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Investigation of the Capillary Structure of Hippocampal Tissue of Healthy Rats after the Use of Urtica Dioica Hydroalcoholic Extract and Endurance Exercise

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ABSTRACT

Background and Objective: The structure and function of the hippocampal capillary are closely related to the regulation of neurogenesis and synaptic plasticity. Maintaining the health and proper organization of the hippocampal capillary network is essential for optimal hippocampal tissue function and cognitive performance. Given the beneficial effects of exercise on the body as well as the antioxidant properties of Urtica Dioica, this study was conducted to investigate the effect of endurance exercise and hydroalcoholic extract of Urtica Dioica on histomorphometric estimates of the capillaries of the hippocampal tissue of healthy rats.

Methods: In the present experimental study, 28 male Wistar rats were divided into 4 groups (n=7): control, endurance exercise (Ex), Urtica Dioica (UD), and endurance exercise+nettle (UD+Ex). After a week of familiarization with the laboratory conditions and treadmill running, endurance exercise was performed 5 days a week and daily gavage of UD hydroalcoholic extract (50 mg/kg) was administered for six weeks. 48 hours after the completion of the research protocol, the rats were sacrificed. Then the hippocampal tissue was removed and placed in 10% formalin for hematoxylin-eosin staining. After tissue staining, histomorphometric estimates, the number of sections, length, volume, and area of capillaries in the rat hippocampal tissue were examined and compared.

Findings: The results showed that the number of vascular sections and capillary volume were not significantly affected by endurance exercise and Urtica Dioica, but the capillary area in the UD+Ex group (1.24±0.21 mm^2) significantly increased compared to the Ex (0.535±0.076 mm^2) (p=0.013) and UD (0.5±0.08 mm^2) (p=0.010) groups, although this increase was not different from the control group (0.815±0.15 mm^2). A decrease in the capillary volume of the hippocampal tissue of the UD group was observed compared to the other groups (p=0.001).

Conclusion: The results of the study showed that the combination of UD and exercise helped maintain the capillary structure and function of hippocampal tissue in healthy rats.

Keywords: *Hippocampus, Endurance Exercise, Urtica Dioica, Vascular Structure*.

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Introduction

Capillaries play a vital role in the body by facilitating the exchange of nutrients and waste products between the blood and tissues (1). They are involved in sensing the tissue environment, coordinating the responses of the microvascular network, and controlling blood flow (2). Capillaries are recognized as a key element in hemodynamic control and are important for metabolic, hormonal, and immune homeostasis (3). They are responsible for transporting hormones throughout the body and regulating transport within the capillary endothelium (4). Capillaries also help match capillary blood flow to the metabolic rate of cells in organs and tissues, ensuring appropriate delivery of nutrients and removal of waste products (5). Pericytes, which surround capillaries, have been identified as key mediators in matching tissue metabolic needs with adequate capillary blood flow. Impaired capillary communication can lead to impaired blood flow, pathophysiology, and disease. In general, capillaries play a central role in maintaining tissue function and homeostasis. Brain capillaries play an important role in maintaining cellular viability and preventing neurodegeneration (6, 7). They provide a constant supply of blood to meet the high metabolic demands of brain tissue (7).

Capillaries have various functions in the brain, including solute transport and diffusion, metabolic homeostasis, vascular hemodynamics, and vascular permeability (8). They also serve as sentinel cells of the brain in the innate immune system and are capable of presenting antigens. Brain capillaries can vary in diameter and undergo changes in response to neuronal activity. The control of capillary diameter involves factors such as pericyte contractility and endothelial wall architecture. Dysfunction of brain capillaries can lead to neuroinflammation and contribute to cerebral small vessel disease. Overall, the importance of capillaries in the brain lies in their role in maintaining brain homeostasis and supporting the proper functioning of brain tissue. In addition, hippocampal capillaries also play an important role in various brain functions and pathologies. They are involved in memory processes and are affected in conditions such as Alzheimer's disease, temporal lobe epilepsy, and chronic limbic encephalitis (9, 10).

It has been shown that hippocampal capillaries are affected by running, which can improve symptoms of depression (11). In one study, running showed positive effects on capillary parameters in the hippocampal CA1 and dentate gyrus, ultimately leading to improvement in depressive symptoms (9). In a study by Kaiser et al., the positive effects of regular exercise, regardless of intensity, were demonstrated by increasing capillary density in CA1 and dentate gyrus, enhancing spatial memory and cognitive function through angiogenesis and regulation of nitric oxide (12).

Vascular endothelial growth factor released during aerobic exercise promotes hippocampal angiogenesis and neurogenesis, which contributes to capillary growth (13). A meta-analysis of controlled trials showed that physical training has a positive effect on total hippocampal volume, especially in older populations and interventions that included more than 24 weeks of moderate-intensity exercise training (14). Although the specific effects of physical training on the number of capillary cross-sections, length, volume, and area in hippocampal tissue have not been specifically investigated, the overall findings suggest that physical training can have beneficial effects on parameters such as angiogenesis and neurogenesis.

On the other hand, Urtica dioica, a plant of the Urticaceae family, commonly known as stinging nettle, has been studied by researchers for its various medicinal activities in recent decades (15-17). This plant is widespread in Europe, Africa, America, and parts of Asia due to its adaptability to different environments and climatic conditions. Bioactive chemical compounds such as flavonoids, phenolic acids, amino acids, carotenoids, and fatty acids have been isolated from this plant (15). Urtica dioica also has excellent pharmacological effects, including antiviral, antimicrobial, anticancer, nephroprotective, hepatoprotective, cardioprotective, antiarthritic, antidiabetic, anti-endometriotic, antioxidant, anti-inflammatory, anti-aging,

and neuroprotective effects, and can potentially be used in the treatment of neurological diseases (16, 17). One study showed that the aqueous extract of Urtica dioica caused a dose-dependent increase in basal tone in isolated rat aorta, indicating vasoconstriction (18). Another study showed that Urtica dioica extract helped reduce plasma glucose levels by increasing insulin secretion and pancreatic beta-cell proliferation (19). This suggests that Urtica dioica may have a dual effect on the vascular network, potentially causing vasoconstriction while also improving glucose control. Further research is needed to fully understand the mechanisms and potential negative effects of Urtica dioica on the vascular network and capillary structure.

Given the importance of the hippocampus in memory and learning, examining the structure of hippocampal capillaries could provide insight into the neural mechanisms underlying these cognitive processes. Both exercise and Urtica dioica have neuroprotective properties, and examining their effects on hippocampal capillary structure could reveal potential benefits for maintaining or improving hippocampal function, particularly in the context of age-related cognitive decline or neurodegenerative disorders. The capillary network in the hippocampus plays an important role in delivering nutrients, oxygen, and other essential components to support neural activity and plasticity. Understanding how exercise and Urtica dioica may modulate capillary structure could shed light on their effects on neuroplasticity and overall hippocampal health. Therefore, conducting a study on the combined effects of exercise and Urtica dioica on hippocampal capillary structure could provide valuable insights into potential therapeutic strategies to enhance brain health and cognitive abilities. Accordingly, this study aimed to investigate the effect of six weeks of endurance exercise and consumption of Urtica dioica on the number of sections, length, volume, and area of capillaries in the hippocampal tissue of healthy rats.

Methods

After approval by the Ethics Committee of Lorestan University, this experimental study was conducted on 28 6-week-old male Wistar rats, randomly selected from the Animal Care Center of Lorestan University of Medical Sciences with the code LU.ECRA.2019.17. After the samples were transferred to the laboratory environment, the rats were kept in transparent polycarbonate cages located in a room with a 12-hour light/dark cycle and a temperature of 25±1 °C. During this period, the rats had free access to water and food. After a one-week period during which the rats adapted to the new laboratory conditions, the familiarization phase of running on a treadmill began. This phase consisted of five sessions, each lasting between 10 and 15 minutes, and the treadmill was set at a speed of 10 to 20 meters per minute (20). Then, the rats were divided into four equal groups of 7 using a random assignment method: Control, Endurance exercise (Ex), Urtica Dioica (UD), and Endurance exercise+Urtica Dioica (Ex+UD).

Urtica Dioica hydroalcoholic extract (collection, preparation, dosage): Urtica Dioica was identified and prepared from the Qalikuh, peaks in the Zagros Mountain range, located in Lorestan province. After confirmation in the herbarium of Agricultural Organization of Lorestan province (sample number: 13/776), the aerial parts of the Urtica Dioica plant were stored, dried and ground in dark and moisture-free conditions. In order to produce Urtica Dioica hydroalcoholic extract, 500 grams of dried plant powder was mixed with 70% ethanol and incubated for 24 hours. The resulting material was filtered twice through a Whatman No. 2 filter. To reduce the volume of solvent and facilitate ethanol evaporation, it was transferred to a rotary device set at 60 °C, and then the extract was placed in an incubator at 60 °C for 24 hours until the ethanol completely evaporated, resulting in a dry plant extract. This extract was subsequently stored in a refrigerator until the time of testing (21). The groups assigned to receive Urtica Dioica were administered a

hydroalcoholic extract of Urtica Dioica at a dose of 50 mg/kg of rat body weight, dissolved in 1 ml of distilled water, which was received orally by gavage daily for 6 weeks (21).

Endurance exercise protocol: The endurance exercise protocol was performed using a treadmill for 5 days per week for a total of 6 weeks, at a moderate intensity level in the groups that were allowed to exercise. The training sessions consisted of three 3-minute warm-up sessions followed by the main exercise that ranged from 10 to 30 minutes, with a 3-minute cool-down period. The speed and duration of the main exercise gradually increased from the first to the sixth week, such that the speed and duration of the exercise in the first week were 10 m/min for 10 minutes and in the second week 10 m/min for 20 minutes. In the third week, it was 14-15 m/min for 20 minutes and in weeks 4 and 5, it was 14-15 m/min and 17-18 m/min for 30 minutes, respectively. To achieve stability, the speed and duration of the treadmill exercise were kept constant in the sixth week (22).

Description: 48 hours after the last endurance exercise session and administration of Urtica dioica hydroalcoholic extract, all rats were anesthetized by inhalation of 2% halothane in a mixture of 30% O_2 and 70% N_2O and perfused with 100 ml of normal saline followed by 250 ml of 0.1% paraformaldehyde in phosphate buffer (pH=7.4) (22). After perfusion, the animals were decapitated and their brains were carefully removed and transferred to a fixing solution (10% formalin) until histological processing.

Histological study: Stereological studies of hippocampal tissue were performed using hematoxylin-eosin staining. To perform hematoxylin-eosin tissue staining, the steps of embedding, dehydrating, clarifying, and molding were performed in order. Then, using a microtome, 5-µm-thick coronal sections were prepared for mounting on slides. In the next step, the slides were stained with hematoxylin and eosin (22). All histological studies were performed using stereological methods in 20 fields, using a microscope with magnifications of 40 and 100, and randomly calculated.

Evaluation of total capillary length in SR (Survival Rate) of hippocampal tissue: Based on the formula ($LV_{(cap/SR)}= 2*\Sigma Q_{(cap/SR)} / \Sigma A$), ΣQ (cap/SR) is the total number of capillary profiles in the hippocampal area counted in each rat. ΣA represents the total area of counting frames used in each rat.

Total capillary volume in SR (Survival Rate) of hippocampal tissue: Each captured image was randomly placed in a transparent dot grid. The capillary volume fraction was analyzed using the formula $VV_{(cap/SR)}= \Sigma P_{(cap/SR)}/ \Sigma P$.

Total capillary area in SR (Survival Rate) of hippocampal tissue: The area occupied by capillaries in SR was estimated using the equation $SV_{(cap/SR)}= 2\times\Sigma L_{(cap)}/\Sigma L$. The expansion of $SV_{(cap/SR)}$ was done through the SR volume (V (SR)) (23, 24).

After data collection, the Shapiro-Wilk test was used to assess the normal distribution of the data. Given the non-normality of the data, the Kruskal-Wallis test was used to examine the significant difference in the mean values between groups in the context of hippocampal tissue stereology studies, and then the Bonferroni-modified post hoc test was used for pairwise comparisons of groups. Data analysis was performed in SPSS version 26, and $p<0.05$ was considered significant.

Results

The results showed that there was no significant difference between the study groups regarding the parameters of the number of vascular sections ($p=0.295$, $\chi2(4)=3.702$) and capillary volume ($p=0.275$, $\chi2(4)=3.879$) in the hippocampal tissue (Figure 1, sections b and d). In contrast, there was a significant difference between the study groups in the parameters of the area ($p=0.026$, $\chi2(4)=9.231$) and capillary length ($p<0.001$, $\chi2(4)=21.143$) of the hippocampal tissue. Comparison of groups showed that capillary length was shorter in the UD group (2.85±0.52 mm) compared to the control group (8.25±0.99 mm)

(p=0.001), but endurance exercise alone and in combination with Urtica dioica did not cause significant changes in capillary length in the Ex (8±1.14 mm) and Ex+UD (8.15±0.77 mm) groups compared to the control group. In addition, the length of the hippocampal capillaries of the UD group was significantly shorter than that of the Ex and Ex+UD groups (p=0.001) (Figure 1, section c).

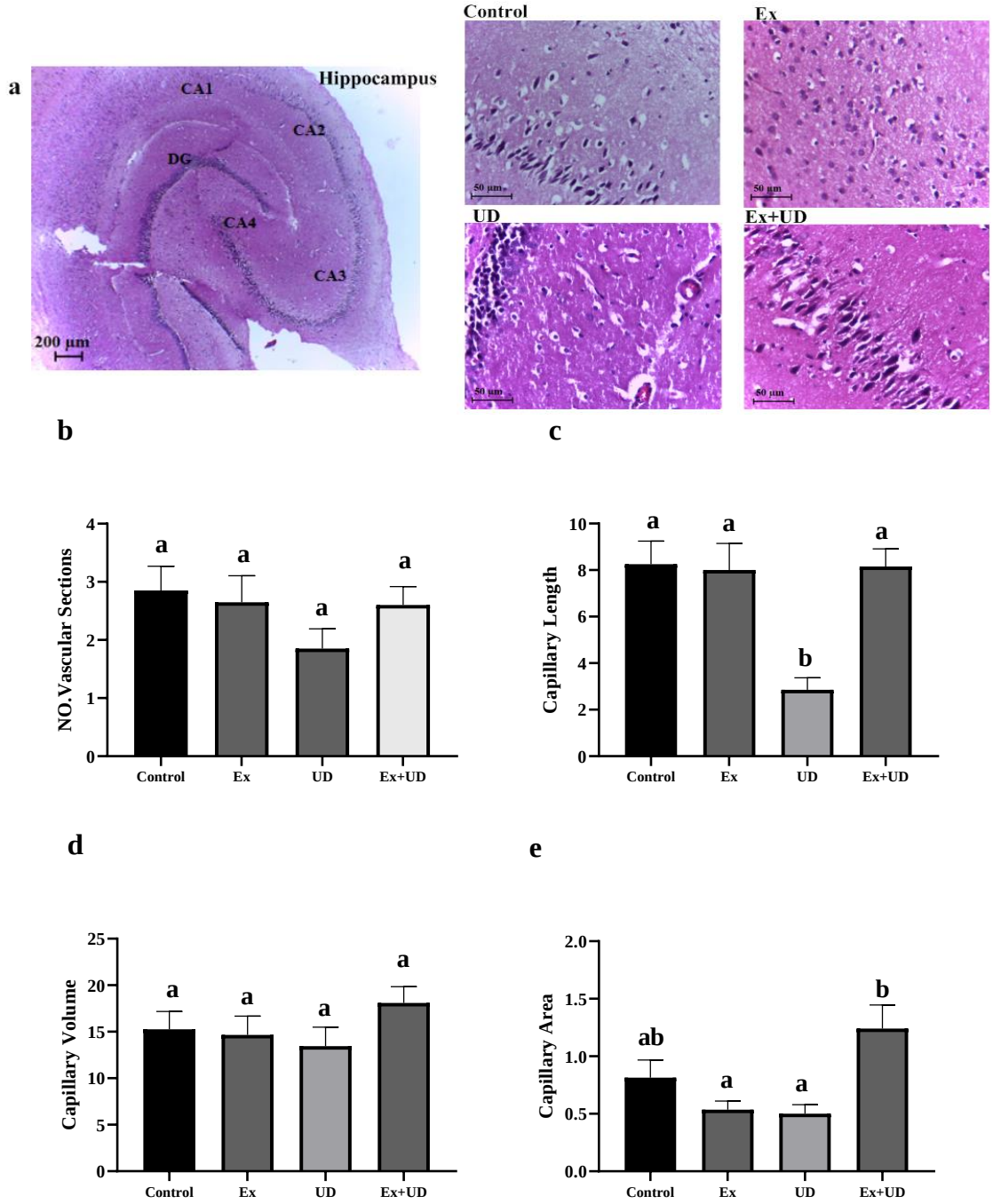

Figure 1. Histomorphometric estimates of vascular cross-sections (number), length (mm), volume (mm^3), and area (mm^2) of rat hippocampal tissue capillaries. (a) H&E staining to reveal the hippocampal partition in the control (Control), endurance exercise (Ex), Urtica dioica (UD), and exercise training+Urtica dioica (Ex+UD) study groups (scale bar=50 µm). Mean±SD of the studied parameters are reported in (b-e).

Furthermore, comparing the groups based on the capillary area showed that the implementation of endurance exercise and Urtica dioica alone and together in the Ex, UD and Ex+UD groups did not differ significantly compared to the control group, but the capillary area in the UD+Ex group (1.24±0.21 mm^2) increased significantly compared to the Ex (0.535±0.076 mm^2) (p=0.013) and UD (0.5±0.08 mm^2) (p=0.010) groups (p<0.05) (Figure 1, section e). The method for calculating the capillary surface area, capillary length and capillary volume of the hippocampal tissue is shown in Figure 2.

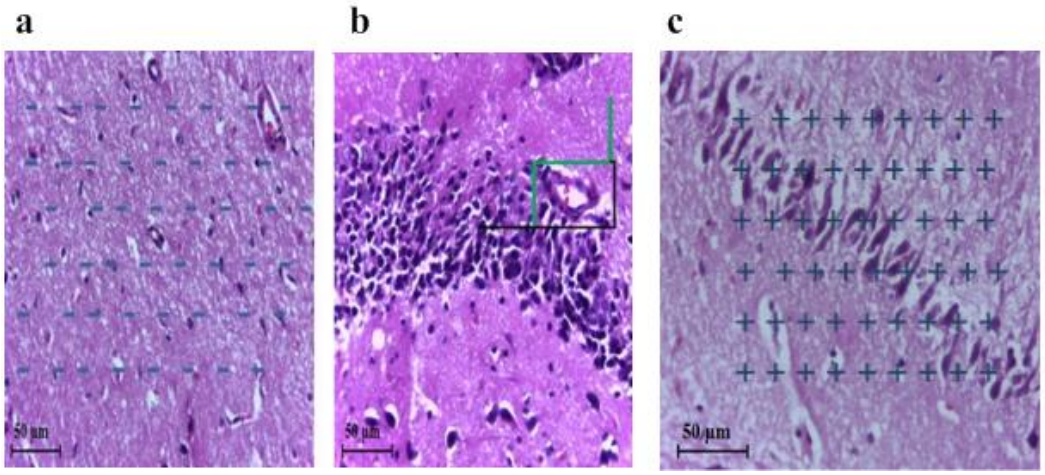

Figure 2. Calculation Method (a) capillary surface area (area occupied by capillaries in a given frame by placing them on the pattern lines (scale bar=50 µm)). (b) capillary length (each image taken is placed on an unbiased counting frame. Only vessels that are in the frame and touch the black line were considered). (c) capillary volume (number of collisions in each capillary was calculated with the within-frame algorithm) in hippocampal tissue.

Discussion

According to the results, the number of vascular sections, length, volume and area of capillaries after six weeks of endurance exercise were not significantly different from the capillaries of the hippocampal tissue of the control group rats. Contrary to expectations, Urtica dioica consumption also had a negative effect on the length of capillaries and caused a non-significant decrease in the number of vascular sections and capillary area. In addition, the combination of endurance exercise with Urtica dioica consumption compared to the single implementation of these protocols caused the number of vascular sections, length, volume and area of capillaries of the hippocampal tissue of the rats in this group to be maintained at the level of the control group and not undergo significant changes. Unfortunately, no specific study has directly investigated the effects of Urtica dioica on the structure of the hippocampal capillary so far. Most of the existing studies have focused on the potential effects of this plant on other aspects of the brain and cognitive function (22, 25), and no study has examined the effect of Urtica dioica on the structure of the hippocampal capillary.

A study by Wodi et al. evaluated the extensive toxicology of commonly used edible plants and their mixtures in home remedies. They found that chronic consumption of ginger, garlic, and lemon extracts resulted in organ-level toxicity, including damage to the liver, kidney, intestinal epithelium, stomach, and pancreas (26). Another parallel study showed that carbon tetrachloride, a chemical that is harmful to the liver, can reduce the hepatoprotective activity of various medicinal plants. Plants contain chemicals with antioxidant properties that can protect liver cells from inflammation and oxidative damage (27). In addition,

a study by Uduchi et al. discussed the potential risks of certain plant extracts on kidney function in diabetes. While some plant extracts may have beneficial effects on kidney function, others can impair kidney function (28). Therefore, caution should be exercised when using medicinal herbs, and more research is needed to determine safe and effective doses for their use (29).

Endurance exercise has been shown to have positive effects on vascular structure in the healthy brain and hippocampus. A six-month endurance exercise program in young sedentary adults resulted in increased brain perfusion in specific regions, particularly in the right superior temporal gyrus and ventral striatum, which was associated with increased maximal oxygen uptake (30). Long-term competitive endurance exercise in middle-aged to older adults also resulted in increased cortical thickness in several brain regions, including the medial prefrontal cortex and the anterior and posterior central gyrus, indicating the development of cortical reserve (31).

Furthermore, a one-week exercise intervention in healthy young men resulted in microstructural changes in the brain, including a reduction in the restricted fraction in the corpus callosum and an increase in fractional anisotropy, indicating improved axonal integrity (32). These findings suggest that short- and long-term endurance exercise can have beneficial effects on the healthy brain and hippocampus.

According to unbiased estimates of these parameters in our study, it is likely that six weeks is a short time to create positive changes in the capillary structure of the hippocampal tissue of healthy rats and that a longer period may be required. It has been reported that brain diseases such as Alzheimer's disease cause vascular changes, and after implementing non-pharmacological interventions such as exercise, the body seeks to stabilize and eliminate negative changes, but in a healthy body, longer periods are needed if we want to see desirable changes. More interestingly, during the same period, contrary to our assumption, Urtica dioica significantly reduced the length of the capillaries of the hippocampus of healthy rats, and only in combination with endurance exercise was the capillary structure preserved.

Finally, the results showed that Urtica dioica consumption in healthy rats did not have a positive effect on histomorphometric parameters of hippocampal tissue. However, in one study, it was found that Urtica dioica extract, when combined with endurance exercise, had a significant effect on increasing the level of heat shock protein in the brain, which may improve neuroprotective conditions in diabetic patients (33, 34). This suggests that the positive effects of Urtica dioica may be enhanced when combined with exercise. In addition, the synergy of endurance exercise and Urtica dioica leaf extract in the study of Haghshenas et al. showed that the unbalanced IDO1-KYN-AHR pathway in the liver of streptozotocin-induced diabetic rats was restored after the combination of endurance exercise and Urtica dioica (35). Although these studies provide valuable insights into the effects of endurance exercise and Urtica dioica on various aspects of brain and organ function and structure, there is no specific research on the synergy of these interventions on the vascular structure of the brain and hippocampus.

Finally, this study showed that the negative effects of Urtica dioica on the capillary structure of the hippocampal tissue of healthy rats were modulated by endurance exercise. Interestingly, the two interventions performed better together than when performed alone, although this combination did not produce significant changes compared to the control group in the capillary structure of the hippocampal tissue of healthy rats. This study shows that well-known medicinal plants should also be used with caution.

Conflict of interest: The authors declare that there are no conflicts of interest.

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