https://doi.org/10.31925/farmacia.2023.6.4 **ORIGINAL ARTICLE**

THE EFFECT OF *VITIS VINIFERA* L EXTRACT ON EMOTIONAL AND BEHAVIORAL STATUS, OXIDATIVE STRESS AND INFLAMMATION IN A RODENT MODEL OF SURGICAL INDUCED MENOPAUSE

MARIA CRISTINA OLĂNESCU VAIDA VOEVOD, IRINA NECHITA, ALEXANDRA-CRISTINA SEVASTRE-BERGHIAN *, CRISTINA BIDIAN, OANA ALINA HOTEIUC, ALINA MIHAELA TOADER, REMUS MOLDOVAN, NICOLETA DECEA, GABRIELA ADRIANA FILIP

Department of Physiology, "Iuliu Hațieganu" University of Medicine and Pharmacy, 1 Clinicilor Street, 400006, Cluj-Napoca, Romania

**corresponding author: berghian.alexandra@umfcluj.ro*

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Abstract

The study aimed to assess the impact of *Vitis vinifera* L. (VV) extract on behavior, oxidative stress and inflammation in the brain, in an experimental model of menopause induced by ovariectomy (OV). For each subset of experiments (I and II), 20 female Wistar rats were divided into 4 groups (n = 5): animals with sham ovariectomy (SOV) treated with vehicle, SOV treated with VV extract, OV treated with vehicle and OV treated with VV. *Vitis vinifera* L. extract (30 mg/kg b.w.) or vehicle were orally administered for 21 days (subset I) and 42 days (subset II), after the surgery. OV had a negative impact on memory, behaviour and motor functions of rats, correlated with an increased redox imbalance and minimal changes in the cytokines. 21 days of VV administration triggered mostly non-significant increases of general locomotion and anxiety while after 42 days, VV extract improved general locomotion and diminished significantly the anxiety. MDA levels and tyrosine oxidation decreased after VV treatment in the frontal lobe, both at 21 days and 42 days, while in hippocampus it had controversial effects, with minimal decreases of MDA, high levels of NTZ and variable changes in interleukin levels, especially after 42 days of treatment. Our findings suggest that *Vitis vinifera* L administration for a long period, may have beneficial effects on both locomotion and emotionality and could also have antioxidant properties in menopause, especially in frontal lobe.

Rezumat

Prezentul studiu are drept scop evaluarea impactului extractului de *Vitis vinifera L* (VV) asupra comportamentului, stresului oxidativ și a inflamației la nivel cerebral, într-un model experimental de menopauză indusă prin ovariectomie chirurgicală (OV). Pentru fiecare subset de experimente (I și II), 20 șobolani Wistar femele, au fost împărțiți în 4 grupe (n = 5): animale cu falsă (*sham*) ovariectomie (SOV) tratate cu vehicul, SOV tratate cu extract de VV, OV tratate cu vehicul, și OV tratate cu extract de VV. Extractul de *Vitis vinifera* L (30 mg/kg corp) sau soluția vehicul au fost administrate oral timp de 21 de zile (subset I) sau 42 de zile (subset II) după operație. Ovariectomia a avut un impact negativ asupra memoriei, comportamentului și asupra funcției motorii a șobolanilor, modificări corelate cu creșterea stresului oxidativ și variații minime în rândul citokinelor. 21 de zile de administrare a extractului de VV au determinat în general ameliorări nesemnificative ale activității locomotorii și anxietății, în timp ce după 42 de zile de administrare continuă s-au remarcat creșteri semnificative ale parametrilor activității locomotorii și scăderea semnificativă a gradului de anxietate. Peroxidarea lipidică și a proteinelor s-a redus în lobul frontal, atât la 21 cât și la 42 de zile, în timp ce la nivelul hipocampului s-au remarcat efecte contradictorii, cu reducerea minimă a MDA, valori crescute de NTZ și modificări variabile ale interleukinelor, în special la 42 de zile. Rezultatele noastre sugerează că administrarea *Vitis vinifera* L., pentru o perioadă îndelungată, ar putea avea efecte benefice atât asupra activității locomotorii cât și asupra statusului emotional, precum și efecte antioxidante în menopauză, mai ales în lobul frontal.

Keywords: *Vitis vinifera* L, ovariectomy, oxidative stress, menopause

Introduction

Menopause is a normal stage of the ageing process in women caused by definitive/permanent cessation of ovarian functions, including the production of ovarian steroid hormones, oestrogens and progesterone [1]. Postmenopausal changes in physiological processes, including cognitive and motor functions, are connected to the major decline in steroid hormones' levels [2]. A key role in learning and memory processes is played by the hippocampus, a brain structure that needs high levels of oestrogen produced during ovarian activity [3, 4].

In order to assess the changes in behaviour, cognitive and motor functions, menopause-like status can be induced by surgical ovariectomy in laboratory rodents [5]. Previous studies of ovariectomized animals showed important alterations in the structure and function of hippocampal and cortical circuits accompanied by poor performance at cognitive tests [6, 7].

The important decline in oestrogen has been shown to have a pro-oxidant like effect [8]. Oxidative stress results from the overproduction of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). As the body ages the antioxidant capacity of the body declines [9]. This decline combined with a gradual loss of oestrogen in the reproductive system is highly associated with the various pathological conditions such as heart disease, vasomotor disturbances and osteoporosis [10].

Oxidative stress actions result in breaks in genetic material, formation of DNA adducts and oxidation of bases [11]. Serum concentrations of inflammatory cytokines and pro-oxidant biomarkers such as glutathione oxidized, 4-hydroxynenal and malonaldehyde were found to be higher in postmenopausal women than in premenopausal women suggesting a prooxidant status in postmenopausal state [12].

Menopausal hormone therapy (MHT) has been used as a potential treatment for the debilitating symptoms and pathological conditions associated with menopause. However, there are a considerable number of co-morbidities associated with MHT, including pulmonary venous embolism, stroke and cardiovascular events as well as oestrogen-dependent breast, ovarian and endometrial cancers [13].

Over past years it has been proven that the consumption of foods rich in antioxidants may have protective effects against oxidative stress on women in menopausal and post-menopausal conditions [14]. *Vitis vinifera L (VV)* extract, also known as grape seed extract, is a natural supplement known for its various health benefits, including anti-oxidant and anti-inflammatory effect, exerted by its high content of polyphenols. Literature high performance liquid chromatography (HPLC) analysis showed that VV extract is rich in procyanidin B, catechin hydrate, epigallocatechin, epicatechin and gallic acid [15].

Recent studies on female rats have demonstrated that the administration of grape extracts exerted protective effects against oxidative stress induced by a pro-oxidant medication and had a great contribution on improvement of memory disorders, learning process, blood pressure [16] and bone calcium retention [17, 18]. Brain-targeting grape-derived polyphenols showed promising anti-neurodegenerative properties [19] through inhibition of neuroinflammation, stimulation of neuro-genesis and improving the memory formation and consolidation [20].

Based on these previous findings, our study aimed to assess the oxidative stress, inflammation and behavioural changes on experimental model of surgical induced menopause by ovariectomy in rats. The impact of grape, *Vitis vinifera* L. extract, on these parameters was also evaluated.

Materials and Methods

Reagents

Trichloroacetic acid, o-phthalaldehyde, t-butyl hydro-peroxide, glutathione reductase, reduced glutathione, Bradford reagent, cytochrome c, xanthine, xanthine-oxidase, β–nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (NADPH) were purchased from Sigma-Aldrich Chemicals GmbH (Munich, Germany). Guanidine hydrochloride, Folin-Ciocâlteu phenol reagent, 2-thiobarbituric acid and EDTA-Na_2 were obtained from Merck KGaA (Darmstadt, Germany), while absolute ethanol, hydrogen peroxide and n-butanol were purchased from Chimopar (Bucharest, Romania). Nitrotyrosine, IL-1α and IL-6 ELISA kits were purchased from BioVendor, (Czech Republic). All reagents were of analytical grade.

Vitis vinifera L. extract

Vitis vinifera L. ethanol extract was obtained from Great Burgund Recas grape seeds, 1:20 in a 50/50 (v/v) mixture of water and ethanol. In order to determine the total amount of polyphenols in the extract, Folin-Ciocalteu method was used [21]. The UVB absorption spectra of *BM* were registered using Jasco V-530 UV/Vis spectrophotometer and the anti-oxidant activity was measured by using the free radical 2,2-diphenyl-l-picryl-hydrazyl (DPHH·) scavenging assay and ABTS test as previously published [22]. The polyphenolic composition of BM extract was evaluated by high-performance liquid chromatography (HPLC) analysis coupled with mass spectrometry (MS) and found in the extract epigallocatechin, epicatechin, catechin hydrate, procyanidin B and gallic acid [23]

Experimental design

40 adult female albino Wistar rats, 12 weeks of age, weighing between 140 - 160 g, provided by the University Animal Facility, were used for the experiments. The animals were hosted in polypropylene lab cages in standard conditions: environmental temperature 21ºC, relative humidity 65% and day-night cycle of 12 hours, had free access to a standard normocaloric pellet diet and received water *ad libitum*. The experiments were performed after a 1-week environmental accommodation period with the approval of the Ethics Committee of "Iuliu Hațieganu" University Cluj-Napoca, Romania, in accordance with Directive 86/609/ EEC on the protection of animals used for scientific purposes.

In order to evaluate the effects of *Vitis vinifera* L. extract administration, the experiment was divided into two subsets, each subset including 4 groups of animals, with five rats per group (n = 5). Each subset consists of group 1, sham ovariectomy and vehicle (SOV + veh), group 2, sham ovariectomy and *Vitis vinifera* L extract (SOV + VV), group 3, experimental ovariectomy treated with vehicle (OV + veh) and group 4, experimental ovariectomy treated with *Vitis vinifera* L extract (OV + VV).

Surgery was performed under general anaesthesia with a cocktail of ketamine (90 mg/kg b.w.) and xylazine (10 mg/kg b.w.). For surgical ovariectomy, after a lumbar dorsal 2 cm incision, the ovaries were identified and dissected from the surrounding tissue and bilateral ovariectomy was performed. For false ovariectomy, the abdominal cavity was opened, the ovaries were exposed and then the abdominal wall was sewn back. *Vitis vinifera* L. extract (30 mg/kg in 0.5 mL) or vehicle (0.5 mL drinking water) were administered by oral gavage for 21 and 42 days, after the surgery, according to the group protocol (subset I and II respectively). After 21 and 42 days, the behavioural tests were conducted [15]. Twenty-four hours after the last behavioural test, under anaesthesia, the blood, hippocampus and frontal lobe were harvested for oxidative stress evaluation. The inflammatory markers form hippocampus and frontal lobe were also quantified at 42 days after surgery.

Behavioural Testing

Two widely used tests to evaluate general locomotion and emotionality – like behaviour in rodents are the open field test (OFT) and the elevated plus maze (EPM). A visual tracking system, (Smart Basic Software version 3.0 Panlab Harvard Apparatus), using specific mazes for rats (Ugo Basil Animal Mazes for Video-Tracking), recorded the animals' behaviour continuously for 5 minutes.

OFT

In OFT the animals were freely allowed to explore an open field arena (100 × 100 × 40 cm) for 5 minutes. The total and peripheral travelled distance and number of entries are common measures for general locomotor activity. High centre travelled distance and number of entries and high centre time ratio (centre/total time) are reported parameters of a low level of anxiety [24]. Therefore, measures of both locomotor activity (total travelled distance, travelled distance in periphery, total number of entries, number of entries in periphery) and anxiety-like behaviour (travelled distance in centre, number of entries in centre, time spent in centre/total time) were recorded in OFT.

EPM

Considered the gold standard for the evaluation of anxiety in the basic research, it also measures the motor activity. The plus-shaped maze consists of two open (10 × 50 cm) and two closed (10 × 50 × 40 cm) arms that are 60 cm elevated above the ground level [25].

Total and closed arm travelled distance and number of entries are variables of motor activity in EPM. High open arm travelled distance and number of entries and high open arms time ratio (open arms/total time) indicate low anxiety-like behaviour. Therefore, behavioural parameters for motor activity (total and closed arm travelled distance and number of entries) and for anxiety-related behaviour (open arm travelled distance and number of entries and open arms time ratio (open arms/total time), were recorded in EPM.

Oxidative stress and inflammation assessment

Malondialdehyde (MDA) and nitrotyrosine (NTZ), were determined in tissue homogenate from hippocampus and frontal lobe. MDA, the end product of lipid peroxidation, was evaluated by spectrofluorometry using Conti method slightly modified, as described: 50 μL brain homogenate was boiled for 1 hour on a water bath with a solution of thiobarbituric acid (10 mM) in K2HPO4 (75 mM) with pH = 3 (1 mL). The reaction product was extracted into n-butanol, followed by centrifugation and separation of fractions, and the concentration was determined in the organic phase. Using a spectrophotometer, the emission intensity was quantified at 534 nm in the synchronous fluorescence system, at a wavelength difference (Δλ) between excitation and emission of 14 nm. A known concentrations MDA curve, was used as a standard. Values were expressed in nmol/mg protein [26]. NTZ, a specific marker of nitrosative stress, was measured using Rat NTZ ELISA kit (BioVendor, Czech Republic) [27] and IL-1α, IL-1β and IL-6 levels in brain homogenate, were also investigated by using ELISA kit (BioVendor, Czech Republic), according to the manufacturer's instructions.

Histological Investigation of the Brain

Frontal lobe slices were harvested for histological investigation. Brain samples were fixed in 10% neutral buffered formalin, then embedded in paraffin in order to produce 5 μm thick sections which were stained with hematoxylin-eosin (HE) for light microscopy (Optika B-383LD2 microscope).

Statistical analysis

The obtained values were statistically analyzed using GraphPad Prism version 5.0 for Windows, GraphPad Software, San Diego, California, USA. One-way ANOVA followed by the post-test Tukey were used to evaluate the effects of *Vitis vinifera* L. on behaviour, oxidative stress and inflammation parameters. The threshold significance level was set at $p < 0.05$. Results are expressed as mean ± SD; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Results and Discussion

VV administration significantly increased the locomotor activity, the total travelled distance ($p < 0.05$, $p < 0.01$ and $p < 0.05$) (Figure 1A), the travelled distance in periphery ($p < 0.05$) (Figure 1B), the zone transition number ($p < 0.001$) (Figure 1C) and the number of entries in periphery ($p < 0.001$) (Figure 1D) in sham operated rats in comparison to sham or ovariectomised rats.

The ovariectomised rats group exhibited significantly reduced number of zone transitions ($p < 0.001$), (Figure 1C) as well as a lower number of entries in the OFT arena ($p < 0.001$), (Figure 1D) as compared to sham operated rats.

VV administration enhanced the travelled distance in closed arms as compared to ovariectomised rats in EPM test ($p < 0.05$) (Figure 2B). The SOV + VV group expressed a significantly high zone transition number and significantly more entries in closed arms in the EPM test as compared to both OV + veh and OV + VV groups ($p < 0.001$) (Figure 2C and Figure 2D). Ovariectomy significantly diminished the locomotor activity in comparison to sham operation ($p < 0.001$) (Figure 2C and Figure 2D).

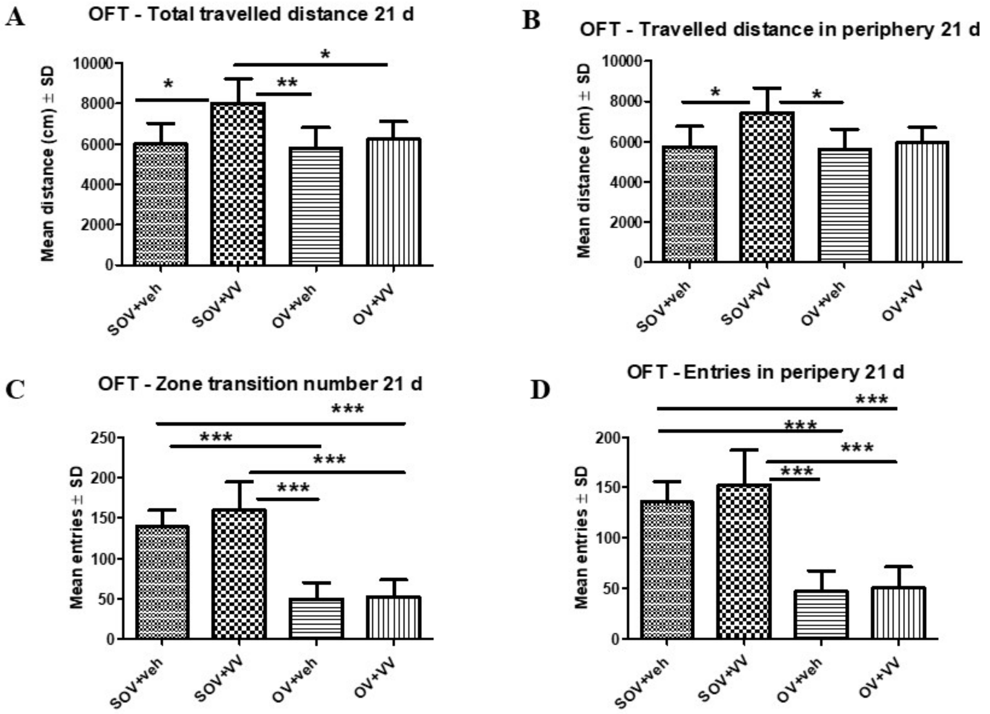

Figure 1.
OFT at 21 days

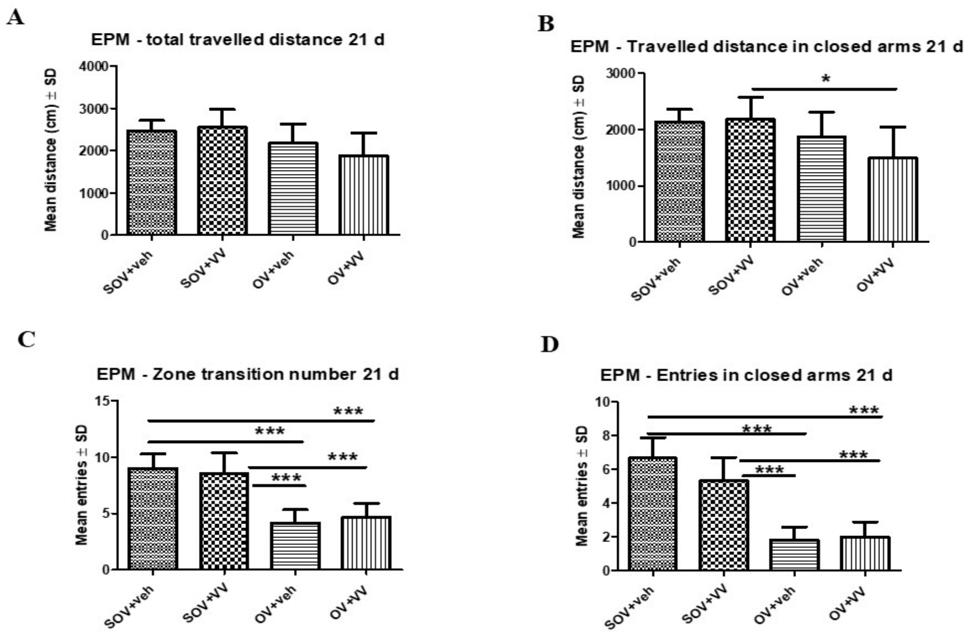

Figure 2.
EPM at 21 days

In OFT, the VV treated rats travelled significantly greater distance (Figure 3A), made more entries (Figure 3B) and spent more time (Figure 3C), in the central part of the OFT arena as compared to both ovariectomised group ($p < 0.01$) and sham operated animals.

In the EPM, the ovariectomised rats spent significantly less time in central part of the maze as compared to sham operated rats ($p < 0.05$) (Figure 3F).

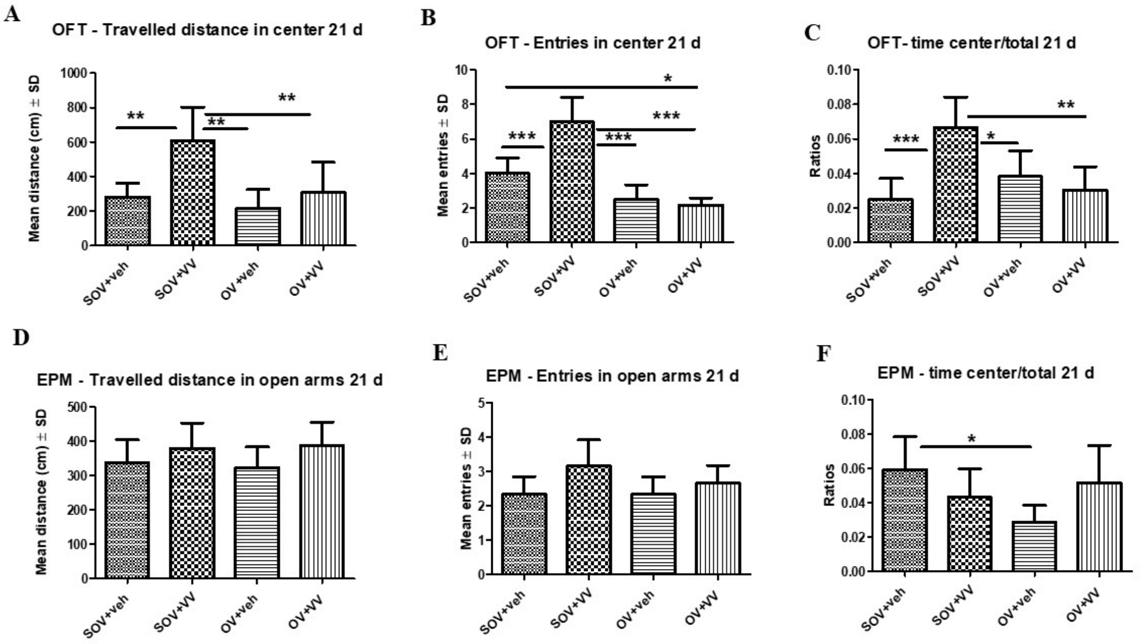

Figure 3.
Anxiety behaviour in OFT and EPM at 21 days

Our results showed that 42 days of VV administration increased significantly the locomotor activity (the total travelled distance, the travelled distance in periphery, the zone transition number and number of entries in periphery) as compared to both ovariectomised and sham operated rats ($p < 0.05$ and $p < 0.01$, $p < 0.05$) (Figure 4Aand Figure 4B**);** ($p < 0.05$) (Figure 4C and Figure 4D**).**

Ovariectomy significantly diminished zone transition number in the ovariectomised animals compared to sham groups ($p < 0.05$) (Figure 4C).

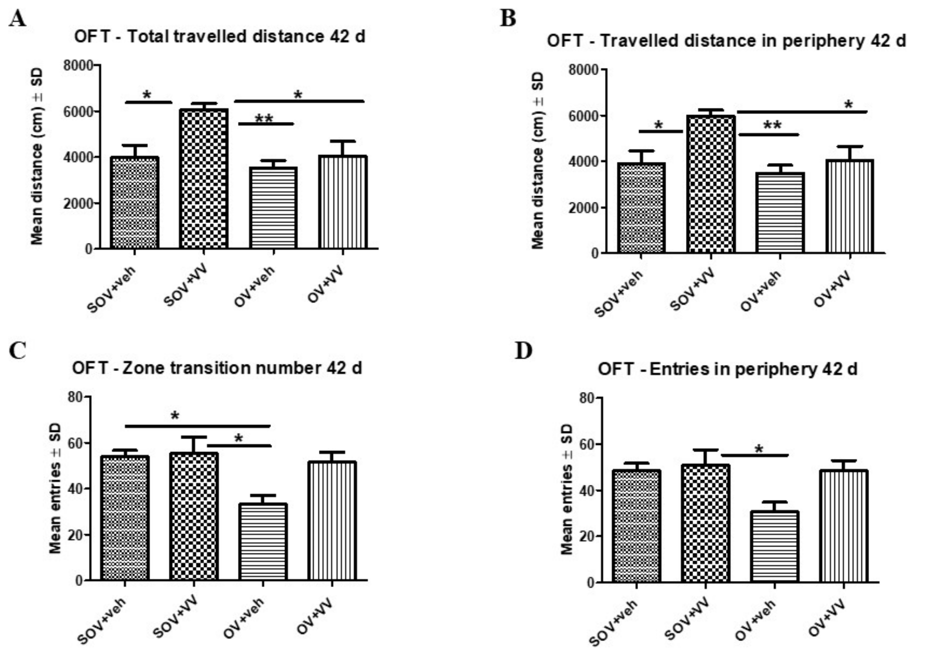

Figure 4.
OFT at 42 days

In the EPM, VV administration in ovariectomized animals significantly improved the general locomotion: total, closed arms travelled distance and zone transition number, ($p < 0.05$) (Figure 5A, 5C); ($p < 0.05$) (Figure 5B).

Regarding the emotionality in OFT, the VV treated rats travelled a significantly greater distance ($p < 0.01$; $p < 0.001$) (Figure 6A) and spent more time (C), in the central part of the OFT arena ($p < 0.05$). The OV group exhibited significantly reduced travelled distance and made fewer entries in the centre of the arena as compared to sham group ($p < 0.01$: $p < 0.001$) (Figure 6A and Figure 6B).

In EPM, our results showed that VV significantly reversed the inhibitory effect of ovariectomy on travelled distance in open arms, entries made in the open arms and the time spent in the centre of the maze ($p < 0.05$) (Figures 6D, 6E and 6F). The ovariectomized group also exhibited diminished activity as compared to the sham operated animals (($p < 0.01$, $p < 0.001$) (Figure 6D and Figure 6F).

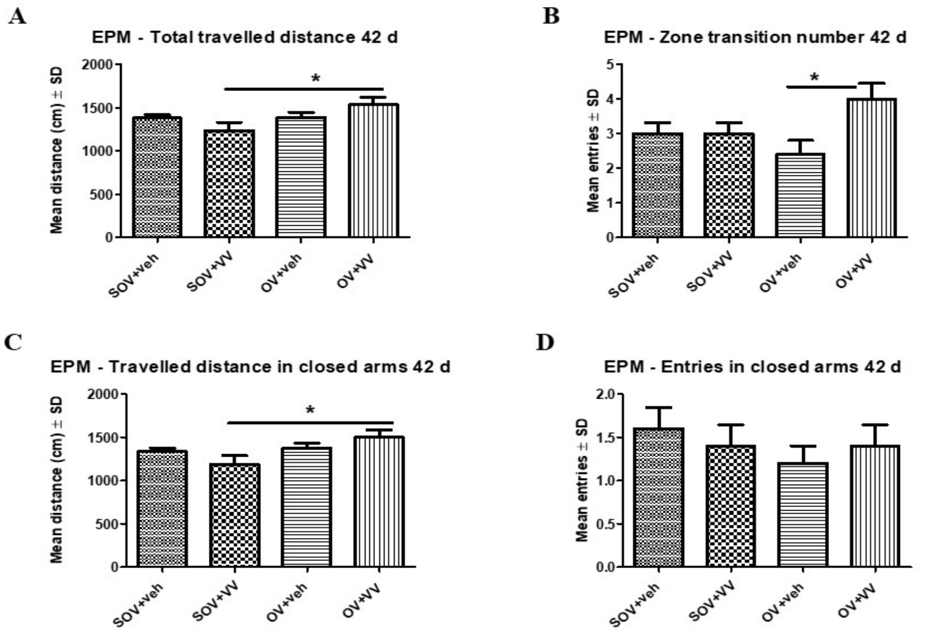

Figure 5.
EPM at 42 days

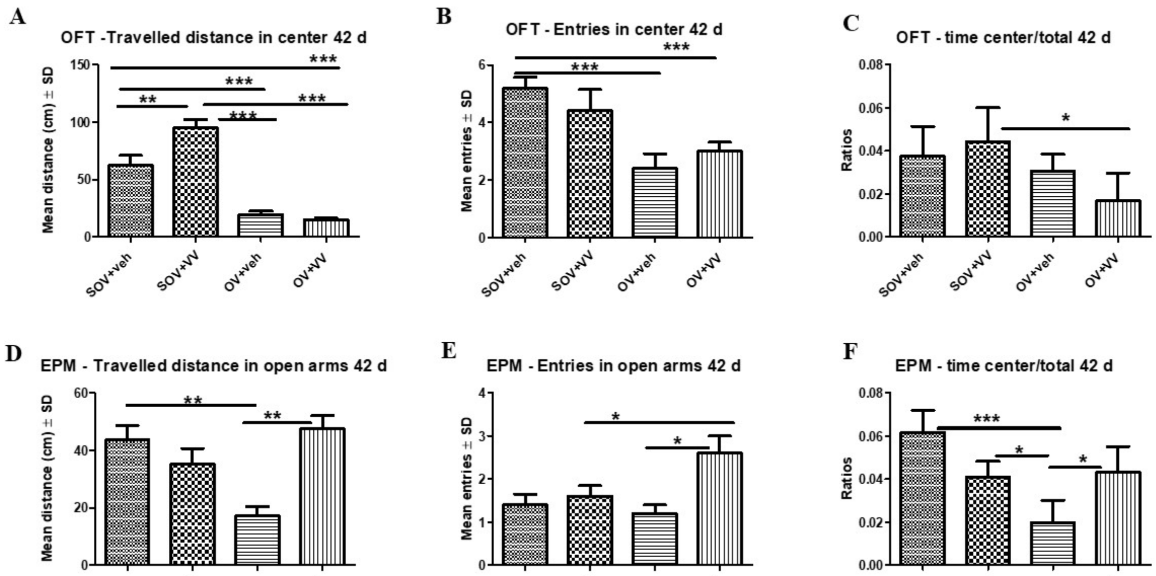

Figure 6.
Anxiety behaviour at 42 days in OFT and EPM

Oxidative stress and inflammation markers in hippocampus and frontal lobe

To evaluate ovariectomy induced oxidative stress we measured the so called effect biomarkers in the hippocampus and frontal tissue homogenates, MDA to quantify the oxidation of the lipids and NTZ to evaluate the nitrosative effect.

MDA levels in the hippocampus had a light increase in all groups compared to the control (SOV + veh) at 21 days, but after 42 days of *Vitis vinifera* administration there was a statistically nonsignificant ($p > 0.05$) decrease in MDA values for ovariectomized treated animals compared to all groups. (Figure 7A and Figure 7C).

In hippocampus tissue homogenate, after 21 days of treatment a nonsignificant decrease of NTZ was noticed in ovariectomized VV treated animals compared to the untreated group ($p < 0.05$) (Figure 7B). At 42 days an increase of NTZ levels was identified for the ovariectomized animals treated with *Vitis vinifera* L. compared to the sham treated ones ($p < 0,05$) (Figure 7D).

High levels of IL-1α were noticed in the hippocampus of ovariectomized treated animals compared to the sham treated group ($p < 0.05$) (Figure 8A). IL-1β presented a significant increase after ovariectomy in untreated group compared to the sham ($p < 0.05$), increase which diminished after *Vitis vinifera* L. administration (Figure 8B). IL-6 increased in a significant manner in treated animals compared to untreated ones in the ovariectomized animals ($p < 0.05$) (Figure 8C).

In the frontal lobe, experimental ovariectomy induced intense oxidative stress stated by the significant increase of MDA at 42 days and significant increase of NTZ at 21 days after the surgery, compared to the sham groups ($p < 0.01$, $p < 0.001$; (Figure 9B and Figure 9C). A statistically significant decrease of MDA was identified at ovariectomized treated animals compared to the untreated group at 21 days ($p < 0.05$). The same effect was seen at 42 days of grape seeds extract administration. ($p < 0.001$) (Figure 9A and Figure 9C). NTZ levels recorded a significant decrease after the grape seed extract at ovariectomized animals compared to the untreated ones ($p < 0.01$, $p < 0.05$). This effect was observed both at 21 and 42 days (Figure 9B and Figure 9D).

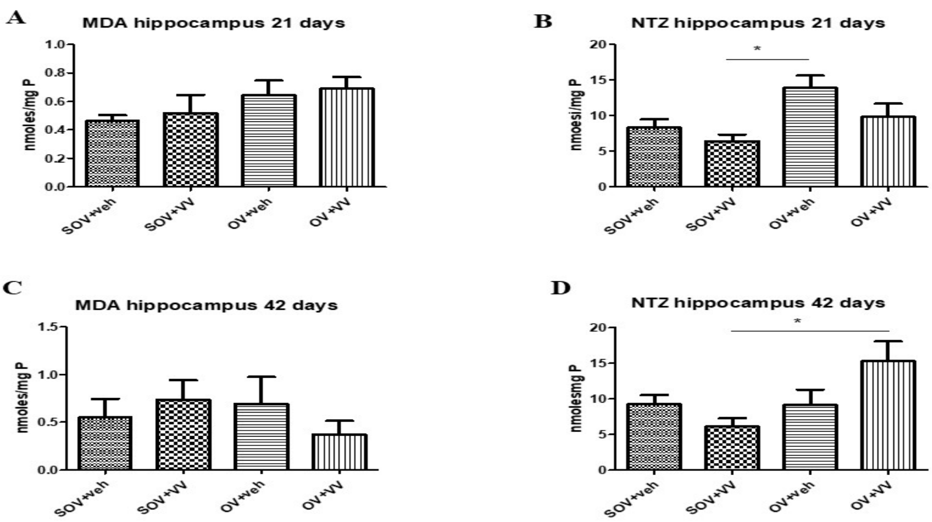

Figure 7.
MDA and NTZ in the hippocampus at 21 and 42 days

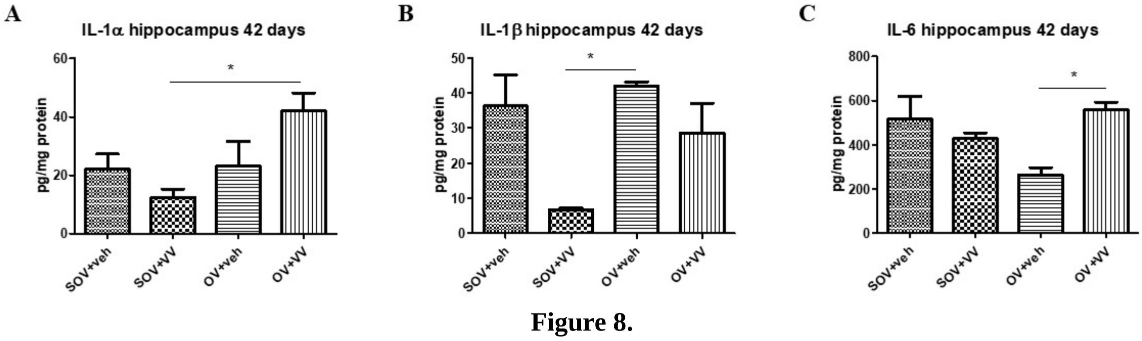

Figure 8.
IL-1α, IL-1β, IL-6 in the hippocampus at 21 and 42 days

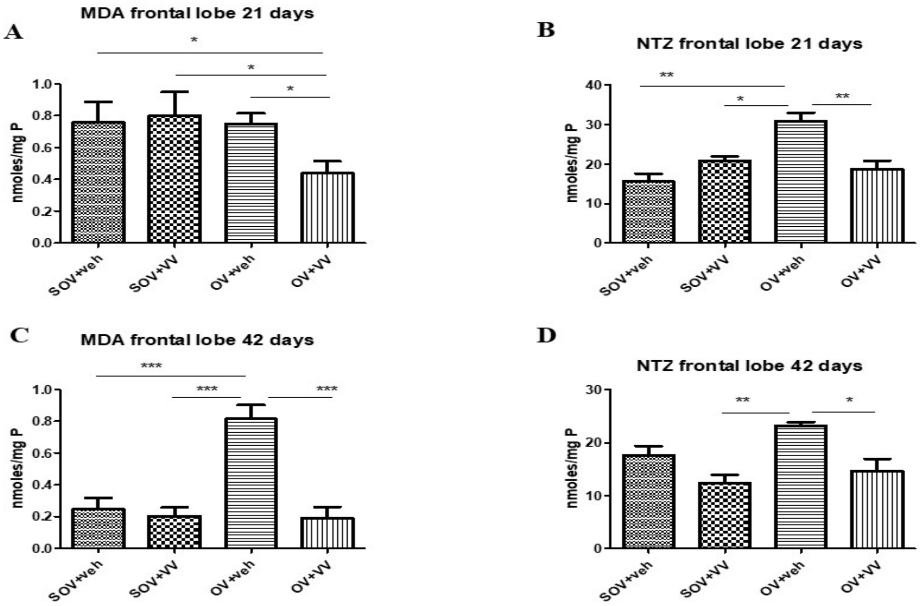

Figure 9.
MDA and NTZ in the frontal lobe at 21 and 42 days

In the frontal lobe *Vitis vinifera* L. administration determined a significant decrease of IL-1α in ovariectomized animals compared to all the other groups ($p < 0.05$, $p < 0.01$; (Figure 10A). IL-1β showed a decrease in treated animals compared to untreated ones in sham groups ($p < 0.01$), a highly significant decrease in ovariectomized animals compared to false ovariectomized ones ($p < 0.0001$), a decrease in treated operated animals compared to the control ($p < 0.05$) and a significant increase in treated ones compared to the untreated ones in the ovariectomized groups ($p < 0.05$) (Figure 10B). IL-6 level was found decreased in the ovariectomized untreated group compared to the control ($p < 0.05$), with the tendency to increase in the treated group (Figure 10C).

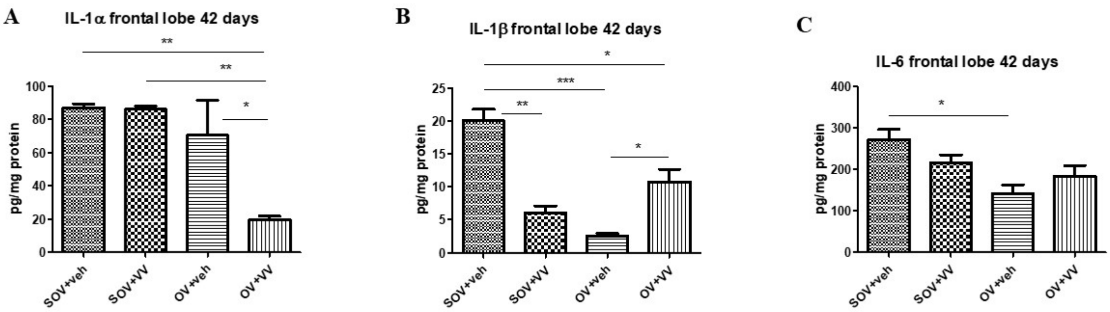

Figure 10.
IL-1α, IL-1β, IL-6 in the frontal lobe at 42 days

The present study brings evidence that *Vitis vinifera* L. administration in surgical induced menopause, in female rats, significantly reduced the oxidative stress in the brain tissue and determined different changes of interleukin levels in the hippocampus and frontal lobe. *Vitis vinifera* L. supplementation also improved the scores for general locomotion and anxiety behaviour, assessed in OFT and EPM, possible due to antioxidant properties of the grape seeds extract.

The general locomotion and anxiety were evaluated by OFT and EPM, both after 21 days and 42 days. Thus, ovariectomy decreased general locomotion in OFT and EPM and reduced the distance travelled in centre, number of entries and time spent in the centre of arena, after 21 days, suggesting an anxiogenic effect of OVX. OVX maintained also a reduced motility of animals after 42 days and induced an anxiogenic behaviour both in OFT and EPM.

VV administration preserved the same levels of anxiety in animals ovariectomized and a reduced motor activity after 21 days of treatment. After 42 days, VV did not improve the motor activity but increased the distance travelled in the centre of arena, number of entries and time spent in centre suggesting anxiolytic effects. In animals without OVX, VV improved the general locomotion and decreased the anxiety suggesting the beneficial effect in normal animals, both after 21 days and 42 days.

The relationship between oestrogens levels and anxiety-like behaviour can be related to redox imbalance in the brain. This mechanism was confirmed by the increase of oxidative stress in ovariectomized animals, especially in frontal lobe, effect attenuated by VV treatment, both at 21 days and 42 days. Our results are in agreement with those of Rahbi *et al*. which have shown important protective effects of polyphenols from Tualang honey on behaviour and cerebral oxidative stress [28, 29] in ovariectomized animals.

The brain consumes a large amount of oxygen and contains high contents of polyunsaturated fatty acids (PUFAs) which increase the susceptibility to oxidative damage, in the presence of relatively low antioxidant defence enzymes [30]. Several *in vitro* and *in vivo* studies evidenced the neuroprotective potential of grape polyphenols attributed to their direct antioxidant and metal-scavenging activities [30, 31]. Polyphenols can act as free radical scavengers and after the transfer of electrons to reactive radicals, render them into un-reactive and more stable species [32, 33]. Polyphenols exert immunomodulatory and anti-inflammatory effects [34] through controlling enzymes and inflammatory mediators and affecting several glial and neuronal signalling pathways, thus, polyphenols can down-regulate the nuclear factor kappa B (NF-κB) pathway, suppress the release of pro-inflammatory cytokines, the production of nitric oxide (NO) and prostaglandins (PGE2), as well as ROS generation in activated glia. In addition, polyphenols may inhibit microglia priming through toll-like receptors (TLR) activation [35, 36] and suppress the activator protein-1 (AP-1), increase the nuclear factor erythroid 2-related factor 2 (Nrf2) expression, down-regulate the apoptotic enzymes [37] and matrix metallopeptidase 9 (MMP-9), in parallel with induction of antioxidant enzymes [38].

The neuroprotective effects of grape polyphenols were explained by the antiinflammatory and anti-oxidant properties as well as by modulatory action on cell signalling pathways, stimulatory effect on synaptic plasticity [39] and anti-amyloidogenic activity [40]. Polyphenols can reduce the alterations of neuronal morphology, enhanced the stress shock proteins, caused the alteration of inflammatory gene expression, induced neuroprotection against excitotoxic stress and reduced the inflammatory mediators such as NF-κB [41].

It is known that low levels of oestrogens during menopause are associated with redox imbalance in rodent subjected to ovariectomy [15]. Surgical ovariectomy can affect the redox status in brain by increasing the oxidative stress [42, 43], and interfering with the mitochondrial antioxidant enzymes including Cu-Zn SOD and Mn SOD [44, 45]. More recent studies have demonstrated that the ablation of the ovarian hormone supply influenced the machinery of transcription, growth factor signalling, channels of synaptic communication, immune and neuroprotective mechanisms involved in neurogenesis, synaptic plasticity and immunomodulation from hippocampus after OVX in middle-aged rats. The authors found an increased number of up-regulated gene expression for macrophage markers, phagocytic receptors, complement system, proinflammatory cytokine IL-1-β that reflect a proinflammatory stage in the absence of gonadal hormone signalling in hippocampus [46]. The results of our study confirmed all these finding and demonstrated that ovariectomy increased the anxiety-like behaviour of animals in parallel with oxidative and nitrozative imbalance and proinflammatory cytokines secretion in cerebral tissues.

Thus, ovariectomy increased the malondialdehyde and NTZ levels, in frontal lobe, especially after 42 days, suggesting the importance of long-term pathological changes in postmenopausal conditions. Many authors found a reduced expression of genes encoding Bcl-2 (Bcl2) and superoxide dismutase (Sod1) after long-term OVX [47-49] associated with increased apoptotic activity and superoxide radical generation. VV administration triggered a significant decrease of MDA, both at 21 and 42 days, in the frontal lobe, demonstrating the protective effects of grape extract in this tissue. The results are different in hippocampus probably because the hippocampus is the brain region most susceptible to ovariectomy-induced oxidative stress and the effect of nutritional agents on its response to oxidative aggression. It is known that cerebral cortex and hippocampus are implicated in anxiety disorders [50] and the depletion of oestrogen levels after ovariectomy leads to GABA activation which transmits anxiety connected information's from amygdala to other brain areas [51]. Consequently, noradrenergic activation occurs and triggers the biochemical changes in the hippocampus responsible for behavioural differences. In the hippocampus the protective effect of VV extract was confirmed only after 42 days demonstrating a different response to oxidative aggression and the importance of long-term administration for natural extracts [41].

In the frontal lobe, NTZ levels increased at 21 and 42 days of surgical induced menopause compared to the sham and decreased after VV therapy at both time points. In hippocampus, NTZ formation was amplified by VV treatment after 42 days suggesting the role of NO generation and nNOS activation in presence of VV extract. It is known that overproduction of reactive nitrogen species (RNS) can lead to oxidative damage of protein and protein function alteration with cellular dysfunction and cell death. RNS inactivated important proteins such as MnSOD, Cu/Zn SOD, actin and tyrosine hydroxylase, and induced cell signalling pathways disturbance [44, 45, 47, 52]. NO can react with the superoxide anion (O_2^-) and form peroxynitrite ($ONOO^-$), known inductor of tyrosine (3-NT) residues nitration [53].

However, the role of NO in brain is controversial. Thus, NO is considered a unique regulator on neurogenesis and synaptogenesis, involved in a number of central nervous diseases such as depression, anxiety and Alzheimer's disease and critic for hippocampus modulation of mood and memories. NO is generated in the hippocampus predominantly as a significant neurotransmitter and participates in LTP and other forms of synaptic plasticity in this area. NOS enzymes are distributed in the hippocampus abundantly. It is well accepted also that oestrogen influences NO system in both peripheral and nervous tissues [54] and increases the expression of eNOS and nNOS, and consequently NO production in brain. In an experimental study, Iravani [55] concluded that learning and memory impairments in OVX rats were accompanied with lower levels of NO_2/NO_3 in serum and hippocampal tissues in comparison with sham-operated rats. In OVX animals, low levels of NTZ were noticed and after VV administration increased NTZ formation. A possible explanation is related to reduced activity of nNOS and eNOS in hippocampus of OVX animals and reduced NO production due to eNOS uncoupling and monomerization [56]. High levels of ROS induced oxidation of cofactor BH4, eNOS became uncoupled, and superoxide was produced instead of NO. [57]. In our study, VV bioavailability in hippocampus probably increased BH4 levels, reduced superoxide anion production and amplified the NO production and NTZ formation. However, higher levels of NO may also have a beneficial effect on the mobility and locomotor activity of the animals. This hypothesis was confirmed by several studies which stated that polyphenols intake increased the synthesis and bioavailability of NO [58, 59]. All these data support the idea of NO production in the hippocampus and its involvement in NTZ formation. The differences between NTZ behaviour in the hippocampus compared to the frontal lobe in OVX and VV treatment can be attributed to the morphological features of the two tissues and differences of their vascularization and enzyme expression.

Our results have shown minimal changes in the cytokines levels, the variability of results may be attributed to the variable responses of different brain regions to oxidative stress and inflammation. Interleukin (IL)-1 is a proinflammatory cytokine expressed in multiple cell types in the brain [60] with major role in coordinating the inflammatory response to the injury and neurodegenerative disease. IL-1β is rapidly synthesized and released, primarily by microglia and astrocytes [61]. In the hippocampus, IL-1 in high concentration acts upon neurons and inhibit synaptic strength and long-term potentiation (LTP) [62]. In contrast, physiological levels of IL-1 promote LTP and memory formation [63]. The distinct functions of IL-1 are mediated through the same type 1 IL-1 receptor (IL-1RI), which is expressed and regulated in both hippocampal neurons and astrocytes but in a different way [64]. The pathways involve the activation of NF-κB or activation of ERK, JNK and P38 [65]. In our study, IL-1α, IL-1β and IL-6 decreased after OVX, especially in frontal lobe for IL-1β and IL-6, while in hippocampus their levels were variable, depending on the type of cytokine. VV treatment increased IL-1α and IL-6 secretion in hippocampus while in frontal lobe their secretion decreased.

In animals with OVX and treated with VV, IL-1α and IL-6 increased in hippocampus while in frontal lobe their levels decreased significantly. Although IL-1β is recognized as a pro inflammatory cytokine, many reports support the possible beneficial effects of this cytokine and point to a possible role of IL-1β and IL-1α in normal memory function [66], particularly in short and long-term plasticity and LTP maintenance [67]. The mechanism and functional consequences for the distinct neuronal responses to IL-1β at physiological and pathophysiological doses remain unclear. Previous studies have demonstrated differential actions of both cytokines in various types of inflammation [68]. In stroke, brain IL-1α expression precedes that of IL-1β and may exert different actions than IL-1β in ischemic stroke [69]. All these data bring arguments for beneficial role of IL-1α in hippocampal function and angiogenesis in presence of VV extract.

IL-6 secretion increased in hippocampus in parallel with NTZ formation and probably NO generation. In frontal lobe IL-6 secretion was reduced in OVX without improvement after VV treatment. The differences between the behaviour of IL-6 can be explained by different role in different brain region. Thus, IL-6 is differently involved in learning and memory in different brain regions, with beneficial effects in the orbitofrontal cortex demonstrated by Donegan *et al*. and no effects on the dorsal striatal learning [70, 71].

Study limitations

The major limitation of the studies on VV consist on the great variety of grape varietals that can be used for the seed extract. Another limitation can be attributed to the influence of natural conditions like soil, sun exposure, climate in the concentration of beneficial constituents in the grapes, which makes standardization more difficult.

Conclusions

Ovariectomy has a negative influence on the locomotor activity and anxiety behaviour which may be a result of the redox status imbalance, certified by the increase of oxidative stress markers especially in a long term evolution. *Vitis vinifera* L administration may have beneficial effects on both locomotion and emotionality and could also have antioxidant properties in menopause, especially in the frontal lobe. According to our results a longer period of administration increases the beneficial effects of the tested grapeseed extract.

Conflict of interest

The authors declare no conflict of interest.

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