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ORIGINAL ARTICLE

The effect of Panax Ginseng on dentate gyrus of ovariectomized albino rats. Histological and Immunohistochemical study

Zedan. omyma ibraheem[1]* and Abd Ellatif. Rasha Abdelaziz[2]
[1]*Anatomy Department, Faculty of Medicine Menoufia University, Egypt
[2]Anatomy Department, Faculty of Medicine Tanta University, Egypt

***Corresponding Author:** Zedan. Omyma Ibraheem
Anatomy Department, Faculty of Medicine Menoufia University
draomymazidan@hotmail.com

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ABSTRACT

Background: Cognitive impairments are common finding with aging particularly in postmenopausal women. Dentate gyrus is the most specialized region in the hippocampus being involved in formation of new memories and neurogenesis. Panax Ginseng is a promising natural compound having many beneficial effects, including anti-inflammatory, antioxidant, antiaging activities. The aim of the current study was to explore the effect of ovariectomy on structure of dentate gyrus and the possible protective role of Panax Ginseng

Methods: 25 adult female Albino rats were divided into three groups control group subdivided into 3 subgroups, ovariectomized (OVX) group and OVX plus Ginseng treated group. Hippocampal sections were subjected to biochemical, histological morphometric and immunohistochemical studies

Results: ovariectomized rats showed significant increase in their weight and decrease in serum estradiol level. Significant increase in malondialdehyde (MDA) with significant decrease in reduced glutathione (GSH) and catalase. Dentate gyrus of ovariectomized rats showed degeneration of granular layer with significant decrease in its thickness. Significant increase in expression of caspase-3, inducible nitric oxide synthase(iNOS), Glial fibrillary acidic protein (GFAP), and significant decrease in, Microtubule-Associated Protein (MAP2), synaptophysin and Ki 67 immunoreactivity were detected. Treatment of ovariectomized rats with Panax Ginseng induced significant reduction in weight and MDA with significant increase in estradiol level, GSH and catalase. Significant decrease in expression of caspase 3, (iNOS), (GFAP) and increase (MAP2), synaptophysin and Ki 67 expression with improvement of the histopathological changes

Conclusions: Panax Ginseng may serve as a promising therapeutic substitute in amelioration of menopausal related cognitive affection

Keywords: Panax Ginseng, Ovariectomy, Dentate gyrus, MAP 2, Neurogenesis

INTRODUCTION

Cognitive impairment is a common finding in both men and women as age advances, this decline in cognitive ability is more obvious in women after menopause. This may be attributed to drop in estrogen level [1].

Dentate gyrus is the most specialized area in the hippocampal formation of limbic system being involved in the formation of new memories and one of the important areas of the brain where neurogenesis takes place [2].

Middle-aged ovariectomized rats were previously used as a model of hormonal deprivation to study memory, behavioral and structural changes related to menopause [3]. One of these hormones is estrogen which has many important functions. Estrogen blocks reactive oxygen species (ROS) formation, lipid peroxidation, oxidative stress and improves the expression of endogenous antioxidant enzymes. Moreover, estrogen deprivation is responsible for some central nervous system diseases such as brain ischemia and Alzheimer's disease [4].

On the other hand hormone replacement therapy (HRT) with female hormones is indicated to overcome various health problems after menopause but recent studies have indicated that (HRT) may have drawbacks on women's health by increasing risk of, blood clots, stroke, heart attack and thromboembolism [5]. Therefore, alternative options as phytoestrogens and nutritional supplements are being investigated as a substitution to HRT.

Ginseng, an Asian traditional herb, used for

different restorative purposes for human health, commonly available in different forms‟ steamed root powder (red ginseng), whole root, root powder (white ginseng), steamed and dried roots (black ginseng), heat processed root powder (sun ginseng) [6].

Panax Ginseng Meyer commonly named Korean red ginseng, a widely used medicinal herbs mainly grown in Korea and China, used in treating many diseases including neurodegenerative disorders and in improving cognitive functions [7].

The main active components of Panax Ginseng are ginsenosides particularly(G-Rg1) these were reported to have many beneficial effects, including anti-inflammatory, antioxidant, anticancer, antimutagenic, antiaging activities, immunomodulatory and adaptogen actions [8].

[9] Reported that ginseng has estrogenic activity, mediated by boosting the biosynthesis of estrogen in circulation and elevating the ERs quantity in the target organs. Confirming that ginseng through its action as an estrogen agonist can treat postmenopausal symptoms.

Regarding menopausal problems and the side effects of the (HRT) the aim of the present study is to investigate the role of panax ginseng in alleviating the deterioration of functions of dentate gyrus.

MATERIALS AND METHODS

Experimental animals:

Twenty-five adult female Albino rats aged 3–4 months with an average weight of 180-200g were used in this study. The experiment was carried out in animal house present in Faculty of Medicine, Menoufia University. The rats were left for 7 days for acclimatization before use in the experiment with food and water were provided ad libitum. Animal care and treatments were administrated according to the guidelines of the Animal Ethics Committee. All animal experiments comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals.

Drugs and Chemicals:

Highly standardized Ginseng extract G115 (100mg) (corresponding to 500mg of ginseng root made from roots of best quality of genuine Panax Ginseng C.A. Meyer) Excip. Q.S.ad (650mg) in the form of capsule was used. It was purchased from Cornell lab company (Cairo- Egypt). The used dose was dissolved in distilled water for oral administration.

Animal grouping and Experiment design:

Twenty-five adult female Albino rats ***were randomly divided into the following groups each of 5 rats:***

1-Group I (control group) included 15 rats which was subdivided into

- Subgroup (1-a) 5 rats were kept without any treatment throughout the experimental period.
- Subgroup (1-b) 5 rats (sham vehicle group) received ginseng, (200 mg/kg) every day by gastric tube once daily.
- Subgroup (1-c) 5 rats (sham operated group) used to explore the ovaries without ligation or crushing.

2- **Group 2: (OVX group)** included 5 rats that were subjected to bilateral ovariectomy operation and left without treatment during experimental period.

3- **Group 3 (OVX + Ginseng group)**: in this group 5 Rats were bilaterally ovariectomized and were given Panax Ginseng ((200 mg/kg/ day) **[10]**. It was dissolved in distilled water and was given by gastric tube once daily two weeks after the ovariectomy operation for 8 weeks. No deaths were recorded in all groups during whole experiment.

All experimental rats were sacrificed at the end of the experiment.

Surgical procedures:

Rats of operative groups were anesthetized with intraperitoneally injection of ketamine hydrochloride 100 mg/kg body weight) and xylazine (10 mg/kg) and underwent a bilateral ovariectomy (OVX). A median abdominal incision was performed to identify the right and left corns uteri and their corresponding ovaries. After suturing the vascular plexus with fine linen thread, both ovaries were removed, followed by closure of the abdominal cavity. Upon recovery from anesthesia, rats were individually housed in ventilated cages one week for recovery from surgery. Postoperative care included the administration of analgesics 10mg/kg) kataflam by intramuscular injection to relief pain and antibiotics flomox 500mg (in dose 10mg/kg body weight) by intramuscular injection for 3days to avoid 2ry bacterial infection **[11].**

Samples Collection

The rats were anesthetized by diethyl ether and sacrificed at the end of the experiment.

Blood samples were collected from the retro-orbital vein in non-heparinized capillary tubes for serum separation to estimate estrogen levels. Serum estradiol levels were estimated using enzyme-linked immunosorbent assay (ELISA) technique **[12].** Estradiol level was expressed as (pg./ml)

The whole brain of each rat was rapidly dissected and washed with isotonic saline. Each brain was sagittally divided into two portions, the hippocampus was gently dissected from the brain, one side was collected for histological study and the other side was collected for biochemical

analysis

Tissue biochemical study

Hippocampus of each rat was removed and rinsed with phosphate-buffered saline (pH 7.4) to remove red blood cells and clots and was homogenized in cold saline (10% w/v) and was kept in − 80 C deep freezer until the estimation of the following markers of oxidative stress:

Determination of malondialdehyde (MDA) level:

Lipid peroxidation represented by malondialdehyde (MDA) was assessed in hippocampal homogenate was assessed according to the method of **[13]** by using for each 0.5 ml homogenate,3 ml of the 1% O-Phosphoric acid solution and 1 ml of the 0.6% thiobarbituric acid (TBA) solution were added, mixed then placed in a boiling water bath for 45 min. After cooling, the pink color formed is extracted by n-butanol and detected at 2 wave lengths (535 and 520) nm and absorbance difference was calculated. MDA levels were expressed as nanomoles per gram tissue (nmol/g tissue protein).

Determination of GSH level:

The reduced glutathione (GSH) of rat hippocampus was determined by the methods of **[14]** using Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid (DNTB)) that reacts with GSH resulting in a product that can be measured at 412 nm**.** GSH levels were expressed as µmol/g tissue.

Determination of Catalase activity:

Catalase (CAT) activity of rat hippocampus was assayed by the method of **[15].** The assay is based on catalase–catalyzed reaction of a known quantity of H2O2 with 3,5-dichloro-2-hydroxy-benzene sulfonic acid DHBS and with 4-aminophenazone (AAP) to form a chromophore, which has a color intensity inversely proportional to the amount of catalase in the original sample which can be measured at 510 nm. Catalase activity was expressed as U/g tissue.

Histological study:

Specimens obtained from each animal were fixed in 10% formalin and processed. Paraffin sections 5 micron in thickness were obtained and were stained by hematoxylin and eosin (H&E**)** and with toluidine blue (TB) to detect Nissl's granules **[16]**

Immunohistochemical study:

Paraffin sections were deparaffinized in xylene then rehydrated in descending grades of ethanol. Then put for 5 minutes in distilled water. Activity of endogenous peroxidase was blocked using 1% hydrogen peroxide for 30 minutes. The slides were washed in phosphate buffered saline (PBS). Antigen retrieval was done by boiling slides in citrate buffer solution (pH 6.0) in a microwave for 20 min. Then, sections were incubated in blocking solution (goat serum) to prevent the nonspecific background staining. The sections were incubated with the following primary anti-body Caspase-3 (Cat. # RB-1197-R7), inducible nitric oxide synthase (iNOS) (Cat. #RB-9242-R7), Glial fibrillary acidic protein (GFAP) (Cat. #MS-280-R7) Microtubule-Associated Protein (MAP2) (Cat#MS-250-P0), synaptophysin (Cat. # RB-SY028-02) and ki67(Cat. # Rabbit Pab NO. A16919) for an hour at room temperature. Sections were rinsed with PBS, followed by 20 min of incubation at room temperature with secondary biotinylated antibody and then washed again in PBS, the sections were incubated with Streptavidin peroxidase complex for 10 min. Secondary antibody binding was visualized using 3,3-diaminobenzoicacid (DAB) to visualize antibody immunostaining areas. Finally, sections were rinsed with PBS, counterstained by hematoxylin and were washed by distilled water. At last, the slides were dehydrated, cleared then covered [16]**.**

Morphometric study:

Five randomly chosen sections stained with H&E and the immunostained (400×) from five rats from every group were analyzed for:

2.9.1. The thickness of the granular layer

2.9.2. The color intensity of toluidine blue

2.9.3. Area percentage of Caspase-3, iNOS, synaptophysin, GFAP and MAP2

2.9.4. Number of ki67 immunopositive cells

2.10. Statistical analysis

The collected data analysis was analyzed using SPSS program for windows version 23 (Inc., Chicago, IL, USA) and were presented as mean ± SD. The acquired data were analyzed by using one way-ANOVA followed by post hoc Bonferroni test. A *P*-value < 0.05 was considered statistically significant **[17].**

RESULTS

Effect on weight of rats in studied groups

There was a highly significant increase in weight of ovariectomized rats in OVX group when compared to control group (194.16 ± 2.73 vs 181.35 ± 2.88) (P< 0.001). On the other hand, a significant reduction of weight of rats in OVX plus Panax Ginseng treated group was noticed when compared to OVX group (183.65 ± 3.61 vs 194.16 ± 2.73) (P< 0.001). (Histogram 1A) (Table1).

Effect on serum estradiol level in rats of studied groups

OVX group showed a highly significant reduction in estrogen level when compared with control group (17.89 ± 0.39 vs 32.28 ± 1.91) (P< 0.001). Meanwhile OVX group treated with Panax Ginseng resulted in significant increase in estrogen level when compared to OVX group (30.92 ± 2.46 vs 17.89 ± 0.39) (P< 0.001) (Histogram 1B)

(Table1).

Effect on oxidative stress biomarkers MDA and GSH content in studied groups

Comparing the MDA content, there was a highly significant increase in OVX group when compared to control group (183.11 ± 3.06 vs154.0 ± 3.77) (P<0.001). On the other hand, OVX + Panax Ginseng treated group showed a significant reduction in MDA content when compared to OVX group (156.67 ± 1.94 vs 183.11 ± 3.06) (P<0.001) (Histogram 1C) (Table1).

GSH content showed a highly significant reduction in OVX group when compared to control (0.91 ± 0.02 vs 1.17 ± 0.05) (P< 0.001). OXV plus Ginseng treated group showed a significant increase in GSH when compared to OVX group (1.12 ± 0.06 vs 0.91 ± 0.02) (P<0.001) (Histogram 1D) (Table1).

Effect of different treatments on Catalase content in studied groups

Comparing the catalase content, a highly significant reduction of catalase content in OVX group when compared to control (4.77 ± 0.17 vs 5.61 ± 0.33) (P<0.001). OXV group treated with Panax Ginseng showed a significant increase in catalase compared to OVX group (5.47 ± 0.43 vs 4.77 ± 0.17) (P<0.001) (Histogram1 E) (Table1).

Histological light microscopic results H&E stain

The hippocampus appeared as a pair of interlocking C-shaped structures, the Cornu Ammonis parts as CA1, CA2, CA3 & CA4 regions and the dentate gyrus (Figure2, A). Dentate gyrus of the control group was formed of three layers, molecular, granular with Granule cells and Polymorphic layer. The molecular layer was a relative cell-free layer containing astrocytes. The granular layer had granule cells with rounded vesicular nuclei. Small spindle shaped stem cells were noticed in the sub-granular zone. Polymorphic layer showed large, dispersed basket cells. (Figures 2B ,3A). Dentate gyrus of OVX group showed apparent decrease in granular cells in granule cell layer, some granule cells of granular layer appeared with distorted nuclei and vacuolated cytoplasm, others showed pyknotic nuclei with vacuolations of their cytoplasm. Areas of cell loss in the sub-granular zone (Figures 2C, 3B), numerous astrocytes in molecular layer could be seen (Figure 2C). Dentate gyrus of OVX + Panax Ginseng group showed increased number of granule cells with preserved normal structure when compared to OVX group (Figures 2D, 3C). Statistically: The thickness of the granular layer was significantly decreased in the ovariectomized group compared with control group (146.09 ± 2.66 vs. 161.91 ± 2.81) (P<0.001). However, it showed a significant increase in ovariectomized group treated with Panax Ginseng when compared with the ovariectomized group (159.82 ± 2.09 vs. 146.09 ± 2.66) (P<0.001) (Figure 2E) (Table2).

Histological light microscopic results Toluidine blue stain

Histological sections of dentate gyrus of control group, revealed granule cell layer with abundant Nissl granules (Figure 4A). On the other hand, dentate gyrus of OVX group, showed less densely packed granule cells with wide inter cellular spaces between cells. Some granule cells are seen with few Nissl granules, the cell margins of damaged granule cells cannot be determined (Figure 4 B). Dentate gyrus of OVX plus panax Ginseng treated group, showed preservation of normal structure of most granule cells, with abundant Nissl granules (Figure 4 C). Comparing the color intensity of Nissl's granules in Toluidine blue sections, there was a highly significant reduction of color intensity of Nissl's granules in OVX group when compared to control group (7.00 ± 1.41 vs 14.50 ± 3.60) (P<0.001), Significant increase in color intensity in OVX + Panax Ginseng treated group when compared to OVX group (13.30 ± 3.02 vs 7.00 ± 1.41) P<0.001 (Figure 4 D) (Table2).

Immunohistochemical expression of Caspase 3

A highly significant increase in caspase-3, apoptosis marker, immunoreaction in OVX group was noticed in granule cell layer, polymorphic and molecular layers of dentate gyrus compared to control group (54.88 ± 2.10 vs 16.29 ± 2.75) (P< 0.001). Significant decrease in caspase 3 immunoreaction in OVX group treated with Panax Ginseng to be localized to some granule cells of dentate gyrus could be detected in comparison to OVX group (34.71 ± 2.29 vs 54.88 ± 2.10) (P< 0.001) (Figure 5 A, B, C, D) (Table2).

. ***Immunohistochemical expression of iNOS***

Inducible nitric oxide synthase (iNOS), oxidative stress marker, revealed a highly significant strong positive immunoreactivity of iNOS of OVX group, when compared to control group (44.29 ± 2.50 vs 8.83 ± 1.72) (P< 0.001). On the other hand a significant decrease iNOS immunoreactivity was noticed in in OVX group treated with Panax Ginseng compared to OVX group(14.38 ± 2.07 vs 44.29 ± 2.50) (P< 0.001)(Figure 5 E, F, G, H)) (Table2).

. ***Immunohistochemical expression of GFAP***

OVX group, showed highly significant increase in GFAP immunoreactivity (astrocyte marker) with increased number and size astrocyte when compared to control group (54.0 ± 2.77 vs 36.29 ± 4.68) (P< 0.001). In comparison OVX + Panax Ginseng group, revealed a significant decrease in GFAP immunoreactivity when compared to OVX group (35.43 ± 3.31vs 54.0 ± 2.77) (P< 0.001). (Figures 6: A, B, C, D) (Table2).

Immunohistochemical expression of Synaptophysin

OVX group, showed highly significant reduction in synaptophysin immunoreactivity (a marker for synaptic plasticity) when compared to control group (25.75 ± 1.67 vs 54.67 ± 2.73) (P < 0.001). On the other hand, treatment of ovariectomized rats with Panax Ginseng resulted in significant increase in synaptophysin expression when compared to OVX group (45.63 ± 1.69 vs 25.75 ± 1.67) (P < 0.001) (Figure 6 E, F, G, H) (Table2).

Immunohistochemical expression of MAP 2

OVX group, showed highly significant reduction in MAP 2 expression (a protein that improves the stability of the dendritic cytoskeleton) when compared to control group (17.00 ± 2.76 vs 41.50 ± 6.89) (P< 0.001). On the other hand, treatment of ovariectomized rats with Panax Ginseng resulted in significant increase in MAP2 immunoreactivity when compared to OVX group (36.83 ± 5.60 vs 17.00 ± 2.76) (P< 0.05). (Figure 7 A, B, C, D) (Table2).

***Immunohistochemical expression of* ki67**

OVX group, showed highly significant reduction in mean number of ki67 positive nuclei (a marker for neurogenesis) when compared to control group (4.36 ± 1.29 vs 21.82 ± 1.78) (P< 0.001). On the other hand treatment of ovariectomized rats with Panax Ginseng resulted in significant increase in mean number of ki67 positive nuclei when compared to OVX group (20.55 ± 1.44 vs 4.36 ± 1.29) (P < 0.05)(Figure 7 E, F, G, H)) (Table2)

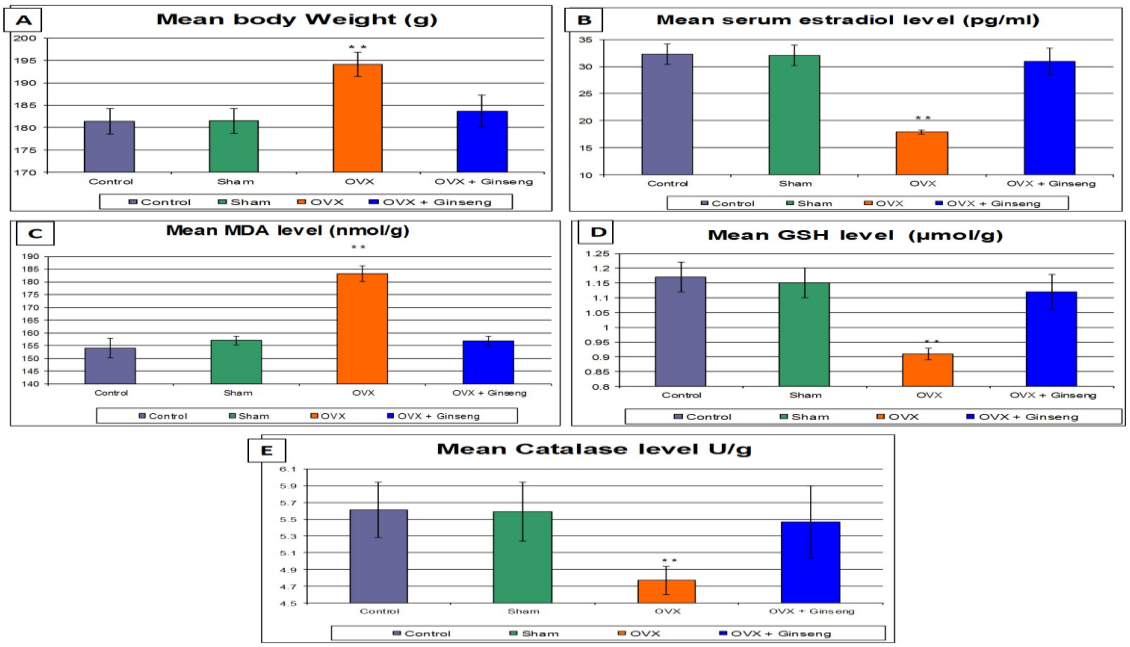

Figure 1 : Histograms showing; A) the mean of body weight (g) , B) the mean of serum estradiol level (pg/ml) C) the mean level of MDA (nmol/g), D) mean level of GSH(µmol/g), E) mean level of Catalase (u/g)of various experimental groups, *P<0.05 is considered significant, **P<0.001 is highly significant.

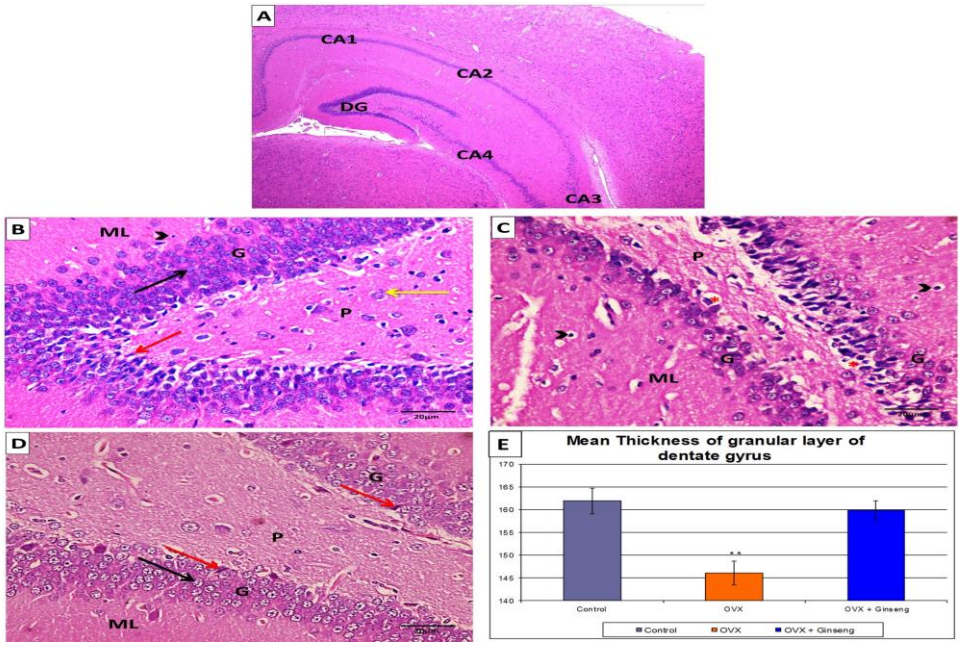

Figure 2: Photomicrographs of the hippocampus and its dentate gyrus showing A) the hippocampus appearing as a pair of interlocking C-shaped structures, the Cornu Ammonis parts as CA1, CA2, CA3 & CA4 regions and the dentate gyrus (DG). B) Dentate gyrus of the control group formed of three layers; molecular (ML), granular cell layer (G) with Granule cells (black arrow), Small spindle shaped stem cells (red arrow) are noticed in the sub-granular zone. Polymorphic layer (P) shows large, dispersed basket cells (Yellow arrow). The molecular layer was a relative cell-free layer containing astrocytes (arrowhead). C) Dentate gyrus of OVX group showing apparent decrease in granular cells in granule cell layer (G), areas of cell loss (*) are noticed in the sub-granular zone. The molecular (ML) layer reveals numerous astrocytes (arrow heads). D) Dentate gyrus of OVX + Ginseng group showing granule cells (black arrow) with rounded vesicular nuclei in the granule cell layer (G). numerous spindle shaped stem cells are detected in the subgranular zone (red arrows). The molecular layer (ML) and the polymorphic layer (P) are noticed. E). Histogram showing mean thickness of granular layer of dentate gyrus. (H &E A x 40 & B, C,D x400).

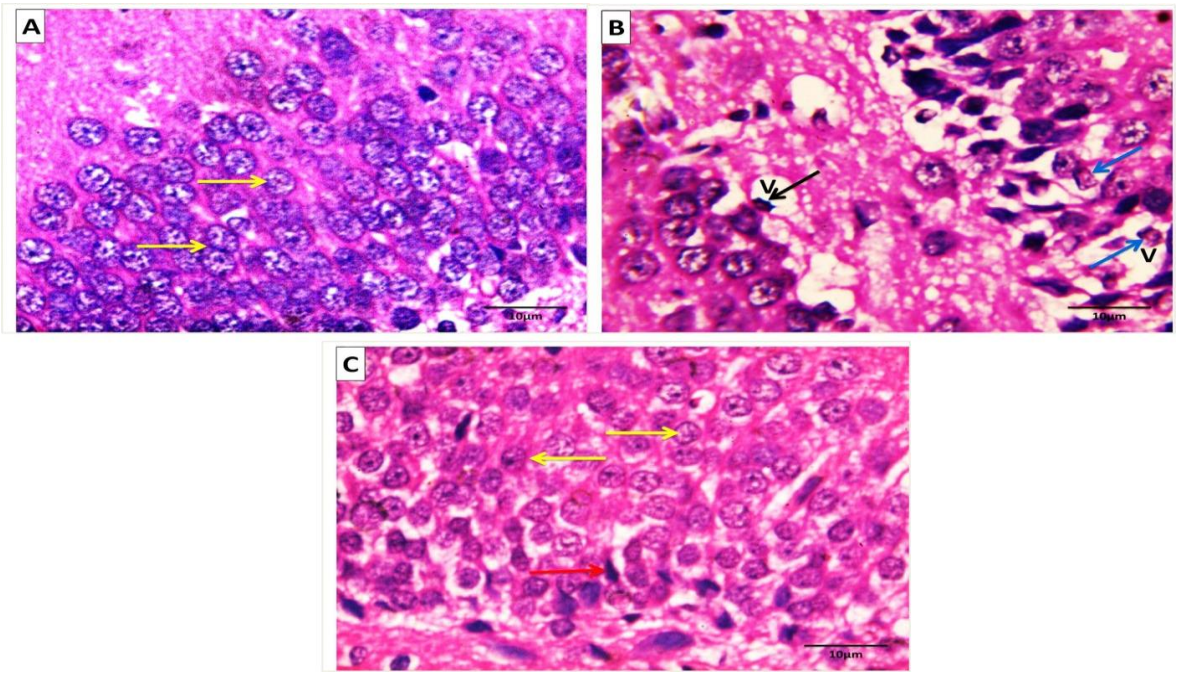

Figure 3: Photomicrographs of higher magnifications of previous sections of dentate gyrus showing A) Control group granule cells (yellow arrows) have rounded vesicular nuclei. B) OVX group, showing some granule cells of granular layer appeared with distorted nuclei (Blue arrows) and vacuolated cytoplasm (V), others showed pyknotic nuclei (Black arrow) with vacuolation (V) of their cytoplasm. C) OVX + Ginseng group, showing apparent normal structure of granule cells (yellow arrows) of granular layer of dentate gyrus.

With prominent spindle shaped stem cells appearing in the subgranular zone (red arrow). (A, B, C. H& E x1000)

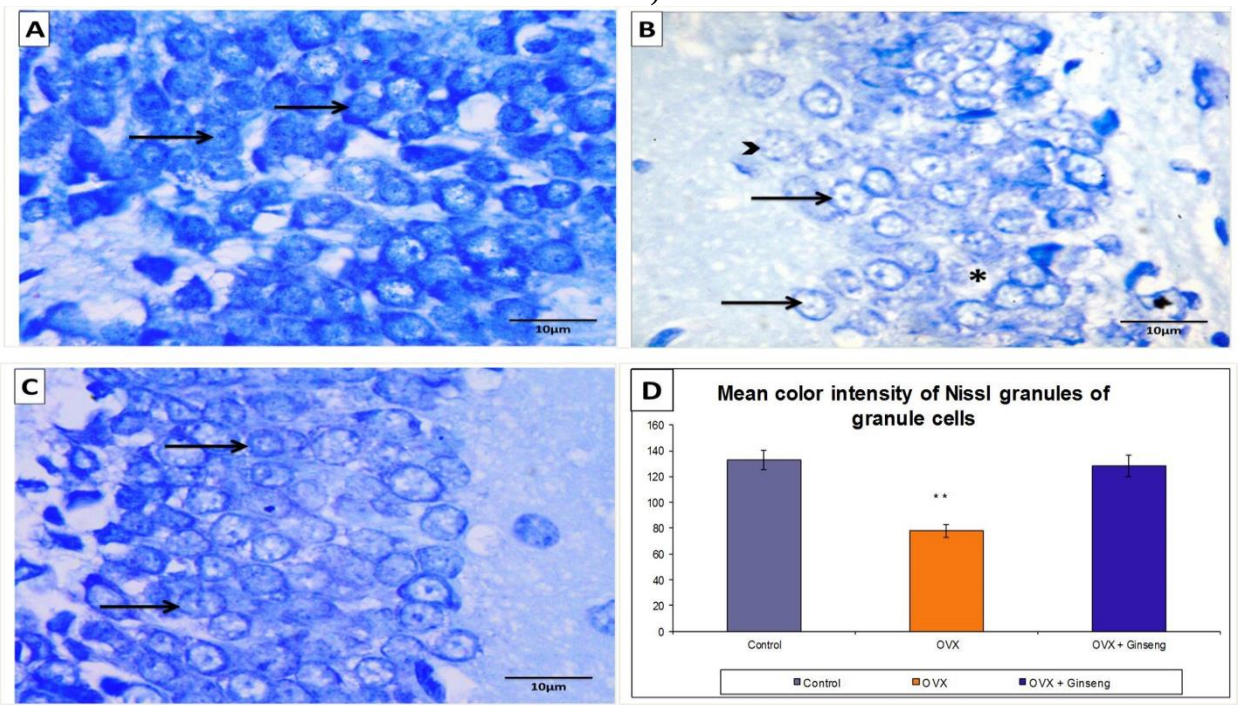

Figure 4: Photomicrographs of dentate gyrus of the hippocampus of adult female rat of the different groups showing A) Dentate gyrus of control group, showing granule cell layer with abundant Nissl`**s** granules (arrows) B) Dentate gyrus of OVX group, showing less densely packed granule cells with wide inter cellular spaces between cells (*). Some granule cells are seen with few Nissl granules (arrows), the Cell margins of damaged granule cells cannot be determined (arrow head).C) Dentate gyrus of OVX + Ginseng group, showing preservation of normal structure of most granule cells, with abundant Nissl granules (arrows); D) Histogram showing mean color intensity of Nissl`s granules in the granule cells Magnifications *P<0.05 is considered significant, **P<0.001 is highly significant. (A, B, C Toluidine blue X 1000).

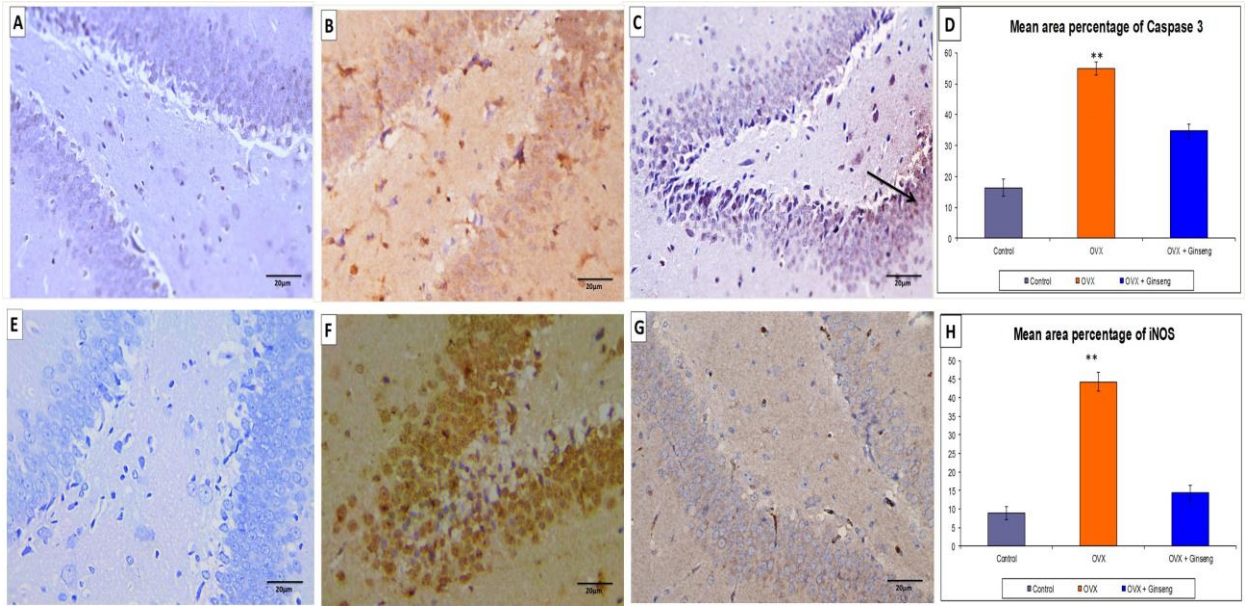

Figure 5: Photomicrographs expression of immunostaining of dentate gyrus of studied groups showing A) control group, showing faint immunoreactivity of caspase 3 localized to granule cell layer of dentate gyrus, B) OVX group, showing strong positive immunoreactivity of caspase 3 expression in granule cell layer, polymorphic and molecular layers of dentate gyrus. C) OVX + Ginseng group, showing weak immunoreaction localized to some granule cells of dentate gyrus (black arrow). E) control group, negative immunoreactivity of iNOS in dentate gyrus; F) OVX group, showing strong positive immunoreactivity of iNOS expression in dentate gyrus G) OVX + Ginseng group, showing Slight immunoreaction in dentate

gyrus; (Immunohistochemical stain of Caspase 3& iNOS x400). D&H) Histograms comparing the mean area percentage of Caspase 3&iNOS expression in dentate gyrus of studied groups respectively. *P<0.05 is considered significant, **P<0.001 is highly significant.

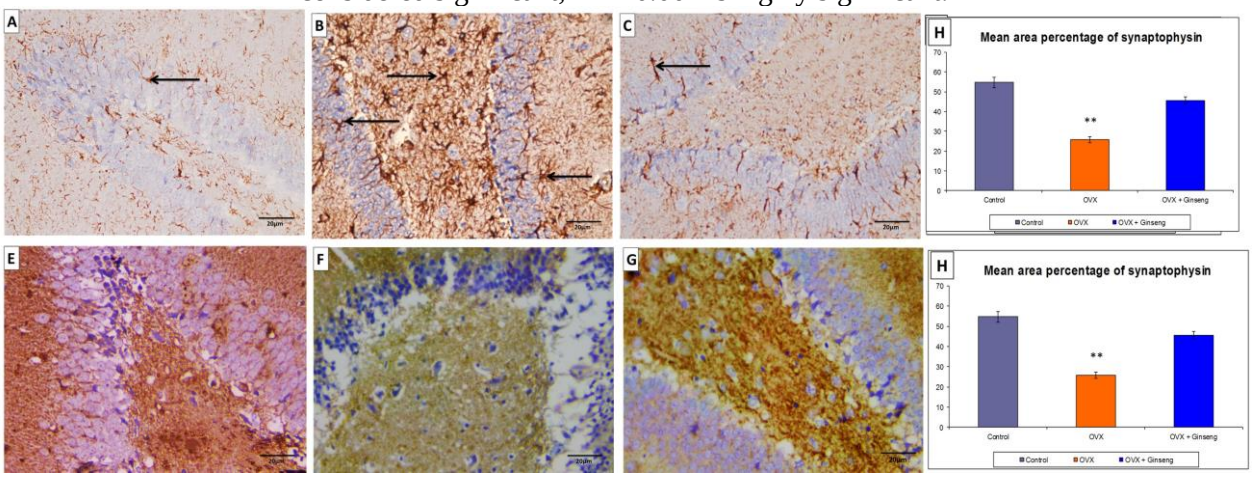

Figure 6: Photomicrographs of immunostaining in dentate gyrus of studied groups showing. A): control group, showing weak positive GFAP immunoreactivity in the cytoplasm of astrocyte and their processes in the form of dark brown granules (arrow). B): OVX group, showing increase in GFAP immunoreactivity the astrocyte appears increased in number and larger in size (arrows), with longer processes. C) OVX + Ginseng group, showing decrease in GFAP immunoreactivity. The astrocytes appear less in number and processes (arrow). E) Control group, showing strong positive immunoreactivity of synaptophysin in dentate gyrus; F) OVX group, showing slight immunoreactivity of synaptophysin expression in dentate gyrus G) OVX + Ginseng group, showing strong positive immunoreaction in dentate gyrus; (Immunohistochemical stain of GFAP & synaptophysin respectively x400). **D&H)** Histograms comparing the mean area percentage of GFAP &synaptophysin expression in the dentate gyrus of studied groups respectively. *P<0.05 is considered significant, **P<0.001 is highly significant.

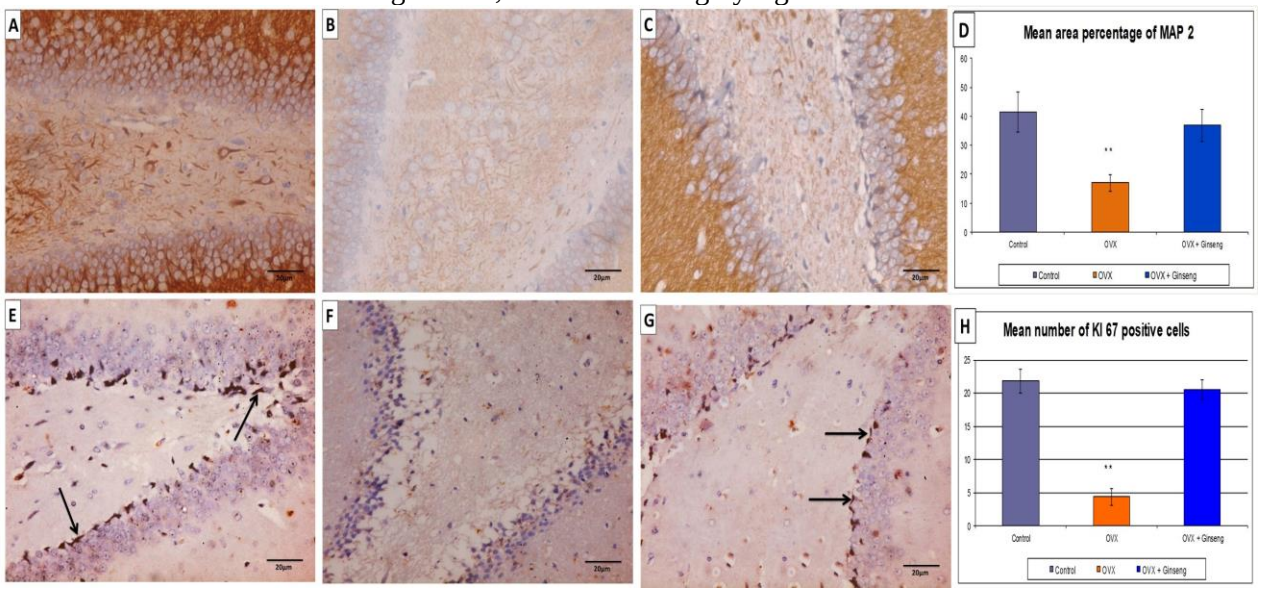

Figure 7: Photomicrographs of immunostaining in dentate gyrus of studied groups showing. A) Control group with strong positive immunoreactivity of MAP 2 B) OVX group, showing weak immunoreactivity C) OVX + Ginseng group, with strong positive immunoreaction of MAP 2 in dentate gyrus;. E) control group, showing Ki 67 positive nuclei (arrows) in the subgranular zone of dentate gyrus; F) OVX group, showing apparent decrease in number of Ki 67 positive nuclei dentate gyrus G) OVX + Ginseng group, showing Ki 67 positive nuclei (arrows)in the subgranular zone of dentate gyrus. (Immunohistochemical stain of MAP-2 & Ki 67 respectively x400). **D&H)** Histograms comparing the mean area percentage of MAP 2 expression & the mean number of Ki positive nuclei in the dentate gyrus of studied groups respectively. *P<0.05 is considered significant, **P<0.001 is highly significant.

DISCUSSION

Menopause, a crucial period in female life, commonly associated with physiological and histological alterations; the most important are changes involving the cognitive functions. The sudden drop in female sex hormones was suggested to be involved in female age-related neurodegenerative changes [18].

Several studies recently paid an attention to the role of Panax Ginseng in attenuating age-related neurodegenerative changes [19] but the exact mechanism was still under appraisal.

The current study was designed to investigate the effect of ovariectomy on the histological structure of dentate gyrus, in adult female albino rats, as a model of menopausal period, with its impact on cognitive ability and the possible protective role of Panax Ginseng.

Considering our observation to the mood and behavior of rats in the present study, our results showed a highly significant increase in weight of ovariectomized rats in OVX group when compared to control group. Similar results were previously reported by **Chen & Heiman [20]** who evidenced that estradiol decreased accumulation of adipose tissue**. Sharma et al.** [21] added that weight gain in ovariectomized rats consequently to depletion of estrogen levels, is attributed to less physical activity and increased food consumption following ovariectomy.

In the present study the ovariectomy performed in (OVX group) resulted in degeneration of most of the granule cells of the dentate gyrus with apparent decrease in granular cells. Same result was reported by **Shoukry et al. [22].** This was also confirmed morphometrically by significant decrease in thickness of granular layer. These results coincide with previous results of **Su et al. [23],** who reported that ovariectomy results in significant decrease in brain volume, thickness of cell layers and density of neurons in hippocampus. **Iivonen et al. [24]** stated that OVX led to significant decrease of sex steroids, as estrogen in rat which has neuroprotective effects. Estrogen enhances outgrowth of neurite, increases dendritic spine density, regulates synaptogenesis, and protects neurons from oxidative stress**.** This was documented immunohistochemically by strong positive caspase 3 expression in OVX group appearing in granule cell layer, polymorphic and molecular layers of dentate gyrus, and was in the same line with **Sales, et al. [**25] who reported the role of estrogen in alleviating apoptotic effects and maintaining hippocampal neuronal viability in ovariectomized rats. Similar results were also reported by **Zhou, et al.** [26] who explained the mechanism by which ovariectomy affects brain neurons and impairs cognitive ability through oxidative stress and neuron apoptosis.

Moreover, in the present work there was significant decrease in the density of Nissl bodies. This was in line with **Chen et al. [27]. Kadar et al. [**28] stated that the density of Nissl bodies which present in the cytoplasm is a special structure to neurons and is used to estimate the neuronal damage.

The results of the present study also revealed few spindles shaped stem cells together with areas of cell loss were noticed in the sub-granular zone in dentate gyrus of OVX group. Moreover, molecular layer revealed numerous astrocytes this agreed with **Park et al. [29].** Aging process decreased neurogenesis by decreasing neural stem cells (NSCs). This reduction in neuronal stem cells (NSCs) was suggested to be caused by increasing systemic pro-aging factors and decreasing extrinsic signals including mitotic signals that support the proliferation of NSCs **[30].**

This was confirmed by a significant decrease in the number of ki67 immunoreactive cells which is a nuclear protein used for detection of adult neurogenesis in ovariectomized group in our study was detected. This was in accordance with El-Mehi & Faried [31] who declared that neurogenesis decreased in the aged rodents. Diminution of the neurogenesis by age occurred due to the alterations occurred in the astrocytes which caused reduction in fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF) and finally affected the maintenance of the pro-genitor cells and decreased neurogenesis and the brain functions

In addition, the results of the current study revealed that synaptophysin expression was markedly decreased in OVX group. This was in line with **Smith et al.** [32] who previously reported that cognitive function is related to alterations in presynaptic proteins, the increase in synaptophysin (SYN) promotes synaptic plasticity and consequently improving learning and memory. It was found that decreased SYN points to synaptic pathology and occurs as tissue degeneration progresses **[33]**. **Yang et al. [**34] declared that estrogen have neuroprotective role as it enhanced synaptogenesis by increasing the expression of brain-derived neurotrophic factor (BDNF) which is essential for synaptic plasticity and memory.

Furthermore, in the present study it was found that significant decrease in the MAP2 expression in ovariectomized rats this coincides with **Stefano et al. [**35] who indicated that MAP2 can be considered a marker for the age-dependent deterioration of dendritic stability and plasticity. Moreover **[36]** observed a rise in MAP2 protein content in the hippocampus of ovariectomized rats after treatments by E2 and P4 hormones at a

posttranscriptional level.

In the same line, it was found that the synaptophysin decreased in aging and reflecting functional disturbances of synaptic transmission. Moreover, Loss of MAP2 correlates with neuronal degeneration and is linked to age-related impairment of synaptic plasticity, cognition, and memory functions **Bruschettini et al.** [37] this was following our results which revealed significant decrease in expression of both synaptophysin and MAP2 in dentate gyrus of ovariectomized rats.

The current results estimated a highly significant reduction in estrogen level in OVX group when compared to control group. Our results came in agreement with **Behling et al. [**4] who postulated that ovariectomy causes changes in the redox profile, increasing oxidative stress and consequently accelerating the process of cellular aging in different tissues. Similar results were also reported by **Sadeghian et al.** [38] who reported learning and memory impairment following ovariectomy and related these to decline in serum estrogen levels. In the same line **Rahman et al.** [39] strongly supported the role of estrogen in maintaining memory, with sever impairment concerning learning, memory and dementia in response to estrogen depletion.

Moreover, **HA et al.** [40] declared that lack of estrogen enhances the production of ROS. The excessive ROS induces oxidative stress which causes cell damage or death, this gave explanation to the significant upregulation of iNOS, an oxidative stress marker in OVX group in our work. Moreover**, Wang et al. [**41] previously described the role of iNOS as it catalyzes the formation and expression of the inflammatory mediator NO in brain mainly through astrocytes and microglia.

Oxidative stress was confirmed also biochemically by the results of the present work which revealed a highly significant increase in MDA and a significant reduction on GSH level and a highly significant reduction in catalase level in OVX group when compared to control group. This was in line with **Désiré, et al.** [42] who stated that estrogen depletion in ovariectomized rats, induces oxidative stress, with increased MDA, and decreased GSH.

Our data showed an increased GFAP immunoreactivity in OVX group, the astrocyte increased in number and appeared larger in size, with longer processes. Our results coincide with the results of **Shoukry et al. [**22] who explained this as reactive gliosis which compensatory reaction to degeneration of adjacent neurons and cell loss is produced by ovariectomy.

Heneka et al. [43] similarly reported that microglia are commonly located in close contact with the dendrites and synapses of adjacent neurons. Being concerned with maintenance of tissue homeostasis by removing accumulating debris from the brain, synaptic remodeling and secretion of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). They also related the increased astrocytes and glial cells in neurodegenerative conditions, to accumulation of misfolded or aggregated proteins in the brain.

In the present work the weight of rats in OVX plus Panax ginseng treated group was significantly reduced this agreed of **Xu et al. [9]** who explained reduction of rats' body weight by enhancing the biosynthesis of estrogen from the adrenal gland which may be mediated through the hypothalamus-pituitary-adrenal axis and increasing the quantity of ERs in the target organs through Ginseng's estrogenic activity. This was online with our results which revealed significant increase in serum estradiol level in ovariectomized group treated with panax ginseng.

Our data revealed that in ovariectomized group treated with panax ginseng MDA was significantly decreased, with significant increase in level of GSH and catalase this was in line with **Ramesh et al. [44]** who declared that utilization of panax ginseng minimizes lipid peroxidation and restores antioxidant capacity by inhibiting oxidative stress in rats through enhancing the enzymatic and nonenzymatic antioxidants status.

Moreover, it has been suggested that ginsenoside Rg2 can increase cell viability, reduce Ca2+, lipid peroxidation the excessive production of MDA, NO and caspase-3 **Li et al. [45].**

In the current work, it was found that the administration of panax ginseng improved the severity of histological alterations; this was in line with Cho [46] who declared that Panax ginseng has neuroprotective effect that prevents the death of vulnerable neurons through several mechanisms. Moreover, significant increase in the Nissl granules was observed in rats treated with panax ginseng this was in line with **Shi et al. [47].**

Furthermore, Ginseng decreased expression of caspase 3 in ovariectomized rats in the present work. This was in accordance with **Cheng et al.** [48] who declared that the antiapoptotic effect of ginseng is due to inhibiting activation of caspase-3 and enhancing the ratio of Bcl-2 to Bax protein through its main active component (G-Rg1).

On the other hand, in dentate gyrus of OVX + Ginseng group many spindles shaped stem cells were detected in the subgranular zone. This was following the results recently reported by **Ryu et al. [**7] who declared significant increase in neuronal stem cell proliferation and expansion of

neuroblasts in granule cell layer of dentate gyrus in ginseng treated groups.

This can be supported by increased ki67 positive cells in ovariectomized rats treated with panax ginseng group in our study. This was in line with Liu et al. [49] who stated that ginsenoside can boost neurogenesis by regulating the expression of caspase-3 and brain-derived neurotrophic factor in rats.

Furthermore, it was found that after panax ginseng treatment there was significant increase in synaptophysin this was in line with **Razgonova et al. [50]**. Ginsenoside Rg1 supplementation improved the performance of behavioral tests in aged mice and significantly increased the expression of synaptic plasticity-associated proteins in the hippocampus, including synaptophysin

Furthermore, our study revealed that MAP2 was preserved by panax ginseng treatment. This agreed with Zhang **et al.** [51] who declared that Ginsenoside Rg1 which is a steroidal saponin presented in ginseng increased MAP2 expression and alleviated neuronal degeneration in the frontal cortex and hippocampus.

Furthermore, iNOS was down regulated by Panax Ginseng in the present study. The same results were reported by **Ye et al [52]** who reported that ginsenosides are capable of inhibiting iNOS, and consequently decreasing NO production and reducing inflammatory processes

In addition, in our study it was noticed that GFAP expression was decreased in ovariectomized rats group treated with ginseng. This was in accordance with Zhu et al. [8] who declared that the treatment of (G-Rg1) which is one of the most active ingredients of Panax ginseng, significantly decreased the GFAP-positive cells number and inhibit excess astrogenesis and increased NSCs/NPCs which decreased by age and enhanced neural stem cells/progenitor cells (NSCs/NPCs) differentiation into neurons.

Panax ginseng can be used in menopause to compensate estrogen deficiency and ameliorate the deleterious effects from aging process to protect dentate gyrus.

In conclusion, Panax Ginseng may serve as a promising therapeutic substitute to overcome the drawbacks of advancing in age particularly menopausal related cognitive affection, by modulating the oxidative stress parameters and structural alterations of dentate gyrus.

Conflicts of interest:

The authors declare no potential conflict of interest concerning this work.

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SUPLLEMENTARY FILE

Table (1): Biochemical analysis and weight of different study groups

weight of rats	181.35 ± 2.88	181.53 ± 2.77	194.16 ± 2.73	183.65 ± 3.61
Serum estradiol	32.28 ± 1.91	32.03 ± 1.91	17.89 ± 0.39	30.92 ± 2.46
MDA	154 ± 3.77	157 ± 1.66	183.11 ± 3.06	156.67 ± 1.94
GSH	1.17 ± 0.05	1.15 ± 0.05	0.91 ± 0.02	1.12 ± 0.06
CAT	5.61 ± 0.33	5.59 ± 0.35	4.77 ± 0.17	5.47 ± 0.43

Table (2): Morphometric analysis of different groups

Mean ± SD (standard deviation) of	Control (GI)	OVX (G II)	OVX plus Ginseng (G III)
The thickness of the granular layer	161.91 ± 2.81	146.09 ± 2.66	159.82 ± 2.09
The color intensity of toluidine blue	14.50 ± 3.60	7.00 ± 1.41	13.30 ± 3.02
Caspase 3	16.29 ± 2.75	54.88 ± 2.10	34.71 ± 2.29
INOS	8.83 ± 1.72	44.29 ± 2.50	14.38 ± 2.07
GFAP	36.29 ± 4.68	54.0 ± 2.77	35.43 ± 3.31
Synaptophysin	54.67 ± 2.73	25.75 ± 1.67	45.63 ± 1.69
Map-2	25.75 ± 1.67	45.63 ± 1.69	36.83 ± 5.60
Ki67	21.82 ± 1.78	4.36 ± 1.29	20.55 ± 1.44

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