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Daidzein improves neuronal health and alleviates inflammation and apoptosis through BDNF and estrogen receptors in the hippocampus of ovariectomized rats

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ABSTRACT

Objective(s): Isoflavone Daidzein (DDZ) has emerged as a promising alternative to hormone replacement therapy (HRT) for ameliorating estrogen deficiency (ED). However, the stereological and molecular mechanism of its effects in the OVX-hippocampus are unclear. We studied the impact of DDZ on stereological changes, estrogen receptor (ERs) expression, BDNF, GSK-3β, and inflammatory and apoptosis-related genes in the hippocampus of ovariectomized rats, compared to 17β-estradiol (E2).

Materials and Methods: OVX rats were treated with DDZ or E2. The stereological analysis assessed the total volume and number of pyramidal and granular neurons in the hippocampus CA1 and DG subregions. Expression of proinflammatory cytokines, apoptotic-related genes, ERs, and BDNF genes was evaluated using Real-Time PCR, and the GSK-3β phosphorylation level was measured by western blot analysis.

Results: DDZ has effectively increased the volume and total number of pyramidal neurons in the CA1 region, the expression of ERα, ERβ, BDNF, and Bcl-2 genes, and the phosphorylation rate of GSK-3β protein. However, the effect of DDZ on the DG region, ERα, and BDNF genes was not significant in comparison with E2; DDZ significantly suppressed the expression of TNF-α, IL-6, and the Bax/Bcl2 ratio compared with OVX rats.

Conclusion: DDZ effectively reversed the stereological changes in the CA1 region by stimulating BDNF gene expression, increasing the phosphorylation ratio of the GSK-3β protein, and modulating inflammatory and apoptotic pathways. Although its effects on the DG region, BDNF, and ERα molecules were less significant than E2, DDZ could still be a promising candidate for ameliorating ED.

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Introduction

17-β estradiol (E2) is a powerful regulator of brain hemostasis and neuronal health. Through binding to its specific nuclear receptors, Estrogen Receptor α (ERα) and Estrogen Receptor β (ERβ), which are differently distributed in various brain regions, E2 initiates a cascade of molecular events that influence vital processes such as synapse formation, cell signaling pathways, neurotrophin systems, and neurogenesis (1, 2). It is now well established that the hippocampus is an early target structure for these effects (1, 3). The human hippocampus is fundamental in creating memories, as well as cognition formation (4, 5), which is carried out by a group of pyramidal cells in the CA1, CA2, and CA3 regions, along with Dentate Gyrus (DG) granule cells (2). Previous research has confirmed that E2 potentially enhances neuronal cell proliferation (6) and synapse formation in all the mentioned regions, especially in hippocampal-CA1 and DG regions (7, 8).

It has been suggested that the neuroprotective effects of E2 can occur through the regulation of a variety of molecules that play a central role in the hippocampus's cognitive function, mood stability, and synapse plasticity, including Brain-Derived Neurotrophic Factor (BDNF)(9) and Glycogen synthase kinase 3β (GSK-3β). The BDNF molecule is a member of the neurotrophic factors family, which is widely distributed throughout the brain in diverse human cell types and promotes axon growth and the survival of various neuron clusters (10). The presence of ERE sequences on the

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shown in Table 3 there was a significant suppression in the Bax/Bcl-2 ratio in the OVX+DDZ group compared with the OVX+E2 rats. Since DDZ had a more significant effect on the expression of the Erβ gene, the significance of this receptor as an anti-apoptotic factor in the hippocampus has been suggested. The key involvement of ERβ in the anti-apoptotic actions of DDZ in Parkinson's disease induced by 6-hydroxydopamine has been previously demonstrated (56). Additionally, a novel regulatory site has been recently discovered in the promoter region of Tnfaip1 (tumor necrosis factor-induced protein 1) that binds to ERβ. This discovery suggests that estrogen or other selective ligands could be targeted to protect against brain inflammation and subsequent apoptosis (57). These findings confirm the data obtained from this study.

A review of previous studies and data obtained from the present study suggests three possible mechanisms through which DDZ may regulate apoptosis: direct interference with Bcl-2-dependent apoptotic processes and a decrease in the Bax/Bcl-2 ratio (58), suppressing the extrinsic death receptor-mediated apoptotic pathway (59), and inhibition of GSK-3β-mediated neuronal cell death (60).

Conclusion

Our data suggests that by enhancing the estrogen receptor gene expression, especially ERβ, and involving two important molecules, BDNF and GSK-3β, the phytoestrogen daidzein could reduce the neuroinflammation, neuro-apoptosis, and stereological changes in the CA1 subregion induced by estrogen deficiency in the hippocampus. Although daidzein was not as effective as estradiol in reversing ovariectomy-induced stereological changes in the DG subregion, changing the dosage or duration of treatment might make it a suitable alternative to HRT.

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Authors' Contributions

A N Visualization, methodology, formal analysis, molecular analysis, stereological analysis, writing original draft, review, editing. Z K Conceptualization, methodology, visualization, review and editing, supervision. F K Methodology, formal analysis, stereological analysis. A S Methodology, visualization, review & editing. Z M Methodology, review & editing, visualization. S A Conceptualization, methodology, visualization, review & editing. F K Methodology, formal analysis, molecular analysis. S S Stereological methodology, visualization, writing - review & editing. F A Methodology, visualization, investigation, formal analysis, supervision, writing – review and editing, resources and funding acquisition.

Conflicts of Interest

All authors firmly declare that they have no conflicts of interest.

Declaration

All the authors declare that the present manuscript has not used AI tools or technologies to prepare this manuscript.

Ethical Approval

Our research project has been approved by the Ethics Committee of Shiraz University of Medical Sciences (Ethic code: IR.SUMS.AEC.1400.021), and the Helsinki declaration was adequately addressed.

Data Availability

Data used in this study are available upon request.

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