 cm in height). The floor is divided into 25 equal squares (20 cm × 20 cm). The rats were brought to the testing room about 5–20 min before the test. Every single rat was removed from the home cage by gently handling from the body and put in the corner square facing the corner. The rat was released just before its front paw touched the floor. Free and uninterrupted movements of the subject rat were allowed throughout the maze for 3 min. The number of squares entered by the rat, the number of rears, the latency to move, the latency to rear,

and the number of fecal boli were recorded (Briones-Aranda et al. [28]).

2.4.2 Modified forced swim test (MFST)

The MFST test is a behavioral test for testing depressive-like behavior in rats. Swim cylinders were tall enough to fill to a depth of 30 cm, leaving space at the top so that the rat could not escape, and at least 20 cm in diameter. A glass mercury thermometer was used to determine the water temperature (23–25 °C). The rats were placed individually into the swim cylinder. A pretest session (not recorded) was done 24 h before the recorded session for 15 min of free swimming. The swim session (recorded) was a session of 5 min of swimming and was recorded. Swimming, climbing, and immobility time were recorded during the swim session (Deacon [29]).

2.4.3 Modified T maze test

The Modified T maze test is a behavioral test for working memory in rats. A T maze is a T-shaped apparatus consisting of a central arm (50 cm × 16 cm) and two goal arms (50 cm × 10 cm), each higher than 30 cm. The maze has two guillotine doors at the entrance of the goal arms and a central partition extended for 10 cm in the central arm. Before the test, a criterion point was determined for the whole animal, including the tail tip, to be on the goal arm. A thin layer of bedding (~ 10 mm thick) was spread over the floor of the maze. For each rat, one sample trial and five choice runs were performed per day for two days, amounting to 12 trials per rat with a duration of 1–2 min for each trial. During the sample run, the rat was placed in the start arm (bottom of the "T") and allowed to choose a goal arm and then trapped for 30 s in the chosen arm by quietly sliding the guillotine door down. While during the choice runs, the central partition was removed, the guillotine door of the goal arm was raised again, and the rat was allowed to choose an arm. A total of ten possible alternations were calculated for each rat (Slattery and Cryan [30]).

$$\frac{\text{Number of correct choices (Alternations)}}{\text{Total possible alternations}} \times 100$$

2.4.4 Social interaction test (Crawley's sociability test)

This test allows the evaluation of two aspects of social behavior, such as social affiliation/motivation and social memory and novelty. The apparatus for Crawley's sociability and preference for social novelty test is comprised of a rectangular, three-chamber box. With an open middle section, which allows free access to each chamber with identical, wire cup-like containers and removable lids that are large enough to hold a single rat. Each rat passed by habituation period for the test followed by two sessions. The first is the Social Affiliation Aspect of the Test (session I) where one of the control rats was placed (Stranger 1) inside a wire containment cup that was in one of the side chambers. The placement of Stranger 1 on the left or right side of the chamber was systematically altered between trials. The walls were removed between the compartments, to allow free access for the "subject" rat to explore each of the three chambers. The second is Social Novelty (Session II) in which we place a second control rat ("Stranger 2") inside an identical wire containment cup in the opposite side chamber (that had been empty during Session I). The same parameters described before were monitored. The behaviors of the subject rat in the presence of Stranger 1 were compared with Stranger 2.

Each session persisted for 10 min and was monitored and recorded by a video camera. Finally, we analyzed the total number of contacts, the total duration of contacts between the experimental rat and empty containment cup vs or cup housing Stranger 1 (in session I), or between the experimental rat and the cup housing Stranger 1 vs Stranger 2 (in session II), the mean duration per contact, the total number and duration of other behaviors (freezing, self-grooming and walking), and finally the total time spent by the subject rat in each compartment. Then, the significant differences were analyzed for all mentioned parameters, by comparing the groups: empty containment cup vs Stranger 1 for the subject rat and "Stranger 1" vs "Stranger 2" for the subject rat (Deacon and Rawlins [31]).

2.5 Collection of blood samples

At the end of the experiment (10 weeks after ovariectomy), overnight fasted rats were initially anesthetized with thiopental, and blood samples were collected from the retro-orbital sinus. Blood was collected on plain tubes for serum preparation and subsequent enzyme-linked immunosorbent assay (ELISA). The serum was stored at − 20 °C.

2.6 Dissection of hippocampus and preparation of homogenate

Then rats were sacrificed, and the brain was removed from the skull, then rinsed in ice-cold saline to remove any surface blood, and then was placed on a cold metal plate and cut into the right and left hemispheres. The olfactory bulb was cut first then the frontal cortex second, by using a blade and forceps (Dumont number.5). Then, the ventral side of the brain was put up and the midbrain was removed to expose the hippocampus. Then the hippocampus was dissected from the cortex using two forceps (Dumont number.5). The left hippocampus was used for homogenization and subsequent biochemical

measurement and determination of gene expression and was stored at − 80 °C. The right hippocampus was preserved in formalin for histopathological examination (Rein et al. [32]).

Left hippocampal samples were homogenized with ice-cold PBS (pH 7.4) (100 mg tissue per 1 mL PBS). Then, the resulting suspensions were centrifuged at 4 °C with 4,000–6,000 rpm for 20 min, and then, the supernatant was collected. The tissue concentration of the hydrogen peroxide, malondialdehyde (MDA), catalase, and total antioxidant capacity (TAC) were measured by a colorimetric technique using Sunostik, China. The kits were provided by Biodiagnostics, Giza, Egypt (Chiu et al. [33]).

2.6.1 Real-time quantitative RT-PCR (qRT-PCR)

RNA extraction from tissue homogenate was performed using GENEzol ™ Reagent (Geneaid). The extracted RNA was measured using Nanodrop spectrophotometry (ND 1000-NanoDrop®). Reverse transcription to synthesize complementary DNA was performed using TOPscript™ cDNA Synthesis Kit (Enzynomics). The real-time PCR was performed on an Mx3005P Real-Time PCR System (Agilent Stratagene, USA) using TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Cat. # P725) (Enzynomics, Korea). The PCR cycling conditions were an initial denaturation at 95 °C for 12 min, followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The oligonucleotide-specific primers were synthesized by Sangon Biotech (Beijing, China) (Table 1). The samples were run in triplicate. The expression level of the target genes was normalized using the mRNA expression of the housekeeping gene, GAPDH. Results were expressed as fold changes compared to the control group following the $2^{-\Delta\Delta CT}$ method.

2.7 Histopathological study

Hippocampal tissue samples were fixed in a 10% neutral buffered formalin solution, followed by alcohol dehydration, embedding in paraffin wax, serial sectioning (5 µm thickness), and H&E staining.

Table 1 The primers of the studied genes

GAPDH	**F: GCATCTTCTTGTGCAGTGCC** **R: GGTAACCAGGCGTCCGATAC**
BAX	F: GAACCATCATGGGCTGGACA R: GGGTCCCGAAGTAGGAAAGG
BCL2	F: GGTGAACTGGGGGAGGATTG R: AGAGCGATGTTGTCCACCAG
P53	F: GTTCGTGTTTGTGCCTGTCC R: TGCTCTCTTTGCACTCCCTG

F forward primer, *R* reverse primer

2.8 ELISA hormonal assay

Rat estradiol (E2) kit (BC-1111, BioCheck, Foster City, CA 94404), rat progesterone kit (BC-1113, BioCheck, Foster City, CA 94404), rat luteinizing hormone (LH) kit (MBS2514287, Bio, BioSource Europe S.A. Rue de l'Industrie, Belgium) and rat follicular-stimulating hormone (FSH) kit (MBS2507988, Bio Source Europe S.A. Rue de l'Industrie, Belgium) were used for the hormonal assay by enzyme-linked immunosorbent assay (ELISA). The samples were run in triplicate (Delrobaei et al. [34]; Tietz [35]).

2.9 Data analysis

SPSS 19 Software (Inc. Chicago, IL, USA) was used for data analysis. The data were presented as mean ± standard deviation (SD), and the difference was assessed by a one-way analysis of variance (ANOVA) test with significance at $P < 0.05$.

3 Results

3.1 The open field (OF) test

The OVX group showed a significant delay to move and to rear compared to other groups ($P < 0.05$), while the K_2OVX group did not show any significant difference compared to the sham-operated groups ($P > 0.05$) (Fig. 2A and B).

The number of squares entered by each rat and the number of rears in the OVX group was significantly less than in the other groups ($P < 0.05$). However, in the K_2OVX group, the number was not significantly different from the sham-operated groups ($P > 0.05$) (Fig. 2C and D).

The OVX group had a significantly higher fecal boli number than the other groups ($P < 0.05$), while in the K_2OVX group, there was no significant difference from the sham-operated groups ($P > 0.05$) (Fig. 3A).

3.2 The modified forced swim test (MFST)

In the OVX group, the swimming time and the climbing time were significantly shorter than the sham-operated groups ($P < 0.05$) and shorter than the (K_2OVX) group ($P < 0.05$). However, the time was not significantly different in the K_2OVX group compared to sham-operated groups ($P > 0.05$) (Fig. 3B and C).

The immobility time was significantly longer in the OVX group than in the other groups ($P < 0.05$), while in the K_2OVX group, the time was not significantly different from the sham-operated ($P > 0.05$) (Fig. 3D).

3.3 Modified T maze

The score of the T maze in the OVX group was significantly lower compared to the sham-operated groups ($P < 0.05$) and lower than the K_2OVX group ($P < 0.05$),

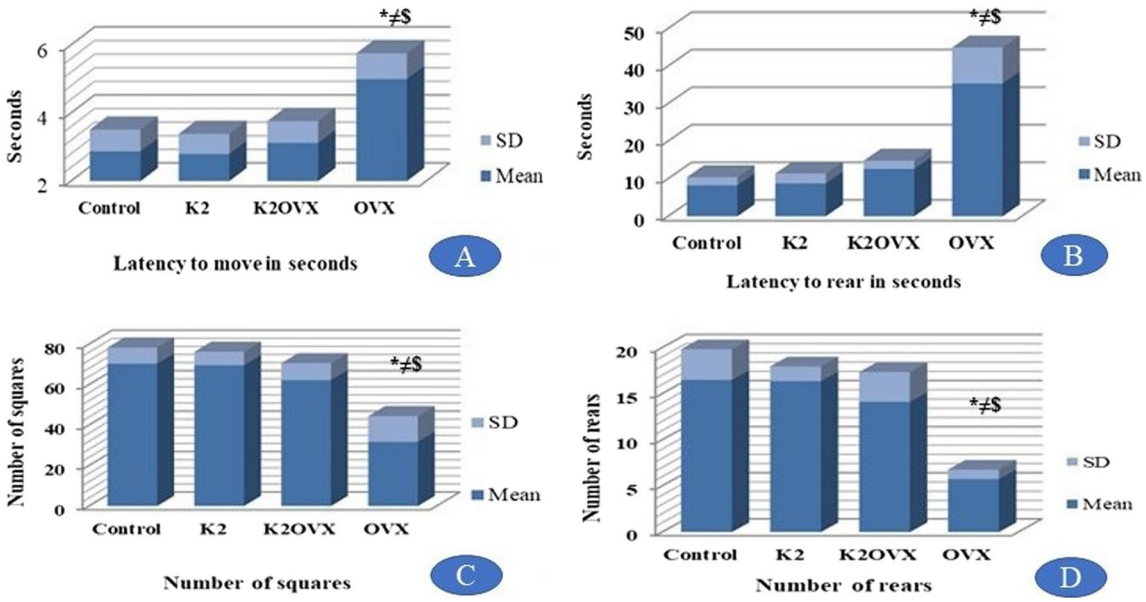

Fig. 2 **A** A histogram shows latency to move in seconds in all the studied groups, **B** A histogram shows latency to rear in seconds in all the studied groups, **C** A histogram shows number of squares entered in all the studied groups, and **D** A histogram shows number of rears in all the studied groups. *: Significant difference to control group, ≠: Significant difference to K_2 group, and $: Significant difference to K_2OVX group

while in the K_2OVX group, the score was significantly higher than the OVX group ($P<0.05$) but still significantly lower compared to the sham-operated groups ($P<0.05$) (Fig. 3E).

3.4 Social interaction test (Crawley's sociability test):

In session I, the time spent in the empty chamber was significantly longer in the OVX group when compared to the sham-operated groups ($P<0.05$), but in the K_2OVX group, this time was significantly shorter than the OVX group ($P<0.05$). However, the time spent in stranger 1 chamber by the OVX group was significantly shorter when compared to the sham-operated groups ($P<0.05$), but in the K_2OVX group, this time was significantly longer than the OVX group ($P<0.05$) (Fig. 4A and B).

In session II, the time spent in the stranger 1 chamber by the OVX group was significantly longer when compared to the sham-operated groups ($P<0.05$), but in the K_2OVX group, this time was significantly shorter than the OVX group ($P<0.05$). However, the time spent in the stranger 2 chambers by the OVX group was significantly shorter when compared to the sham-operated groups ($P<0.05$), but in the K_2OVX group, this time was significantly longer than the OVX group ($P<0.05$) (Fig. 4C and D).

3.5 Biochemical results

Hydrogen peroxide and MDA activity were significantly higher in the OVX group compared to the sham-operated groups ($P<0.05$), while in the K_2OVX group, their activity showed a significant decrease in comparison with the OVX group ($P<0.05$). However, catalase and total antioxidant state were significantly lower in the OVX group compared to the sham-operated group ($P<0.05$), while in the K_2OVX group, their activity showed a significant increase in comparison with the OVX group ($P<0.05$) (Table 2).

3.6 Gene expression results

BAX and P53 apoptotic genes expression and BAX/BCL2 ratio were significantly higher in the OVX group compared to the sham-operated group and the K2 group ($P<0.01$) and the K_2OVX group ($P<0.05$). Additionally, in the K_2OVX group, the expression of the genes and the ratio were significantly higher than in the sham-operated group ($P<0.01$) but still significantly lower than in the OVX group ($P<0.05$). On the other hand, the expression of the anti-apoptotic BCL2 gene was significantly lower in the OVX group compared to the sham-operated and K_2 groups ($P<0.01$) and the K_2OVX group ($P<0.05$), while in the K_2OVX groups, the BCL_2 expression was significantly higher than the OVX group ($P<0.5$) but still lower than the sham-operated groups ($P<0.05$) (Fig. 5).

3.7 Hormonal results

The ovariectomized groups had significantly lower serum levels of E_2 and progesterone than the sham-operated groups ($P<0.05$). On the other hand, the ovariectomized

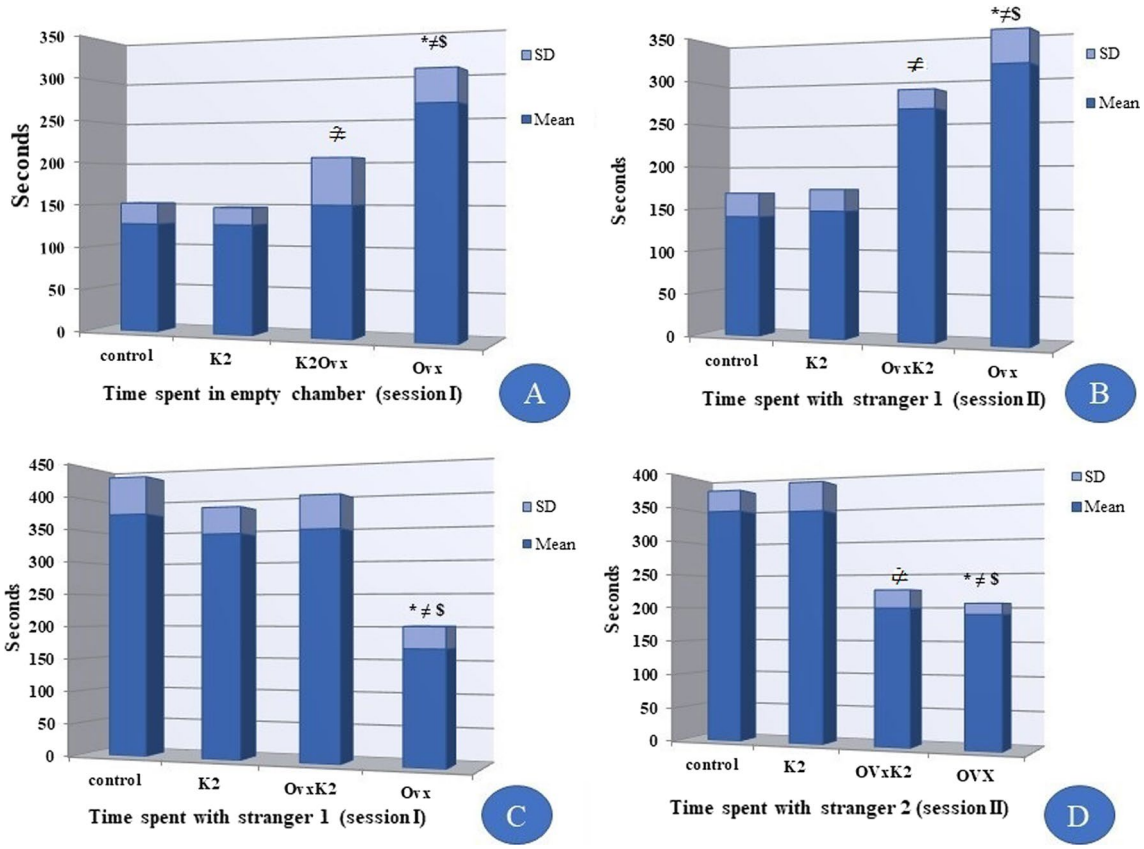

Fig. 3 **A** A histogram shows number of fecal boli in all the studied groups, **B** A histogram shows time of swimming in seconds in all the studied groups, **C** A histogram shows the time of climbing in all the studied groups, **D** A histogram shows time of immobility in seconds in all the studied groups, and **E** A histogram shows score of T maze in percentage in all the studied groups. *: Significant difference to control group, ≠ : Significant difference to K_2 group, and $: Significant difference to K_2OVX group

groups had significantly higher serum levels of FSH and LH than the sham-operated groups ($P < 0.05$) (Fig. 6).

3.8 Histopathological results

Hematoxylin and eosin staining of the sections of the hippocampus in the control group showed normal cells with multiple nuclei with no signs of necrosis or engorged blood vessels. The OVX group showed few pyramidal cells with shrunken apoptotic deeply stained nuclei with pericellular vacuolation with engorged blood vessels. The K_2OVX group showed mostly normal pyramidal cells with very few deeply stained nuclei as a sign of degeneration (Fig. 7).

4 Discussion

Menopause is commonly associated with changes in behavior, brain molecular parameters, and synaptic transmission. That leads to cognitive changes including anxiety, depression-like behaviors, and memory deterioration that can be reversed by hormone replacement therapy (HRT) and proves that this impairment is a consequence of menopause due to ovarian hormone depletion (Chiu et al. [33]; Rebar et al. [36]).

In our study, the OF test was used for assessing anxiety behavior. The OVX group without MK-7 intake showed a significantly prolonged latency to move and to rear time and an increase in the number of fecal boli. However, the number of squares entered by each rat and the number of rears significantly decreased in this group compared to other groups, including the OVX group which received MK-7. These findings indicate increased anxiety-like behavior according to Deacon, 2006 protocol (Briones-Aranda et al. [28]). In agreement with our findings, similar studies on rats showed increased anxiety-like behavior shortly after ovariectomy (Puga-Olguín et al. [8]; Djiogue et al. [15]; Ajayi and Akhigbe [27]; Briones-Aranda et al. [28]). In addition, it showed that the intake of MK-7 reversed anxiety-like behavior in the OVX group.

For the evaluation of depressive-like behavior, we performed the MFST. There was a significant decrease in the time of swimming and climbing with an increase in immobility time in OVX rats without MK-7 intake

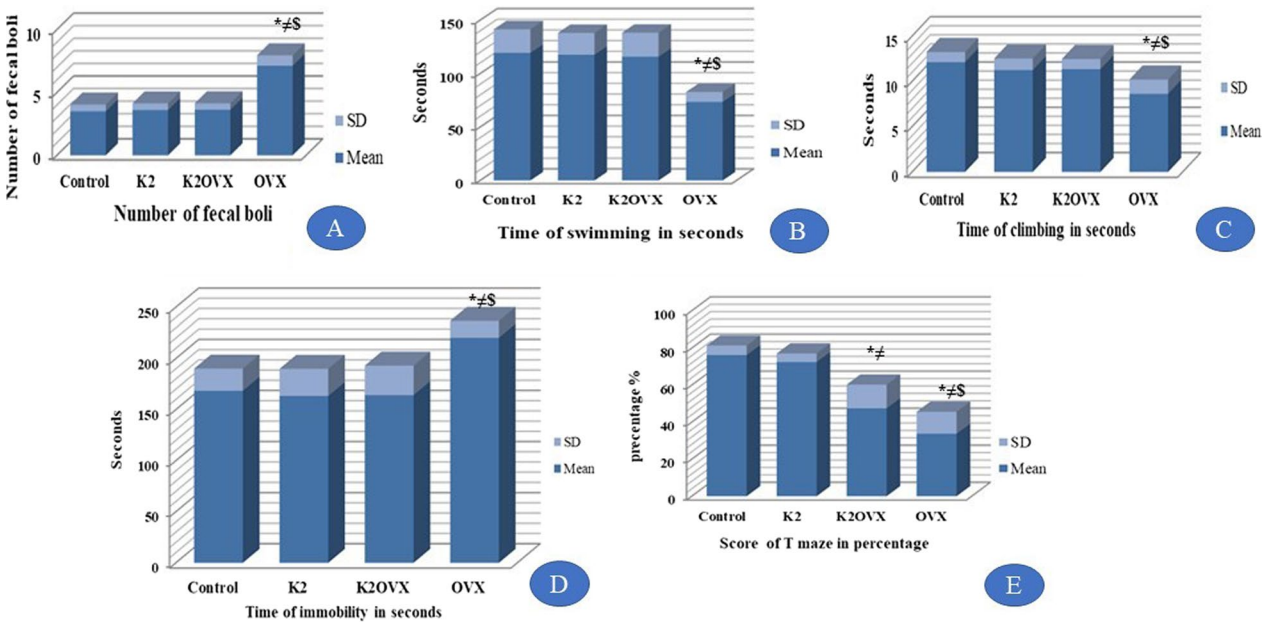

Fig. 4 Histograms A, B, C, D show time spent in different chambers in seconds by Crawley's sociability test in all the studied groups. *: Significant difference to control group, ≠ : Significant difference to K_2 group, and $: Significant difference to K_2OVX group

Table 2 Biochemical results in the studied groups

Oxidative markers	Control group	K_2 group	K_2OVX group	OVX group
Hydrogen peroxide (H_20_2) mmol/gm	4.8 ± 0.3	4.7 ± 0.2	$6.8 \pm 0.5^{\neq}$	$9.9 \pm 0.6^{*\neq\$}$
MDA activity (nmol/gm)	9.2 ± 0.6	9.2 ± 0.5	10.87 ± 1.3	$12.8 \pm 2.8^{*\neq\$}$
Total antioxidant capacity (TAC) (μ M/mg tissue)	2.2 ± 0.1	2.4 ± 0.2	$1.9 \pm 0.2^{\neq}$	$1.4 \pm 0.1^{*\neq\$}$
Catalase (U/gm tissue)	4.2 ± 0.3	4.8 ± 0.1	3.7 ± 0.2	$2.1 \pm 0.1^{*\neq\$}$

*significant to control

$^{\neq}$: significant to K_2 group

$^{\$}$: significant to K_2OVX group

compared to rats in other groups, including the ovariectomy rats that received MK-7. These observations suggest that ovariectomy is followed by mood changes, including depressive-like behavior. On the other hand, the OVX rats that received MK-7 showed no significant difference in mood compared to the sham-operated groups. This indicates that MK-7 has a positive effect on depression behavior following ovariectomy.

For the evaluation of sociability, social interaction test was used for assessing sociability and social preference. Sociability is the tendency to spend time with another rat, as compared to time spent alone in an identical empty chamber. However, preference is the tendency to spend time with a previously unfamiliar rat rather than with a familiar rat (Adu-Nti et al. [37]). In our study, the OVX rats tended to stay a significantly longer duration in the empty chamber compared to other groups in which the subject rat tended to stay longer duration with the stranger 1 chamber during the social affiliation session of the test, which indicates disturbed social behavior (Kaidanovich-Beilin et al. [38]). Moreover, in the social novelty session of this test, the OVX rats showed a preference to stay a significantly longer duration with the stranger1 rat compared to the sham-operated groups in which rats preferred to stay longer duration in the stranger 2 chambers. In agreement with our findings, Adu-Nti et al. and Renczés et al. showed disturbed social behavior in the OVX rats. These abnormal behaviors in both sessions were significantly improved by MK-7 treatment (Leite et al. [39]; Adu-Nti et al. [40]).

In agreement with our findings, previous studies proved that depression is a common chronic complication occurring after ovariectomy and associated with other cognitive impairments (Renczés et al. [41]; Vega

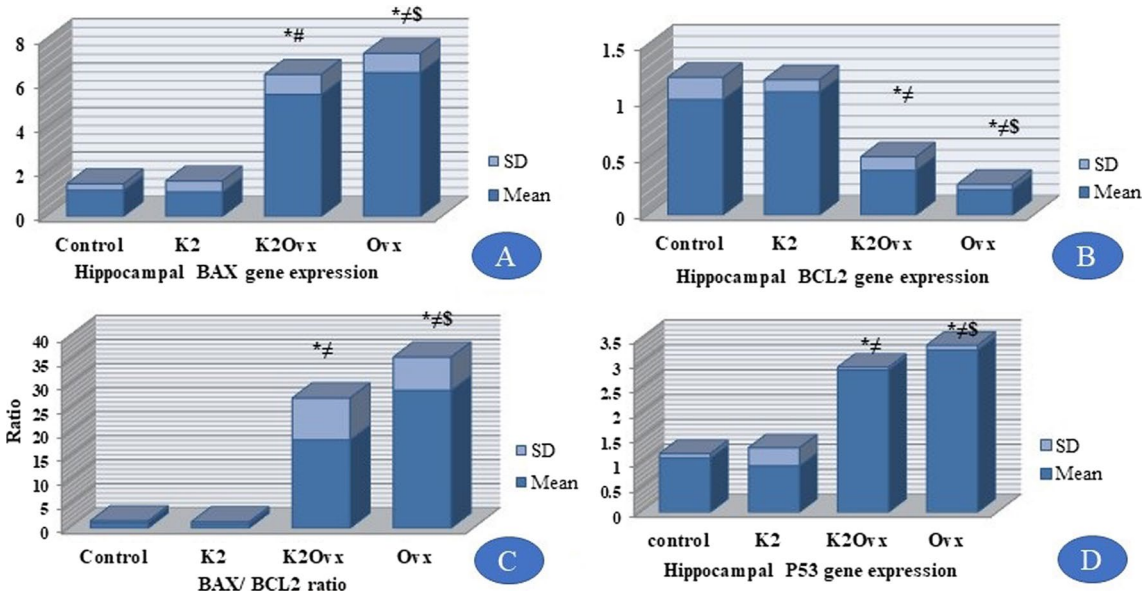

Fig. 5 **A** A histogram shows hippocampus BAX gene expression in all the studied groups, **B** A histogram shows hippocampus BCL2 gene expression in all the studied groups, **C** A histogram shows hippocampus BAX/BCL2 ratio in all the studied groups, and **D** A histogram shows hippocampus P53 gene expression in all the studied groups. *: Significant difference to control group, ≠ : Significant difference to K_2 group, and $: Significant difference to K_2OVX group

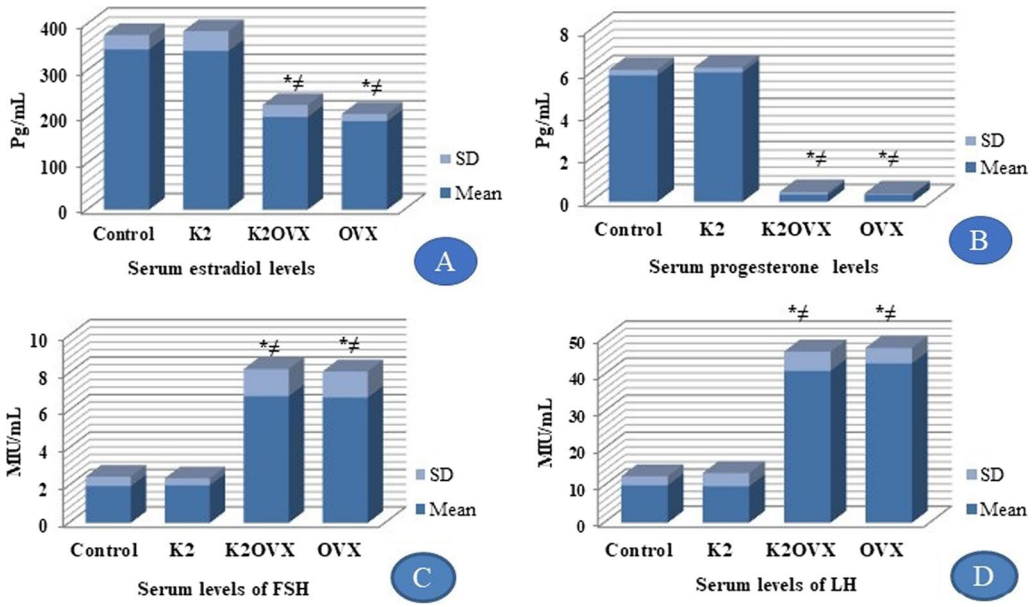

Fig. 6 **A** A histogram shows serum estradiol level in all the studied groups, **B** A histogram shows serum progesterone level in all the studied groups, **C** A histogram shows serum level of FSH in all the studied groups, and **D** A histogram shows serum levels of LH in all the studied groups. *: Significant difference to control group, ≠ : Significant difference to K_2 group, and $: Significant difference to K_2OVX group

Rivera et al. [42]; Khayum et al. [43]; Wu et al. [44]). On the other hand, other studies did not show any significant changes in mood by using the forced swim test either shortly after 3 weeks or long duration after 12 weeks and 4 months after ovariectomy (Banin et al. [45]; Estrada-Camarena et al. [46]). Regarding the effect of MK-7 on depressive behavior and in agreement with our findings, Gancheva and Zhelyazkova-Savova (Gancheva and Zhelyazkova-Savova [25]) showed that MK-7 can

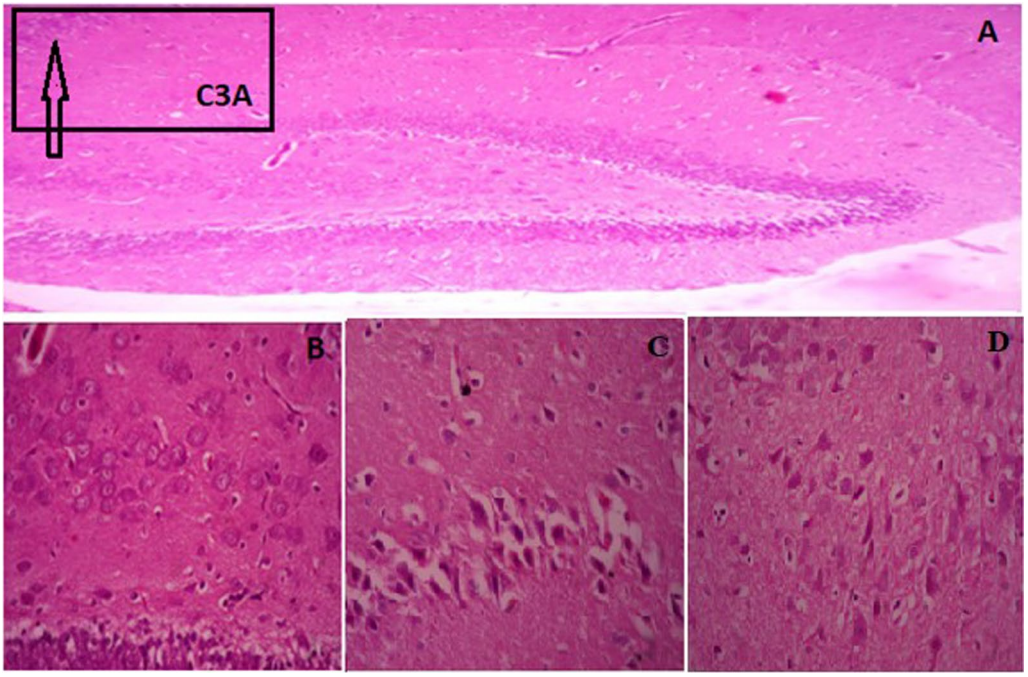

Fig. 7 **A** Photomicrograph: Hematoxylin and eosin staining of section of hippocampus at a magnification of 100 . (**B**, **C**, **D**) Higher magnification of C3A: B) Control group shows normal cells with multiple nuclei and no signs of necrosis or engorged blood vessels. **C** OVX group shows few pyramidal cells with shrunken apoptotic deeply stained nuclei with pericellular vacuolation with engorged blood vessels. **D** K_2OVX group shows mostly normal pyramidal cells, very few deeply stained nuclei as a sign of degeneration. H&E × 400

improve the mood and anxiety-like behavior in rats with metabolic syndrome by improving the glycemic profile.

In our study, the OVX rats showed a significant decline in T maze scores compared to the sham-operated groups. However, the OVX rats that received MK-7 had a significantly higher score than the group not received it. These findings indicate that ovariectomy resulted in a decline in working memory and that MK-7 minimized this decline.

Similarly, Djiouge et al. (Djiogue et al. [15])and Egashira et al. (Egashira et al. [9])showed impairment of short-term, long-term, and spatial memory after ovariectomy as estrogen is necessary for normal hippocampal functions. Moreover, Adu-Nti et al. (Rebar et al. [36])proved that short-term memory disturbance is a common finding following ovariectomy and is associated with mood disorders. In addition, Zhao et al. (Zhao et al. [47])and Tao et al. (Tao et al. [48])proved that ovariectomy leads to memory decline for a short and long duration after the procedure. Controversy, a previous study did not prove any effect of MK-7 on the memory of rats (Gancheva and Zhelyazkova-Savova [25]).

The data of the present study could be explained by apoptosis occurring in the brain after ovariectomy. Following ovariectomy, there is a downregulation of NADH dehydrogenase ubiquinone oxidoreductase subunit A11 (NDUFA11) in the mitochondrial respiratory complex I (Andrews et al. [49]). Moreover, adenylate kinase2 (AK2), was slightly decreased in the hippocampus leading to neuronal apoptosis (Peng et al. [50]). In addition, hexokinase binding to the outer mitochondrial membrane and inhibiting BAX-induced cytochrome c release and apoptosis was down-regulated in the hippocampus after ovariectomy (Azoulay-Zohar et al. [51]).

The dopaminergic-like action of estrogen (E2) and its involvement in dopamine metabolism is also a common cause of disturbed brain function. E2 can modulate dopamine function through estrogen receptors (ER α and ER β) in pre- and postsynaptic membranes. E2 deficiency following ovariectomy could lead to disturbed dopamine function and metabolism which affect sociability and exploratory behavior in animals (Nadal et al. [52]; Morgan et al. [53]). Recently, estradiol had been proven to increase tryptophan hydroxylase-2 and serotonin transporter expression but reduce serotonin 1A receptor expression. In addition, E2 could reduce the expression of monoamine oxidase A and B and modulate the function of serotonin, which can protect women against depression (Hernández-Hernández et al. [54]).

Moreover, E2 has a rapid action on phosphatidylinositol 3-kinase (PI3 K) and extracellular signal-regulated

kinase (ERK) (Fernandez et al. [55]). It stimulates the synthesis of mTOR proteins (mammalian target of rapamycin) that regulate intercellular signaling. There is also an interaction between ERs and N-methyl D-aspartate (NMDA) receptors at the neuron plasma membrane and synaptic terminals that plays a role in the regulation of the function of excitatory chemical transmitters (Boulware et al. [56]).

Moreover, progesterone deficiency in ovariectomy leads to a decrease in allopregnanolone (ALLO) which regulates dopamine and $GABA_{(a)}$receptors involved in normal sociability behavior (Guo et al. [57]). ALLO as a metabolite of progesterone has a stimulatory effect on $GABA_{(A)}$receptors producing barbiturate-like effects and acting as an anxiolytic, anticonvulsant, and sedative/hypnotic (Belelli and Lambert [58]; Fujii et al. [59]). In addition, the increase in LH hormone levels occurring in menopause is associated with an increased plasma level of $amyloid_{B1\text{-}40}$ and $amyloid_{B1\text{-}42}$that leads to impaired memory function (Bhatta et al. [60]).

In this line, some hippocampal oxidative markers were measured in our study, and the level of MDA activity and hydrogen peroxide was significantly higher in the OVX group, but catalase and total antioxidant state were significantly lower. However, the intake of MK-7 in the OVX rats led to a significant improvement in these parameters. This approves the role of MK-7 as an antioxidant agent that helps as a mechanism for decreasing apoptosis. In accordance with our results, MK-7 supplementation protected the astrocytes from hypoxic damage and reduced ROS levels (Yang et al. [61]).

Furthermore, gene expression analysis for BAX, BCL2, and P53 in the hippocampus was performed in our study. The OVX group showed a significant increase in BAX, BAX/BCL2 ratio, and P53 gene expression, but the BCL2 was significantly decreased compared to the sham-operated groups, while the OVX rats that received MK-7 showed a significant improvement in this apoptotic profile.

In agreement with our results, previous studies demonstrated similar results by using the western blot technique in the hippocampus of OVX rats for assessing the apoptotic markers, and the result was increased BAX, P53, and decreased BCL2 compared to the sham-operated group (Sharma and Mehra [62]; Sales et al. [63]; Fang et al. [64]).

Few studies discussed the apoptotic and anti-apoptotic effects of MK-7. A previous study supported our findings by proving that MK-7 protects the neural cells against amyloid B (AB) apoptosis in Alzheimer's disease. Moreover, MK-7 favors the expression of the Gas gene and carboxylation of Gas residues in the brain, which is a vitamin K-dependent protein and necessary for cognitive functions (Huang et al. [65]).

On the other hand, Duan et al. (Duan et al. [66]) reported that MK-7 induces apoptosis and apoptotic genes like BAX while decreasing BCL2 in human cancer cells but not in normal cells and stated that the release of ROS is necessary for this mechanism. Tokita et al. (Tokita et al. [67])showed the same finding in gastric cancer, while the mechanism is not understood. Nishimaki et al. (Nishimaki et al. [68])and Miyazawa et al. (Miyazawa et al. [69]) proved that MK-7 supplementation in leukemia and cancer patients increases BAX and decreases BCL2. We could explain this controversy by saying that MK-7 has both apoptotic and anti-apoptotic effects, and its main role is the regulation of the cell cycle.

5 Conclusions

In our study, we showed that ovariectomy results in significant anxiety, depressive-like behavior, and working memory deterioration, and the intake of MK-7 can prevent these complications. It seems that MK-7 has an anti-apoptotic function, and this could be the underlying mechanism, since MK-7 reduces the apoptotic and increases the anti-apoptotic genes' expression. However, further studies are recommended to detect other possible mechanisms.

Abbreviations

OVX	Ovariectomy
MK-7	Menaquinone-7
FSH	Follicle-stimulating hormone
LH	Luteinizing hormone
PBS	Phosphate-buffered saline
OF	Open field
MFST	Modified forced swim test
ELISA	Enzyme-linked immunosorbent assay
E2	Estradiol
MDA	Malondialdehyde
TAC	Total antioxidant capacity
ANOVA	One-way analysis of variance

Acknowledgements
The authors are thankful to the members of the Physiology Animal Behavior Laboratory, Faculty of Medicine, Zagazig University.

Author contributions
SWM and SAS were responsible for conception and revision, and SAS, SH, and ERA were responsible for interpretation and analysis of data. SAS and SH wrote the manuscript that was revised and approved by all coauthors.

Funding
Not applicable.

Availability of data and materials
Available upon a reasonable request.

Declarations

Ethics approval and consent to participate
The experimental protocol was approved by the Physiology Department and by the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC/3/F/42/2019).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 24 September 2022 Accepted: 1 February 2023
Published online: 19 February 2023

References

1. Burger HG, Dudley EC, Hopper JL, Shelley JM, Green A, Smith A, Dennerstein L, Morse C (1995) The endocrinology of the menopausal transition: a cross-sectional study of a population-based sample. J Clin Endocrinol Metab 80(12):3537–3545
2. Hogervorst E, Bandelow S (2009) Brain and cognition. Is there any case for improving cognitive function in menopausal women using estrogen treatment? Min Ginecol 61(6):499–515
3. Reed SD, Lampe JW, Qu C, Copeland WK, Gundersen G, Fuller S, Newton KM (2014) Premenopausal vasomotor symptoms in an ethnically diverse population. Menopause 21(2):153–158
4. Koothirezhi R, Ranganathan S (2021) Postmenopausal syndrome. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC.
5. Yoshida T, Takahashi K, Yamatani H, Takata K, Kurachi H (2011) Impact of surgical menopause on lipid and bone metabolism. Climacteric 14(4):445–452
6. Miller VM, Lahr BD, Bailey KR, Heit JA, Harman SM, Jayachandran M (2016) Longitudinal effects of menopausal hormone treatments on platelet characteristics and cell-derived microvesicles. Platelets 27(1):32–42
7. Baker FC, de Zambotti M, Colrain IM, Bei B (2018) Sleep problems during the menopausal transition: prevalence, impact, and management challenges. Nat Sci Sleep 9(10):73–95
8. Puga-Olguín A, Rodríguez-Landa JF, Rovirosa-Hernández MJ, Germán-Ponciano LJ, Caba M, Meza E, Guillén-Ruiz G, Olmos-Vázquez OJ (2019) Long-term ovariectomy increases anxiety- and despair-like behaviors associated with lower Fos immunoreactivity in the lateral septal nucleus in rats. Behav Brain Res 15(360):185–195
9. Egashira N, Akiyoshi Y, Iba H, Arai T, Hatip-Al-Khatib I, Mishima K, Iwasaki K (2018) Tokishakuyakusan ameliorates spatial memory deficits induced by ovariectomy combined with β-amyloid in rats. J Pharmacol Sci 136(3):149–154
10. Sarrel PM, Sullivan SD, Nelson LM (2016) Hormone replacement therapy in young women with surgical primary ovarian insufficiency. Fertil Steril 106(7):1580–1587
11. Freeman EW, Sammel MD, Lin H (2009) Temporal associations of hot flashes and depression in the transition to menopause. Menopause 16(4):728–734
12. Bromberger JT, Epperson CN (2018) Depression during and after the perimenopause: impact of hormones, genetics, and environmental determinants of disease. Obstet Gynecol Clin North Am 45(4):663–678
13. Faubion SS, Kuhle CL, Shuster LT, Rocca WA (2015) Long-term health consequences of premature or early menopause and considerations for management. Climacteric 18(4):483–491
14. Karishma KJ, Sahni RK, Singh A, Grewal S (2020) Effect of menopause on cognition in post-menopausal women. Brain Disord Ther 9:257
15. Djiogue S, Djiyou Djeuda AB, Seke Etet PF, Ketcha Wanda GJM, Djikem Tadah RN, Njamen D (2018) Memory and exploratory behavior impairment in ovariectomized Wistar rats. Behav Brain Funct 14(1):14
16. Zemla R, Basu J (2017) Hippocampal function in rodents. Curr Opin Neurobiol 43:187–197
17. Schwalfenberg GK (2017) Vitamins K1 and K2: the emerging group of vitamins required for human health. J Nutr Metab 2017:6254836
18. Zhang Y, Weng S, Yin J, Ding H, Zhang C, Gao Y (2017) Vitamin K2 promotes mesenchymal stem cell differentiation by inhibiting miR-133a expression. Mol Med Rep 15(5):2473–2480
19. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G (2007) Endocrine regulation of energy metabolism by the skeleton. Cell 130(3):456–469
20. Ferron M, McKee MD, Levine RL, Ducy P, Karsenty G (2012) Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. Bone 50(2):568–575
21. Hadipour E, Tayarani-Najaran Z, Fereidoni M (2020) Vitamin K2 protects PC12 cells against $A\beta_{(1-42)}$ and H_2O_2-induced apoptosis via p38 MAP kinase pathway. Nutr Neurosci 23(5):343–352
22. Farhadi Moghadam B, Fereidoni M (2020) Neuroprotective effect of menaquinone-4 (MK-4) on transient global cerebral ischemia/reperfusion injury in rat. PLoS ONE 15(3):e0229769
23. Rufini A, Tucci P, Celardo I, Melino G (2013) Senescence and aging: the critical roles of p53. Oncogene 32(43):5129–5143
24. Mosconi L, Berti V, Dyke J, Schelbaum E, Jett S, Loughlin L, Jang G, Rahman A, Hristov H, Pahlajani S, Andrews R, Matthews D, Etingin O, Ganzer C, de Leon M, Isaacson R, Brinton RD (2021) Menopause impacts human brain structure, connectivity, energy metabolism, and amyloid-beta deposition. Sci Rep 11(1):10867
25. Gancheva SM, Zhelyazkova-Savova MD (2016) Vitamin K2 improves anxiety and depression but not cognition in rats with metabolic Syndrome: a role of blood glucose? Folia Med 58(4):264–272
26. Auta T, Hassan AT (2016) Alteration in oestrus cycle and implantation in Mus musculus administered aqueous wood ash extract of Azadirachta indica (neem). Asian Pacific J Reprod 5(3):188–192
27. Ajayi AF, Akhigbe RE (2020) Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil Res Pract 14(6):5
28. Briones-Aranda A, Castellanos-Pérez M, Villa VMV, Picazo O (2020) Impact of exposure to environmental enrichment on the anxiety-like behavior of ovariectomized mice. Iran J psychiatry 15(1):88–95
29. Deacon RM (2006) Housing, husbandry and handling of rodents for behavioral experiments. Nat Protoc 1(2):936–946
30. Slattery DA, Cryan JF (2012) Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat Protoc 7(6):1009–1014
31. Deacon RM, Rawlins JN (2006) T-maze alternation in the rodent. Nat Protoc 1(1):7–12
32. Rein B, Ma K, Yan Z (2020) A standardized social preference protocol for measuring social deficits in mouse models of autism. Nat Protoc 15(10):3464–3477
33. Chiu K, Lau WM, Lau HT, So KF, Chang RC (2007) Micro-dissection of rat brain for RNA or protein extraction from specific brain region. J Vis Exp 7:269
34. Delrobaei F, Fatemi I, Shamsizadeh A, Allahtavakoli M (2019) Ascorbic acid attenuates cognitive impairment and brain oxidative stress in ovariectomized mice. Pharmacol Rep 71(1):133–138
35. Tietz NW, Cook T, Mc-Niven MA (1995) Clinical guide to laboratory tests, 3rd edn. Pbl WB Saunders, Co, Philadelphia, pp 509:12.
36. Rebar RW, Morandini IC, Petze JE, Erickson GF (1982) Hormonal basis of reproductive defects in athymic mice: reduced gonadotropins and testosterone in males. Biol Reprod 27(5):1267–1276
37. Adu-Nti F, Gao X, Wu JM, Li J, Iqbal J, Ahmad R, Ma XM (2021) Osthole ameliorates estrogen deficiency-induced cognitive impairment in female mice. Front Pharmacol 6(12):641909
38. Kaidanovich-Beilin O, Lipina T, Vukobradovic I, Roder J, Woodgett JR (2011) Assessment of social interaction behaviors. J Vis Exp 48:2473
39. Leite IS, Castelhano AS, Cysneiros RM (2016) Effect of diazepam on sociability of rats submitted to neonatal seizures. Data Brief 12(7):686–691
40. Adu-Nti F, Ghartey-Kwansah G, Aboagye B (2019) Sex differences in the antidepressant effects of ketamine in animal models of depression. Int J Depress Anxiety 2:013
41. Renczés E, Borbélyová V, Steinhardt M, Höpfner T, Stehle T, Ostatníková D, Celec P (2020) The role of estrogen in anxiety-like behavior and memory of middle-aged female rats. Front Endocrinol 7(11):570560

42. Vega Rivera NM, Gallardo Tenorio A, Fernández-Guasti A, Estrada CE (2016) The post-ovariectomy interval affects the antidepressant-like action of citalopram combined with ethynyl-estradiol in the forced swim test in middle aged rats. Pharmaceuticals 9(2):21
43. Khayum MA, Moraga-Amaro R, Buwalda B, Koole M, den Boer JA, Dierckx R et al (2020) Ovariectomy-induced depressive-like behavior and brain glucose metabolism changes in female rats are not affected by chronic mild stress. Psychoneuroendocrinology 115:104610
44. Wu B, Song Q, Zhang Y, Wang C, Yang M, Zhang J, Han W, Jiang P (2020) Antidepressant activity of ω-3 polyunsaturated fatty acids in ovariectomized rats: role of neuroinflammation and microglial polarization. Lipids Health Dis 19(1):4
45. Banin RM, Machado MMF, de Andrade IS, Carvalho LOT, Hirata BKS, de Andrade HM, Júlio VDS, Ribeiro JSFB, Cerutti SM, Oyama LM, Ribeiro EB, Telles MM (2021) Ginkgo biloba extract (GbE) attenuates obesity and anxious/depressive-like behaviours induced by ovariectomy. Sci Rep 11(1):44
46. Estrada-Camarena E, López-Rubalcava C, Hernández-Aragón A, Mejía-Mauries S, Picazo O (2011) Long-term ovariectomy modulates the antidepressant-like action of estrogens, but not of antidepressants. J Psychopharmacol 25(10):1365–1377
47. Zhao H, Niu Q, Li X, Liu T, Xu Y, Han H, Wang W, Fan N, Tian Q, Zhang H, Wang Z (2012) Long-term resveratrol consumption protects ovariectomized rats chronically treated with D-galactose from developing memory decline without effects on the uterus. Brain Res 27(1467):67–80
48. Tao X, Yan M, Wang L, Zhou Y, Wang Z, Xia T, Liu X, Pan R, Chang Q (2020) Effects of estrogen deprivation on memory and expression of related proteins in ovariectomized mice. Ann Transl Med 8(6):356
49. Andrews B, Carroll J, Ding S, Fearnley IM, Walker JE (2013) Assembly factors for the membrane arm of human complex I. Proc Natl Acad Sci U S A 110(47):18934–18939
50. Peng Y, Jiang B, Wu H, Dai R, Tan L (2012) Effects of genistein on neuronal apoptosis, and expression of Bcl-2 and Bax proteins in the hippocampus of ovariectomized rats. Neural Regen Res 7(36):2874–2881
51. Azoulay-Zohar H, Israelson A, Abu-Hamad S, Shoshan-Barmatz V (2004) In self-defence: hexokinase promotes voltage-dependent anion channel closure and prevents mitochondria-mediated apoptotic cell death. Biochem J 377(Pt 2):347–355
52. Nadal A, Ropero AB, Laribi O, Maillet M, Fuentes E, Soria B (2000) Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor alpha and estrogen receptor beta. Proc Natl Acad Sci U S A 97(21):11603–11608
53. Morgan MA, Schulkin J, Pfaff DW (2004) Estrogens and non-reproductive behaviors related to activity and fear. Neurosci Biobehav Rev 28(1):55–63
54. Hernández-Hernández OT, Martínez-Mota L, Herrera-Pérez JJ, Jiménez-Rubio G (2019) Role of estradiol in the expression of genes involved in serotonin neurotransmission: implications for female depression. Curr Neuropharmacol 17(5):459–471
55. Fernandez SM, Lewis MC, Pechenino AS, Harburger LL, Orr PT, Gresack JE, Schafe GE, Frick KM (2008) Estradiol-induced enhancement of object memory consolidation involves hippocampal extracellular signal-regulated kinase activation and membrane-bound estrogen receptors. J Neurosci 28(35):8660–8667
56. Boulware MI, Heisler JD, Frick KM (2013) The memory-enhancing effects of hippocampal estrogen receptor activation involve metabotropic glutamate receptor signaling. J Neurosci 33(38):15184–15194
57. Guo Q, Ebihara K, Fujiwara H, Toume K, Awale S, Araki R, Yabe T, Dong E, Matsumoto K (2019) Kami-shoyo-san ameliorates sociability deficits in ovariectomized mice, a putative female model of autism spectrum disorder, via facilitating dopamine D(1) and GABA(A) receptor functions. J Ethnopharmacol 23(236):231–239
58. Belelli D, Lambert JJ (2005) Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 6(7):565–575
59. Fujii M, Ohgami S, Asano E, Nakayama T, Toda T, Nabe T, Ohya S (2019) Brain allopregnanolone induces marked scratching behaviour in diet-induced atopic dermatitis mouse model. Sci Rep 9(1):2364
60. Bhatta S, Blair JA, Casadesus G (2018) Luteinizing hormone involvement in aging female cognition: not all is estrogen loss. Front Endocrinol (Lausanne) 24(9):544
61. Yang RY, Pan JY, Chen Y, Li Y, Wu J, Wang XD (2020) Menaquinone-7 protects astrocytes by regulating mitochondrial function and inflammatory response under hypoxic conditions. Eur Rev Med Pharmacol Sci 24(19):10181–10193
62. Sharma K, Mehra RD (2008) Long-term administration of estrogen or tamoxifen to ovariectomized rats affords neuroprotection to hippocampal neurons by modulating the expression of Bcl-2 and Bax. Brain Res 14(1204):1–15
63. Sales S, Ureshino RP, Pereira RT, Luna MS, Pires de Oliveira M, Yamanouye N, Godinho RO, Smaili SS, Porto CS, Abdalla FM (2010) Effects of 17 beta-estradiol replacement on the apoptotic effects caused by ovariectomy in the rat hippocampus. Life Sci 86(21–22):832–838
64. Fang YY, Zeng P, Qu N, Ning LN, Chu J, Zhang T, Zhou XW, Tian Q (2018) Evidence of altered depression and dementia-related proteins in the brains of young rats after ovariectomy. J Neurochem 146(6):703–721
65. Huang SH, Fang ST, Chen YC (2021) Molecular mechanism of vitamin K2 protection against amyloid-β-induced cytotoxicity. Biomolecules 11(3):423
66. Duan F, Yu Y, Guan R, Xu Z, Liang H, Hong L (2016) Vitamin K2 induces mitochondria-related apoptosis in human bladder cancer cells via ROS and JNK/p38 MAPK signal pathways. PLoS ONE 11(8):e0161886
67. Tokita H, Tsuchida A, Miyazawa K, Ohyashiki K, Katayanagi S, Sudo H, Enomoto M, Takagi Y, Aoki T (2006) Vitamin K2-induced antitumor effects via cell-cycle arrest and apoptosis in gastric cancer cell lines. Int J Mol Med 17(2):235–243
68. Nishimaki J, Miyazawa K, Yaguchi M, Katagiri T, Kawanishi Y, Toyama K, Ohyashiki K, Hashimoto S, Nakaya K, Takiguchi T (1999) Vitamin K2 induces apoptosis of a novel cell line established from a patient with myelodysplastic syndrome in blastic transformation. Leukemia 13(9):1399–1405
69. Miyazawa K, Yaguchi M, Funato K, Gotoh A, Kawanishi Y, Nishizawa Y et al (2001) Apoptosis/differentiation-inducing effects of vitamin K2 on HL-60 cells: dichotomous nature of vitamin K2 in leukemia cells. Leukemia 15(7):1111–1117

Publisher's Note