Exp Biomed Res 2025; 8(4): 247-257 **https://doi.org/ 10.30714/j-ebr.2025.254**

Experimental Biomedical Research *Original article*

Modulatory effects of saffron and ginseng on phoenixin-20 levels and oxidative stress markers in wistar rats

Müşteba Bayramzadeh*, Eda Özturan Özer

Department of Medical Biochemistry, Baskent University, Ankara, Türkiye

ABSTRACT

Aim: Phoenixin-20 (PNX-20), a neuropeptide implicated in stress regulation via the hypothalamic-pituitary-adrenal (HPA) axis, plays a critical role in neuroprotection and antioxidant defense. This study investigates the effects of two traditional medicinal herbs, saffron and ginseng, on PNX-20 levels, oxidative stress markers, and acetylcholinesterase (AChE) activity in Wistar rats.

Methods: Thirty-two male Wistar rats were randomly assigned to four groups: a control group (saline-treated), a dimethyl sulfoxide (DMSO)-treated group (vehicle group), a ginseng-treated group, and a saffron-treated group. They were treated intraperitoneally for five days.

Results: Results revealed a significant increase in brain PNX-20 and glutathione (GSH) levels in both the saffron and ginseng groups, suggesting enhanced antioxidant capacity. Conversely, malondialdehyde (MDA) levels remained stable compared to the DMSO control group, indicating protection against lipid peroxidation. Importantly, both saffron and ginseng treatment mitigated the DMSO-induced increase in brain AChE activity, suggesting improvement in cholinergic signaling and neuroprotection. No significant changes in serum parameters, including uric acid and AChE activity, were observed, supporting the central nervous system specificity of these treatments.

Conclusion: These findings suggest that saffron and ginseng may hold therapeutic potential for oxidative stress-related neurodegenerative disorders.

Keywords: Phoenixin-20, saffron, ginseng, malondialdehyde, glutathione, acetylcholinesterase.

Müşteba Bayramzadeh *
Department of Medical Biochemistry, Baskent University, Ankara, Türkiye
E-mail: mustebab@baskent.edu.tr
Received: 2025-04-25 / Revisions: 2025-06-17
Accepted: 2025-08-04 / Published: 2025-09-15

1. Introduction

Phoenixin is a newly discovered neuropeptide generated from a small integral membrane protein that has two active isoforms, phoenixin-14 (PNX-14) and phoenixin-20 (PNX-20). It is a ligand for the G protein-coupled receptor 173 (GPR173) and has been expressed and detected in the central and peripheral tissues of humans, rats, mice, bovine, and zebrafish [1,2]. Initially, it was found to regulate reproductive functions by stimulating luteinizing hormone release from pituitary cells via increased gonadotropin-releasing hormone levels [3,4]. Recently, various functions of phoenixin have been identified, including the regulation of reproductive function, food intake, memory improvement, effects on Alzheimer's disease, anxiety, inflammation, neuronal and microglial activity, energy metabolism, body fluid balance, cardiovascular function, and endocrine activity [2-6]. PNX-20

neuropeptides are found in the hypothalamus, a crucial area for the stress response. Research suggests that PNX-20 might be involved in regulating the hypothalamic-pituitary-adrenal (HPA) axis, the body's primary stress response system, with levels that may fluctuate during stress responses [6-9]. Based on this information, antioxidant compounds such as saffron and ginseng may potentially alter PNX-20 levels and enhance its effects in the brain.

Saffron, derived from the stigmas of the plant Crocus sativus L., is a spice commonly used in cooking. It contains four main constituents: safranal, crocin, crocetin, and picrocrocin [10]. Traditionally, saffron is utilized for gingival sedation, catarrhal healing, expectoration, appetite and digestion enhancement, nerve sedation, anticonvulsant effects, and as an antispasmodic. Over the past two decades, various clinical and experimental studies have revealed that saffron possesses therapeutic functions, including cardioprotective, anti-atherosclerotic, anticancer, antidiabetic, antioxidant, antiparasitic, anti-inflammatory, analgesic, and immunomodulatory properties [10-14]. Saffron has also been reported to have antidepressant, anticonvulsant, and anti-anxiety effects, with additional evidence supporting its potential in reducing dopamine levels, suppressing neurotoxicity, and protecting retinal ganglion cells in neurological impairments such as Parkinson's disease. In Vivo studies show that saffron can alleviate memory impairments induced by ethanol, aluminum, morphine, ketamine, and arsenic [11,15].

The antioxidant properties of saffron are linked to its ability to modulate oxidative stress within cellular structures and molecules [16]. Stressed individuals exhibit increased malondialdehyde (MDA) levels and reduced total antioxidant capacity [17]. Elevated corticosterone levels confirm the role of glucocorticoids in chronic stress-related oxidative damage [17,18].

Ginseng is a plant derived from the roots of species belonging to the Panax genus and has been used in traditional medicine. As a well-known medicinal herb, Panax ginseng is abundant in antioxidants [19]. These compounds play a crucial role in regulating cellular pathways influenced by oxidative stress. The potent antioxidant properties of ginseng and its constituent ginsenosides combat oxidative damage by effectively neutralizing reactive oxygen species (ROS). Studies in both laboratory and in vivo settings have demonstrated that ginseng and ginsenosides enhance the activity of antioxidant enzymes, mitigating ROS's harmful effects [19,20]. This protective effect extends across various cell types, where ginseng acts as a shield against oxidative stress. Furthermore, studies have shown that ginseng has anti-inflammatory properties and can increase antioxidant enzyme levels while reducing the production of ROS and malondialdehyde (MDA), underscoring Panax ginseng’s significant role in reducing oxidative stress and promoting overall health, with protective effects against diabetes and cancer [20-23].

2. Materials and methods

2.1. Chemicals: All chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA), and Merck (Darmstadt, Germany). Phoenixin-20 ELISA kit (Cat No: E3427Ra) was purchased from BT-LAB (Zhejiang, China). Ginseng root was from Panax quinquefolium (supplier: sigma, cat No: G7253). *Saffron* was a commercial product and the saffron sample used in this study was selected based on its verified physical and

chemical composition, documented in an official test report (Test No: 1417). The analysis confirmed high purity and quality in accordance with international standards (ISO 259-2 and related specifications). Based on these chemical quality parameters and traceability, this saffron was deemed suitable and appropriate for scientific investigation.

2.2. Animals: Male albino rats of Wistar strain (*n*=32) were assigned to four groups eight in each group as:

1. **Control**: Saline.
2. **Group I (DMSO)**: 10% DMSO (vehicle for ginseng).
3. **Group II (Ginseng)**: 50 mg/kg in DMSO.
4. **Group III (Saffron)**: 50 mg/kg in water.

While *saffron* is water soluble; *Panax ginseng root* was dissolved in DMSO (10%) and therefore Group I (DMSO) was added as the sham control group (vehicle for ginseng).

Rats were housed in Başkent University, Medical and Surgical Experimental Research Center (temperature 20 ± 2ºC, humidity 50 ± 10% and 12h light: 12h dark cycle) and were supplied with standard laboratory diet and tab water *ad libitum*. All experimental procedures involving animals were approved by Başkent University Institutional Review Board and Ethics Committee, Ankara, Turkey with the project number DA23/22. All treatments were done intraperitoneally (IP) for five days and after 48 h of last treatment tissue and serum samples were obtained. Sacrifice was performed by intracardiac puncture after rats were anesthetized i.p. with ketamine (50 mg/kg) /xylazine (10 mg/kg). Following sacrification, tissue and serum samples were were kept at -80°C for biochemical analysis. All biochemical studies were performed in duplicate.

2.3. Analysis of serum biochemical parameters: Serum uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine levels were determined by Roche Hitachi modular system (Mannheim, Germany) using Roche Diagnostic reagents.

2.4. Determination of Tissue Malondialdehyde (MDA) and Reduced Glutathione (GSH) Concentrations: Brain homogenates were prepared in saline (10%, w/v) using all-glass homogenizer for the determination of tissue MDA and GSH concentrations.

MDA levels were determined in brain homogenates according to the method of Buege and Aust **[**24] two volumes of the thiobarbituric acid reagent was added to one volume of sample and mixture was incubated in a boiling water bath for 15 min. After cooling, centrifugation was achieved at 1000x g for 10 min. The absorbance was measured at 535 nm against a reagent blank by using a spectrophotometer (Shimadzu UV-1601, Japan). Concentrations were quantified by using molar extinction coefficient of 1.56 x 105 M-1cm-1. The results were expressed as nmole MDA/g tissue. GSH levels were assayed in tissue homogenates according to the method of Ellman (25). After deproteinization of the samples, Ellman's color reagent was added into the supernatants and then the absorbance of generated color complex was measured immediately at 412 nm against a reagent blank with a spectrophotometer (Shimadzu UV-1601, Japan). By using GSH standard curve, concentrations were calculated and results were expressed as μmole GSH/g tissue.

2.5. Determination of tissue and serum Acetylcholinesterase Activity: Brain tissues were homogenized in 0.1 M potassium phosphate buffer pH 7.4 using all glass

homogenizer and then centrifuged at 10000xg for 10 min at 4 °C. Supernatants were used as an enzyme source. Acetylcholinesterase (AChE) activity was determined by the method of Ellman et al. [26] which is based on monitoring the rate of color complex formation at 412 nm for 5 min against a sample blank by using a spectrophotometer (Shimadzu UV-1601, Japan). Assays were carried out at 25^0 C in 84 mM potassium phosphate buffer pH 7.4, 0.1 mM 5, 5'-dithiobis-2-nitrobenzoic acid and 0.84 ml of enzyme source. Reaction was initiated by the addition 0.4 mM acetylthiocholine (AcSCh). Activity was defined as µmole AcSCh utilized /min/gram tissue (U/g). Serum AChE activites were determined according to method of Elmann and activity was defined as (U/ml)

2.6. Determination of brain and serum PNX-20 levels: PNX-20 levels of samples were determined by commercially available ELISA kit (BT-Lab, Cat No: E3427Ra, Zhejiangi, China). Brain homogenates were prepared according to the instructions of the kit. Standards and samples were pipetted onto monoclonal antibody coated wells of microtiter strips and the assay was carried out as indicated in the instructions of the manufacturer. The optical densities were measured at 450 nm by microplate reader (Bio-Tek Instruments, INC.ELX 800, USA). Quantitation was carried out by standard curve and concentrations were expressed as ng/g tissue for brain homogenates and ng/L for serum samples.

2.7. Statistical Analysis: Analyses were evaluated by with SPSS, Version 17.0 Software. Univariate analysis of variance (ANOVA) coupled with Duncan's post-hoc test was performed. Data were expressed as means ± Standard error of mean (SEM) and *p*-values less than 0.05 were considered as statistically significant.

3. Results

3.1. Serum parameters: Serum AST and ALT levels were evaluated to determine the effect of chemicals on liver functions wheras BUN and creatinine levels were determined as the indicator of renal function. Uric concentrations were analyzed to evaluate the change of total antioxidant capacity of the rats with respect to treatments. As given in Table 1. Saffron and ginseng treatment did not significantly affect serum markers.

3.2. Tissue MDA and GSH concentrations: In order to investigate the probable effect of chemicals on brain oxidative stress, lipid peroxidation index, in terms of MDA, were analyzed. Tissue GSH levels were evaluated as one index of tissue redox status (Table 2). MDA levels did not change significantly but all treatments caused to enhance GSH concentrations in brain tissue.

3.3. Tissue and serum phoenixin-20 levels: The alterations of brain and serum PNX-20 levels with the respect to treatments are given in Figure 1, 2 respectively. As given in Figure 1. Both saffron and ginseng injection caused to

Table 1. Serum parameters.

Groups	AST (U/L)	ALT (U/L)	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control (n:8)	116.00 ± 12.452	54.50 ± 1.546	21.27 ± 1.254	0.37 ± 0.051	1.41± 0.089
Group I (n:8)	107.25 ± 8.643	53.87 ± 5.143	19.53 ± 0.637	0.38 ± 0.025	1.35 ± 0.059
Group II (n:8)	106.12 ± 8.329	49.75 ± 1.739	20.18 ± 0.506	0.33 ± 0.014	1.55 ± 0.140
Group III (n:8)	115.00 ± 12.361	51.25 ± 4.953	19.71 ± 0.267	0.33 ± 0.018	1.56 ± 0.311

Data is given as Mean ± SEM. ALT: alanine aminotransferase, AST: aspartate aminotransferase, BUN: blood urea nitrogen.

Table 2. Brain MDA and GSH levels.

Groups	MDA (nmol/g tissue)	GSH (µmol/g tissue)
Control	29.43 ± 0.667	2.98 ± 0.304
Group I	31.36 ± 0.818	3.34 ± 0.203*, **
Group II	27.50 ± 1.364	3.77 ± 0.280*
Group III	29.13 ± 1.079	3.80 ± 0.246*

*Data is given as Mean ±SEM. The sample size of each group is 8. * Groups I,II,III vs Control .p<0.05, ** Group II vs Groups III,IV.*

increase brain PNX-20 concentrations. Serum PNX-20 levels did not change significantly with treatments (Figure 2).

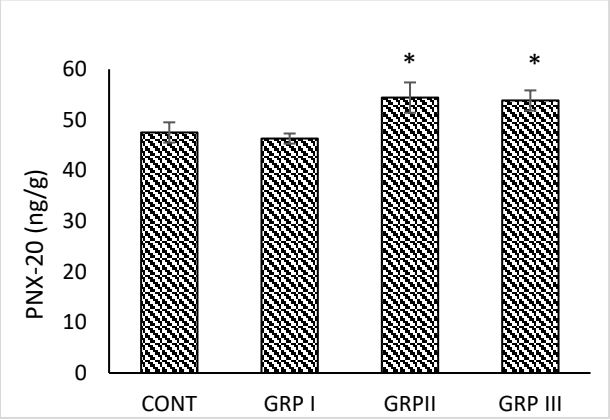

Figure 1. Brain PNX-20 concentrations.
Treatments are detailed under experimental procedures section. *Values are mean ± SEM. The sample size of each group is 8.*** p<0.05; GRPII and GRP III vs Control.*

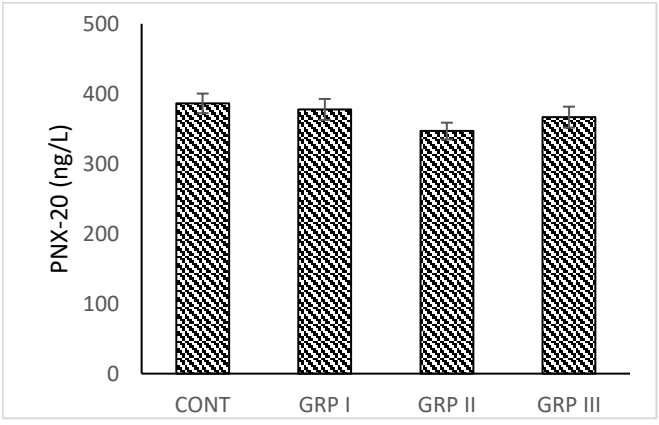

Figure 2. Serum PNX-20 concentrations.
Treatments are detailed under experimental procedures section. Values are mean ± SEM. The sample size of each group is 8.

3.4. Tissue and serum AChE activities: AChE activities were investigated to determine the formation of a possible neurodegenetion caused by treatments. As shown in Figure 3, DMSO admistration caused an increase brain AChE activities and the increase was ameliorated with saffron and ginseng enrichment. Treatments did not alter serum AchE activities as shown in Figure 4.

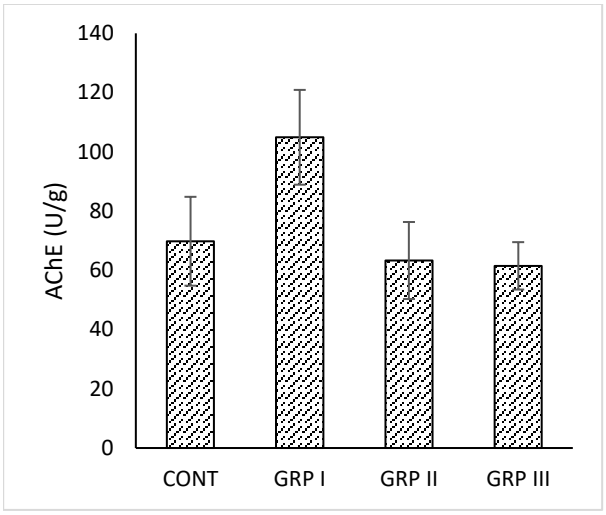

Figure 3. Brain AChE activities.
*Treatments are detailed under experimental procedures section. Values are mean ± SEM. The sample size of each group is 8. *GRP I vs CONT, GRP II and GRP III. p<0.05*

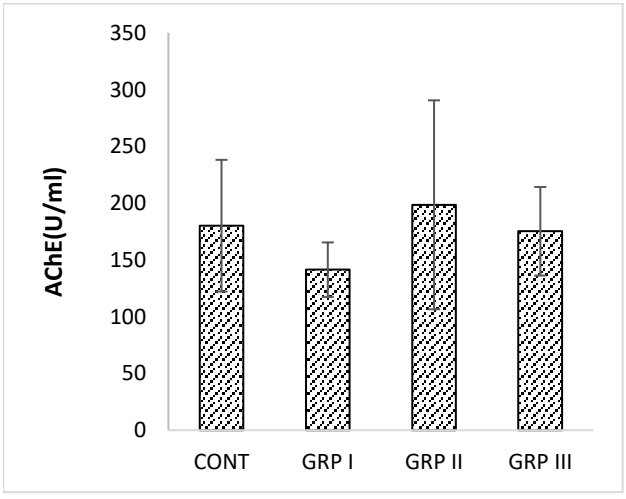

Figure 4. Serum AChE.
Treatments are detailed under experimental procedures section. Values are mean ± SEM. The sample size of each group is 8.

4. Discussion

This study was conducted to evaluate the potential therapeutic benefits of the traditional medicinal herbs (saffron and ginseng) on the levels of the neuromodulator phonexin-20 peptide (PNX-20) and their secondary role of inducing oxidative capacity under normal physiological conditions.

In the following study, serum biochemical parameters were evaluated to investigate the physiological response of organism to treatments (Table 1). As shown in the Table 1, treatments did not affect the serum parameters significantly in the means of kidney and hepatic function. Also the uric acid levels, which were evaluated as the marker of total antioxidant status of the rats [27] did not significantly change within this period of treatment.

Oxidative stress is accepted as the response of cells to any exogeneous / endogeneous stimulation [17]. In order to investigate the role of treatment on the progression of a probable oxidative stress, brain MDA and GSH levels were determined.

The significant increase in brain GSH levels reflects the potent antioxidative properties of saffron and ginseng [28,29]. Saffron's active components, including crocin and safranal, stimulate glutathione reductase activity and facilitate the recycling of oxidized glutathione (GSSG) to its reduced form (GSH), thereby maintaining intracellular antioxidant capacity [28]. These mechanisms are vital for protecting neurons from oxidative damage, particularly under stress conditions [28, 30]. The findings of this study reinforce and expand upon existing literature. Zhang et al. and Rastogi et al. [31, 29] highlighted ginseng's efficacy in reducing neuroinflammation and oxidative stress. The observed elevation in GSH levels underscores the potent antioxidant properties of both herbs, consistent with their roles in reducing oxidative stress and enhancing neuronal resilience [16, 17, 19, 28].

Despite the significant increase in GSH levels, brain MDA levels, a marker of lipid peroxidation, remained unchanged. This stability suggests that saffron and ginseng exhibited a protective antioxidant role and behaved as a ROS scavenger before significant lipid damage occurred. Similar findings were reported by Lee et al. [20], who noted that ginseng's antioxidative effects often prevent the accumulation of lipid peroxidation markers under mild oxidative stress conditions. However, the unchanged MDA levels in our study may also reflect the short treatment duration, as longer interventions have been shown to result in measurable reductions in MDA levels [30]. Additionally, saffron and ginseng's bioactive compounds may act selectively on cellular targets, preserving membrane integrity and preventing lipid peroxidation at early stages, as suggested by Bian et al. [32, 33].

PNX-20 is a neuropeptide known to regulate neuroendocrine balance, particularly through its involvement in the hypothalamic-pituitary-adrenal (HPA) axis [1, 2]. Besides these regulatory functions, several roles of peptide including antioxidant or neuroprotective effects are widely reported (2,3).

In our model, both saffron and ginseng treatments significantly elevated PNX-20 levels in the brain, while serum levels remained unchanged. This localized response supports the hypothesis of brain-specific modulation of PNX-20, aligning with previous studies [2,3]. The significant increase in brain PNX-20 levels after saffron and ginseng treatment underscores their potential role in enhancing stress adaptation and neuroprotection. Increased PNX-20 levels in this study likely reflect

enhanced hypothalamic signaling, supported by saffron's bioactives, such as crocin and safranal, and ginsenosides in ginseng, which are known to modulate stress-related pathways [9, 15].

The adaptogenic effects of saffron and ginseng may be mediated through GPR173 receptor upregulation, a mechanism proposed by Mcllwraith et al. [34]. This receptor, essential for PNX-20 function, is implicated in stress adaptation and neuroendocrine regulation. The role of mitochondrial energy regulation in the hypothalamus, as proposed by Friedrich and Stengel [35], further supports the observed localized increase in brain PNX-20 levels, suggesting that these herbs may act synergistically to enhance neuropeptide expression and function.

Interestingly, serum PNX-20 levels remained unchanged, suggesting brain-specific modulation. This observation aligns with Yosten et al. and Schalla et al. who reported that PNX isoforms primarily exert central effects with limited peripheral involvement [36, 37]. This central specificity highlights the targeted action of saffron and ginseng on hypothalamic pathways, which are critical for maintaining neuroendocrine homeostasis and stress resilience. Furthermore, Zhang et al. [38], demonstrated PNX-20's role in mitigating cognitive dysfunction through PKA/CREB signaling, future studies should investigate whether this mechanism is also activated by the bioactive compounds in saffron and ginseng.

AChE is a key enzyme responsible for acetylcholine breakdown, and its inhibition has been linked to improved synaptic transmission and neuroprotection in neurodegenerative conditions and enhanced cognitive function [39, 40].

In our model, Critically, DMSO administration alone significantly increased brain AChE activity compared to the saline control group (Figure 3), indicating a mild neurotoxic or stressor effect of the solvent itself. This finding warrants a more cautious and thorough reevaluation of the pharmacological effects of the herbal treatments. The increase in AChE activity by DMSO is associated with impaired cholinergic signaling and may worsen neurodegenerative processes [40]. For ginseng—dissolved in DMSO—the observed reduction in AChE activity may primarily reflect a reversal of its solvent's adverse effects rather than a direct neuroprotective action under physiological conditions. In contrast, saffron (solved in water) also counteracted DMSO-induced AChE elevation, suggesting a broader neuroprotective capacity. The amelioration of AChE hyperactivity by both herbs highlights their potential to mitigate solvent-induced neurotoxicity, but the confounding role of DMSO in ginseng's effects must be acknowledged [41-43].

Interestingly, serum AChE activity remained unaffected, highlighting the central specificity of saffron and ginseng's effects. This observation is consistent with the localized action of PNX-20 and its receptor-mediated pathways, as noted by Mcllwraith et al. [34] the adaptogenic properties of these herbs, including their ability to modulate stress-related neuropeptides and enhance neuronal resilience, may explain the selective reduction in brain AChE activity. Additionally, the observed neuroprotective effects are supported by studies demonstrating saffron's anti-inflammatory properties and ginseng's ability to enhance cholinergic function through ginsenosides [42, 44, 45].

While the reduction in brain AChE activity suggests improved cholinergic signaling, this effect should be interpreted within the context

of DMSO's potential confounding influence. Specifically, ginseng's activity may represent a corrective response to solvent-induced neurotoxicity rather than inherent neuroprotection. Nevertheless, enhanced cholinergic signaling remains a key factor in supporting cognitive function and protecting against neurodegenerative processes [39, 46-48].

This study demonstrates that saffron and ginseng counteract DMSO-induced neurotoxicity, as evidenced by increased brain PNX-20 levels, enhanced antioxidant capacity (elevated GSH), and reversal of DMSO-driven AChE hyperactivity. However, the neuroprotective effect claims for ginseng require qualification: its effects may be partially attributable to mitigating its solvent (DMSO) rather than solely direct bioactive actions. The observed elevation in GSH levels underscores the potent antioxidant properties of both herbs, consistent with their roles in reducing oxidative stress and enhancing neuronal resilience [14, 19, 28]. Elevated PNX-20 levels suggest that saffron and ginseng may modulate stress adaptation mechanisms, possibly through the HPA axis and could involve GPR173 receptor pathways [32-34].

The antioxidant actions of saffron and ginseng may modulate PNX-20 levels by counteracting oxidative stress, a known repressor of PNX-20 expression [6]. This is supported by evidence that other antioxidants (e.g., sulforaphane, resveratrol) upregulate PNX-20 through Nrf2 and SIRT1 pathways [34, 35]—pathways similarly activated by saffron's crocin [28] and ginseng's ginsenosides [23]. Thus, PNX-20 may act as a downstream effector of these herbs' neuroprotective effects.

The elevation in brain PNX-20 levels and GSH concentrations observed in this study suggest that saffron and ginseng hold significant potential for the treatment of conditions characterized by oxidative stress and neuroinflammation, such as neurodegenerative diseases.

4.1. Conclusion

In conclusion, the findings of this study highlight the neuroprotective effects of saffron and ginseng treatments, particularly in relation to central PNX-20 levels, oxidative stress markers, and AChE activity. These results contribute to the growing evidence supporting the therapeutic potential of traditional medicinal herbs in managing oxidative stress-related neurodegenerative disorders. Further research is needed to address the limitations of this study, including longer treatment durations and mechanistic analyses, to fully elucidate the neuroprotective mechanisms of saffron and ginseng and their potential clinical applications. Saffron and ginseng demonstrate significant neuroprotective effects by modulating PNX-20 levels, enhancing antioxidant defenses, and reducing AChE activity.

Funding: *The authors received no financial support for the research, authorship, and/or publication of this article.*

Conflict of Interest: *The authors declared no conflict of interest.*

Ethical Statement: *All experimental procedures involving animals were approved by Başkent University Institutional Review Board and Ethics Committee, Ankara, Turkey with the project number DA23/22.*

References

[1]Yuan T, Sun Z, Zhao W, et al. Phoenixin: A Newly Discovered Peptide with Multi-Functions. Protein Pept Lett. 2017;24(6):472-475.

[2]Liang H, Zhao Q, Lv S, et al. Regulation and physiological functions of phoenixin. Front Mol Biosci. 2022; 9:956500.

[3]Billert M, Rak A, Nowak KW, et al. Phoenixin: More than Reproductive Peptide. Int J Mol Sci. 2020; 8: 21(21):8378.

[4]McIlwraith EK, Loganathan N, Mak KWY, et al. Phoenixin knockout mice show no impairment in fertility or differences in metabolic response to a high-fat diet, but exhibit behavioral differences in an open field test. J Neuroendocrinol. 2024;36(10):13398.

[5]Yilmaz N, Hudaykulıyeva J, Gul S. Phoenixin-14 may ameliorate testicular damage caused by torsion-detorsion by reducing oxidative stress and inflammation in prepubertal rats. Tissue Cell. 2024; 88:102405.

[6]Wang S, Liang R, Liu H. Phoenixin-20 ameliorates brain infarction by promoting microglia M2 polarization in an ischemic stroke model. Metab Brain Dis. 2022;37(5):1517-1526.

[7]McIlwraith EK, Zhang N, Belsham DD. The Regulation of Phoenixin: A Fascinating Multidimensional Peptide. J Endocr Soc. 2021; 6(2):192.

[8]Rajeswari JJ, Unniappan S. Phoenixin-20 Stimulates mRNAs Encoding Hypothalamo-Pituitary-Gonadal Hormones, is Pro-Vitellogenic, and Promotes Oocyte Maturation in Zebrafish. Sci Rep. 2020;10(1):6264.

[9]Rajabian A, Rameshrad M, Hosseinzadeh H. Therapeutic potential of Panax ginseng and its constituents, ginsenosides and gintonin, in neurological and neurodegenerative disorders: a patent review. Expert Opin Ther Pat. 2019;29(1):55-72.

[10]Leone S, Recinella L, Chiavaroli A, et al. Phytotherapic use of the Crocus sativus L. (Saffron) and its potential applications: A brief overview. Phytother Res. 2018;32(12):2364-2375.

[11]Fernández-Albarral JA, de Hoz R. et al. Beneficial effects of saffron (Crocus sativus L.) in ocular pathologies, particularly neurodegenerative retinal diseases. Neural Regen Res. 2020;15(8):1408-1416.

[12]Sani A, Tajik A, Seiiedi SS, et al. A review of the anti-diabetic potential of saffron. Nutr Metab Insights. 2022; 15:11786388221095223.

[13]Moradzadeh M, Kalani MR, Avan A. The antileukemic effects of saffron (Crocus sativus L.) and its related molecular targets: A mini review. J Cell Biochem. 2019; 4:4732-4738.

[14]Heitmar R, Brown J, Kyrou I. Saffron (Crocus sativus L.) in Ocular Diseases: A Narrative Review of the Existing Evidence from Clinical Studies. Nutrients. 2019;11(3):649

[15]Cho IH. Effects of Panax ginseng in Neurodegenerative Diseases. J Ginseng Res. 2012;36(4):342-53.

[16] Lee YM, Yoon H, Park HM, et al. Implications of red Panax ginseng in oxidative stress associated chronic diseases. J Ginseng Res. 2017;41(2):113-119.

[17] Rustamov, N.; Ma, Y.; Park, J.-S, et al. Korean Red Ginseng Improves Oxidative Stress-Induced Hepatic Insulin Resistance via Enhancing Mitophagy. Foods 2024; 13: 2137.

[18] Olufunmilayo EO, Gerke-Duncan MB, Holsinger RMD. Oxidative Stress and Antioxidants in Neurodegenerative Disorders. Antioxidants (Basel). 2023 18;12(2):517.

[19] Chaudhary P, Janmeda P, Docea AO, et al. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front Chem. 2023; 11:1158198.

[20] He S, Shi J, Chai H, et al. Mechanisms with network pharmacology approach of Ginsenosides in Alzheimer's disease. Heliyon. 2024;10(5):26642.

[21] Fazalyar N, Ede Pazarbaşı S, Dörücü D, et al. The Effects of Panax ginseng on Serum Oxidative Stress Following Bisphenol A Exposure. Experimed. 2024;14(2):68-72.

[22] Zhang M, Niu H, Li Q, et al. Active Compounds of Panax ginseng in the Improvement of Alzheimer's Disease and Application of Spatial Metabolomics. Pharmaceuticals (Basel). 2023 26;17(1):38.

[23] Hyun SH, Bhilare KD, In G, et al. Effects of Panax ginseng and ginsenosides on oxidative stress and cardiovascular diseases: pharmacological and therapeutic roles. J Ginseng Res. 2022;46(1):33-38.

[24] Buege, J.A. ve Aust, A.D. Microsomal lipid peroxidation. Methods in Enzymology, 1978; 302-310.

[25] Ellmann, G.L. Tissue sulphydryl groups. Archieves of biochemistry and biophysics. 1959; 82: 70-77.

[26] Ellman GL, Courtney KD, Andres V, et al. A new and, rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 1961;7: 88-90.

[27] Qiao M, Chen C, Liang Y, et al. The influence of serum uric acid level on Alzheimer's disease: A narrative review. Biomed Res Int. 2021; 5525710-5525718.

[28] Cerdá-Bernad D, Valero-Cases E, Pastor JJ, et al. Saffron bioactives crocin, crocetin and safranal: effect on oxidative stress and mechanisms of action. Crit Rev Food Sci Nutr. 2022;62(12):3232-3249.

[29] Rastogi V, Santiago-Moreno J, Doré S. Ginseng: a promising neuroprotective strategy in stroke. Front Cell Neurosci. 2015; 8:457.

[30] Gupta RK, Patel AK, Shah N, et al. Oxidative stress and antioxidants in disease and cancer: a review. Asian Pac J Cancer Prev 2014; 15:4405-4409.

[31] Bian Y, Zhao C, Lee SM. Neuroprotective Potency of Saffron Against Neuropsychiatric Diseases, Neurodegenerative Diseases, and Other Brain Disorders: Front Pharmacol. 2020; 11:579052.

[32] McIlwraith EK, Loganathan N, Belsham DD. Regulation of Gpr173 expression, a putative phoenixin receptor, by saturated fatty acid palmitate and endocrine-disrupting chemical bisphenol A through a p38-mediated mechanism in immortalized hypothalamic neurons. Mol Cell Endocrinol. 2019; 485:54-60.

[33] Friedrich T, Stengel A. Current state of phoenixin-the implications of the pleiotropic peptide in stress and its potential as a

therapeutic target. Front Pharmacol. 2023; 14:1076800.

[34] Yosten GL, Lyu RM, Hsueh AJ, et al. A novel reproductive peptide, phoenixin. J Neuroendocrinol. 2013; 25:206-15.

[35] Schalla M, Prinz P, Friedrich T, et al. Phoenixin-14 injected intracerebroventricularly but not intraperitoneally stimulates food intake in rats. Peptides. 2017; 96:53-60.

[36] Zhang J, Wang Z, Cong K, et al. Phoenixin-20 ameliorates Sevoflurane inhalation-induced post-operative cognitive dysfunction in rats via activation of the PKA/CREB signaling. Aging (Albany NY). 2023;15(24):14666-14676

[37] Mehri S, Abnous K, Mousavi S, et al. Neuroprotective effect of crocin on acrylamide-induced cytotoxicity in PC12 cells. Cell Mol Neurobiol. 2012;32(2):227–35.

[38] Obara K, Matsuoka Y, Iwata N, et al. Dimethyl Sulfoxide Enhances Acetylcholine-Induced Contractions in Rat Urinary Bladder Smooth Muscle by Inhibiting Acetylcholinesterase Activities. Biol Pharm Bull. 2023;46(2):354-358.

[39] Palasz, A. and Ve Ciez, D. In Search of Uracil Derivatives as Bioactive Agents. Uracils and Fused Uracils: Synthesis, Biological Activity and Applications. Eur J Med Chem. 2015; 97: 582-611.

[40] Milić M, Žunec S, Micek V, et al. Oxidative stress, cholinesterase activity, and DNA damage in the liver, whole blood, and plasma of Wistar rats following a 28-day exposure to glyphosate. Arh Hig Rada Toksikol. 2018; 69(2):154-168.

[41] Friedrich T, Stengel A. Role of the Novel Peptide Phoenixin in Stress Response and Possible Interactions with Nesfatin-1. Int J Mol Sci. 2021;22(17):9156.

[42] Bej E, Volpe AR, Cesare P, Cimini A, d'Angelo M, Castelli V. Therapeutic potential of saffron in brain disorders: From bench to bedside. Phytother Res. 2024;38(5):2482-2495.