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Effect of Icariin on Pentylenetetrazole-Induced Seizures in Male Mice with Gonadoctomy

running title: The effect of Icariin on epilepsy

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Abstract

Background:in epileptic patients and humans treated with antiepileptic drugs, androgen concentration is significantly reduced, leading to an increase in seizures, followed by the destruction of hippocampal neurons and memory and learning disorders.

Objectives: the purpose of this study was the effect of icariin extract on epileptic rats with gonadectomy.

methods: 84 male mice were used for this study. The mice were randomly divided into 12 groups, including control, pentylenetetrazole, dimethyl sulfoxide, icariin, gonadectomy, pentylenetetrazole + icariin, pentylenetetrazole + gonadectomy, dimethyl sulfoxide + icariin, dimethyl sulfoxide + gonadectomy, icariin + gonadectomy, pentylenetetrazole + icariin + gonadectomy, and dimethyl sulfoxide + icariin + gonadectomy. After inducing epilepsy with pentylenetetrazole, the shuttle box test and then the tissue parameters were evaluated.

Results: The results of this study showed that both epilepsy-induced seizures and gonadectomy caused a decrease in neuronal density in the hippocampus, a decrease in nestin expression, an increase in degenerated cells and an increase in inflammation, an increase in the number of monocytes, and then a decrease in memory and learning ability. The icariin extract improved this condition to a certain extent.

Conclusion: The use of herbal medicines such as icariin in the treatment of epilepsy patients by increasing testosterone levels and its neuroprotective properties may open up new horizons in the treatment of patients with epilepsy.

Key words: epilepsy ،gonadectomy ، Icariin, pentylene tetrazole , seizure

Introduction

Epilepsy is a group of brain diseases characterized by an enduring predisposition to generate epileptic seizures(Sumadewi et al ., 2013). Accordingly, seizures constitute the primary symptom of epilepsy. Epileptic seizures can also occur as isolated events not associated with the enduring predisposition, and as such, the occurrence of a seizure may not necessarily imply a diagnosis of epilepsy(Perucca et al ., 2020). The important thing about epileptic seizures is that these seizures are variable and can be partial or severe, short or long, frequent or infrequent (Kamali *et al*., 2018). The most important symptoms of people with epilepsy are headache, speech problems, memory and concentration disorders, or cognitive disorders in general (Novak *et al*.,2022). Cognitive disorders in epilepsy are due to the loss of neurons in the hippocampus or entorhinal cortex (Holmes., 2015). The CA1 regions of the hippocampus and dentate gyrus are involved in the development of seizures and hippocampal sclerosis (Pires *et al*., 2021). Men and women of all ages can suffer from epilepsy. However, most clinical studies focus on men (Bahramnejad *et al*., 2023), experimental models of susceptibility to seizures and epilepsy show gender differences, and in general, seizures and epilepsy are more common in men than in women (Scharfman *et al*., 2014), and this gender difference may be related to factors such as body weight, steroid hormones, cytochrome P450 activity, and biological differences in the neuronal network (Behmanesh *et al*., 2017). In men, removal of the testicles (gonadectomy), which are the main source of androgens, leads to an increase in seizures. On the other hand, the occurrence of inflammatory reactions followed by cell apoptosis leading to astrogliosis can effectively influence the severity of the disease. (Hassanpour, S., & Ghasemi ., 2024), antiepileptic drugs cause changes in plasma levels of sex hormones, and this complication is particularly evident in men and the change in their androgen levels. Among androgens, the decrease in testosterone in epileptic patients causes an increase in hippocampal neuron disorders and an increase in seizures (Andréen *et al*., 2005) Thus, considering that antiepileptic drugs can cause changes in plasma levels of sex hormones, the aim of this study is the use of Icariin in the treatment of epilepsy caused by injection of pentylenetetrazole (PTZ). Pentylenetetrazole is a convulsion-inducing substance that can cause convulsions by injecting a certain concentration of this drug, and even

the use of a single dose of pentylenetetrazole can cause memory and learning impairment (Alachkar *et al*., 2020). Icariine is a flavonol found in several species of plants in the Epidemium family, commonly known as bugleweed or yin yang hu. Icariine powder is an extract from the stem and leaves of Epimedium. This product is a light blue to light yellow crystalline powder with a molecular weight of 676.65. Icariin powder can increase blood flow to the heart, blood vessels and bone marrow, and Icariin can also increase testosterone production and raise estrogen levels, especially in postmenopausal women (Nian *et al*., 2009). It can therefore be said that Icarin as an antiepileptic can help to reduce plasma levels of sex hormones and improve brain structures by increasing blood flow to the heart and brain.

Materials and methods

Animal grouping

Experiments were done according to the Ethical Committee (by Ethical code: IR.ILAM.REC.1403.006) for Animal Care Use of Laboratory Animals at the ilam University. In this study, 84 mice weighing 30-35 gr were used. Animals were housed in a controlled environmental condition with 12-h light/dark cycles with standard rat food and water ad libitum، The animals were then randomly divided into 12 groups of 7 animals each.

First group: Control (Intact)

Second group: Pentylenetetrazole (PTZ)

Third group: Dimethyl sulfoxide (DMSO)

Fourth group: Icariin (E)

Fifth group: Gonadectomy (GO)

Sixth group: Pentylenetetrazole+Ikarin (PTZ+E)

Seventh group: Pentylenetetrazole + Gonadectomy (PTZ + GO)

Eighth group: Dimethyl sulfoxide + icariin (DMSO + E)

9. Group: Dimethyl sulfoxide + Gonadectomy (DMSO + GO)

10. Group: Icariin + Gonadectomy (E+ GO)

11. Group: Pentylenetetrazole + Icariin + Gonadectomy (PTZ+ E+ GO)

12. Group: Dimethyl sulfoxide + Icariin + Gonadectomy (DMSO+ E+ GO)

Surgical procedures

In the gonadectomy groups، For gonadectomy, the male mouse was placed in the prone position and a 1 cm median incision was made in the scrotum and the skin pulled back to expose the

tunica. The tunica was then punctured to remove the testes individually. The testicles were lifted to expose the underlying spermatic cord. This was clamped and ligated at the confluence of blood vessels and epididymis.

Two weeks after gonadectomy, the experiments were performed on mice, with all injections made intraperitoneally. Half an hour before the injection of pentylenetetrazole (PTZ) (70 mg/kg) (Shimada, T., & Yamagata, K, *et al* ., 2018) icariin (50 mg/kg)(Guo, Y *et al*., 2020) was injected. Immediately after the injection of PTZ, the time of the different seizure stages was examined and recorded for 20 minutes.

Examination of the seizure stages:

The seizure stages are:

1- Zero stage: threshold stage or onset of seizure (no response)

2- Stage one: contraction of the facial and ear muscles 3- Stage two: spread of the contraction wave over the whole body 4- Stage three: myoclonic jumping while standing on two feet 5- Stage four: falling on the side 6- Stage five: falling on the back and generalised tonic and clonic seizures (Sharma, S *et al* ., 2018)

The functional assessments

The shuttle box device was used to measure the learning and memory capacity of adult rats. This device, manufactured in Iran, consists of two light-colored rooms measuring 20 x 20 cm made of transparent and dark plastic, the walls of which are covered with opaque plastic. Between the two rooms is a sliding door (8 x 8 cm) that is opened and closed with a wire. The floor of both rooms is covered with stainless steel bars. The bars are each 2 mm thick and are spaced one

centimeter apart. The floor of the darkroom can be supplied with electricity by connecting it to a power supply. The amount of current received and the time can be set and the test is carried out on 2 consecutive days. The first day is regarded as the training day and the second day as the test day.

The initial adaptation stage: the target animal is placed in the bright part of the device and after 20 seconds, the guillotine valve is opened and according to the animal's natural tendency to the dark environment, it goes to the dark part, and after the mouse enters the dark part, the guillotine valve is closed. The pack and the mouse remain in the dark area for 30 seconds, and then the mouse is taken out of the dark area and returned to its cage.

Secondary reconciliation stage: After half an hour, the primary reconciliation stage is repeated. The training phase includes the following steps:

A: After half an hour of secondary adaptation, this stage is performed as follows: As in the stage of adaptation, the desired animal is placed in the lighted part of the device, 20 seconds later, the guillotine valve is opened and after the mouse enters the part Tarik closed the valve and pressed the start button of the device to apply the shock, so that the electric shock with the intensity of 0.4 milliamps in 1.5 seconds was delivered. Then, after 20 seconds, the door of the dark part is opened and the mouse is returned to the cage.

B: Step A is repeated after two minutes. If the mouse does not enter the dark part of the device after 120 seconds from the opening of the valve between the two parts, the mouse is removed and the experiment is over. In this case, receiving a shock will be recorded for the mouse. If the mouse enters the dark area, the valve is closed and the shock is applied and the previous step is repeated.

C: Step b is repeated after 2 minutes. The repetition of the training phase (shocking) continues until the mouse stays in the light area for 120 seconds and does not enter the dark area, and the number of shocks received by the mouse is recorded.(Zavvari, F., & Karimzadeh, F., 2017).

second day

24 hours after the training phase, the recall phase (memory test) of the mouse should be evaluated. In this way, the trained mouse is placed in the light box for 20 seconds, and then the doors of the two boxes are closed and the time of entering the dark part is recorded. This time should be up to 300 seconds, and if the mouse does not go to the dark part by this time, the same 300 seconds will be recorded for it. After the mouse went to the dark area, the elapsed time is measured and considered as the time spent in the dark box, 24 hours after the training and application of the shock(Resae, A *et al* ., 2022)

Histological assessment of hippocampus

After Euthanasia of mice with ketamine-xylazine, the brains were slowly and carefully removed and fixed in 10% formalin for one week, and after tissue passage, they were cut with a thickness of 5 microns.

Cresyl fast violet

To prepare Cresyl Fast Violet solution, 990 milliliters of distilled water was heated to 45 degrees and then one gram of Cresyl Fast Violet powder was dissolved in distilled water. Then 10% acetic acid was slowly added to the obtained solution. Before using for dyeing, the solution was filtered through a filter. In order to stain the tissue, the slices were placed next to the dye for 6 minutes and after staining, they were washed several times with distilled water to remove the dye from the cells. The cresyl fast violet staining technique is used to study the tissue in terms of the division of nerve cells in the changed area(Otify, D. Y *et al*., 2014).

Hematoxylin-eosin (E&H) staining

A non-specific staining is very common in which hematoxylin and eosin are used. Eosin is an acidic dye and has a negative charge. This dye makes basophilic structures red or pink. These

structures are sometimes called eosinophilic. Hematoxylin is considered as a basophilic dye. This dye is used to stain acidic structures (basophils). Purple color is used, therefore, in this staining method, DNA in the nucleus and RNA in ribosomes are colored purple, and the rest of the cytoplasm components are pink(Bancroft, J. D., & Gamble, M., 2008).

Immunohistochemistry staining

For immunohistochemistry staining, sections were permeated with triton x-100 (0.3%) blocked in goat serum 10%. Samples were treated overnight with primary antibodies, and mouse anti-nestin monoclonal antibody (1:500; Abcam). After 24 h these were washed in PBS and incubated with a secondary antibody (rabbit anti-mouse antibody conjugated with FITC) for 45 min at room temperature. Nuclei were stained with propidium iodide (PI, Sigma, 1:10,000) for 3 min (Nooraei et al., 2022)

Statistical analysis:

Statistical analysis of the data obtained from the behavioral test was evaluated using repeated measures analysis of variance. The microscopic parameters were evaluated using two-way analysis of variance and Tukey's complementary test, and $p \leq 0.05$ was considered a significant difference.

Results

The results:

Results of the shuttle box test

The results of this study showed that, in the shuttle box test, the duration in which the mice entered the darkroom(STL) was shorter in the pentylenetetrazole (PTZ) and pentylenetetrazole + icariin + gonadectomy (PTZ + E + GO) groups than in the other groups ،This decrease in the time of entering the dark chamber was statistically significant compared to other groups (Graph 1), indicating memory impairment and hippocampal involvement in these groups,

Also, the survival time of mice in the dark chamber (TDC)in the pentylenetetrazole (PTZ) and pentylene tetrazole + icariin + gonadectomy (PTZ + E + GO) groups is more than the other groups, and this increase in the survival time in the dark chamber is compared to the other studied groups. It was also statistically significant (Graph 2).

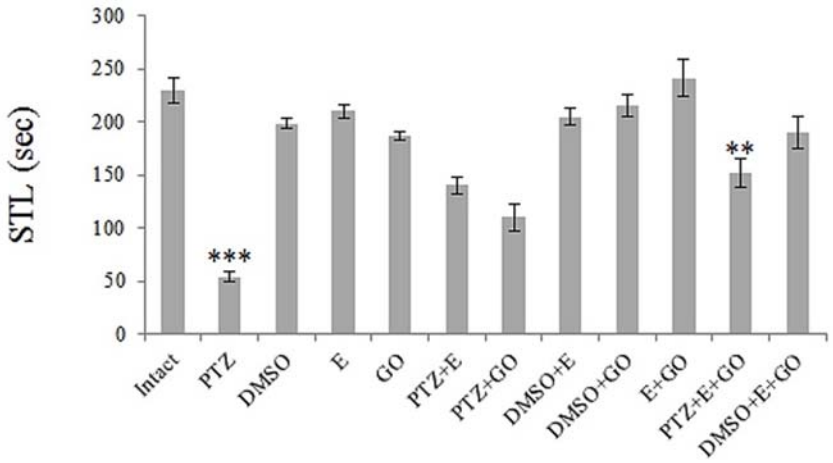
Results of tissue studies

In Nestin staining, it was shown that in the Pentylenetetrazole (PTZ) Pentylenetetrazole + Gonadectomy (PTZ + GO) groups, the level of nestin expression was greatly reduced and endogenous cells and neural stem cells had less division. In the Pentylenetetrazole + Icariin (PTZ+ E) group, Icariin has been able to increase the expression of nestin to some extent (picture 2), in other groups the expression of nestin is normal. (Figure 3)

In cresyl fast violet staining, dark and pyknotic cells with reduced cytoplasmic level were examined, and cell viability and survival were examined in all groups. In the pentylenetetrazole (PTZ) and pentylenetetrazole + icariin + gonadectomy (PTZ+E+ GO) groups, the number of pyknotic cells increased with a decrease in cytoplasmic level, indicating dark cells (Figure 4), even when cell survival was counted in the CA1 region of the hippocampus. It was found that

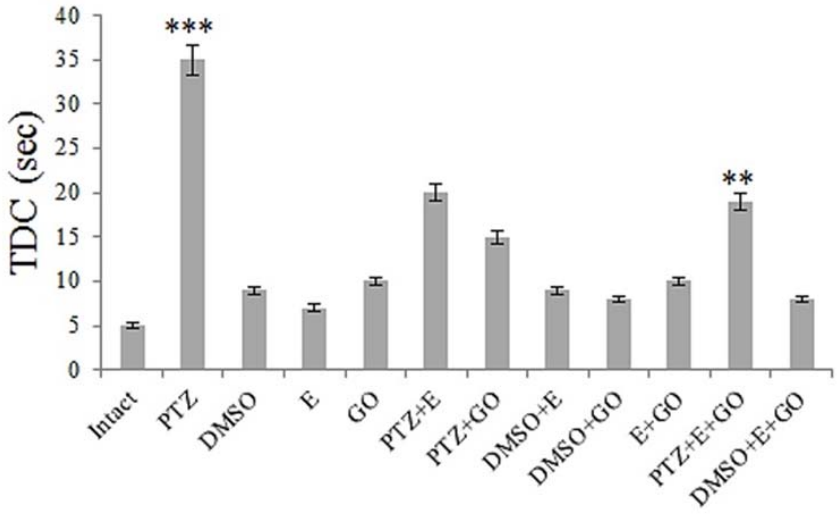
cell survival and survival rate decreased in the pentylenetetrazole (PTZ) and pentylenetetrazole + icariin + gonadectomy (PTZ + E + GO) groups compared to the other groups, which was statistically significant (Figure 5).

E&H staining examined the trend of inflammation and the presence of monocytes. In the pentylenetetrazole group, the nuclei were highly stained due to pyknosis and cell death, and the inflammation and presence of monocytes were also extremely high in this group. The pentylenetetrazole + gonadectomy group also showed a high rate of inflammation and cell death. In the pentylenetetrazole + icariin group, this trend was slightly lower compared to the other two groups (Figure 6).



Graph 1: Shuttle box test. In this test, the duration of the animal's entry into the darkroom (step-through latency) was measured. The results showed that there was a significant difference between the group receiving pentylenetetrazole (PTZ) and other groups*** ($p < .001$) and also between the group receiving pentylenetetrazole + icariin + gonadectomy (PTZ + E + GO) and other groups.

A



significant difference was found** ($p < .01$)

Graph 2: Shuttle box test. In this test, the survival time of mice was measured in the darkroom (TDC), and the longest survival time belonged to the pentylenetetrazole group and the pentylenetetrazole + gonadectomy + icariin group, which has a statistically significant difference compared to the other groups. was *** ($p < .001$). **($p < .01$).

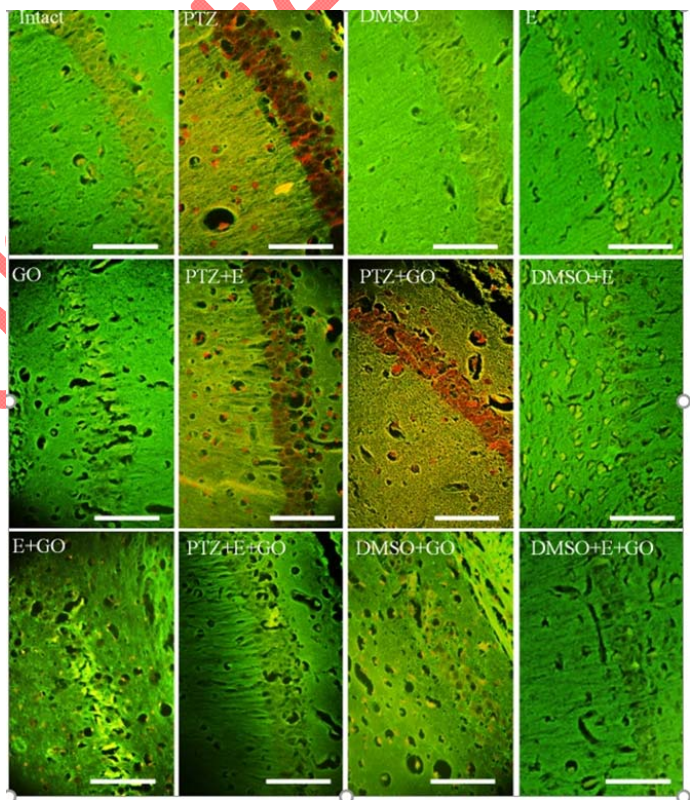


Figure 3: Nestin staining. As a neuro-fundamental marker: Nestin expression in pentylene tetrazole and pentylenetetrazole + gonadectomy group has decreased drastically, in Icarin and pentylenetetrazole group the expression rate has increased compared to the previous two groups, in other groups nestin expression is normal is.

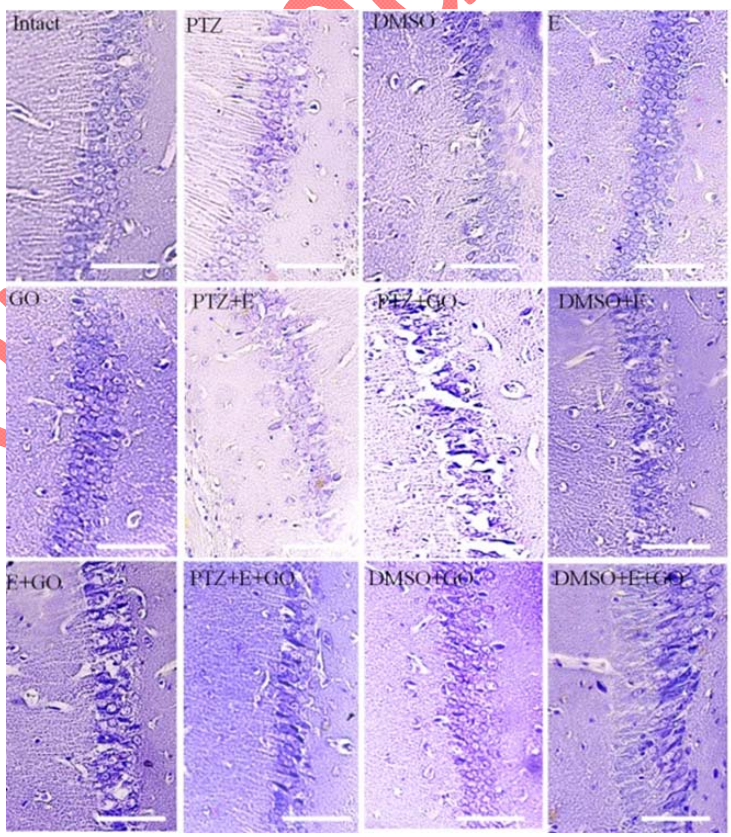


figure 4: Cresyl fast violet staining: In the pentylenetetrazole and pentylenetetrazole + gonadectomy groups, the number of pyknotic cells increased with a decrease in cytoplasm, i.e. dark cells, and the survival rate of nerve cells decreased, but in the pentylenetetrazole + and icariin group, the number of dark cells decreased compared to the previous two groups, in the other groups, the number of dark cells and cell viability are normal.

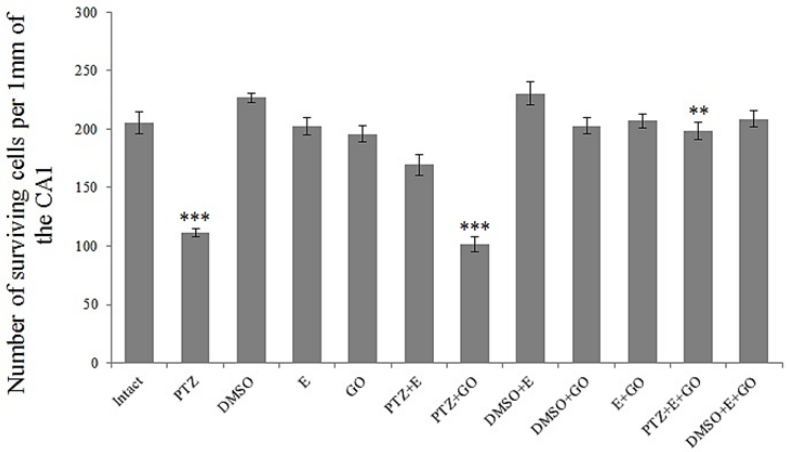


figure 5: Cell viability count in the CA1 region of the hippocampus, the number of viable cells in the pentylenetetrazole, pentylenetetrazole + gonadectomy and pentylenetetrazole + gonadectomy + icariin groups decreased compared to the other groups, which is statistically significant. ***($p < .001$). **($p < .01$).

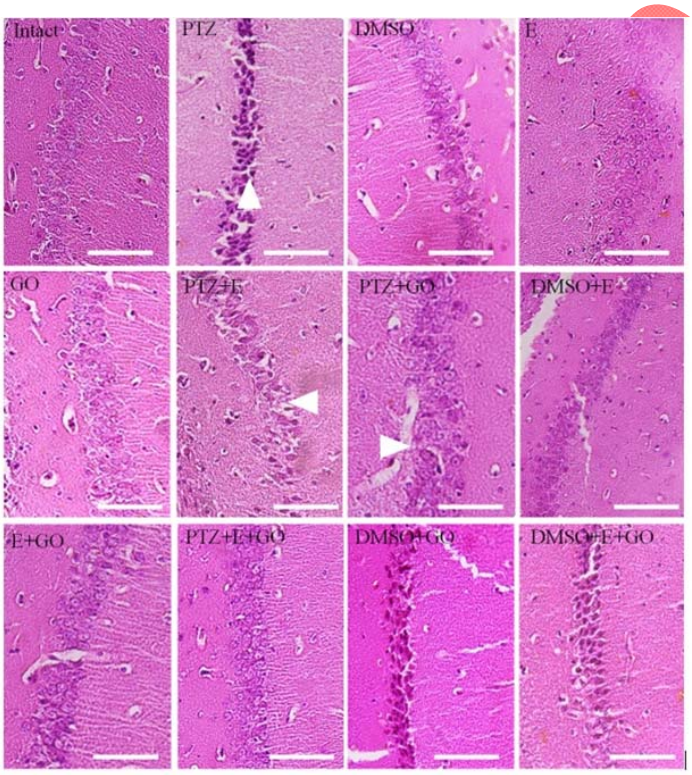


Figure 6: H&E staining. In the pentylenetetrazole group, the number of inflammatory monocytes and the inflammation are strongly increased (white arrow). In the pentylenetetrazole + gonadectomy and pentylenetetrazole + icariin groups, inflammation is also observed (white arrow), but the extent of inflammation is lower. No inflammation and no increase in monocytes were observed in the other groups.

Discussion

Epilepsy is one of the most important neurological diseases. One of the most important features of epilepsy is seizures (Milligan., 2015). In men with epilepsy, a decrease in plasma levels of testosterone is observed, and a decrease in testosterone levels leads to an increase in seizures (Kim., 2023). Many synthetic antiepileptic drugs cause a decrease in plasma testosterone levels (Moazzami *et al*., 2013), after this disorder memory and learning are impaired due to the destruction of neurons in the hippocampus region (Zaitsev *et al*., 2021). The results of this study in the shuttle box test showed that in the swallowing group (PTZ) and the swallowing and gonadectomy and icariin receiving group (PTZ+E+GO), the time the animal entered the dark area (SLT) decreased, indicating impairment. However, in the icariin group (E), the icariin and gonadectomy group (E+G) and also the icariin and epilepsy group (E+PTZ), this period is normal. This suggests that Icarin may prevent memory impairment by increasing androgen levels and improving hippocampal neurons by reducing inflammation in the hippocampus. Epileptic seizures cause memory impairment by damaging hippocampal neurons (Solati *et al*., 2019). Agarwal et al. showed that PTZ-induced epilepsy in rats impairs passive avoidance memory and significantly reduces the latency to enter the dark compartment in the shuttle box test, which is consistent with our results. In addition, studies have reported that PTZ-induced epileptic seizures impair spatial memory, cause learning impairment, and impair passive avoidance behaviour (Agarwal *et al*., 2011). The results of our study showed that memory and learning performance

was lower in the gonadectomy group (G) than in the gonadectomy-icariin group (G+E). This shows that testosterone levels are related to memory and learning performance, which is consistent with the present study by Roshanaei et al. showing that the decrease in testosterone levels in gonadectomized male rats leads to a decrease in memory and learning performance (Roshanaei *et al*., 2013). Ghahramani, P et al. investigated the memory of gonadectomized rats using the shuttle box test and reported that gonadectomy of rats increases the persistence of this group of rats in the darkroom (TDC), indicating a decrease in memory and learning of rats. Gonadectomy is being pursued (Ghahramani *et al*., 2018). The absence or lack of sex hormones has been shown to have important effects on the hippocampus, which is one of the most important structures playing a role in memory and learning (Mosleh., 2013), and it has also been shown that testosterone affects the growth of dendritic spines and increases synaptic density. In the pyramidal neurons of the hippocampus, it increases memory and learning performance. gonadectomy causes a decrease in dendritic spines and a decrease in hippocampal neurons, especially in the CA1 area of the hippocampus, which is due to these interactions that decrease memory and learning performance (Leranth *et al*., 2003). In recent years, it has been reported that the decrease of testosterone level increases the level of beta-amyloid protein, and the increase of beta-amyloid is more evident in Alzheimer's patients suffering from memory loss (Gillett *et al*., 2003). Erasmus et al. reported that icariin increases testosterone levels (Erasmus *et al*., 2021). Thus, assuming that antiepileptic drugs decrease testosterone levels, icariin may increase testosterone levels and thus enhance learning and memory performance. Asgharzade et al. reported that epileptic rats with PTZ showed memory impairment in the shuttle box test compared to other groups, which is consistent with this study. They believed that these memory impairments were due to The death of hippocampal neurons (Asgharzade *et al*., 2020). The histologic results of this study showed that the survival rate of neurons in the CA1 region decreased dramatically in the castration and gonadectomy and castration-gonadectomy and icariin groups, but in the other groups, especially in the groups that received icariin, this proportion is normal and increases. Lopim et al., after counting neurons in the hippocampal area on consecutive days in epileptic rats, showed that the number of neurons decreased drastically in epileptic rats, which is consistent with the present study (Lopim *et al*., 2016). Several studies in

animal models have shown that epilepsy is characterized by various changes in the hippocampus, including the destruction of neurons in the CA1 and CA3 regions of the hippocampus, a decrease in gamma-aminobutyric neurons, and the disappearance of calcium-binding proteins in dentate granule cells. be (Rao *et al*.,2005. Shetty et al., 2007). Moghadami et al. reported that gonadectomy decreases the density of androgen receptor-immunoreactive neurons in the hippocampus and that injection of testosterone and subsequent elevation of testosterone levels increases the density of these neurons (Moghadami *et al*., 2016). It appears that androgens, particularly testosterone, have nutritional effects on the dendrites of the hippocampus, thereby enhancing neurogenesis in the hippocampal region (McMahon.,2014). Liu et al. have shown in a study that icariin can improve the function of hippocampal neurons through the mechanism of anti-inflammation (Liu *et al*., 2020). The results of our study showed that the expression of nestin decreased dramatically in the epilepsy group and in the group with epilepsy and gonadectomy. Consistent with this study, Cho et al. also decreased (Cho., 2015). In this study, nestin staining showed that icariin extract can enhance neurogenesis in the icariin-receiving group and increase nestin expression compared with the icariin group, demonstrating the neuroprotective properties of icariin. Liu and colleagues reported that icariin has neuroprotective effects and can also significantly increase the survival rate of hippocampal neurons treated with corticotropin (Liu *et al*., 2020). The anti-apoptotic role of icariin has also been established in several studies. Icarin inhibits CORT-induced neuronal apoptosis in rat hippocampus by inhibiting the p38 MAPK signaling pathway and suppresses ER stress-induced neuronal apoptosis by activating the PI3K/Akt signaling pathway. (Li *et al*., 2015), in this study and hematoxylin-eosin staining shows an increase in the number of monocytes and inflammation in the epilepsy group, but icariin greatly reduced inflammation in the epilepsy group receiving icariin, neuroinflammation mediated by microglia has a significant contribution It has epilepsy in the pathophysiology (Wang *et al*., 2023). Icarin prevents inflammation by suppressing pro-inflammatory signals such as NF-kB and MAPK. In addition, icariin prevents inflammation by activating anti-inflammatory signaling GR and Nrf2 (Luo *et al*., 2020). Cong et al. have shown that icariin prevents acute demyelination and regulates the number of microglia, astrocytes and oligodendrocytes (Cong *et al*. 2021), which is consistent with the results of this study.

Conclusion

it can be concluded that epilepsy due to frequent seizures and gonadectomy due to the decrease in testosterone levels can cause memory and learning disorders, reduce the density of hippocampal neurons and increase inflammation in the brain structures, whereas the icariin extract with its anti-inflammatory and neuroprotective properties can prevent the complications of epilepsy and also has the property of increasing testosterone.

Ethical Considerations

Compliance with ethical guidelines

All steps of this study were done according to the ethical guidelines for working with laboratory animals

code of ethics IR.ILAM.REC.1403.006

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Authors' contributions

Salman Soltani, Aref Nooraei and Marzieh Darvish, the main design of the article and the writing of the article. Javad Cheraghi, Marzieh Havasi, data analysis and article editing

Conflict of interest

The authors declared no conflict of interest

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References

Alachkar, A., Ojha, S. K., Sadeq, A., Adem, A., Frank, A., Stark, H., & Sadek, B. (2020). Experimental models for the discovery of novel anticonvulsant drugs: focus on

pentylenetetrazole-induced seizures and associated memory deficits. *Current pharmaceutical design*, *26*(15), 1693-1711. DOI: 10.2174/1381612826666200131105324[PMID: 32003682]

Andréen, L., Sundström-Poromaa, I., Bixo, M., Andersson, A., Nyberg, S., & Bäckström, T. (2005). Relationship between allopregnanolone and negative mood in postmenopausal women taking sequential hormone replacement therapy with vaginal progesterone. *Psychoneuroendocrinology*, *30*(2), 212-224. DOI: 10.1016/j.psyneuen.2004.07.003[PMID: 15471618]

Anwar, H., Khan, Q. U., Nadeem, N., Pervaiz, I., Ali, M., & Cheema, F. F. (2020). Epileptic seizures. *Discoveries*, *8*(2). DOI: 10.15190/d.2020.7.[PMID: 32577498]

Asgharzade, S., Rabiei, Z., Rabiei, S., Bijad, E., & Rafieian-Kopaei, M. (2020). Therapeutic effects of oleuropein in improving seizure, oxidative stress and cognitive disorder in pentylenetetrazole kindling model of epilepsy in mice. *Iranian Journal of Pharmaceutical Research: IJPR*, *19*(1), 98. DOI : 10.22037/ijpr.2019.14212.12209.[PMCID: PMC7462478]

Bahramnejad, E., Barney, E. R., Lester, S., Hurtado, A., Thompson, T., Watkins, J. C., & Hammer, M. F. (2023). Greater female than male resilience to mortality and morbidity in the Scn8a mouse model of pediatric epilepsy. *International Journal of Neuroscience*, 1-13. DOI: 10.1080/00207454.2023.2279497.[PMID: 37929583]

Bancroft, J. D., & Gamble, M. (Eds.). (2008). Theory and practice of histological techniques. Elsevier health sciences.

Behmanesh, M. A., Efani Majd, N., Shahriari, A., & Nnajafzadeh, H. (2017). Evaluation of antioxidant potential of Aloe vera and pituitary sexual hormones after experimental diabetes in male rats. Iran J Vet Med, 11(2), 164-74. DOI: 10.22059/ijvm.2018.252228.1004877

Cho, K. O., Lybrand, Z. R., Ito, N., Brulet, R., Tafacory, F., Zhang, L., ... & Hsieh, J. (2015). Aberrant hippocampal neurogenesis contributes to epilepsy and associated cognitive decline. *Nature communications*, *6*(1), 6606. DOI: 10.1038/ncomms7606.[PMID: 25808087]

Cong, H., Liang, M., Wang, Y., Chang, H., Du, L., Zhang, X., & Yin, L. (2021). Icariin ameliorates the cuprizone-induced acute brain demyelination and modulates the number of oligodendrocytes, microglia and astrocytes in the brain of C57BL/6J mice. *Brain Research Bulletin*, *175*, 37-47. DOI: 10.1016/j.brainresbull.2021.07.010.[PMID: 34274431]

Erasmus, L. J. C., & Leisegang, K. (2021). Herbal medicines (Zingiber officinale and Epimedium grandiflorum) used to treat andrological problems: Asia and Indian subcontinent. In *Herbal Medicine in Andrology* (pp. 123-128). Academic Press. DOI: 10.1016/B978-0-12-815565-3.00018-7

Fisher, R. S., Cross, J. H., French, J. A., Higurashi, N., Hirsch, E., Jansen, F. E., ... & Zuberi, S. M. (2017). Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia*, *58*(4), 522-530. DOI: 10.1111/epi.13670.[PMID: 28276060]

Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., ... & Wiebe, S. (2014). ILAE official report: a practical clinical definition of epilepsy. *Epilepsia*, *55*(4), 475-482. DOI: 10.1111/epi.12550.[PMID: 24730690]

Ghahramani, P., Harooni, H. E., Fatemi Tabatabaei, S. R., & Moazedi, A. A. (2018). Effects of zinc chloride on passive avoidance memory of male rats, in gonadectomized peri-pubertaly. *Studies in Medical Sciences*, *28*(11), 708-714.

Gillett, M. J., Martins, R. N., Clarnette, R. M., Chubb, S. A. P., Bruce, D. G., & Yeap, B. B. (2003). Relationship between testosterone, sex hormone binding globulin and plasma amyloid beta peptide 40 in older men with subjective memory loss or dementia. *Journal of Alzheimer's Disease*, *5*(4), 267-269. DOI: 10.3233/jad-2003-5401.[PMID: 14624021]

Guo, Y., Cai, Y., & Zhang, X. (2020). Icariin ameliorates the cognitive function in an epilepsy neonatal rat model by blocking the GluR2/ERK I/II pathway. *Folia Neuropathologica*, *58*(3), 245-252. DOI :10.5114/fn.2020.100067

Hassanpour, S., & Ghasemi, Z. (2024). Effects on Betaine on Ameliorating Depression in Gonadectomized Male Rats.in press

Holmes, G. L. (2015). Cognitive impairment in epilepsy: the role of network abnormalities. *Epileptic Disorders*, *17*(2), 101-116. DOI: 10.1684/epd.2015.0739.[PMID: 25905906]

Kamali, M., Zendehdel, M., Babapour, V., & Heshmatian, B. (2018). The effect of gestational exposure of sodium cromoglycate on epileptiform activities in the rat offspring. DOI: 10.22059/ijvm.2018.252228.1004877.

Karoly, P. J., Rao, V. R., Gregg, N. M., Worrell, G. A., Bernard, C., Cook, M. J., & Baud, M. O. (2021). Cycles in epilepsy. *Nature Reviews Neurology*, *17*(5), 267-284. DOI: 10.1038/s41582-021-00464-1.[PMID: 33723459]

Kim, H. H., Goetz, T. G., Grieve, V., & Keuroghlian, A. S. (2023). Psychopharmacological considerations for gender-affirming hormone therapy. *Harvard review of psychiatry*, *31*(4), 183-194. DOI: 10.1097/HRP.0000000000000373.[PMID: 37437250]

Leranth, C., Petnehazy, O., & MacLusky, N. J. (2003). Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *Journal of Neuroscience*, *23*(5), 1588-1592. DOI: 10.1523/JNEUROSCI.23-05-01588.2003

Li, F., Gao, B., Dong, H., Shi, J., & Fang, D. (2015). Icariin induces synoviolin expression through NFE2L1 to protect neurons from ER stress-induced apoptosis. *PloS one*, *10*(3), e0119955. DOI: 10.1371/journal.pone.0119955.[PMID: 25806530]

Liu, J., Liu, L., Sun, J., Luo, Q., Yan, C., Zhang, H., ... & Dong, J. (2020). Icariin protects hippocampal neurons from endoplasmic reticulum stress and NF-κB mediated apoptosis in fetal rat hippocampal neurons and asthma rats. *Frontiers in pharmacology*, *10*, 1660. DOI: 10.3389/fphar.2019.01660.[PMID: 32082160]

Luo, Z., Dong, J., & Wu, J. (2022). Impact of Icariin and its derivatives on inflammatory diseases and relevant signaling pathways. *International Immunopharmacology*, *108*, 108861. DOI: 10.1016/j.intimp.2022.108861.[PMID: 35597118]

Lopim, G. M., Campos, D. V., da Silva, S. G., de Almeida, A. A., Lent, R., Cavalheiro, E. A., & Arida, R. M. (2016). Relationship between seizure frequency and number of neuronal and non-neuronal cells in the hippocampus throughout the life of rats with epilepsy. *Brain research*, *1634*, 179-186. DOI: 10.1016/j.brainres.2015.12.055.[PMID: 26764534]

McMahon, D. (2014). *The effects of adult gonadectomy on hippocampal neuron morphology in male mice* (Doctoral dissertation, University of Guelph).

Milligan, T. A. (2021). Epilepsy: a clinical overview. *The American Journal of Medicine*, *134*(7), 840-847. DOI: 10.1016/j.amjmed.2021.01.038.[PMID: 33775643]

Moazzami, K., Emamzadeh-Fard, S., & Shabani, M. (2013). Anticonvulsive effect of atorvastatin on pentylenetetrazole-induced seizures in mice: the role of nitric oxide pathway. *Fundamental & clinical pharmacology*, *27*(4), 387-392. DOI: 10.1111/j.1472-8206.2012.01038.x

Moghadami, S., Jahanshahi, M., Sepehri, H., & Amini, H. (2016). Gonadectomy reduces the density of androgen receptor-immunoreactive neurons in male rat's hippocampus: testosterone replacement compensates it. *Behavioral and Brain Functions*, *12*, 1-10.[PMID: 26822779]

Mosleh, M., & Palizvan, M. (2013). Effect of sumatriptan on the field potentials of the CA1 region of hippocampus in male rats. *Journal of Arak University of Medical Sciences*, *15*(10), 77-84.

Nian, H., Ma, M. H., Nian, S. S., & Xu, L. L. (2009). Antiosteoporotic activity of icariin in ovariectomized rats. *Phytomedicine*, *16*(4), 320-326. DOI: 10.1016/j.phymed.2008.12.006.[PMID: 31849666]

Novak, A., Vizjak, K., & Rakusa, M. (2022). Cognitive impairment in people with epilepsy. *Journal of Clinical Medicine*, *11*(1), 267. DOI: 10.3390/jcm11010267.[PMID: 35012007]

Nooraei, A., Khazaeel, K., Darvishi, M., Ghotbeddin, Z., & Basir, Z. (2022). Dimorphic evaluation of hippocampal changes in rat model of demyelination: A comparative functional, morphometric, and histological study. Brain and Behavior, 12(8), e32723.DOI. /10.1002/brb3.2723

Otify, D. Y., Youssef, E., Nagy, N. B., Marei, M. K., & Youssif, M. I. (2014). Transdifferentiation of bone marrow mesenchymal stem cells into neural cells via cerebrospinal fluid. Biomed Biotechnol, 2(4), 66-79. DOI: 10.12691/bb-2-4-2.

Perucca, P., Bahlo, M., & Berkovic, S. F. (2020). The genetics of epilepsy. *Annual review of genomics and human genetics*, *21*(1), 205-230. DOI: 10.1146/annurev-genom-120219-074937 [PMID: 32339036]

Pires, G., Leitner, D., Drummond, E., Kanshin, E., Nayak, S., Askenazi, M., ... & Devinsky, O. (2021). Proteomic differences in the hippocampus and cortex of epilepsy brain tissue. *Brain Communications*, *3*(2), fcab021. DOI : 10.1093/braincomms/fcab021.[PMID: 34159317]

Rao, M. S., Hattiangady, B., Abdel-Rahman, A., Stanley, D. P., & Shetty, A. K. (2005). Newly born cells in the ageing dentate gyrus display normal migration, survival and neuronal fate choice but endure retarded early maturation. *European Journal of Neuroscience*, *21*(2), 464-476. DOI: 10.1111/j.1460-9568.2005.03853.x.[PMID: 15673445]

Resae, A., Yousefi, M., Naeimi, S., & Mahdavi, A. (2022). Effects of Occupational Formaldehyde Exposure on Passive Avoidance Conditioning and Anxiety Levels in Wistar rats. Iran J Vet Med. DOI.org/10.32598/ijvm.17.1.1005241

Roshanaei, K., Rezazadeh Amin, S. A., & Heidarieh, N. (2013). Effects of Valeriana officinalis extract on passive avoidance learning in Wistar rats gonadectomized. In *First National Conference on Medicinal Plants and Sustainable Agriculture*.

Samba Reddy, D. (2017). Sex differences in the anticonvulsant activity of neurosteroids. *Journal of neuroscience research*, *95*(1-2), 661-670. DOI 10.1002/jnr.23853.[PMID: 27870400]

Scharfman, H. E., & MacLusky, N. J. (2014). Sex differences in the neurobiology of epilepsy: a preclinical perspective. *Neurobiology of disease*, *72*, 180-192. DOI: 10.1016/j.nbd.2014.07.004.[PMID: 25058745]

Shetty, A. K., & Hattiangady, B. (2007). Restoration of calbindin after fetal hippocampal CA3 cell grafting into the injured hippocampus in a rat model of temporal lobe epilepsy. *Hippocampus*, *17*(10), 943-956. DOI: 10.1002/hipo.20311.[PMID: 17604349]

Shimada, T., & Yamagata, K. (2018). Pentylenetetrazole-induced kindling mouse model. *Journal of visualized experiments: JoVE*, (136). DOI: 10.3791/56573.[PMID: 29985308]

Sharma, S., Puttachary, S., Thippeswamy, A., Kanthasamy, A. G., & Thippeswamy, T. (2018). Status epilepticus: behavioral and electroencephalography seizure correlates in kainate experimental models. *Frontiers in neurology*, *9*, 7. DOI: 10.3389/fneur.2018.00007.[PMID. 29410648]

Solati, K., Rabiei, Z., Asgharzade, S., Amini-Khoei, H., Hassanpour, A., Abbasiyan, Z., ... & Rafieian-Kopaei, M. (2019). The effect of pretreatment with hydroalcoholic extract of Alpinia officinarum rhizome on seizure severity and memory impairment in pentylenetetrazol-induced kindling model of seizure in rat. *AIMS neuroscience*, *6*(3), 128. DOI: 10.3934/Neuroscience.2019.3.128.[PMID: 32341973]

Sumadewi, K. T., Harkitasari, S., & Tjandra, D. C. (2023). Biomolecular mechanisms of epileptic seizures and epilepsy: a review. Acta Epileptologica, 5(1), 28. DOI: 10.1186/s42494-024-00157-4

Wang, J., Liu, Y., Wu, Y., Yang, K., Yang, K., Yan, L., & Feng, L. (2023). Anti-inflammatory effects of icariin in the acute and chronic phases of the mouse pilocarpine model of epilepsy. *European Journal of Pharmacology*, *960*, 176141. DOI: 10.1016/j.ejphar.2023.176141.[PMID: 37866741]

Zaitsev, A. V., Amakhin, D. V., Dyomina, A. V., Zakharova, M. V., Ergina, J. L., Postnikova, T. Y., ... & Magazanik, L. G. (2021). Synaptic dysfunction in epilepsy. *Journal of Evolutionary Biochemistry and Physiology*, *57*(3), 542-563. DOI: 10.1134/S002209302103008XS.[PMID: 30618633]

Zavvari, F., & Karimzadeh, F. (2017). A review on the behavioral tests for learning and memory assessments in rat. *The Neuroscience Journal of Shefaye Khatam*, *5*(4), 110-24. DOI: 10.18869/acadpub.shefa.5.4.110.

اثر ایکارین بر تشنج ناشی از پنتیلن تترازول در موش هاس سوری نر گنادوتومی شده

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چکیده:

زمینه مطالعه : در مبتلایان به صرع و افرادتحت درمان با داروهای ضد صرع غلظت آندروژن به طور قابل توجهی کاهش می یابد که منجر به افزایش تشنج و به دنبال آن تخریب نورون های هیپوکامپ و اختلالات حافظه و یادگیری می شود.

هدف: هدف از این مطالعه تأثیر عصاره ایکارین بر موش‌های صحرایی مبتلا به گنادکتومی بود

روش کار: در این مطالعه تعداد84 سر موش سوری نر استفاده شد، موش ها به صورت تصادفی به 12 گروه شامل کنترل، پنتیلن تترازول، دی متیل سولفواکسید، ایکارین، گنادکتومی، پنتیلن تترازول + ایکارین، پنتیلن تترازول + گنادکتومی ، دی متیل سولفواکسید + ایکارین، دی متیل سولفواکسید + گنادکتومی، ایکارین + گنادکتومی ، پنتیلن تترازول + ایکارین + گنادکتومی و گروه دی متیل سولفواکسید + ایکارین + گنادکتومی، که پس از القای صرع با پنتیلن تترازول آزمون شاتل باکس و سپس پارامترهای بافتی مورد ارزیابی قرار گرفتند.

نتایج: نتایج این مطالعه نشان داد که تشنج ناشی از صرع و همچنین گنادکتومی سبب کاهش تراکم نورونی در هیپوکامپ، کاهش میزان بیان نستین، افزایش سلول های دژنره و افزایش التهاب ، افزایش تعدادمونوسیت ها و به دنبال آن کاهش میزان حافظه و یادگیری می شود که عصاره ایکارین تا حدودی سبب بهبود این وضعیت شد.

نتیجه گیری نهایی: استفاده از داروهای گیاهی مانند ایکارین در درمان مبتلایان به صرع از طریق افزایش میزان سطح تستوسترون و همچنین خاصیت نوروپروتکتیو بودن آن می تواند افق های جدیدی در درمان افراد مصروع با این عصاره را بگشاید.

کلمات کلیدی: ایکارین، پنتیلن تترازول، تشنج، گنادکتومی، صرع