Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

High-Salt Diet Differentially Affects Anxiety-Depression-Like Behaviours and Cognitive Functions in Mice: Gender Difference

Hasan ŞİMŞEK[1*], Mehmet ÖZ[1], Nurhan AKARAS[3]

[*1]*Department of Physiology, Faculty of Medicine, Aksaray University, Aksaray, Türkiye*

[2]*Department of Histology and Embryology, Faculty of Medicine, Aksaray University, Aksaray, Türkiye*

Cite: *Şimşek H, Öz M, Akaras N. High-Salt Diet Differentially Affects Anxiety-Depression-Like Behaviours and Cognitive Functions in Mice: Gender Difference. Eurasian Mol Biochem Sci 2024;3(1): 1-9.*

Received: 27 November 2023, Accepted: 26 December 2023

Abstract

Objective: Salt is a compound used for taste and flavor in the daily diet. Although the World Health Organization (WHO) recommends salt consumption below 5g/day, the average worldwide is 15.24g. A high salt diet (HSD) causes cognitive impairment by increasing oxidative stress and inflammation. This study aimed to determine the effect of HSD on anxiety-depression-like behaviors and cognitive functions in female and male mice. Materials and Methods: Twenty-eight mice (14 females, 14 males) were divided into four groups depending on gender (n=7): Male Control, Male HSD, Female Control, and Female HSD. Mice were fed an HSD for 16 days. Open-field (OFT), tail-suspension (TST), forced swimming (FST), and novel object recognition (NORT) tests were used. In addition, biochemical and histopathological analyses were made from brain tissues. Results: Compared to their control in females with HSD, the time spent in the center ($p<0.01$) and the number of rearing ($p<0.01$) decreased in the OFT. Also, an increase in sedentary time in the TST ($p=0.027$) and a decreased memory index ($p<0.01$) in the NORT, were determined. There was no difference in all tests in males with HSD compared to their control group. Oxidative stress increased in both genders (male: $p=0.021$, female: $p<0.001$) but was higher in females. Inflammation was increased in both genders. The damage to the brain region was increased in both genders, and this increase was more in females. Conclusions: HSD affects female mice more through the cumulative effect of oxidative stress and inflammation, increasing depression and anxiety, and decreasing memory index.

Keywords: Anxiety, Depression, Cognitive, High Salt Diet, Oxidative Stress

Introduction

Salt is the most prominent seasoning commonly used in food processing and flavoring (1). Salt is a compound of sodium (40%) and chlorine (60%) and is used in the daily diet (2). Salt is an essential compound for maintaining physiological processes in the body and has become an integral part of our daily lives due to our dietary habits (3). Sodium, an extracellular cation, is essential for cellular functions by controlling water distribution, electrolyte balance, and osmotic pressure.

[*]**Correspondence**: Hasan ŞİMŞEK
Department of Physiology, Faculty of Medicine, Aksaray University, Aksaray, Türkiye E-mail: hasansimsek@aksaray.edu.tr
Tel: +90 532 158 43 47

The human body requires approximately 0.5 g/day of sodium to maintain healthy physiological processes. However, most food preservatives used in the food industry have a high sodium content and are the leading cause of increased dietary sodium intake (2). A high salt diet (HSD) causes hypertension and heart disease, which can lead to death (4,5). When only deaths caused by cardiovascular disorders are considered, HSD is the fourth risk factor (6). HSD was the first cause of 1.89 million deaths worldwide in per year. Reducing salt intake is one of the most efficient public health management strategies for reducing morbidity and mortality from non-communicable diseases (7). For all these reasons, the World Health Organization (WHO) recommends salt intake to be below five g/day, but salt consumption worldwide is much higher than ten g/day (8,9).

HSD can negatively affect brain health and cause deficiencies in physiological processes such as behavior and cognitive function (10). HSD causes cognitive impairment by increasing blood pressure and inducing oxidative stress damage in the hippocampus (11,12). However, in addition to these effects, HSD alone has also been associated with cognitive impairment (13).

Cognitive function is one of the significant factors that ensure the continuity of life comfort with aging. Factors that confer risk for cardiovascular disease (CVD) may also be associated with risk for cognitive impairment and dementia (14). Neuropsychiatric disorders caused by stress, such as anxiety and depression, and CVD can co-occur. Salt intake, a controllable risk factor, poses a risk for CVD. For these reasons, there are hypotheses that HSD is also associated with neuropsychiatric diseases (15). Studies have shown that females who sometimes add salt to their food have good cognitive functions, while males who often add salt to their food have poor cognitive functions (16). Studies on experimental animals have linked HSD to increased risks of endothelial dysfunction, anxiety, metabolic disturbances, cerebrovascular disorders, and dementia (13,17).

Despite growing evidence that females and males respond differently to sodium, research on sodium has generally been limited to males (18). Therefore, this study aimed to investigate the effects of HSD on oxidative stress and inflammation damage and learning, depression, and anxiety in a gender-dependent.

Our study is based on the following hypothesis;

H1. Because there are physiological differences between genders, the impact of HSD on behavioral and cognitive dysfunctions may differ between genders.

Materials and Methods

Ethical Statement: The ethics committee approval of this study was obtained from Aksaray University Animal Experiments Local Ethics Committee (Date:18.05.2022, No:2022/4-10). This study was conducted according to NIH guidelines for handling laboratory animals.

Experimental Groups: Twenty-eight Mus musculus mice (10-12 weeks old, weighing 30-40 g) obtained from Aksaray University Experimental Animal Research and Application Center were used in the current study. The mice were housed under standard laboratory conditions in a ventilated room with constant temperature, 50%±5 humidity, and a 12-hour light±dark cycle. Mice were randomly divided into four groups (seven mice in each group): Male Control (MC), Male High Salt Diet (MHSD), Female Control (FC), and Female High Salt Diet (FHSD). Body weights of all groups at the beginning (day 0) and end (day 17) of the experiment and total water consumption at the end of the experiment (day 17) were calculated.

In the current study, an open field test was performed to evaluate anxiety-like behaviors, forced swimming and tail suspension tests for depression-like behaviors, and a new object recognition test for learning.

High Salt Diet: Mice only had ad libitum access to their cage-assigned drinking water and feed: normal tap water and 0.4% salt chow to control groups; HSD groups were given 4% NaCl-containing feed and 1% NaCl-containing tap water. It was applied for 16 days (19).

Open Field Test (OFT): A wooden box (40cm x 40cm x 40cm) was divided into 16 equal compartments. The central area was defined as a 20cm x 20cm square in the center, and the other region was defined as the peripheral area. Entry into the central area was defined as the placement of all four claws into the middle area. In our study, mice were observed in the open field for 5 minutes, and the time spent in the central area, the number of line crossings, and the number of rearing were recorded. The box was cleaned with 10% ethanol between all trials (20).

Forced Swimming Test (FST): Mice were placed in a plexiglas cylinder (height = 25 cm; diameter = 20 cm) in 15 cm high water (24 °C) at a height at which they could not climb. A 6-minute video recording was taken for each animal. The last 4 minute of the 6-minute test were analyzed (21). The animals' immobility time (swimming time when only the head was above the water but still immobilized) was recorded.

Tail Suspension Test (TST): Mice were suspended by the tail end with adhesive tape with their heads 15 cm above the ground. All animals were videotaped for 6 minutes: an adaptation phase for the first 2 minutes and a period of immobility for the last 4 minutes. The criteria for mice to be considered immobilized were passive hanging and/or no movement (22).

Novel Object Recognition Test (NORT): A NORT was applied to evaluate the memory index (MI) (23). Between trials, the objects and box were cleaned with 10% ethanol. Twenty-four hours after the completion of the salt diet (day 17), each animal was placed in a 40x40x40 cm black wooden box for area exploration for 5 min. Twenty-four hours (day 18) after the area exploration, two objects (Objects A1 and A2) identical in size, color, shape, and material were placed at two consecutive corners and a distance of 9 cm from the edges. The mice were allowed to recognize the objects for 5 min.

Ninety minutes after object recognition, one of the objects was taken and replaced with another object (Object B) that was the same in terms of material and size but had a different shape and color. It was analyzed by taking a 5-minute video recording. The result obtained by multiplying the ratio of the time spent with the new object (B) to the total time spent with these objects (A+B) by 100 was used as the MI. The higher the MI, the better the memory is considered.

Tissue Homogenization for Biochemical Analysis: To obtain 1:10 (w/v) homogenate from brain tissues, they were homogenized in 1.15% potassium chloride (KCl) with a homogenizer (MiuLab, Zhejiang, China). Homogenates were centrifuged (+4°C, 1000 × g', 15 min), and the supernatant obtained was used for biochemical analysis. Inflammation and oxidative stress markers were analyzed from the obtained supernatants.

Measurement of Inflammation Markers: To determine the level of inflammation, Nuclear factor kappa B (NF-κB) (MyBioSource, USA Cat no: MBS2023542) and tumor necrosis factor-α (TNF-α) (BT Lab, China; Cat no: E0117Mo) levels and cyclooxygenase-2 (COX-2) (BT Lab, China; Cat no: E0605Mo) activity were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits at 450nm.

Oxidative Stress Index: Oxidant capacity (TOS) and antioxidant capacity (TAS) levels were analyzed from blood sera using commercial kits and according to the manufacturer's instructions (Rel Assay Diagnostic, Gaziantep, Turkey). The measurement method of the kits was developed by Erel. TAS was expressed as mmol Trolox Eq/mg protein and TOS as μmol H_2O_2 Eq/mg

protein (24,25). Determination of oxidative stress index (OSI) was performed according to the previous study (26).

Light microscopy examination: Brain tissues were kept in a 10% neutral formalin buffer for 24 hours. After tissue tracing, five µm thick sections were taken from the paraffin blocks with a microtome. The slices were placed on slides and stained with hematoxylin and eosin (H&E) stains. The stained samples were examined and photographed with an Olympus Cx 43 microscope (Japan). Neuron counting was also performed.

Statistical analysis: Statistical analysis of the data obtained from brain tissues was performed with SPSS 20.0 (IBM, NY) program. One-way ANOVA and Tukey's post hoc tests were used for group comparison. Data are presented as mean±SE. Statistical significance was accepted at three levels; $p<0.05$, $p<0.01$ and $p<0.001$**.**

Results

Body weights and Water Consumption: There was no difference in body weight between control and HSD in both female ($p=0.981$, $p=0.675$) and male ($p=0.748$, $p=1.000$, respectively) mice. According to the water consumption calculated at the end of the experiment, it was determined that there was more water consumption (respectively; $p=0.001$, $p<0.001$) in female and male mice treated with HSD compared to their control (Figure 1).

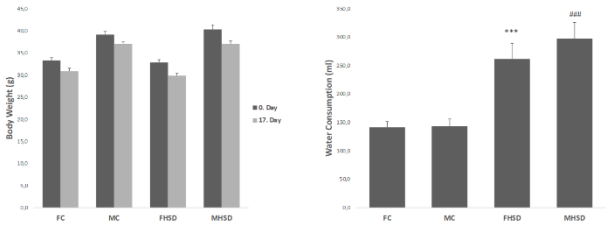

Figure 1. The results of body weight and water consumption. FHSD vs FC; ***p = 0.001, MHSD vs MC: ###p < 0.001. FC: female control, MC: male control, FHSD: female high salt diet, MHSD: male high salt diet.

Open Field Test: A significant decrease was found in the time spent in the center ($p<0.001$) and the rearing numbers ($p<0.001$) in the FHSD compared to the FC. No difference in the line crossing numbers ($p=0.663$) in the FHSD compared to the FC. No significant difference was found in the time spent in the center ($p=0.565$), the number of rearing ($p=0.86$), and the number of line crossing ($p=0.924$) in the MHSD compared to the MC (Figure 2).

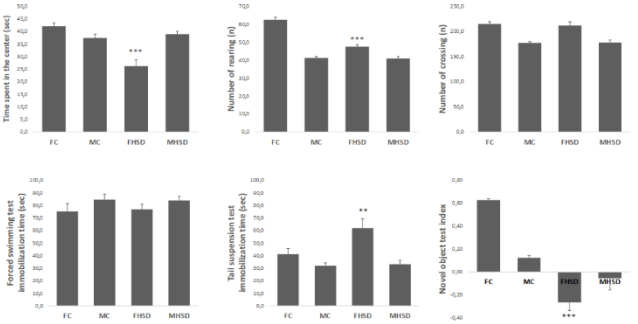

Figure 2. The results of anxiety, depression, and memory index. FHSD vs FC; **p < 0.01, *** p < 0.001. FC: female control, MC: male control, FHSD: female high salt diet, MHSD: male high salt diet.

Forced Swimming Test: According to the analysis made from four-minute recordings, no difference was found in the immobility times of HSD-treated female ($p = 0.813$) and male ($p = 0.914$) mice compared to the control groups (Figure 2).

Tail Suspension Test: There was an increase in sedentary time in the FHSD compared to the FC ($p=0.003$). There was no difference in sedentary time in the MHSD compared to the MC ($p=0.861$) (Figure 2).

Memory Index: According to the memory index data, there was a significant decrease in the FHSD compared to the FC ($p<0.001$). No difference was found in MHSD compared to MC ($p=0.228$) (Figure 2).

Inflammation Findings: NF-κB, TNF-α levels, and COX-2 activity were measured to determine the level of

inflammation in brain tissue. NF-κB level and COX-2 activity were significantly increased in FHSD compared to FC ($p<0.05$). There was an increase in all parameters ($p<0.05$) in the MHSD compared to the MC (Figure 3).

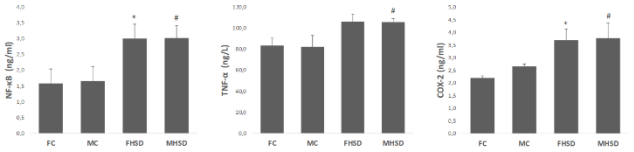

Figure 3. Effects of HSD on NF-κB, TNF-α and COX-2 in brain tissues of mice. FHSD vs FC; *p < 0.05, MHSD vs MC: #p < 0.05. FC: female control, MC: male control, FHSD: female high salt diet, MHSD: male high salt diet, NF-κB: Nuclear factor kappa B, TNF-α: tumor necrosis factor-α, COX-2: cyclooxygenase-2.

Oxidative Stress Index: According to oxidative stress findings, there was an increase in TOS and OSI (respectively; $p=0.003$, $p<0.001$) in FHSD compared to FC, while there was a decrease in TAS ($p=0.009$). Compared to MC, there was an increase in OSI ($p=0.021$) and a decrease in TAS ($p=0.045$) in MHSD (Figure 4).

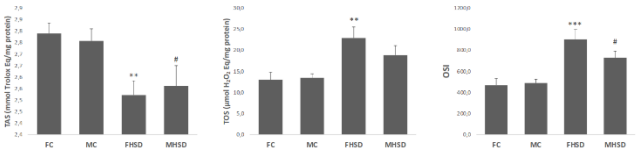

Figure 4. Effects of HSD on oxidative stress in brain tissues of mice. FHSD vs FC; *p < 0.05, **p < 0.01, MHSD vs MC: #p < 0.05. FC: female control, MC: male control, FHSD: female high salt diet, MHSD: male high salt diet, TAS: antioxidant capacity, TOS: oxidant capacity, OSI: oxidative stress index.

Histopathological examination: It was observed that the neurons were compactly arranged and had normal morphology in the H&E-stained hippocampus of the control groups of female and male mice. In FHSD, neurons were found to be more abnormal due to nuclear condensation, and nuclei were found to be karyopycnosis. The arrangement of neurons was found to be abnormal. According to the number of neurons in the hippocampus CA1, CA3, and dentate gyrus areas, the number of neurons in all three regions decreased in FHSD compared to FC. Neurons are more loosely arranged in MHSD compared to MC. According to MC, there was a significant reduction in the number of neurons in CA1 and CA3. There was no difference in the number of neurons in the dentate gyrus region. Pathological changes in MHSD were milder than in FHSD (Figure 5).

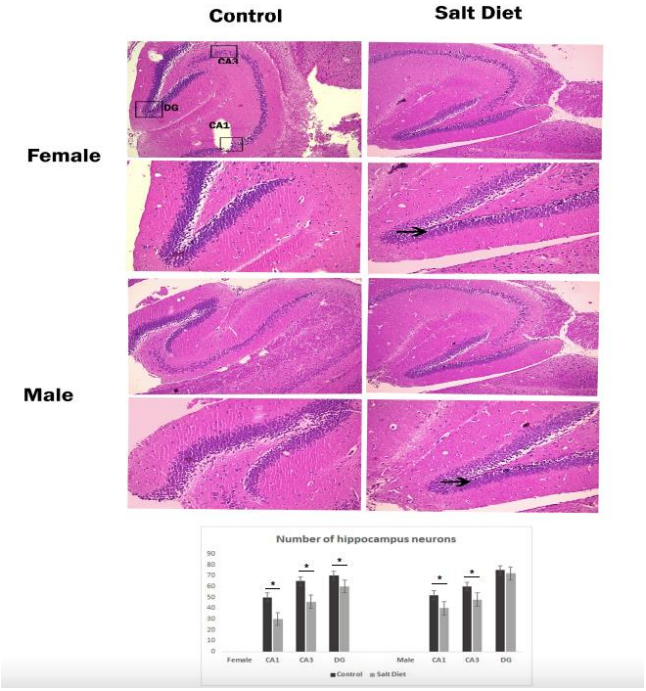

Figure 5. Histological changes in brain tissues. FC: female control, MC: male control, FHSD: female high salt diet, MHSD: male high salt diet, DG; dentate gyrus. *p < 0.05.

Discussion

Diet and its elements are significant environmental factors that affect body health. Salt is a frequently used nutrient, especially for sweetening, and when used in high amounts, significant disorders such as hypertension, cardiovascular complications, and increased inflammation occur (27). Because increased blood pressure contributes significantly to the development of chronic cardiovascular diseases (28). Due to dietary habits, the amount of salt taken daily is

increased by about 10% just for taste in the diet (29). Reducing salt is part of WHO's 2025 targets to reduce death rates from preventable diseases, and it is estimated that 2.5 million deaths could be prevented in line with these global targets (29,30).

Studies in mice make it much more convenient to control salt intake and exposure to specific stressors and allow further research. Salt loading studies in the literature are generally performed in males. This is because males are more likely to be affected by cardiovascular conditions (15). But, CVD is the leading cause of death and disability for women worldwide (31). When we reviewed the literature, we could not find many studies investigating the effect of gender-linked HSD on anxiety, depression, and cognitive functions in mice. Therefore, we planned to determine the potential underlying mechanism and to what extent a high-salt diet would affect anxiety, depression, and cognitive functions under the same conditions in both genders.

While HSD does not affect body weight, it significantly increases water consumption (32). In this current study, there was an increase in water intake while no change in body weights after HSD. This proves that animals are exposed to HSD and provides a conceptual framework to elucidate the cognitive dysfunction caused by HSD.

HSD may have a negative effect on brain tissue. Because it may also affect cognitive function through its effects on cerebrovascular function and cerebral blood flow. HSD is associated with impaired vascular endothelial function, which is associated with cerebral small vessel disease, a significant risk factor for cognitive impairment with increasing age (33). Studies in rodents with HSD have reported that high salt (2-8%) added to feed and/or water impairs cognitive activity, increases cerebral ischemia damage, and creates high stress (1). Studies on mice have shown that poorer cognitive functions and limited blood flow to the brain are due to higher salt intake (34). According to NORT results, the decrease in the time spent with the new object in females is higher than in males treated with HSD. This decrease is significant in females while not in males, compared to their control groups. When compared with the control groups in AFT for anxiety, time spent at the center, number of rearings, and reduction in line crossing were greater in HSD-treated females than in males. The time spent sedentary in the TST and FST for depression was higher in females than males with HSD. Interestingly, the changes in all these parameters were not significant in MHSD compared to MC. Similar to this study, Gilman et al. (15) could not detect a relationship with anxiety in male rats treated with HSD. On the other hand, Hu et al. (32) found impaired learning and memory levels in female mice treated with HSD, similar to our study.

There is strong evidence to suggest that HSD can directly trigger superoxide generation and secretion of proinflammatory cytokines (35). Reactive oxygen species (ROS), the source of oxidative stress, directly interact with cellular lipids, proteins and DNA, causing cell damage and death (36). Oxidative stress is a result of the balance between oxidant and antioxidant systems being disrupted in favor of the oxidant (37). Oxidative stress is the possible mechanism for HSD-related behavioral and cognitive impairments. It is known that HSD triggers oxidative stress in the hypothalamus, hippocampus, and cerebellum, which are significant regions in the brain for both behavioral and cognitive functions (1). The brain tissue is more sensitive to oxidative stress due to its high oxygen consumption and has relatively less antioxidant defense than other tissues (38). OSI (TOS/TAS ratio) is an indicator of oxidative stress and indicates anti-oxidation and oxidation redox balance (39). In this study, it was determined that the oxidant level increased, the antioxidant level decreased, and the OSI value increased in the brain tissue of female and male mice treated with HSD. When the gender was compared, the increase in the oxidant level, the

decrease in the antioxidant status, and the increase in the OSI value were higher in females than in males. When evaluating the deterioration in behavior and cognitive functions and oxidative stress in females, it can be stated that as the oxidative stress level increases, there will be more deterioration in behavior and cognitive functions.

Studies have reported that inflammation is effective in tissue damage caused by HSD (40). NF-κB, an important marker of inflammatory damage, plays a key role in many biological processes such as expression of chemokines, activation of cytokines, immune response and cell differentiation (41). Activation of proinflammatory cytokine genes (TNF-α and COX-2) begins with the activation of NF-κB (42,43). COX-2 is a proinflammatory cytokine and is triggered by NF-κB. COX-2 is also associated with reactive oxygen species (44,45). In this study inflammation-related NF-κB and TNF-α levels and COX-2 activity in brain tissues were analyzed. These parameters were increased in female and male mice treated with HSD compared to controls (more so in males). In particular, the increase in TNF-α is not significant in females but significant in males. This may indicate that males are more susceptible to salt-induced inflammation.

The hippocampus is a critical brain area for learning and memory processes. The hippocampus is estimated to be the first brain region affected by HSD-induced oxidative stress (38). When we evaluated histopathologically the hippocampus CA1, CA3, and DT regions, a decrease in neuron numbers was detected in female and male mice treated with HSD (females were more affected). We think that the total effect of increased oxidative stress and inflammation factors as the reason for this situation affects the hippocampus more in females.

Conclusion

In conclusion, the current study has shown that HSD increases levels of depression and anxiety and decreases memory in female and male mice. Female mice were more affected by HSD than males. This is mainly because the cumulative effect of increased oxidative stress and inflammation in the brain affects the hippocampus more in females. We conclude that HSD may increase oxidative stress and inflammation in females in a gender-dependent manner, resulting in more adverse outcomes in depression, anxiety, and cognitive function.

Acknowledgments: The authors thank the members of Aksaray University Scientific and Technological Application and Research Center (ASÜBTAM) for making this study possible.

Declaration of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

Authorship Contributions: Concept – H.Ş.; Design - H.Ş., M.Ö.; Supervision - H.Ş., M.Ö.; Materials - H.Ş., M.Ö., N.A.; Data Collection and/or Processing - H.Ş., M.Ö., N.A.; Analysis and/or Interpretation - H.Ş., M.Ö., N.A.; Literature Search - H.Ş., M.Ö.; Writing Manuscript - H.Ş., Critical Review - H.Ş., M.Ö., N.A.

ORCID:

Hasan ŞİMŞEK 0000-0001-5573-4923

Mehmet ÖZ 0000-0003-4167-2623

Nurhan AKARAS 0000-0002-8457-9448

References

1. Leal PEPT, da Silva AA, Rocha-Gomes A, Riul TR, Cunha RA, Reichetzeder C, et al. High-salt diet in the pre- and postweaning periods leads to amygdala oxidative stress and changes in locomotion and anxiety-like behaviors of male wistar rats. Front Behav Neurosci. 2022; 15: 779080.
2. Patel Y, Joseph J. Sodium ıntake and heart failure. Int J Mol Sci. 2020; 21(24): 9474.
3. Li Y, Lyu Y, Huang J, Huang K, Yu J. Transcriptome sequencing reveals high-salt diet-induced abnormal liver metabolic pathways in mice. BMC Gastroenterol. 2021; 21(1): 335.
4. Stern N, Buch A, Goldsmith R, Nitsan L, Margaliot M, Endevelt R, et al. The role of caloric intake in the association of high salt intake with high blood pressure. Sci Rep. 2021; 11(1): 15803.
5. Wang Z, Cheng C, Yang X, Zhang C. L-phenylalanine attenuates high salt-induced hypertension in Dahl SS rats through activation of GCH1-BH4. PLoS One. 2021; 16(4): e0250126.
6. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and

injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the global burden of disease study 2010. Lancet. 2012; 380(9859): 2224-60.
7. WHO global report on sodium intake reduction. Geneva: World Health Organization; 2023. Available from: https://www.who.int/publications/i/item/9789240069985. Retrieved; 13 Dec 2023
8. Salt reduction: World Health Organization; 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/salt-reduction. Retrieved; 13 Dec 2023
9. Wang Y, Liu X, Zhang C, Wang Z. High salt diet induces metabolic alterations in multiple biological processes of dahl salt-sensitive rats. J Nutr Biochem. 2018; 56: 133-141.
10. Du X, Yu L, Ling S, Xie J, Chen W. High-salt diet ımpairs the neurons plasticity and the neurotransmitters-related biological processes. Nutrients. 2021; 13(11): 4123.
11. Husain I, Akhtar M, Madaan T, Vohora D, Abdin MZ, Islamuddin M, et al. Tannins enriched fraction of emblica officinalis fruits alleviates high-salt and cholesterol diet-ınduced cognitive impairment in rats via Nrf2-ARE pathway. Front Pharmacol. 2018; 9: 23.
12. Husain I, Akhtar M, Madaan T, Abdin MZ, Islamuddin M, Najmi AK. Rosuvastatin alleviates high-salt and cholesterol diet-induced cognitive impairment in rats via Nrf2-ARE pathway. Redox Rep. 2018; 23(1): 168-179.
13. Santisteban MM, Iadecola C. Hypertension, dietary salt and cognitive impairment. J Cereb Blood Flow Metab. 2018; 38(12): 2112-28.
14. Mogi M, Tsukuda K, Li JM, Iwanami J, Min LJ, Sakata A, et al. Inhibition of cognitive decline in mice fed a high-salt and cholesterol diet by the angiotensin receptor blocker, olmesartan. Neuropharmacology. 2007; 53(8): 899-905.
15. Gilman TL, George CM, Andrade MA, Mitchell NC, Toney GM, Daws LC. High salt intake lowers behavioral inhibition. Front Behav Neurosci. 2019; 13: 271.
16. Milte CM, Ball K, Crawford D, McNaughton SA. Diet quality and cognitive function in mid-aged and older men and women. BMC Geriatr 2019;19(1):361.
17. Ge Q, Wang Z, Wu Y, Huo Q, Qian Z, Tian Z, et al. High salt diet impairs memory-related synaptic plasticity via increased oxidative stress and suppressed synaptic protein expression. Mol Nutr Food Res. 2017; 61(10): 1700134.
18. Gohar EY, De Miguel C, Obi IE, Daugherty EM, Hyndman KA, Becker BK, et al. Acclimation to a high-salt diet is sex dependent. J Am Heart Assoc. 2022; 11(5): e020450.
19. He W, Xu J, Mu R, Li Q, Lv DL, Huang Z, et al. High-salt diet inhibits tumour growth in mice via regulating myeloid-derived suppressor cell differentiation. Nat Commun. 2020; 11(1): 1732.
20. Sun J, Li H, Jin Y, Yu J, Mao S, Su KP, et al. Probiotic Clostridium butyricum ameliorated motor deficits in a mouse model of parkinson's disease via gut microbiota-GLP-1 pathway. Brain Behav Immun. 2021; 91: 703-715.
21. Koek W, Sandoval TL, Daws LC. Effects of the antidepressants desipramine and fluvoxamine on latency to immobility and duration of immobility in the forced swim test in adult male C57BL/6J mice. Behav Pharmacol. 2018; 29(5): 453-456.
22. Dang R, Wang M, Li X, Wang H, Liu L, Wu Q, et al. Edaravone ameliorates depressive and anxiety-like behaviors via Sirt1/Nrf2/HO-1/Gpx4 pathway. J Neuroinflammation. 2022; 19(1): 41.
23. Cechella JL, Leite MR, Rosario AR, Sampaio TB, Zeni G. Diphenyl diselenide-supplemented diet and swimming exercise enhance novel object recognition memory in old rats. Age (Dordr). 2014; 36(4): 9666.
24. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem. 2004; 37(2): 112-119.
25. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005; 38(12): 1103-1111.
26. Zeren S, Bayhan Z, Kocak FE, Kocak C, Akcılar R, Bayat Z, et al. Gastroprotective effects of sulforaphane and thymoquinone against acetylsalicylic acid-induced gastric ulcer in rats. J Surg Res. 2016; 203(2): 348-359.
27. Rizvi ZA, Dalal R, Sadhu S, Kumar Y, Kumar S, Gupta SK, et al. High-salt diet mediates interplay between NK cells and gut microbiota to induce potent tumor immunity. Sci Adv. 2021; 7(37): eabg5016.
28. Akcılar R, Akçer S, Şimşek H, Akcılar A, Bayat Z, Genç O. The effect of ozone on blood pressure in DOCA-salt-induced hypertensive rats. Int J Clin Exp Med 2015;8(8):12783-12791.
29. Li Z, Hu L, Rong X, Luo J, Xu X, Zhao Y. Role of no table salt on hypertension and stroke based on large sample size from national health and nutrition examination survey database. BMC Public Health. 2022; 22(1): 1292.
30. Arsang-Jang S, Mansourian M, Mohammadifard N, Khosravi A, Oveis-Gharan S, Nouri F, et al. Temporal trend analysis of stroke and salt intake: a 15-year population-based study. Nutr Neurosci. 2021; 24(5): 384-394.
31. McKenzie, B., Santos, J. A., Trieu, K., Thout, S. R., Johnson, C., Arcand, J., ... & McLean, R. (2018). The Science of Salt: a focused review on salt-related knowledge, attitudes and behaviors, and gender differences. The Journal of Clinical Hypertension, 20(5), 850-866.
32. Hu L, Zhu S, Peng X, Li K, Peng W, Zhong Y, et al. High salt elicits brain ınflammation and cognitive dysfunction, accompanied by alternations in the gut microbiota and decreased SCFA production. J Alzheimers Dis. 2020; 77(2): 629-640.
33. Nowak KL, Fried L, Jovanovich A, Ix J, Yaffe K, You Z, Chonchol M. Dietary Sodium/Potassium Intake Does Not Affect Cognitive Function or Brain Imaging Indices. Am J Nephrol 2018;47(1):57-65.
34. Faraco G, Brea D, Garcia-Bonilla L, Wang G, Racchumi G, Chang H, et al. Dietary salt promotes neurovascular and cognitive dysfunction through a gut-initiated TH17 response. Nat Neurosci. 2018; 21(2): 240-249.
35. Costa AP, de Paula RC, Carvalho GF, Araújo JP, Andrade JM, de Almeida OL, de Faria EC, Freitas WM, Coelho OR, Ramires JA, Quinaglia e Silva JC, Sposito AC; Brasilia Heart Study Group. High sodium intake adversely affects oxidative-inflammatory response, cardiac remodelling and mortality after myocardial infarction. Atherosclerosis 2012;222(1):284-91.
36. Kocak C, Kocak FE, Akcilar R, Isiklar OO, Kocak H, Bayat Z, Simsek H, Taser F, Altuntas I. Molecular and biochemical evidence on the protective effects of embelin and carnosic acid in isoproterenol-induced acute myocardial injury in rats. Life Sci 2016; 147:15-23.
37. Ileriturk M, Kandemir O, Akaras N, Simsek H, Genc A, Kandemir FM. Hesperidin has a protective effect on paclitaxel-induced testicular toxicity through regulating oxidative stress, apoptosis, inflammation and endoplasmic reticulum stress. Reprod Toxicol. 2023; 118:108369.
38. Liu YZ, Chen JK, Li ZP, Zhao T, Ni M, Li DJ, et al. High-salt diet enhances hippocampal oxidative stress and cognitive impairment in mice. Neurobiol Learn Mem. 2014; 114: 10-15.
39. Akcılar R, Akcılar A, Şimşek H, Koçak FE, Koçak C, Yümün G, Bayat Z. Hyperbaric oxygen treatment ameliorates lung injury in paraquat intoxicated rats. Int J Clin Exp Pathol. 2015;8(10):13034-13042.
40. Liu F, Mu J, Yuan Z, Wu G, Liu E, Zheng S, et al. High salt intake fails to enhance plasma adiponectin in normotensive salt-sensitive subjects. Nutrition. 2012; 28(4): 422-425.
41. Şimşek H, Küçükler S, Gür C, Akaras N, Kandemir FM. Protective effects of sinapic acid against lead acetate-induced nephrotoxicity: a multi-biomarker approach. Environ Sci Pollut Res Int 2023;30(45):101208-101222.
42. Şimşek H, Akaras N, Gür C, Küçükler S, Kandemir FM. Beneficial effects of chrysin on cadmium-induced nephrotoxicity in rats: modulating the levels of Nrf2/HO-1, RAGE/NLRP3, and Caspase-3/Bax/Bcl-2 signaling pathways. Gene. 2023; 875: 147502.
43. Kandemir FM, Yıldırım S, Kucukler S, Caglayan C, Darendelioğlu E, Dortbudak MB. Protective effects of morin against acrylamide-induced hepatotoxicity and nephrotoxicity: A multi-biomarker approach. Food Chem Toxicol. 2020; 138: 111190.
44. Simsek H, Akaras N. Acacetin ameliorates acetylsalicylic acid-induced gastric ulcer in rats by interfering with oxidative stress, inflammation, and apoptosis. Int J Med Biochem. 2023; 6(2): 96-103.
45. Şimşek H, Küçükler S, Gür C, İleritürk M, Aygörmez S, Kandemir F.M. Protective effects of zingerone against sodium arsenite-

induced lung toxicity: A multi-biomarker approach. Iran J Basic Med Sci. 2023; 26(9): 1098-1106.