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Effect of Mushroom Extract *Hericium erinaceus* on Spatial Memory and Morphology of Neurons in the CA1 and CA3 Regions of the Hippocampus in Ovariectomized Rats

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Abstract

Various studies indicate that low levels of estradiol negatively impact cognitive abilities. Extracts from the fungus *Hericium erinaceus* (HE) contain bioactive components that promote the proper functioning of the nervous system and potential effects on protection against neurodegenerative diseases, including dementia and motor dysfunctions. The objective was to evaluate the effects of the administration of the HE mushroom extract on visuospatial memory and morphology of neurons in the CA1 and CA3 regions of the hippocampus in ovariectomized rats. 40 young Wistar rats weighing 90 ± 10 g BW were used, which were distributed into four groups of 10 animals; Control Group, non-ovariectomized and untreated rats; Group E2, ovariectomized rats treated with estradiol (2 µg/kg/body weight); Group HE, ovariectomized rats treated with the extract of the fungus Hericium erinaceus (0.5 mg/kg body weight) and Group Ovx/ST, ovariectomized rats, without treatment. The animals were tested in the Barnes and Open Field maze, then they were sacrificed, and their brains were obtained to perform a histological analysis of neuronal morphology in the CA1 and CA3 areas of the hippocampus. The most outstanding results showed that the Ovx/ST group recorded the longest time to arrive at the escape box and stay in the Barnes maze. A correlation was observed between neuronal damage and function; in the groups that did not present satisfactory performance in the maze tests, morphological alterations were identified such as the presence of some neuronal somata with degeneration characteristics such as pyknosis, nuclear ba-

sophilia and shrinkage of the cells. Its soma, as well as a decrease in the nuclear area of CA1 and CA3 neurons. It is concluded that the fungus Hericium erinaceus exerted a neuroprotective effect on the neuronal bodies of the hippocampus, associated with better performance in the visuospatial recognition memory test.

Keywords

Ovariectomized Rats, Ovarian Hypofunction, Estradiol, *Hericium erinaceus*, Barnes Circular Labyrinth, Open Field Labyrinth

1. Introduction

Ovarian hypofunction (OH) is generally described as a failure in the production of ovarian hormones [1], and is characterized as a syndrome consisting of amenorrhea, sex steroid deficiency, and elevated levels of gonadotropins [2].

Its etiology can be multifactorial, among which are: metabolic, environmental, autoimmune, genetic and iatrogenic; being idiopathic in around 90% of cases [3]. OH can be primary (spontaneous) or secondary (induced by radiation, chemotherapy or surgery).

This condition can be classified into three categories, based on biochemical assays of follicle-stimulating hormone (FSH) and estradiol (E2) in peripheral blood. One of them is the combination of high concentrations of FSH and low E2 indicates that the primary defect is at the ovarian level (classified in WHO group 3, that is, early menopause or premature ovarian failure), with the ovaries failing to produce estrogen despite maximal stimulation by endogenous FSH [1].

Clinical research suggests that the type of loss of ovarian hormones influences the cognition process, mainly the memory and learning process. Until recently, ovariectomy (Ovx) has been the main model in rodents to examine the effects of ovarian hormone loss on the aforementioned processes [4].

It has been observed that estrogens are one of the most powerful compounds that contribute to cognitive improvement in animals and humans. The action of estrogen contributes to neuronal processes related to cognitive function such as memory, learning and neuronal plasticity in animal models and in humans. Other actions that estrogen can generate are alterations that include morphological, neurochemical and electrophysiological characteristics of neurons; as part of the morphological changes, estrogen alters the size and arborization of neurons [5].

The most common treatment in OH is hormone therapy (HT) such as estradiol, progesterone and allopregnanolone, which can alleviate some of these alterations, however, studies suggest that the use of HT can generate serious health risks, such as cardiovascular conditions, strokes, blood clots and even a high incidence of cancer in different tissues such as the breast., uterus, ovary, among others. Understanding the impact of the use of female steroids is key to disco-

vering new treatments that promote health in women caused by this condition [4].

An alternative treatment for OH is the use of natural products such as those derived from plants that contain compounds similar to estrogens (phytoestrogens).), such as those of soybeans, flax seeds, clover, some legumes and fruits [6]. There is evidence which indicates the effects of phytoestrogens. They can offer an alternative treatment for several pathologies related to memory and learning functions. Evidence from epidemiological studies shows a comparison between Western and Asian populations, where clinical studies suggest that people from Asian countries who consume a greater amount of phytoestrogens present a decrease in clinical cases related to neurological problems associated with OH, which suggests that the consumption of phytoestrogens could represent an alternative treatment to improve cognitive functions, particularly memory and learning [6].

A resource of natural origin with possible phytoestrogenic effects may be the extract of *Hericium erinaceus* (HE). This is a medicinal mushroom capable of inducing a large number of modulating effects on human physiology, ranging from strengthening the immune system to improving cognitive functions [6]. In addition, A recent study conducted in a murine rodent model has shown that oral supplementation with HE*,* induces a significant improvement in recognition memory and an increase in spontaneous and directed excitatory synaptic current in fibers of the CA3 region of the hippocampus [7]. Therefore, the purpose of the present study is to evaluate the phytoestrogenic and neuroprotective effect of an HE extract on spatial memory and neurons in the CA1 and CA3 regions of the hippocampus of ovariectomized rats.

2. Materials and Methods

The present study was carried out in the Morphophysiology Laboratory of the Department of Veterinary Medicine of the University of Guadalajara. 40 young adult female Wistar strain rats weighing 90 ± 10 g were used, which were housed in vivarium conditions with water and food. *optional* and 12-h light/dark cycles, with climate and controlled ventilation, safeguarding the regulations in the surgical management and sacrifice of animals of the Mexican regulations NOM-062-ZOO-1999 [8].

2.1. Experimental Design

Once the rats had adapted to the vivarium conditions, they were distributed into four groups of 10 animals each; Group 1 (Control) non-ovariectomized and untreated rats; Group 2 (Estradiol), ovariectomized rats treated with estradiol (2 μg/kg/weight) every 2 days for 6 weeks; Group 3 (*Hericium erinaceus,* HE) ovariectomized rats with treatment of the *Hericium erinaceus* fungus extract for 6 weeks (Ovx + 0.5 mg/kg body weight); Group 4 (Ovx/ST), ovariectomized rats without treatment.

2.2. Obtaining the Extract *Hericium erinaceus*

The fungus was planted in a matrix of oak sawdust, corn stubble and sorghum, with a humidity of 75%, it was sterilized in plastic bags at 121˚C, 1.5 pounds of pressure for 50 minutes. After 12 h, it was inoculated with the fungus and incubated at 25˚C. When the primordia appeared, the bags were placed in the fruiting room (relative humidity of 80%), then the fungi were harvested, washed, and crushed in a mill. high speed and macerated in 96˚ alcohol for 48 hours. The alcohol was removed using a water bath and the residue was placed in an autoclave at a temperature of 121˚C, 5 min, filtered and stabilized with polyalcohol at a ratio of 1:1. By weight difference, the solids in the extract were quantified (0.0031 g of solids/5 g), so the dose to be administered orally was 0.5 mg/kg of CP, daily per animal.

2.3. Oophorectomy Technique

The animals were anesthetized intraperitoneally with xylazine 10 mg/kg and ketamine 90 mg/kg body weight, to achieve a degree of deep anesthesia. A double longitudinal dorsal incision of 1 cm was made, with a number 3 scalpel handle and a number 15 knife, to identify and expose the ovaries, they were ligated with absorbable suture (000 polyglycolic acid) at the level of the meso-ovary, they removed each of the ovaries completely without any risk to the animal. Subsequently, the peritoneal edges and dorsal muscles were sutured with absorbable suture of the same caliber, the skin was sutured with 000 nylon to avoid dead spaces between the tissues. It was finally placed on a heat mat for recovery.

2.4. Treatment Administration

Once ten days had passed after surgery, the administration of the treatments began for each of the groups, previously identified and separated in cages with two rats each to facilitate handling and avoid stress. The treatments used and application time are shown in **Figure 1**.

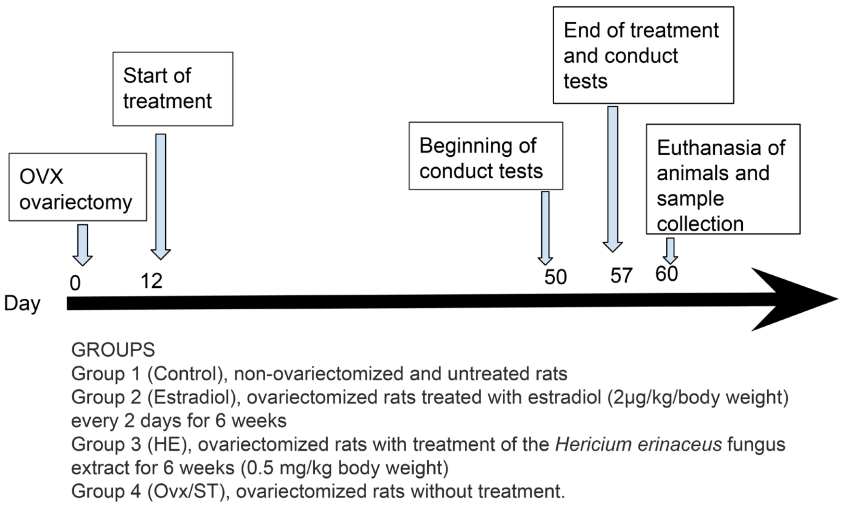

Figure 1. Schematic representation of group handling time.

2.5. Tests to Evaluate Spatial Memory

After 6 weeks of treatment, the animals were subjected to the Barnes maze test to evaluate visuospatial memory in the different groups of animals [9] [10]. The procedure that was carried out was the following:

Day 1: Habituation or memory encoding, in which each of the rats in each group was taken to the escape box, where it remained for one minute, in a single trial. Day 2: 2 trials were carried out with each of the rats in which they were placed in the middle of the maze and given 3 minutes to reach the escape box, to finally spend one minute inside it; On days 3 and 4, the same procedure was performed. Day 5: All rats were given rest on this day to reinforce learning in the last trial. Day 6: a single trial was performed with each of the rats in the Barnes maze, to observe long-term memory. The parameters evaluated were: arrival time to the escape zone, arrival time to the escape box, total time spent in the maze and number of errors. All trials were recorded with a high-definition camera (Logitech lws280®) for subsequent analysis of the video recordings.

2.6. Open Field Test

The open field test is used as an unconditioned response model, which aims to measure behavioral or physiological responses to stressful or new situations [11]. This test is one of the most popular in psychology, which allows evaluating the reaction of subjects under a stress condition [12], and was developed in 1934 to observe the emotions that rodents can present [13]. This test was applied 24 h after finishing the Barnes Maze test (day 7). On this day, a single open field test was performed per rat, which was carried out in a 60 × 60 cm dark box and each of the rats was left for 5 minutes. The trials were video recorded for the analysis of their behavior and the parameters evaluated were: percentage of immobility time, time in the center of the open field, total number of excretions, number of rearing and number of grooming.

Perfusion Technique and Tissue Collection for Morphological Analysis

Once the behavioral tests were completed, the animals were anesthetized with pentobarbital sodium at a dose of 63 mg/ml/kg body weight, via IP, and immediately afterward an intracardiac perfusion with formalin was performed, the entire brain was removed and postfixed by immersion for 24 hours. Subsequently, five of the brains from each group were subjected to the paraffin technique to make 10-micron-thick coronal sections of the dorsal hippocampal formation of the brain on a rotating microtome (Leica®). Subsequently, the sections were mounted on slides and stained with the hematoxylin-eosin technique [14].

2.7. Morphological Analysis of Areas CA1 and CA3

For this analysis, 10 different fields from the CA1 and CA3 regions of the dorsal hippocampus, both right and left, were selected from the slices of each brain. Its appearance and cyto-architecture were analyzed and photomicrographs were obtained with the 40× objective using a brand microscope (Leica®) coupled with

image analyzer software (QWin500®). From these images, the cyto-architecture of the regions mentioned above was analyzed, thus quantifying the nuclear area of its pyramidal neurons.

2.8. Statistic Analysis

The data were statistically analyzed with the univariate ANOVA test, the design was completely randomized, with four groups, 10 animals per group and 7 behavioral trials in behavioral tests. For the Post-Hoc analysis, the Tukey test was used. The differences were considered significant at $p \leq 0.05$. Data are presented as the mean ± standard error. The analysis was performed with GraphPad Prism V6.0 software.

2.9. Ethical Aspects

For the care of the animals and in all the procedures that were carried out, the guidelines specified by the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (NIH) were followed [15], in addition to the ethical guidelines indicated in NOM-062-ZOO-1999 [8].

3. Results

In general, the results showed that ovariectomized females showed decreased learning and visuospatial memory, They did not show changes in anxiety-type behaviors and through histological analysis of the hippocampus, neurons with some degenerative changes and findings of mild inflammation of the neuropil and a decrease in the nuclear area of these CA1 and CA3 neurons of the animals in the Ovx group were observed. In contrast, animals that received the HE extract showed significantly decreased behavioral and histological changes.

3.1. Test in the Barnes Maze

When comparing the means of arrival time to the escape zone (latency), there were no significant differences ($F_{4,6} = 1.945$; $p = 0.1194$) between the groups. However, the intra-trial analysis showed differences ($F_{4,6} = 24.24$; $p < 0.0001$); The post hoc test indicated a difference in the arrival time in trial 1, the OVX/ST group recorded the longest arrival time (15.1 ± 1.5) compared to the control groups (11.42 ± 2.06), estradiol (8.75 ± 1.09) and HE (9.88 ± 1.14) ($p < 0.01$, $p < 0.001$ and $p < 0.001$, respectively). On the other hand, when the cumulative means of the total trials of each group were analyzed, there were no differences between the groups ($F_4 = 1.535$, $p = 0.1916$).

In the parameter time of arrival at the escape box, the statistical analysis, between trials, showed no difference between the groups ($F_{4,6} = 2.297$; $p = 0.073$). On the other hand, the intratrial analysis showed that in trial 1 there were differences between groups ($F_{4,6} = 15.36$; $p < 0.0001$). The post hoc test indicated that the time of arrival of the OVX/ST group (65.13 ± 2071) was higher than that recorded by the control group (26.13 ± 5.76) ($p < 0.001$). Likewise, in trial 2, the

OVX/ST group (48.35 ± 12.48) presented a longer arrival time than the estradiol group (19.80 ± 3.39) ($p < 0.05$, $p < 0.01$, respectively) and the HE group (20.60 ± 4.09) ($p < 0.05$, $p < 0.05$, respectively). In the analysis of the cumulative means, a significant difference was also observed in this parameter between the groups ($F4, 5 = 2.416$, $p < 0.05$); of which the OVX/ST group (29.24 ± 0.72) had a longer time to reach the escape box than the rest of the groups (Figure 2).

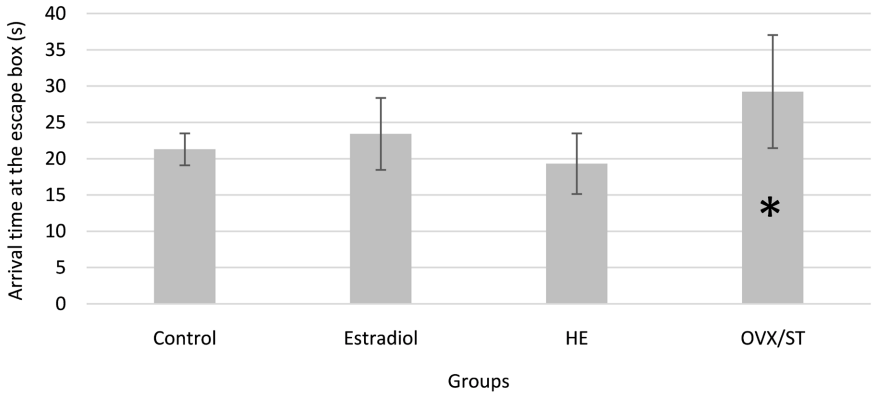

Figure 2. The means ± standard error of the arrival time at the escape box of the different groups are presented. This parameter represents the time it took the rats to reach the escape box and was obtained from 7 trials. The statistical analysis showed a significant difference ($F4,5 = 2.416$, $p < 0.05$) between groups. The OVX/ST group showed the longest arrival time to the escape zone compared to the rest of the groups. (*) indicates statistical difference ($p < 0.05$) between groups.

The inter-trial analysis of the **total time spent in the maze.** There was no significant difference between the groups ($F4,6 = 2.027$, $p = 0.1066$), however, in the intra-trial comparison, differences were observed; in trial 1 the estradiol (59.38 ± 12.09) and OVX/ST (67.70 ± 19.47) groups remained longer compared to the control group (33.07 ± 6.68) ($p < 0.05$, $p < 0.01$ respectively); and in trial 2 the OVX/ST group remained longer than the estradiol group ($p < 0.05$); In this same trial, a longer residence time was also observed in the OVX/ST group (50.76 ± 12.51) compared to the HE group (21.83 ± 4.06) ($p < 0.05$). In the analysis of the cumulative means of the different groups, no significant differences were observed ($F4,5 = 2.153$, $p = 0.074$).

In the analysis of the **number of errors** between the different trials, no significant differences were found between the groups ($F4,6 = 0.608$, $p = 0.6586$). For the analysis of cumulative means, no significant differences were observed between the groups ($F4,5 = 0.769$, $p = 0.545$).

3.2. Open Field Test

The result of the analysis **immobility time** of the behavioral test in the open field presented a significant difference in the comparison between groups ($F4.1 = 6.117$, $p < 0.05$); Tukey's post hoc analysis showed intra-trial differences, in which the Estradiol group (103.40 ± 11.42), HE (109.10 ± 17.98) and OVX/ST

(90.11 ± 16.46) had a longer immobility time in the open field compared to the control group (49.41 ± 10.10) ($p < 0.05$) (Figure 3).

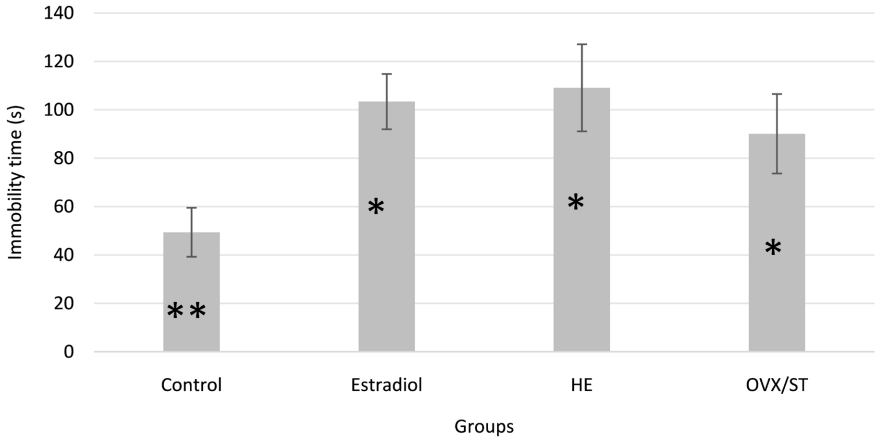

Figure 3. The means ± standard error of immobility time in seconds of the different groups in the Open Field test. Significant differences were observed between groups ($F4 = 6.117$, $p < 0.05$). The result showed an increase in immobility time in the experimental groups compared to the control group. (*) indicates statistical difference ($p < 0.05$) between groups, (**) indicates statistical difference ($p < 0.01$) between groups.

On the other hand, in the analysis of the time in the center of the field, no significant differences were found between the groups ($F4,1 = 1.298$, $p = 0.2852$). Only a trend towards a shorter time was observed for the Estradiol and HE groups. In the analysis of the parameter grooming time, no significant differences were observed ($F4,1 = 1.924$, $p = 0.1228$). The analysis of the number of stools There were no significant differences between the values of the four groups ($F4,1 = 1.753$, $p = 0.1552$). Only a trend was observed towards a lower number of times the HE group defecated. As for the Rearing number performed by the subjects in the open field, in the analysis of variance, no significant difference was identified between groups ($F4,1 = 2.517$, $p = 0.0544$). Finally, in the analysis of the number of crosses to the center of the field, there were no significant differences between the study groups ($F4,1 = 6.196$, $p < 0.05$).

3.3. Morphological Analysis of Neurons in the CA1 and CA3 Regions of the Hippocampus

Qualitative Analysis of the Hippocampus

In the CA1 and CA3 regions of the group Control Profiles of neuronal somata were observed that showed oval (almost round) nuclei with finely granular chromatin and a prominent nucleolus. In some, a slightly acidophilic cytoplasmic halo was perceived. The neuropil was seen to have a uniform, slightly acidophilic granular texture. On the other hand, in the OVX group in the CA1 region, some neuronal somas with degeneration characteristics such as pyknosis, nuclear basophilia, shrinkage of their soma and marked spherosis in the neuropil corresponding to the Oriens and Radiatum strata (mild inflammation of

axons) were observed. In the observation of the CA3 region, the basophilia of its nuclei was evident, with a greater number of cells with pyknosis and shrunken, in which the distribution of its chromatin or its nucleolus was not appreciated. His neuropil appeared lumpy and spherotic. When the tissues of the group were observed OVX + HE, the lower number of cells with degenerative characteristics was evident in CA1 and CA3, in addition, a tintoreal change of pyramidal neurons with mild acidophilia; The neuropil was observed with a lower degree of spherosis than the OVX group. In the Estradiol group, histological characteristics more similar to those of the Control group were identified (Figure 4).

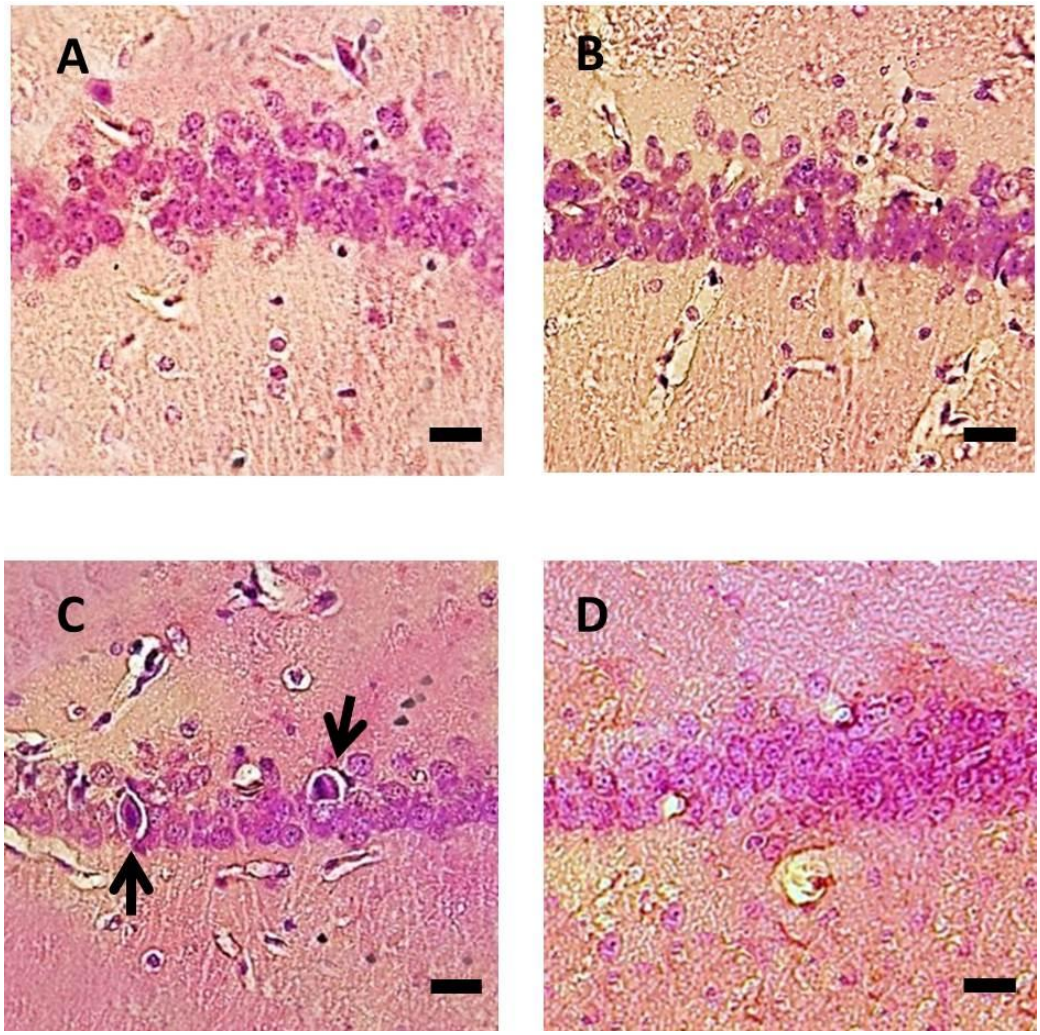

Figure 4. Representative photomicrographs of the hippocampal CA1 region of the different groups. (A) It shows nuclear profiles of the pyramidal neurons of the Control group, these nuclei are oval nuclear with finely granular chromatin and a prominent nucleolus. (B) Similar nuclear profiles are seen that correspond to the Estradiol group. (C) A high number of nuclear profiles similar to those of the control group is shown, however neuronal somata with morphological characteristics of degeneration are also observed, such as pyknosis, peri-neuronal cavitation and nuclear acidophilia, this image corresponds to the OVX/ST group. (D) The nuclear profiles of neurons similar to the control and estradiol-treated groups can be seen. This image corresponds to the HE group. HE stain, bar indicates 30 microns, 40× magnification.

In morphometric analysis applied to the nuclei of the pyramidal neurons of the two selected regions of the hippocampus of each of the study groups, the following findings were identified: significant decrease in the nuclear area of the CA1 neurons in the OVX/ST group (133.36 ± 2.25) in comparison of the Control (179.55 ± 5.04), Estradiol (174.69 ± 2.72), HE (176.32 ± 5.95) groups ($p < 0.0001$) (Figure 5).

On the other hand, in the CA3 region, it was observed that the OVX/ST group (166.28 ± 9.64) presented a decrease in the nuclear area, with significant differences in relation to the Control (194.28 ± 3.23), Estradiol (206.62 ± 2.82) groups. and HE (199.39 ± 2.24). It was also observed that between these latter groups there was no difference with respect to the control group (Figure 6).

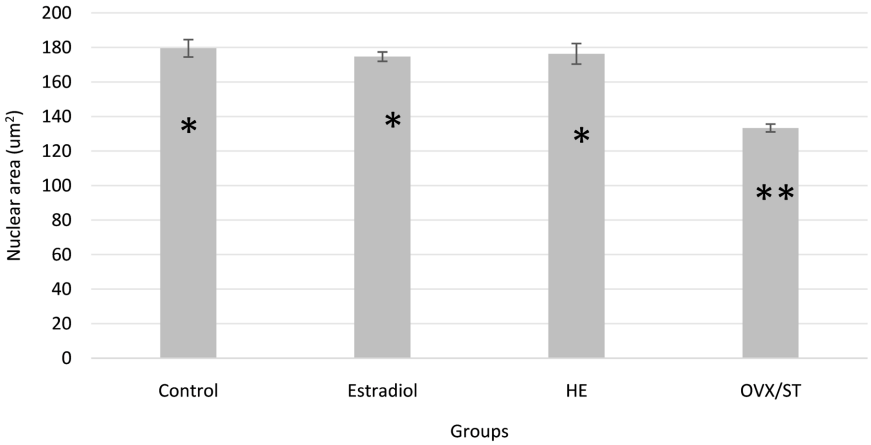

Figure 5. The means ± standard error of the nuclear area of the neurons in the CA1 region of the Hippocampus are presented. Statistical analysis showed significant differences between groups; The OVX/ST group showed nuclei with a smaller area than the rest of the groups. (*) indicates statistical difference ($p < 0.05$) between groups, (**) indicates statistical difference ($p < 0.01$) between groups.

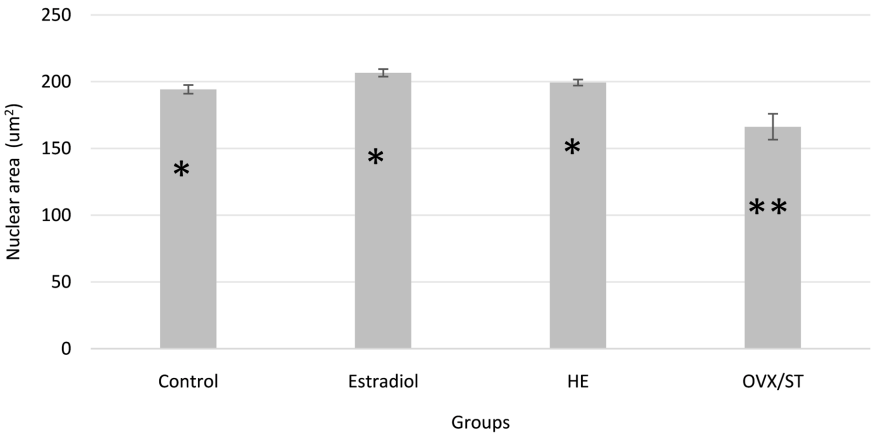

Figure 6. The means ± SE of the nuclear area of the pyramidal neurons of the CA3 region of the hippocampus are presented. The statistical analysis showed significant differences between groups; The OVX/ST group showed nuclei with a smaller area than the rest of the groups. (*) indicates statistical difference ($p < 0.05$) between groups, (**) indicates statistical difference ($p < 0.01$) between groups.

4. Discussion

4.1. Barnes Maze Test

In this study, the results obtained in the evaluation of spatial memory using the Barnes Maze showed that the decrease in gonadal estrogen concentrations in ovariectomized rats affected the cognitive ability related to spatial memory and learning, this can be observed in the latency of arrival at the escape box of the OVX/ST group, compared to the rest of the groups. Similar findings have been obtained in other studies that have used this model [16]-[18]. Likewise, they coincide with reports that have used other similar paradigms, such as the study by Ziegler and Gallagher [19] who administered estradiol benzoate to ovariectomized rats and evaluated memory in the Radial Aquatic Maze (RAM), recording an improvement in the latency times in animals treated with exogenous estradiol compared to the group of females that were only ovariectomized.

On the other hand, when another group of OVX rats was administered the HE extract at a dose of 0.5 mg/kg, this decrease in visuospatial memory capacity was notably prevented, and was evident during the first days of testing. It should be noted that this is the first study in which the capacity of visuospatial memory and learning in the Barnes maze is assessed using the model of ovariectomized rats to which an extract of HE was administered orally. In the few studies currently carried out on cognitive impairment, promising results have been reported with the consumption of diets added with this mushroom. For example, in a study of mice that consumed a diet with 5% of a HE spray for three weeks and their performance in the Y-maze test was evaluated, their results describe that the deterioration of spatial memory capacity was prevented. short-term and visual recognition induced by amyloid β peptide [20]. In another study with wild-type mice, oral supplementation with HE led to significant improvement in recognition memory, in addition to an increase in the spontaneous excitatory capacity of mossy fibers at their synapses in the CA3 region of the hippocampus [7]. In another project carried out, but in a trial with senescent adult humans who were given a dietary supplement containing 0.8 grams of a dehydrated HE for 12 weeks, improvement in their human cognitive abilities was observed [21].

In the results obtained from the current study in which the Barnes maze test was used, it was found that the groups of animals treated with Estradiol or HE showed better performance than the ovariectomized animals that were not administered treatment. Likewise, the animals in these treated groups showed tendencies to decrease the arrival time to the escape zone. In the group analysis, it was observed that the OVX/ST group had the longest time to reach the escape zone on day one and in the second trial, compared to the control group. The data obtained in this phase of the experiment contrasts with what was pointed out by Gawel [22], where he reports that even on the third day of the test the animals need more time to find the escape hole. In our study, the results showed that the differences were no longer statistically significant.

4.2. Open Field Test

The Open Field Test (OFT) is well known and applied in the aspect of animal behavior in laboratory rodents [23], and has also been used to identify motor changes. In the present study, the following parameters were taken as a model to identify anxious behaviors of the experimental subjects: time in the center of the maze, grooming, number of feces, immobility time, rearing, and number of crossings through the center, which yielded a pattern of similar results. In previous studies using the OFT model or another model to identify anxiety-type behaviors, some particular behaviors have been highlighted; however, in this work, no results were observed with relevant significant differences in the parameters that were considered to be analyzed. This result can be related to the dose of the extract used, since previous studies do show anxiolytic effect with the use of different oral administrations of compounds from this fungus, although at much higher doses than those used in the present study [24].

It is worth mentioning that in this test no significant relevant data is reported, however, as mentioned by Saavedra *et al.* [25], the motor decrease and exploratory activity in the OFT may be related to the neuronal decrease in the hippocampal area, due to surgical manipulation and administration of pharmacological treatments.

4.3. Morphological and Morphometric Aspects of Hippocampal Neurons

In the present study, in addition to evaluating spatial memory and possible anxiety behavior, special attention was paid to the morphology of the neuron. It is known that morphological changes are the slowest to occur in the nervous system, but it is also known that they provide a lot of information about the health of a particular circuit. These morphological changes were evaluated in the present work through the anatomopathological analysis of the neurons and neuropil of the evaluated regions, in addition to the quantification of the nuclear area, which is related to the transcriptional state of the cell [26]. The results obtained from the evaluation of the nuclear area revealed that oophorectomy had an unfavorable effect on the nuclear surface of neurons in the CA1 and CA3 regions of the hippocampus. Neurons in the OVX group showed a smaller nuclear surface and some neurons with degenerative characteristics (pyknotic nuclei) compared to the rest of the groups. These changes are related directly to the effect of ovarian hormone deprivation in these animals, since previous studies have described the effect of ovariectomy on cytoplasmic and nuclear morphology of hippocampal neurons [18]. The nuclear area has been classically considered as an indicator of the metabolic, transcriptional and functional activity of cells [27] [28]. The reduction in the nuclear area of neurons in the CA1 and CA3 regions due to the effect of ovariectomy has already been reported. in a previous study by our group [26].

Based on the morphological and cognitive results of the present work, it is

possible to suggest that this cellular mechanism was replicated after the administration of the HE extract and its possible interaction with estrogen receptors, which probably triggered an activation of the regulated intracellular signaling chain. by these receptors, which resulted in the maintenance of the nuclear surface of CA1 and CA3 neurons. This approach is supported by a recent study in which it is reported that two isoflavones; daidzein and genistein, have been isolated from HE mycelium [29]. Therefore, it is possible that after oral administration of the HE extract, high absorption of the components of the extract occurred at the intestinal level despite the low dose used. It is also worth mentioning that natural polyphenols, such as flavonoids, have potentially beneficial effects due to their antioxidant effect and, more importantly, their interactions with various cellular signaling pathways such as BDNF that are important in the normal functioning of the cells. CNS neurons. The experimental data show that globally the different parameters evaluated during this study (behavioral and morphological) improved in the HE group. Several explanations for this dose-dependent effect could be related to: 1) A good bioavailability of the "biologically active molecules" at the relatively low dose used in this study; 2) A possible effect on estrogen receptors, as has already been demonstrated in other studies where the competition for the ER of different phytoestrogens was evaluated [30]; 3) In addition, future studies are necessary to elucidate how the administration of these HE extracts guides the expression and accumulation of one or more neurotrophins (NGF and possibly BDNF), where each of these can present different biological effects related to the cognition, neuronal morphology and neuro-protection. The above suggests that the expression of these neurotrophins may not only be modified at the transcriptional level.

4.4. Mechanism of Action of Bioactive Compounds *Hericium erinaceus*

Numerous studies have described that hericenones (meroterpenoids) and erinacins (cyathane diterpenoids) are the two main bioactive compounds isolated from HE and tested to induce nerve growth (NGF) biosynthesis [31] [32]. Furthermore, it has been widely documented that HE possesses a range of therapeutic properties such as antioxidant activity [33], hypolipidemic activity [34], antimicrobial activity [35], immunity modulation [36], anticancer activity [37] [38] and the improvement of mild cognitive impairment [39]. The proposed mechanisms of action of the compounds of this fungus for these effects can be summarized in its capacity, first as a carrier of ROS, since it has been reported that its extracts inhibit the accumulation of free radicals, by reducing oxidative stress by protecting antioxidant enzymes, and it has also been demonstrated to increase the concentration of endogenous antioxidants [40] [41]. As a second mechanism, its ability is to inhibit one of the main apoptotic pathways (iNOS/p38 MAPK) [42]. Therefore, HE extracts are considered to have neuroprotective potential by blocking oxidative stress through the JNK1/2, p38 pathways,

which inhibits the positive regulation of Fas and Bax, and decreases the apoptosis cascade [43]. These assumed protective properties of HE extracts were evident in our study, first when the spatial memory capacity in the Barnes Maze of rodents was evaluated, and second, when histological preparations of the hippocampus were analyzed. The behavioral test showed, although slight, an improvement in the short-term memory capacity in the treated animals, and the morphological analysis showed that in these same animals, there was an increase in the nuclear surface of the pyramidal cells of the CA1 and CA3 regions. which together are objective evidence of an improvement in the transcriptional and functional activities of these cells (28). Likewise, it is worth mentioning that the morphological changes observed in the nucleus and cytoplasm (shrinkage) of the OVX/ST group are indicative of degenerative changes possibly caused by the activation of apoptotic pathways in these cells, changes that were counteracted by the administration of HE. In conclusion, it can be said that the ovariectomy model used showed that female rats showed a decrease in visuospatial memory capacity and morphological changes in the pyramidal neurons of CA1 and CA3, such as a decrease in the size of their nuclei and the presence of some degenerative changes (pyknosis). And that the continuous administration of the mushroom extract *HE*. At the dose used, this effect of ovariectomy on visuospatial memory capacity and degenerative findings in the hippocampal neurons analyzed decreased.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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