Exploring the neuroprotective effects of black garlic ethanol extract on acrylamide-induced brain damage through apoptotic and neurodegenerative pathways

Arzu GEZER[1,a,✉], Ebru KARADAĞ SARI[2,b], Volkan GELEN[3,c], Sevda ELİŞ YILDIZ[4,d], Mustafa ÖZKARACA[5,e], Gürsel BEDİR[6,f], Fatma ÇALIK[7,g], İsa ELİŞ[2,h]

[1]Atatürk University, Vocational School of Health Services, Erzurum, Türkiye; [2]Kafkas University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Kars, Türkiye; [3]Kafkas University, Faculty of Veterinary Medicine, Department of Physiology, Kars, Türkiye; [4]Kafkas University, Faculty of Health Sciences, Department of Midwifery, Kars, Türkiye; Sivas [5]Cumhuriyet University, Faculty of Veterinary Medicine, Department of Pathology, Sivas, Türkiye; [6]Atatürk University, School of Medicine, Department of Histology and Embryology, Erzurum, Türkiye; [7]Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum, Türkiye

[a]ORCID: 0000-0002-1658-2098; [b]ORCID: 0000-0001-7581-6109; [c]ORCID: 0000-0002-5091-1262; [d]ORCID: 0000-0002-3585-6648
[e]ORCID: 0000-0002-6359-6249; [f]ORCID: 0000-0001-8859-7814; [g]ORCID: 0000-0003-1548-8689; [h]ORCID: 0000-0003-3212-7842

ARTICLE INFO

Article History
Received : 01.11.2023
Accepted : 08.03.2024
DOI: 10.33988/auvfd.1384531

Keywords
Acrylamide
Apoptosis
Black Garlic
Ethanol
Neurodegeneration
Neurotoxicity

✉Corresponding author
a.gezer25@hotmail.com
arzu.gezer@atauni.edu.tr

How to cite this article: Gezer A, Karadağ Sarı E, Gelen V, Eliş Yıldız S, Özkaraca M, Bedir G, Çalık F, Eliş İ (XXXX): Exploring the neuroprotective effects of black garlic ethanol extract on acrylamide-induced brain damage through apoptotic and neurodegenerative pathways. Ankara Univ Vet Fak Derg, XX (X), 000-000. DOI: 10.33988/auvfd.1384531.

ABSTRACT

This research focused on exploring the therapeutic impact of black garlic ethanol extract (BGE) on the brain tissue of rats exposed to acrylamide (ACR). Twenty-four female rats were divided into four groups. Rats in the control group were given 1 ml of saline by oral gavage for 14 days. The BG group received 5 mg/200 g of BGE extract on a daily basis. The ACR group was administered 40 mg/kg of ACR daily. Rats in the BGE+ACR group received both 5 mg/200 g of BG extract and 40 mg/kg of ACR daily. Brain tissue samples were collected at the study's conclusion for histopathological, immunohistochemical, and biochemical analyses. Hematoxylin-eosin staining was performed to examine the general structure of the brain tissue. Erk1/2, p-ERK1/2, and c-fos were analyzed immunohistochemically; Bcl-2, Caspase-3, ATF6, CREB, and NfkB-p65 protein levels were analyzed by Western blotting; and MDA, SOD, CAT, GSH, TNF-α, IL-1β, and IL-6 activities and levels were analyzed using ELISA kits. It was determined that ACR application raised the levels of Erk1/2, p-ERK1/2, c-Fos, NfkB-p65, caspase-3, MDA, IL-6, IL-1-β, and TNF-α, and BGE supplementation decreased this increase. ACR exposure caused a decrease in Bcl-2, ATF6, CREB, CAT, GSH, and SOD expressions, and BGE supplementation prevented or increased this decrease. Based on the findings obtained, it can be said that the ethanol extract of black garlic has antioxidative and anti-inflammatory effects, prevents cell damage, and has positive effects on apoptosis in rat brain tissue.

Introduction

Acrylamide (ACR), which is widely exposed in daily life through environmental pollution and nutrition, is a crystalline, vinylic small molecule, a synthetic substance with high water solubility, and no specific color and odor. It was classified as a probable carcinogenic compound for humans by the International Agency for Research on Cancer in 1994 (15). As a result of various studies, it has been discovered that some heat-treated foods such as bread, coffee, and French fries contain high amounts of Acrylamide. The main way Acrylamide is formed in foods is the Maillard reaction between reducing sugars and the amino acid asparagine during heat treatments such as frying, baking, and roasting at temperatures above 120°C

(6). Many health problems, including immunotoxic, hepatotoxic, neurotoxic, genotoxic, carcinogenic, and teratogenic effects, can develop because of acrylamide toxicity (14). It has been observed that plant-based compounds with antioxidant, anti-inflammatory, and antiapoptotic effects are used to minimize the harmful effects of ACR or to treat the damage, and garlic extract has strong anti-inflammatory, antioxidant, and antiapoptotic effects (20).

Fresh garlic contains large amounts of sulfur compounds such as alliin, allicin and S-allyl cysteine (SAC) (3). In recent years we have witnessed the implementation of methods like heat treatment, drying, and fermentation to eliminate the unpleasant odor and taste associated with garlic (38). Thanks to these methods, irritating properties are eliminated, and the garlic is given a more delicious form. The product called "black garlic" was obtained by exposing the garlic heads to high temperatures (60°C and above), maintaining 85-90% relative humidity for 30-40 days, and then softening and darkening the garlic heads. During fermentation, the enzyme alliinase is broken down, thereby alliin cannot be converted into allicin (8). As a result of this event, the specific odor of garlic decreases. Alliin is converted into SAC and odorless components are formed. While the amount of SAC increases in the formation of black garlic, alliin, allicin, and polysaccharides decrease. In this way, the bitterness and pungent odor of garlic is reduced (5).

Erk1/2 plays significant roles in cell proliferation, differentiation, and survival, signaling from the cell surface to the nucleus and detecting cellular stress. It exerts crucial effects on inflammation, and there is communication between cell receptors and different nuclear transcription factors (48). p-ERK1/2 is a member of the MAPK family and contains an ERK protein within this family (32). c-fos is a protooncogene that is a member of the IEG (Immediate Early Gene) group, and the increase of its accumulation depends on the effect of various stimuli (24). Bcl-2 is one of the most important regulators of the intrinsic apoptosis pathway (45). Caspase-3, located at the end of caspase cascades activated by apoptotic pathways acts as a primary protein for apoptosis (30). ATF6 is an endoplasmic reticulum transmembrane glycoprotein, transcription factor, and ER stress sensor. It detects protein misfolding in the endoplasmic reticulum and triggers the Unfolded protein response (UPR) to maintain homeostasis (36). CREB, a general transcription factor, acts as an inhibitor for the cell survival mechanism, thus leading the cell to programmed death (44). NfkB-p65 is an essential transcription factor and translocates from the cytosol to the nucleus upon activation and controls the expression of numerous genes related to cell cycle, growth, viability, specialization, movement, bonding, and inflammation (9). MDA, a widely recognized result of lipid peroxidation, elevates in response to heightened levels of free radicals. Hence, the concentration of MDA serves as an indicator of oxidative stress. Within the category of antioxidants, SOD, GSH, and CAT are substances that impede the formation of free radicals (4). TNF-α is a transmembrane protein and a crucial proinflammatory cytokine produced by various cells. Dysregulation of TNF-α has been linked to such several pathological conditions as autoimmune disease, atherosclerosis, cancer, Alzheimer's disease, infection, and inflammatory bowel disease. TNF-α also has various functions in the regulation of different developmental and immunological processes, such as inflammation, differentiation, lipid metabolism, and apoptosis (28). IL-1-β is a cytokine that has a potent proinflammatory effect when inducing inflammation. IL-1β damages neuronal synapses during inflammation and causes neurodegeneration (40). IL-6 is a proinflammatory cytokine that triggers inflammation; the uncontrolled, heightened, and continuous production of IL-6 has adverse effects on both acute systemic inflammatory response syndrome and chronic immune-mediated disorders (11).

After being consumed through food products, acrylamide can be rapidly absorbed from the gastrointestinal system and transferred to all body tissues (such as the liver, thymus, brain, heart, and kidneys) through the bloodstream. Therefore, this experimental study aims to determine the effects of BGE on oxidative stress, inflammation, and apoptosis in ameliorating the adverse effects of ACR on brain tissue.

Material and Methods

Collection and Extract Preparation of BGE: The black garlic used in the study was obtained from Edovital (Kastamonu, Turkey). The peeled black garlic cloves were cut into small pieces and dried on blotting papers in a dark area with air circulation and out of the sun. The dried black garlic parts were ground with the help of a grinder. The soxhlet device cartridge was washed with extraction solvent by taking 50 g of the ground garlic samples and placing them in a 500 ml soxhlet extractor. Ethanol was used as an extraction solvent. 650 ml of ethanol solvent was added to the boiling flask. The solvent was subjected to extraction (using 10-15 siphons) for roughly 10 hours until the solvent became transparent. At the end of the 10th hour, the liquid extracts obtained were filtered through blue band filter paper and the particles were removed. The filtrated extract sample underwent evaporation using a rotary evaporator at temperatures between 35-45ºC. The garlic extract left in the flask was placed inside a desiccator for 12 hours. After removing all traces of solvent from the garlic extract, it was weighed with a precision of 0,1 mg, transferred to an extraction container, and stored at +4ºC for subsequent analysis (43).

DOI: 10.33988/auvfd.1384531

Animal and Experimental Groups: The study utilized rats sourced from the Kafkas University Medical Experimental Research and Application Center, where the experimental phase of the study was also conducted. The research adhered to the principles outlined in the Declaration of Helsinki. A total of 24 female Wistar albino rats were utilized in the study; these rats were in the estrous phase, aged 3-4 months, and weighed between 250-350 grams. It was calculated with the G*Power program that at least 24 rats for 4 groups (at least 6 rats for each group) should be included in the study at 80% power and 95% confidence level for the case where there is an effect size (f = 0.45) compared to the reference study (27). The groups were randomly formed. They were fed *ad libitum* with tap water in an environment with 12 hours of darkness and 12 hours of lighting and kept in standard cages. Control group were given 1 ml of saline by oral gavage for 14 days. Rats in the BGE group were administered 5mg/200g black garlic extract dissolved in saline by oral gavage for 14 days (26). The rats in the ACR group were administered 40 mg/kg acrylamide dissolved in saline i.p. once a day for 14 days (39). Rats in the BGE+ACR group were administered 5 mg/200 g black garlic extract by oral gavage and 40 mg/kg Acrylamide i.p. as a daily dose for 14 days.

Following the conclusion of the experiment, rats were deeply sedated using sevoflurane (Sevorane®, Abbott Lab. Istanbul, Turkey), cervical dislocation was then performed, and brain tissue samples were collected. Some of the obtained brain tissues were stored at -80^{0}C for ELISA and Western blot examinations. Brain tissue specimens were immersed in a 10% buffered neutral formalin solution, and following standard histological processes, they were embedded in paraffin wax.

Histopathological Examination: The 5µm sections taken on polylysine slides were stained with hematoxylin-eosin (H&E) and semiquantitatively evaluated as absent (-), mild (+), moderate (++), and severe (+++) in terms of pyknotic and degenerative changes seen in neurons in the cortex parts (22).

Immunohistochemical examination: The 5µm sections taken on polylysine slides were passed through xylol and alcohol series, washed with PBS, and then kept in 3% H_2O_2 for 10 min to inactivate endogenous peroxidase. To release the antigen in the tissues, they were treated with antigen retrieval solution at 500 watts for 2x5 min. The tissues were then washed with PBS and incubated with Erk1/2 (Santa Cruz, Catalog No. sc-514302), p-ERK1/2 (Affbiotech, Catalog No. AF1015), and c-fos (Santa Cruz, Catalog No. sc-166940) primary antibodies at a dilution ratio of 1/150 at +4ºC overnight. As secondary; The Large Volume Detection System employed anti-polyvalent, HRP (Thermofischer, Catalog no: TP-125-HL) as per the manufacturer's instructions. DAB (3,3′-diaminobenzidine) served as the chromogen. Subsequently, following counterstaining with Mayer's hematoxylin, the slides were sealed with entellan and inspected using a light microscope. In the examination, immunopositivity was evaluated semiquantitatively as absent (-), mild (+), moderate (++), severe (++++), and very severe (++++) (21).

Western Blot Analysis: The brain tissue samples were weighted and crushed in nitrogen gas, treated with radioimmunoprecipitation (RIPA buffer, Ecotech Bio, Turkey) supplemented with protease and phosphatase inhibitors, and homogenized using a tissue lyser device (Qiagen, USA) at 30 Hz for 30 sec to determine the relative protein expressions of Bcl-2, Caspase-3, ATF6, CREB, and NfkB-p65. A protein assay kit was used to quantify brain tissue's total protein (Pierce BCA, Thermo Sci., USA). 25 ug of protein were then put into the PVDF membrane after being separated by 10% SDS-PAGE. First, at room temperature, 5% bovine serum albumin was used to block the membranes for 90 minutes. Then, the membranes were incubated at 4°C overnight with the appropriate primary antibodies NfkB-p65 (AF5006, Affinity Biotech), ATF6 (DF6009, Affinity Biotech), CREB antibody (AF6188, Affinity Biotech), Caspase-3 (sc-56053, Santa Cruz), Bcl-2 (sc-7382, Santa Cruz), and Beta-actin (sc-47778, Santa Cruz). After primary antibody incubation, the PVDF membranes were washed with TBST and then incubated for an additional 90 minutes at room temperature with the second antibody (Santa Cruz, sc-2004/sc-2005) coupled to horseradish peroxidase. Afterward, the protein bands were recorded using the Western ECL substrate, an enhanced chemiluminescence reagent (Thermo, 3405), visualized and analyzed by Image Lab™ Software (Bio-Rad, Hercules, CA, USA).

Biochemical Examination: Brain tissue homogenates required for oxidative stress and inflammation biomarkers analyses were obtained as described in our previous study. Malondialdehyde (MDA), glutathione (GSH) levels, superoxide dismutase (SOD), and catalase (CAT) activity were determined in brain tissue. Moreover, the concentrations of interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α) were evaluated. Oxidative stress parameters and cytokine levels in brain tissue supernatants obtained from rats were quantified using a rat ELISA kit following the provided guidelines from the manufacturer. The assessments were conducted utilizing an ELISA Plate Reader (Bio-Tek, Winooski, VT, USA) following the standard instructions provided by the manufacturer, and the absorbance was recorded at 450 nm.

Statistical Analysis: Statistical analysis was performed using the SPSS (version 25.0; IBM SPSS Inc, Chicago, IL, USA) package program. The normality of data was determined with the Kolmogorov-Smirnov test. Descriptive statistical analyses (mean ± standard deviation) were used. One-way ANOVA test and post-hoc Tukey test were performed to compare groups. P values less than 0.05 at the 95% confidence interval were considered statistically significant.

Results

Histopathological Results: Histopathologic evaluation revealed statistically significant differences between the groups (Table 1, P<0.05).

The neurons in the cortex of the brain of rats in the control and BGE groups had a normal histologic appearance. While severe pyknotic and degenerative changes were observed in neurons in the ACR group, these changes were found to be moderate in the BGE+ACR group. Microscopically, the nuclei were dark and shrunken in pyknotic and degenerative neurons (Figure 1).

Immunohistochemical Results: Immunohistochemical staining for Erk1/2, p-ERK1/2, and c-Fos revealed statistically significant differences between the groups (Table 2), (P<0.05).

While Erk1/2 immunopositivity was very severe in the control and BGE group, it was mild in the ACR group. There was a correlation between p-ERK1/2 and c-Fos immunopositivity and Erk1/2 immunopositivity in the same direction. p-ERK1/2 and c-Fos immunopositivity were mild in the control and BGE groups, severe in the ACR group, and moderate in the BGE+ACR group (Figure 2). Immunohistochemical staining with Erk1/2, p-ERK1/2, and c-Fos showed neuron localization (Figure 2, 3, 4).

Table 1. Pyknotic and degenerative changes in neurons.

Groups	Cortex
Control	0.33 ± 0.21^{a}
BGE	0.33 ± 0.21^{a}
ACR	2.83 ± 0.16^{b}
BGE+ACR	1.66 ± 0.21^{c}

[a,b,c] The difference between groups in the same column (P<0.05).

Table 2. Comparison of the groups in terms of Erk1/2, p-ERK1/2 and c-Fos.

Groups	Erk1/2	p-ERK1/2	c-Fos
Control	1.16 ± 0.40^{a}	1.00 ± 0.00^{a}	0.83 ± 0.40^{a}
BGE	1.66 ± 0.51^{a}	1.16 ± 0.40^{a}	1.00 ± 0.00^{a}
ACR	3.83 ± 0.40^{b}	2.83 ± 0.40^{b}	2.83 ± 0.40^{b}
BGE+ACR	2.83 ± 0.40^{c}	1.83 ± 0.40^{c}	2.00 ± 0.00^{c}

[a,b,c] The difference between groups in the same column (P<0.05).

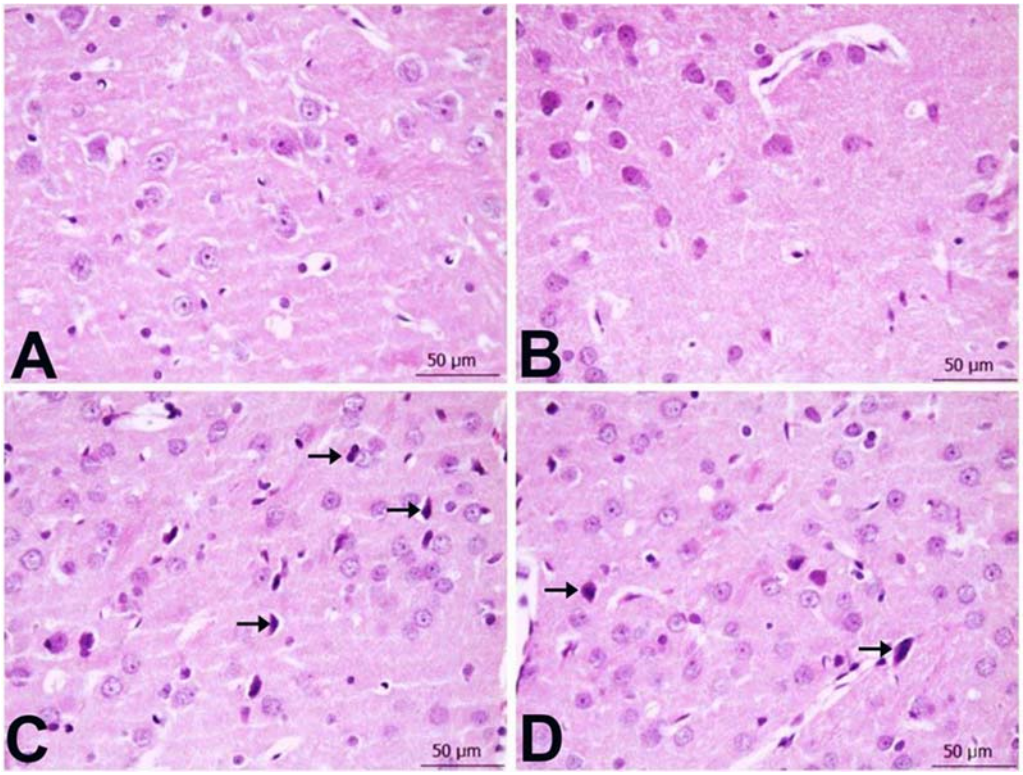

Figure 1. Histological changes in rat brain cortex.

A) Control group, B) BGE group; in normal histologic appearance, C) ACR group; severely pyknotic and degenerative neurons, D) BGE+ACR group; moderately pyknotic and degenerative neurons (arrows). Bar: 50 µm, H&E.

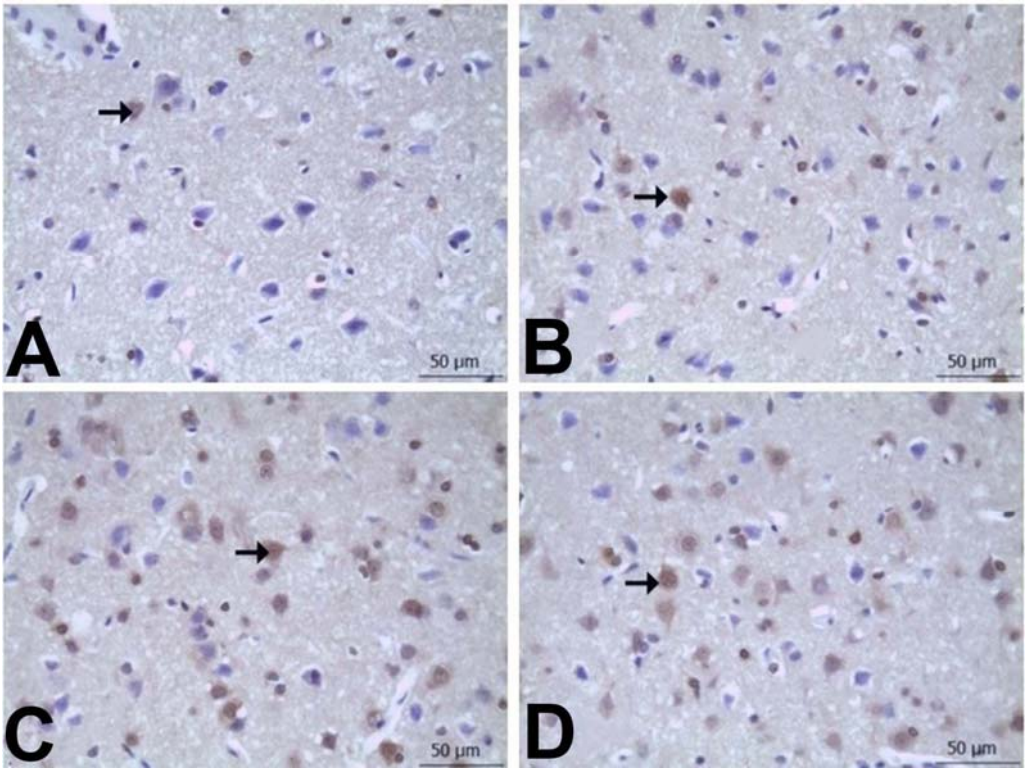

Figure 2. Erk1/2 immunohistochemistry.
A) Control group; mild level, B) BGE group; mild level, C) ACR group; very severe level, D) BGE+ACR group; moderate level Erk1/2 immunopositivity (arrows). Bar: 50 µm, IHC.

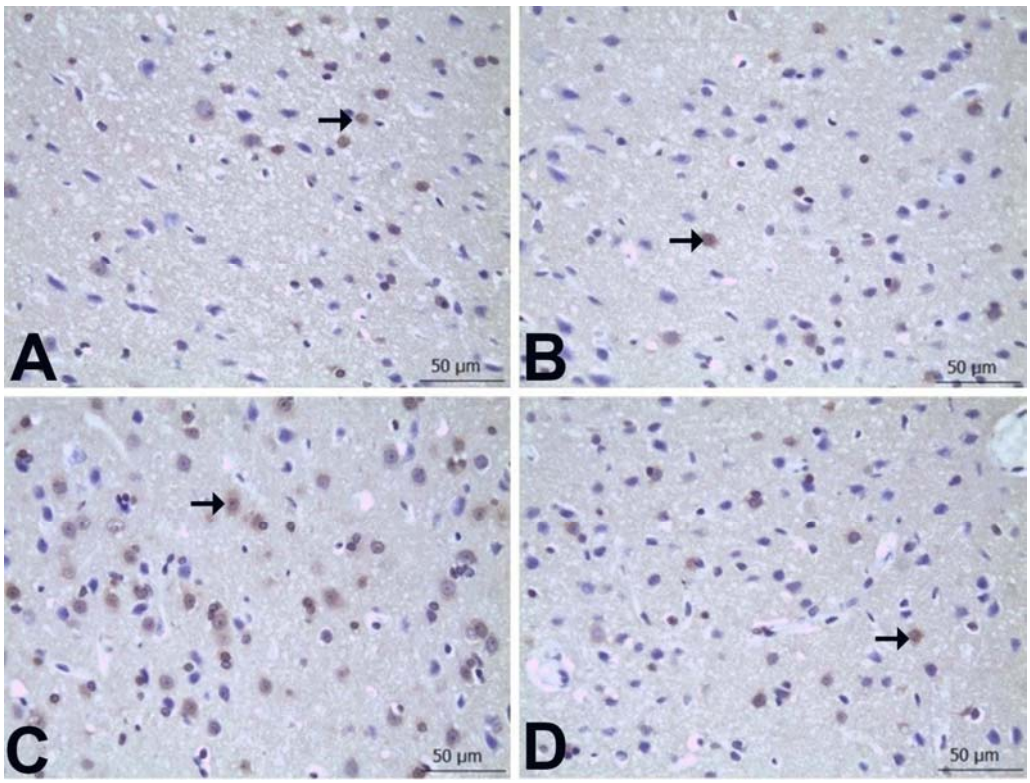

Figure 3. p-ERK1/2 immunohistochemistry.
A) Control group; mild level, B) BGE group; mild level, C) ACR group; severe level, D) BGE+ACR group; moderate level p-ERK1/2 immunopositivity (arrows). Bar: 50 µm, IHC.

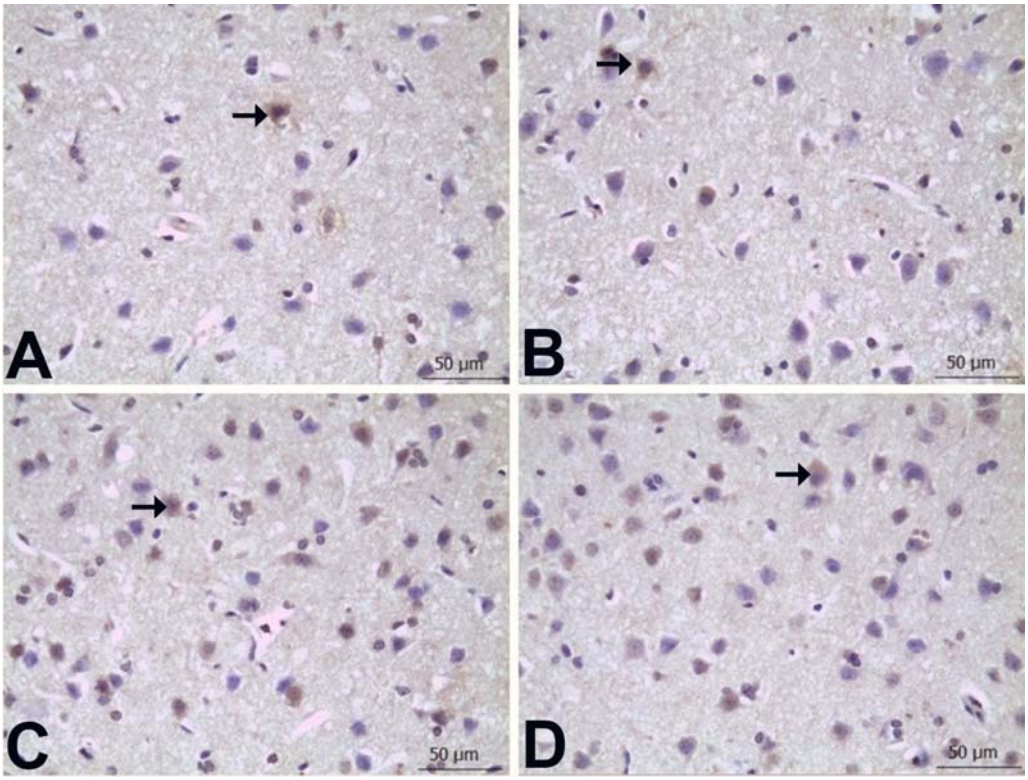

Figure 4. c-Fos immunohistochemistry.
A) Control group; mild level, B) BGE group; mild level, C) ACR group; severe level, D) BGE+ACR group; moderate level c-Fos immunopositivity (arrows). Bar: 50 µm, IHC.

Relative expressions of Bcl-2, Caspase-3, ATF6, CREB, and NfkB-p65 proteins: In the relative protein expression analysis, the CREB and Bcl-2 protein expression levels were found as the highest in the control group, while a dramatic decrease was detected in the ACR group. However, an increase was observed in the BGE+ACR group compared to the ACR group. In the analysis of Nf-kB, Caspase-3, and ATF6 protein expression levels, a significant increase was seen in the ACR group, while a relative decrease was achieved in the BGE+ACR groups. The lowest protein expression level was determined in the Control and BGE groups compared to other groups. Protein gel images and analysis graphics for all groups are presented in (Figure 5).

Biochemical Results

Effects of Black Garlic Ethanol Extract on ACR-Induced Brain Tissue Oxidative Stress: The effects of BGE administration on ACR-induced brain tissue oxidative stress are summarized in Figure 6. The MDA level was significantly higher in the ACR group than in the control group ($P<0.05$). BGE significantly ($P<0.05$) prevented ACR-induced lipid peroxidation. BGE administration alone did not cause any change in MDA level ($P>0.05$). SOD and CAT enzyme activities were significantly decreased in the ACR group compared to the control, BGE+ACR and BGE groups ($P<0.05$). In addition, GSH levels decreased in the ACR group compared to the BGE+ACR group, but this decrease was not statistically significant ($P>0.05$).

Effects of Black Garlic Ethanol Extract on ACR-Induced Inflammation in Brain Tissue: The effects of BGE administration on inflammation in ACR-induced brain tissue are summarized in (Figure 7). TNF-α, IL-1-β, and IL-6 levels were significantly increased in the ACR group compared to control, BGE, and BGE+ACR groups ($P<0.05$). BGE administration appears to prevent ACR-induced cytokine increase.

DOI: 10.33988/auvfd.1384531

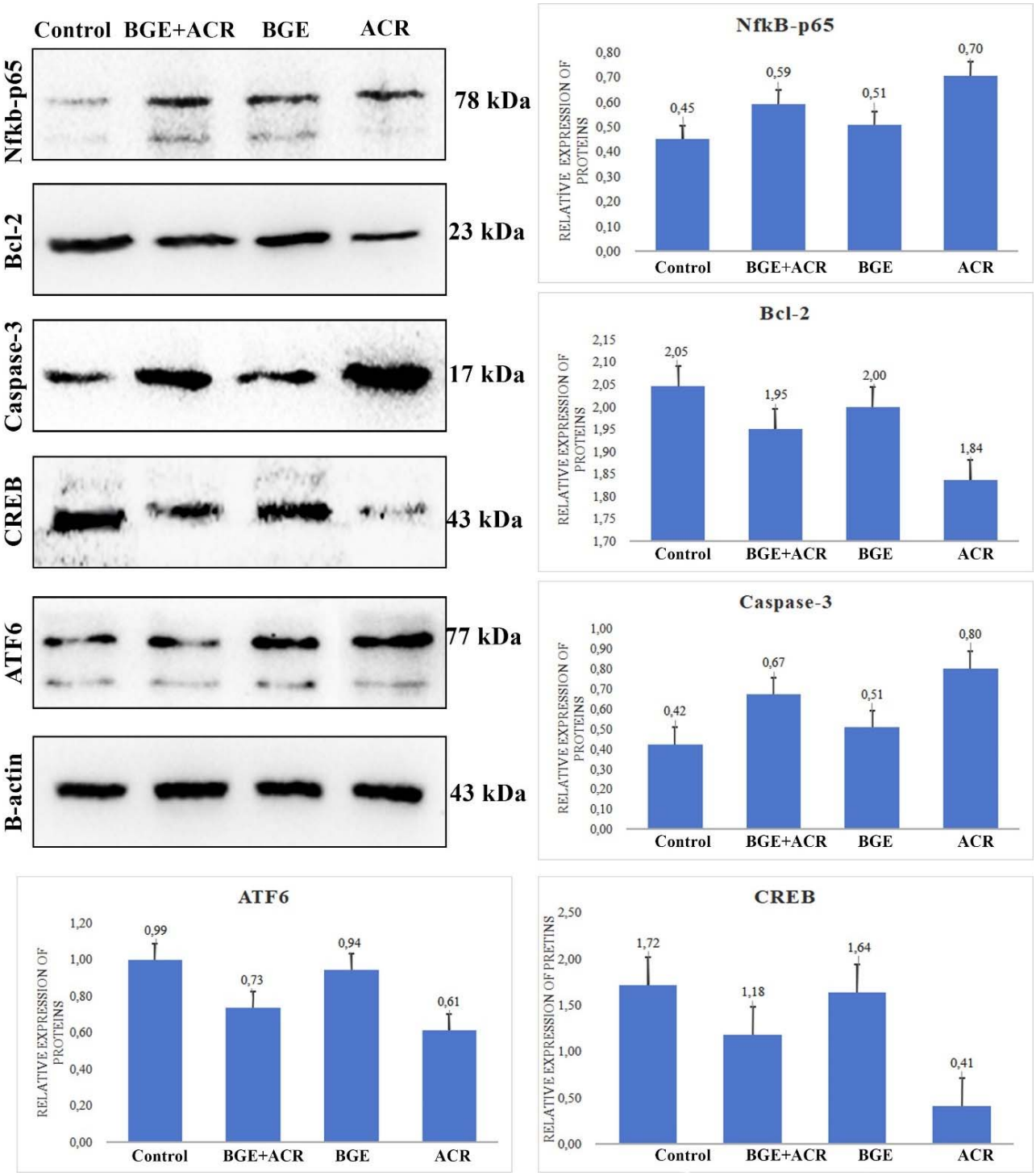

Figure 5. Relative expression of proteins for ATF6, Bcl-2, CREB, Caspase-3, NfkB-p65.

The values are given as mean ± SD (n=6) and analyzed by one-way ANOVA followed by the Tukey test. Distinct letters denote statistically significant variances, ($P<0.05$).

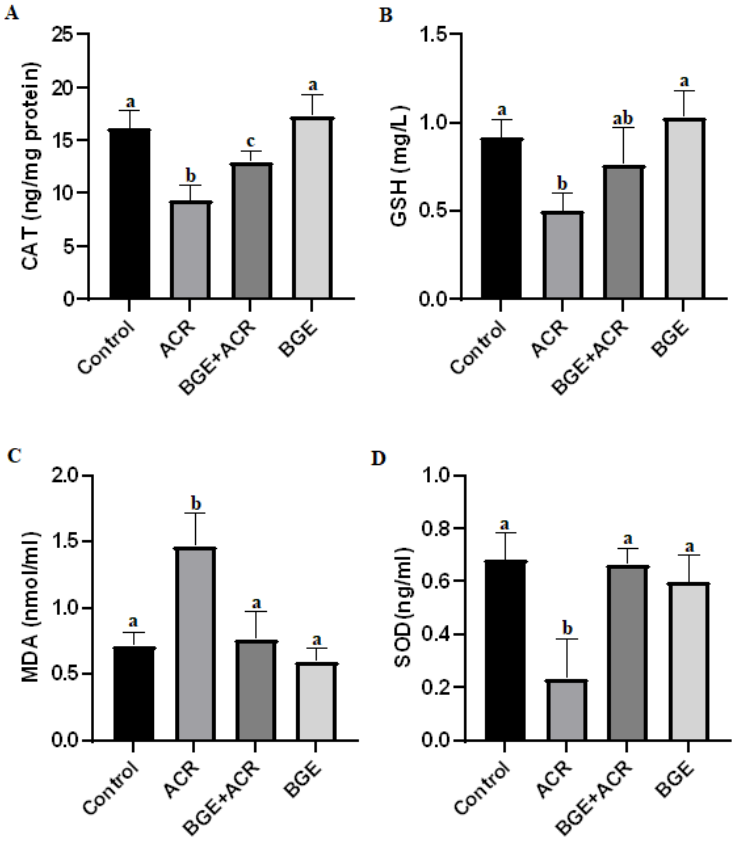

Figure 6. Effects of BGE against ACR-induced brain tissue oxidative stress in rats.

Values are expressed as mean ± SD. Different letters in columns (a-c) indicate statistical difference (P<0.05).

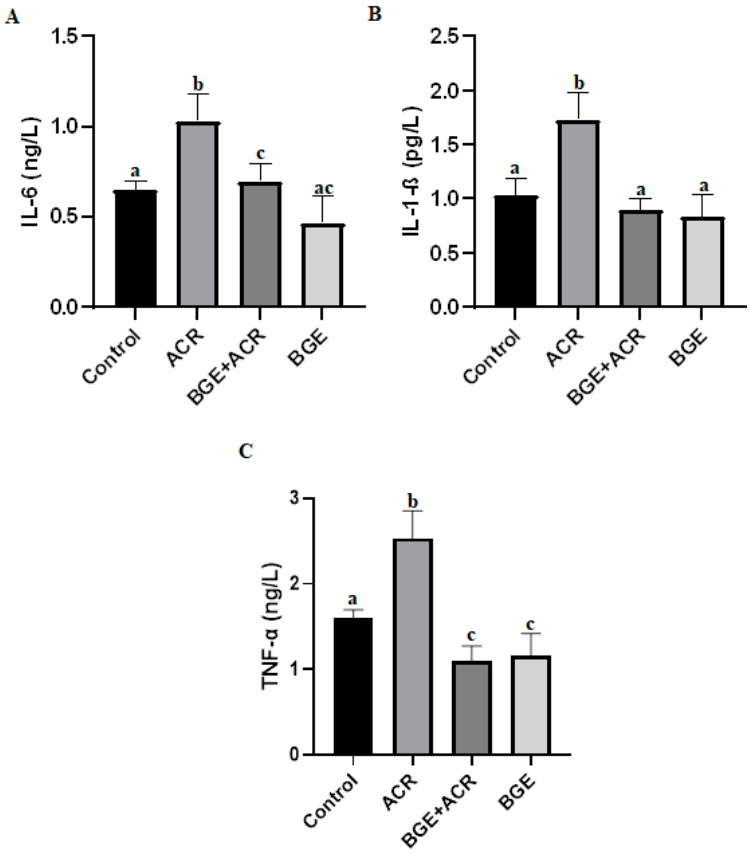

Figure7. Effects of BGE against ACR-induced brain tissue inflammation in rats.

Values are expressed as mean ± SD. Different letters in columns (a-c) indicate statistical difference (P<0.05).

Discussion and Conclusion

ACR, despite its toxic effects, finds application as an industrial chemical in various fields, including polymers, paper, cosmetics, fabrics, wastewater treatment, and the manufacture of laboratory gels. It is also formed during the high-temperature cooking of starchy foods (15). ACR exposure has been recorded through consumption of ACR-containing foods, drinking water contaminated with polyacrylamide coagulants, and occupational exposure through dermal contact or inhalation of industrial production dust (42). Adults typically have an average daily ACR intake of 1 µg/kg body weight/day. Exposure to ACR has been documented to induce diverse toxic effects in experimental animals (23). Exposure to ACR can notably trigger apoptosis and oxidative stress in pertinent tissues and cells and has been suggested to disrupt the intracellular oxidant and antioxidant balance system in cells (20). Various doses of ACR have been demonstrated to lead to neurotoxicity, reproductive toxicity, genotoxicity, and embryonic toxicity (47).

It has been documented that ACR results in severe damage to brain tissue, and the utilization of substances possessing antioxidant and anti-inflammatory properties are increasingly prevalent to mitigate or address this damage (46). In line with this information, in this study, we investigated the protective effects of BGE administration against ACR-induced neurotoxicity in rats.

It was determined that ACR caused abnormal histopathological findings in the cerebral cortex and meninges in rats. It was reported that there was significant occlusion in the submeningeal blood vessels and degeneration in the cerebral cortex (12). In our study, ACR application caused severe pyknotic-degenerative changes in neurons and BGE had a protective effect. Hermawati et al. (2015) reported that BGE was protective against monosodium glutamate (MSG)-induced brain damage. The fact that BGE administration was effective against ACR-induced brain tissue damage in the histopathological findings we obtained in our study suggests that BGE may have a multifaceted protective effect when evaluated with the findings of Hermawati et al. (2015). Because it has been reported that BGE may be protective against neurodegenerative diseases in the brain. For example, it has been reported that BGE application against oxidative stress caused by MSG in experimentally induced Alzheimer's disease is a powerful antioxidant that has a positive effect on memory (34) and helps to increase pyramidal neurons in hippocampus regions (7).

Immunohistochemically, the levels of Erk1/2, p-ERK1/2, and c-fos in the brain were examined. It was found that while these protein levels increased with ACR application, their levels decreased with BGE application. It is known that high ROS activates the MAPK pathway and thus Erk1/2 in response to glutamate-induced oxidative stress (32). Furthermore, Erk1/2 activation has been demonstrated in response to neurological injuries (35) and phosphorylated as p-ERK1/2 to provide neuroprotection. BGE significantly enhances Erk signaling suppressed by acrylamide in the hippocampus (13). The present study revealed that ACR administration increased Erk1/2, p-ERK1/2, and c-Fos expression in brain tissue and that BGE administration prevented this increase.

There is a close relationship between oxidative stress caused by ROS and the apoptosis mechanism. ROS has a significant role in stimulating the apoptotic mechanism in the cell (19). Apoptosis occurs through a mechanism induced by members of the Bcl-2 protein family, including Caspase-3 and Bcl-2 (10). In ACR-induced brain toxicity, increased Caspase-3 expression and decreased Bcl-2 expression were reported. BGE has been reported to suppress apoptosis by decreasing Caspase-3 expression and increasing Bcl-2 expression in various tissues (49). In this study, ACR significantly increased Caspase-3 expression and decreased Bcl-2 expression in brain tissue, while BGE decreased Caspase-3 expression and increased Bcl-2 expression. CREB serves as a significant nuclear transcription factor involved in the nervous system. CREB activation serves as a protective factor of brain tissue against damage (17, 31). It has been reported that ACR treatment decreased CREB expression in human neuroblastoma cell lines (29), while garlic extract increased CREB levels (13). The data we obtained in our study are consistent with this and show that while ACR application decreases CREB expression in brain tissue, BGE application prevents this decrease. It has been reported that garlic extract dropped the elevated NfkB-p65 level in Alzheimer's disease (37) and decreased ATF 6 level in this Alzheimer's disease increased with garlic extract (41).

In addition, the effect of BGE against inflammation induced by ACR was examined in the present study. Inflammation is associated with many chronic diseases, especially cancer, diabetes, cardiovascular, and neurological diseases. It has been emphasized that ACR induces inflammation by increasing proinflammatory cytokine levels and decreasing anti-inflammatory cytokine levels (1). Garlic has been found to reduce proinflammatory cytokines such as IL-1β, IL-6, and TNF-α (41). In addition, BGE was found to prevent/reduce inflammation experimentally (2). It was determined that neuronal inflammation induced by ACR increased proinflammatory cytokine levels and decreased anti-inflammatory cytokine levels. BGE prevented ACR-induced neuronal inflammation with an anti-inflammatory effect.

Studies in experimental animals have reported that ACR causes oxidative stress and cell damage (18, 33). Gur et al. found that SOD, CAT activity, and GSH levels decreased, and MDA levels increased in ACR-induced brain damage (25). It was reported that ACR application significantly decreased SOD and GSH activities and significantly increased MDA levels (16). BGE administration significantly reduced ACR-induced lipid peroxidation by decreasing MDA levels and increased SOD, GSH, and CAT expression levels (13). In our study, we determined that ACR administration stimulated oxidative stress in brain tissue, MDA level increased, GSH levels, SOD and CAT activities decreased as a result of induction of lipid peroxidation. BGE is an agent with a proven protective effect against various tissue toxicities caused by numerous agents on the brain (7, 26). We found that BGE administration significantly prevented ACR-induced oxidative stress in rats in our study. These findings were consistent with the literature.

The findings of the current study provide robust evidence for the anti-inflammatory properties of BGE, given its ability to suppress both NFKB and pro-inflammatory cytokines. We found that BGE attenuates oxidative damage after ACR exposure, resulting in reduced neuroinflammation and signaling expression, and neurological improvement. It provides an effective intervention strategy against neurotoxins and neurological diseases by providing anthocyanin-enriched foods. Our results suggest that BGE is a promising agent to eliminate ROS and thus may alleviate brain injuries mediated by oxidative stress. We think the findings will contribute to future research, but further studies are needed.

Financial Support

This research received no grant from any funding.

Conflict of Interest

The authors declare that all data were generated in-house and that no paper mill was used.

Author Contributions

AG, EKS, VG, SEY, MÖ, GB, FÇ and İE experiment design, experiment application, samples collection. AG, EKS, SEY, MÖ, GB and İE histopathological and immunohistochemical, investigation. VG and FÇ western blot analysis, determination antioxidant enzyme activities and of inflammation markers. All the authors contributed to the writing and editing, and they read and approved the manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

The approval was obtained by the Animal Experiments Local Ethics Committee of Kafkas University, Faculty of Veterinary Medicine, on the date of 03.10.2023 with the ethics committee number 2023/11 and decision no 108 for this study.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

References

1. **Abdel-Daim MM, Abd Eldaim MA, Hassan AG** (2015): *Trigonella foenum-graecum ameliorates acrylamide-induced toxicity in rats: Roles of oxidative stress, proinflammatory cytokines, and DNA damage.* Biochem Cell Biol, **93**, 192-198.
2. **Ahmed RA** (2018): *Hepatoprotective and antiapoptotic role of aged black garlic against hepatotoxicity induced by cyclophosphamide.* JOBAZ, **79**, 1-8.
3. **Ahmed T, Wang C-K** (2021): *Black garlic and its bioactive compounds on human health diseases: A review.* Molecules, **26**, 5028.
4. **Alizadeh M, Kheirouri S** (2019): *Curcumin reduces malondialdehyde and improves antioxidants in humans with diseased conditions: A comprehensive meta-analysis of randomized controlled trials.* BioMedicine, **9**.
5. **Al-Shehri SA** (2021): *Efficacy of black garlic extract on anti-tumor and anti-oxidant activity enhancement in rats.* Clin Nutr Open Sci, **36**, 126-139.
6. **Alturki HA, Elsawy HA, Famurewa AC** (2022): *Silymarin abrogates acrylamide-induced oxidative stress-mediated testicular toxicity via modulation of antioxidant mechanism, DNA damage, endocrine deficit and sperm quality in rats.* Andrologia, **54**, e14491.
7. **Aminuddin M, Partadiredja G, Sari D** (2015): *The effects of black garlic (Allium sativum L.) ethanol extract on the estimated total number of Purkinje cells and motor coordination of male adolescent Wistar rats treated with monosodium glutamate.* Anat Sci Int, **90**, 75-81.
8. **Aoudeh E, Oz E, Oz F** (2023): *Effect of beef patties fortification with black garlic on the polycyclic aromatic hydrocarbons (PAHs) content and toxic potency.* Food Chem, **428**, 136763.
9. **Begum SFM, Hemalatha S** (2022): *Gelidiella acerosa compounds target NFκB cascade in lung adenocarcinoma.* Appl Biochem Biotechnol, 1-14.
10. **Chen Y, Zhang W, Guo X et al** (2019): *The crosstalk between autophagy and apoptosis was mediated by phosphorylation of Bcl-2 and beclin1 in benzene-induced hematotoxicity.* Cell Death Dis, **10**, 772.
11. **Conti P, Ronconi G, Caraffa A, et al** (2020): *Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies.* J Biol Regul Homeost Agents, **34**, 327-331.
12. **Edres HA, Taha NM, Lebda MA, et al** (2021): *The potential neuroprotective effect of allicin and melatonin in*

acrylamide-induced brain damage in rats. Environ Sci Pollut Res, **28**, 58768-58780.

13. **Fang Z, Luo Y, Ma C** (2022): Blueberry anthocyanins extract attenuates acrylamide-induced oxidative stress and neuroinflammation in rats. Oxid Med Cell Longev, 2022.
14. **Farag OM, Abd-Elsalam RM, El Badawy SA, et al** (2021): *Portulaca oleracea seeds' extract alleviates acrylamide-induced testicular dysfunction by promoting oxidative status and steroidogenic pathway in rats.* BMC Complement Altern Med, **21**, 1-15.
15. **für Gesundheit HKBL** (2023): Lebensmittel: Acrylamid.
16. **Gao J-G, Jiang Y, Zheng J-T, et al** (2022): *Pubertal exposure to acrylamide disrupts spermatogenesis by interfering with meiotic progression in male mice.* Toxicol Lett, **358,** 80-87.
17. **Gelen V, Özkanlar S, Kara A, et al** (2023): *Citrate-coated silver nanoparticles loaded with agomelatine provide neuronal therapy in acute cerebral ischemia/reperfusion of rats by inhibiting the oxidative stress, endoplasmic reticulum stress, and P2X7 receptor-mediated inflammasome.* Environ Toxicol, **39,** 1-13.
18. **Gelen V, Sengul E, Yildirim S, et al** (2023): *The role of GRP78/ATF6/IRE1 and caspase-3/Bax/Bcl2 signaling pathways in the protective effects of gallic acid against cadmium-induced liver damage in rats.* Iran J Basic Med Sci, **26,** 1326-1333.
19. **Gelen V, Şengül E, Yıldırım S, et al** (2021): *The protective effects of hesperidin and curcumin on 5-fluorouracil–induced nephrotoxicity in mice.* Environ Sci Pollut Res, **28**, 47046-47055.
20. **Gelen V, Yıldırım S, Şengül E, et al** (2022): *Naringin attenuates oxidative stress, inflammation, apoptosis, and oxidative DNA damage in acrylamide-induced nephrotoxicity in rats.* Asian Pac J Trop Biomed, 12.
21. **Gezer A, Karadag-Sari E** (2022): *The role of amifostine in preventing radiotherapy-induced testicular tissue damage in rats.* Biotech Histochem, **97**, 215-221.
22. **Gezer A, Sari EK** (2023): *Investigation of apoptotic and autophagic effects of chronic roflumilast use on testicular tissue in rats by immunohistochemical and immunofluorescence methods*. Iran J Basic Med Sci, **26**, 276.
23. **Goudarzi M, Mombeini MA, Fatemi I, et al** (2019): *Neuroprotective effects of Ellagic acid against acrylamide-induced neurotoxicity in rats.* Neurol Res, **41**, 419-428.
24. **Gu X, Nardone C, Kamitaki N, et al** (2023): *The midnolin-proteasome pathway catches proteins for ubiquitination-independent degradation.* Science, **381**, eadh5021.
25. **Gur C, Kandemir FM, Darendelioglu E, et al** (2021): *Morin protects against acrylamide-induced neurotoxicity in rats: an investigation into different signal pathways.* Environ Sci Pollut Res, **28**, 49808-49819.
26. **Hermawati E, Sari DCR, Partadiredja G** (2015): *The effects of black garlic ethanol extract on the spatial memory and estimated total number of pyramidal cells of the hippocampus of monosodium glutamate-exposed adolescent male Wistar rats.* Anat Sci Int, **90**, 275-286.
27. **Hosseini E, Farid Habibi M, Babri S, et al** (2022): *Maternal stress induced anxiety-like behavior exacerbated by electromagnetic fields radiation in female rats offspring.* PLoS One, **23,** 17-18.
28. **Huang S-L, Chang T-C, Sun N-K** (2023): *Curcumin reduces paclitaxel resistance in ovarian carcinoma cells by upregulating SNIP1 and inhibiting NFκB activity.* Biochem Pharmacol, **212**, 115581.
29. **Kacar S, Sahinturk V** (2021): *The protective agents used against acrylamide toxicity: An in vitro cell culture study-based review.* Cell Journal (Yakhteh), **23**, 367.
30. **Kayacan S, Sener L, Melikoglu G, et al** (2018): *Induction of apoptosis by Centaurea nerimaniae extract in HeLa and MDA-MB-231 cells by a caspase-3 pathway.* Biotech Histochem, **93**, 311-319.
31. **Khakha N, Khan H, Kaur A et al** (2023): *Therapeutic implications of phosphorylation-and dephosphorylation-dependent factors of cAMP-response element-binding protein (CREB) in neurodegeneration.* Pharmacol Rep, 1-14.
32. **Kim J-J, Kang Y-J, Shin S-A, et al** (2016): *Phlorofucofuroeckol improves glutamate-induced neurotoxicity through modulation of oxidative stress-mediated mitochondrial dysfunction in PC12 cells.* PLoS One, **11**, e0163433.
33. **Kunle-Alabi OT, Akindele OO, Odoh MI, et al** (2017): *Comparative effects of coconut water and N-Acetyl cysteine on the hypothalamo-pituitary-gonadal axis of male rats.* Songklanakarin J Sci Technol, **39**, 759-764.
34. **Li F, Kim MR** (2019): *Effect of aged garlic ethyl acetate extract on oxidative stress and cholinergic function of scopolamine-Induced cognitive impairment in mice.* Prev Nutr Food Sc, **24**, 165.
35. **Lu K, Cho C-L, Liang C-L, et al** (2007): *Inhibition of the MEK/ERK pathway reduces microglial activation and interleukin-1-beta expression in spinal cord ischemia/reperfusion injury in rats.* J Thorac Cardiovasc Surg, **133**, 934-941.
36. **Nagar P, Sharma P, Dhapola R, et al** (2023): *Endoplasmic reticulum stress in Alzheimer's disease: Molecular mechanisms and therapeutic prospects.* Life Sci, 121983.
37. **Paul BD, Pieper AA** (2023): *Protective Roles of Hydrogen Sulfide in Alzheimer's Disease and Traumatic Brain Injury.* Antioxidants, **12**, 1095.
38. **Ryu JH, Kang D** (2017): *Physicochemical properties, biological activity, health benefits, and general limitations of aged black garlic: A review.* Molecules, **22**, 919.
39. **Sengul E, Gelen V, Yildirim S, et al** (2023): *Effects of naringin on oxidative stress, inflammation, some reproductive parameters, and apoptosis in acrylamide-induced testis toxicity in rat.* Environ Toxicol, **38**, 798-808.
40. **Sun Y, Yu H, Guan Y** (2023): *Glia connect inflammation and neurodegeneration in multiple sclerosis.* Neurosci Bull, **39**, 466-478.
41. **Tedeschi P, Nigro M, Travagli A, et al** (2022): *Therapeutic potential of allicin and aged garlic extract in Alzheimer's disease.* Int J Mol Sci, **23**, 6950.
42. **Thabet NM, Moustafa EM** (2018): *Protective effect of rutin against brain injury induced by acrylamide or gamma radiation: role of PI3K/AKT/GSK-3β/NRF-2 signalling pathway.* Arch Physiol Biochem, **124**, 185-193.
43. **Uluman E, Kilicle PA** (2020): *The investigation of the possible antigenotoxic in vivo effects of pomegranate (Punica granatum L.) peel extract on mitomycin-C genotoxicity.* Turkish J Vet Anim Sci, **44**, 382-390.

44. **Wang Y, Che M, Xin J, et al** (2020): *The role of IL-1β and TNF-α in intervertebral disc degeneration.* Biomed Pharmacother, **131**, 110660.
45. **White K** (2023): *Sayonara to some evolutionary puzzles in the Bcl-2 family.* EMBO J, **42**, e113980.
46. **Yassa HA, George SM, Refaiy AERM, et al** (2014): *Camellia sinensis (green tea) extract attenuate acrylamide induced testicular damage in albino rats.* Environ Toxicol, **29**, 1155-1161.
47. **Yu D, Jiang X, Ge W, et al** (2022): *Gestational exposure to acrylamide suppresses luteal endocrine function through dysregulation of ovarian angiogenesis, oxidative stress, and apoptosis in mice.* Food Chem Toxicol, **159**, 112766.
48. **Yu S-M, Kim S-J** (2015): *The thymoquinone-induced production of reactive oxygen species promotes dedifferentiation through the ERK pathway and inflammation through the p38 and PI3K pathways in rabbit articular chondrocytes*. Int J Mol Med, **35**, 325-332.
49. **Zhang J, Zhu X, Xu W, et al** (2023): *Exposure to acrylamide inhibits testosterone production in mice testes and Leydig cells by activating ERK1/2 phosphorylation.* Food Chem Toxicol, **172**, 113576.