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The effect *Eremurus spectabilis* M. Bieb. on diethylnitrosamine-induced neurotoxicity in hippocampus (cornu ammonis) of rat

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Abstract

This study aimed to histopathologically investigate the protective effect of *Eremurus spectabilis* M. Bieb's on Cornu Ammonis (CA) in diethylnitrosamine (DEN)-induced neurotoxicity. In the study, rats were equally divided into (n=8); Control Group: Rats in this group were not exposed to any treatment, DEN Group: Rats in this group were treated with 150 mg DEN/kg body weight dissolved in 0.9% physiological water once a month and thioacetamide (TAA) 200 mg /kg body weight once a week intraperitoneally (i.p.), Lyophilized plant extract (LPE) Group: The rats in this group were given 150 mg DEN/kg once a month dissolved in 0.9% physiological water, 200 mg TAA/kg once a week and 100 mg/kg body weight LPE daily by oral gavage method and fed with standard rat chow and tap water, Nanoparticle plant extract (NPE) Group: Rats in this group were given 150 mg DEN/kg once a month and TAA 200 mg/kg once a week (i.p.) and 100 mg/kg body weight nanoparticle plant extract dissolved in 0.9% physiological water and fed with standard rat chow and tap water by oral gavage method. In our findings, a significant reduction in granular layer cells and significant dissociations in CA regions were observed in DEN group rats. There was a slight alleviation in the lesions in the groups to which we gave lyophilized plant extract. In rats treated with nanoparticle plant extract, CA gave an appearance very close to the control group.

In conclusion, *Eremurus spectabilis* M. Bieb. may exhibit a protective effect against neurotoxicity as well as Alzheimer's disease and Parkinson's disease, which are closely associated with the CA regions. Also, it was reported as an important finding that the nanoparticle extract of *E. spectabilis* was more effective than the lyophilized extract.

Keywords: Diethylnitrosamine, Neurotoxicity, *Eremurus spectabilis*, Cornu Ammonis, Rat.

Introduction

Nitrosamines are formed by chemical reactions with nitrites and secondary amines or proteins [1]. Diethylnitrosamine (DEN) is a chemical agent that triggers various oncogenic mutations [2]. DEN is an identified potential carcinogen for humans and animals. Processed and canned foods, beer, tobacco, personal care products, water and some medicines may contain DEN [3]. Furthermore, nitrosamines are readily released under strong acid conditions in the stomach or at high temperatures associated with frying or flame cooking [4].

Metabolic biotransformation of DEN by cytochrome P450 enzymes is known to induce oxidative stress and cellular damage by releasing free radicals that form reactive electrophiles [5]. These radicals initiate lipid peroxidation in the cell membrane of the endoplasmic reticulum and cause a chain reaction that can oxidise DNA, proteins and lipids [6]. Nitrosamines produce reactive oxygen species such as superoxide ($O^{2\cdot-}$) and hydrogen peroxide (H_2O_2) and, in this context, increase oxidative stress (OS), DNA damage, lipid peroxidation and the formation of protein adducts [7,8,9]. DEN is known to cause problems in the central nervous system and symptoms such as Alzheimer's disease (AD) [3]. DEN-induced oxidative stress and DNA damage promote insulin resistance, a key element of experimental neurodegeneration in the pathogenesis of AD [10,11,12]. The International Agency for Research on Cancer reviewed the first biological DEN experiments in various animal, fish and plant species and reported that they could cause liver, kidney and brain tumours in pigs [13]. Although research on nitrosamines is mostly on mutagenesis, cellular damage and oxidative stress caused by nitrosamine exposure are associated with neurotoxicity, ageing and AD [10].

DEN, which is a complete carcinogen, requires a tumor-stimulating substance to maintain its effect in repeated applications. For this purpose, thioacetamide (TAA), carbon tetrachloride, phenobarbital, and recently high-fat diet feeding methods have been used as both stimulants and accelerators [14]. It has been reported that immunoreactive foci and apoptotic cells increase when TAA is used as a promoter of DEN [15].

The sum of biochemical processes that cause an imbalance in the functioning of antioxidants and oxidants in living organisms is considered OS [16]. This stress occurs due to insufficient antioxidant levels or overproduction of reactive oxygen species [17]. OS increases cell damage through mechanisms such as DNA, lipid and protein damage and aberrations in signalling pathways and gene expression [18]. Recent studies have reported the prevalence of OS in dozens of neurodegenerative diseases such as Parkinson's disease (PD), AD and amyotrophic lateral sclerosis [17,19]. The OS and DNA damage activate apoptotic pathways and cause neurodegeneration [20].

The hippocampus is a very important brain region, characterised by the dentate gyrus (DG) and cornu ammonis (CA) regions, responsible for storing and retrieving short and long memory. The DG contains a V-shaped recess, enclosing the CA4 region between its wings (supra pyramidal wing, infra pyramidal wing) and the CA is anatomically and functionally subdivided into CA1, CA2, CA3 and CA4 [21,22].

The hippocampus sends signals to the limbic system and many brain regions [23,24,25]. Thus, before movements are transformed into behaviours, the hippocampus stimulates the limbic system and influences the shaping of behaviours [26]. The hippocampus is known to be responsible for storing and retrieving short- and long-term memory [22]. Regardless of the mechanism, it is not possible for long or short-term memories to be permanent without the right and left hippocampus. Connections in the CA3 region of the hippocampus are known to play an important role in forming episodic memory [27]. On the other hand, the right side of the hippocampus shows more activity in functions related to visual, and the left side of the hippocampus shows more activity in functions related to verbal memory, and lesions in these regions lead to loss of related memories [26].

The hippocampal CA is considered one of the most sensitive regions, vulnerable to many stimuli [28]. It is also one of the three brain regions most susceptible to oxidative stress and is the first to be disrupted in its functioning [29].

In Turkey, which has a very rich plant population, *E. spectabilis* M. Bieb. is widely consumed as a wild vegetable and is used in traditional medicine to strengthen the immune system and for treatments [30]. The root of *E. spectabilis* has been used in conventional medicine to treat liver disorders, stomach problems, skin diseases and hyperlipidemia [31]. It was reported that water and hexane-ethanol extracts of *E. spectabilis* exhibited antiproliferative effects against different cancer cell lines with their bioactive compounds [32]. It was reported that water extracts did not show as high free radical scavenging activity as standard antioxidants, but boiled extract was noticeably more effective than cold water extract [33]. These effects of *E. spectabilis* are thought to be due to its richness in phenolics and flavonoids [34]. Published reports on the properties of *E. spectabilis* are limited to hot and cold-water extracts, acetone and alcohol extracts. Therefore, this study investigated the protective activity of lyophilised and

nanoparticle extracts of *E. spectabilis* on CA.

In this study, it was aimed to contribute to the literature on neurotoxicity induced by DEN and supported by TAA and to histopathologically examine the protective-therapeutic efficacy of lyophilised and nanoparticle extracts of *E. spectabilis* in the CA region in terms of morphological changes, cell density, regeneration, inflammation and degenerative-necrotic changes.

Methods

Dose Determination and Formation of Experimental Groups

Before Wistar rats (2-3 months, female) were divided into groups, an acute toxicity test was performed to determine the appropriate doses. The rats were observed during this process to determine the appropriate dose. According to Organization for Economic Corporation and Development guideline 425, the acute toxicity test was performed. Two groups were formed, with 3 rats in each group, and the live weights of the rats were weighed and placed in 2 separate cages. One group received 2000 mg/kg *E. spectabilis* ethanol extract (LPE); the other group received 2000 mg/kg *E. spectabilis* nanoparticle ethanol extract (NPE) by oral gavage, and rats were observed for 72 hours. No mortality was observed in the rats due to the toxicity test. The toxicity test was terminated, and the rats used in the study were divided into 4 groups, with 8 rats in each group. The study was terminated after three months.

Groups

Control Group: Rats in this group were not exposed to any treatment and were fed with standard rat chow and tap water.

DEN Group: Rats in this group were treated with 150 mg DEN (Sigma)/kg body weight dissolved in 0.9% physiological water once a month and TAA 200 mg/kg body weight once a week intraperitoneally (i.p.) and fed with standard rat chow and tap water.

LPE (Lyophilized plant extract) Group: The rats in this group were given 150 mg DEN /kg once a month, TAA 200 mg/kg once a week and 100 mg /kg body weight lyophilised plant extract dissolved in 0.9% physiological water once a month by oral gavage method and fed with standard rat chow and tap water.

NPE (Nanoparticle plant extract) Group: Rats in this group were given 150 mg DEN/kg once a month and TAA 200 mg/kg once a week (i.p.) and 100 mg/kg body weight nanoparticle plant extract dissolved in 0.9% physiological water and fed with standard rat chow and tap water by oral gavage method.

DEN and TAA applications continued for 90 days in total. LPE and NPE applications continued every day during this study period.

Histopathologic examination

At the end of the experiment, tissue samples were taken from the brains of the sacrificed rats to cover the CA region, fixed in buffered 10% formaldehyde solution, and embedded in paraffin blocks after tissue tracing, 4 μm thick sections were taken from the blocks, the sections were stained with hematoxylin-eosin staining method, examined under light microscope and the pathological changes observed were photographed (Nikon ECLIPSE 80i–DS-Ri2). The histopathologic examination evaluated morphologic changes regarding cell density (cell densities were assessed semi-quantitatively), regeneration, inflammation and degenerative-necrotic changes and photographed.

Results

Control Group: Normal histologic structure of the CA was observed. The CA comprises five regions: CA1, CA2, CA3, CA4 and DG. CA1 and CA2 regions comprise small pyramidal cells with three to four rows of vesicular nuclei. CA3 and CA4 regions are composed of large pyramidal cells with vesicular nuclei. CA1, CA2, CA3, and CA4 regions consist of molecular, pyramidal, and polymorphous layers. The molecular layer of the CA consists of organised neuronal structures, as well as glial and nerve cells. The dentate gyrus consists of a supra pyramidal wing, infrapyramidal wing, molecular cell layer, granule cell layer, and polymorphic cell layer on both sides of the CA4 area. The part of the dentate gyrus facing the CA4 area surrounds the CA4 area in a V-shape (Figure 1, 2A).

DEN Group: In this group of rats, no morphologic changes were observed in the CA1, CA2, CA3 and CA4 regions compared to the control group. However, diffuse dissociation and reduction of granular layer cells in the dentate gyrus region were observed (Figure 2B).

LPE Group: Morphological changes were not detected in CA regions as in the other groups. In the dentate gyrus region, there was less dissociation in the granular layer cells than in the DEN group, and cell numbers were close to normal in some regions (Figure 2C).

NPE Group: Morphologic changes were not detected in the CA regions as in the other groups. However, compared to the LPE group, granular layer cells in the DG Dentate gyrus region were similar to the normal arrangement tendency in the control group. They were significantly more numerous (Figure 2D).

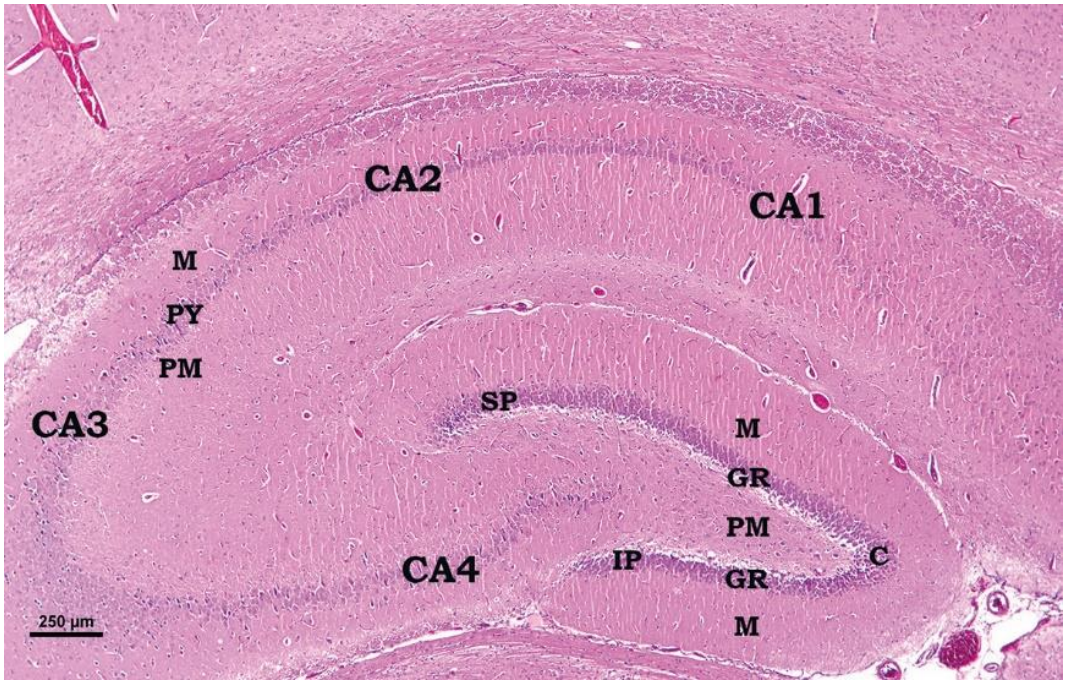

Figure 1. Normal histologic appearance of Cornu Ammonis and Dentate gyrus. Cornu Ammonis; *CA1, CA2, CA3, CA4 regions. Pyramidal layer (PY), Polymorphous layer (PM), and Molecular layer (M).* ***Dentate Gyrus*** *Suprapyramidal wing (SP), Infrapiramidal wing (IP), Molecular layer (M), Granular layer (GR), Polymorphic layer (PM), Crest (C). H&E, (Bar=250 µm).*

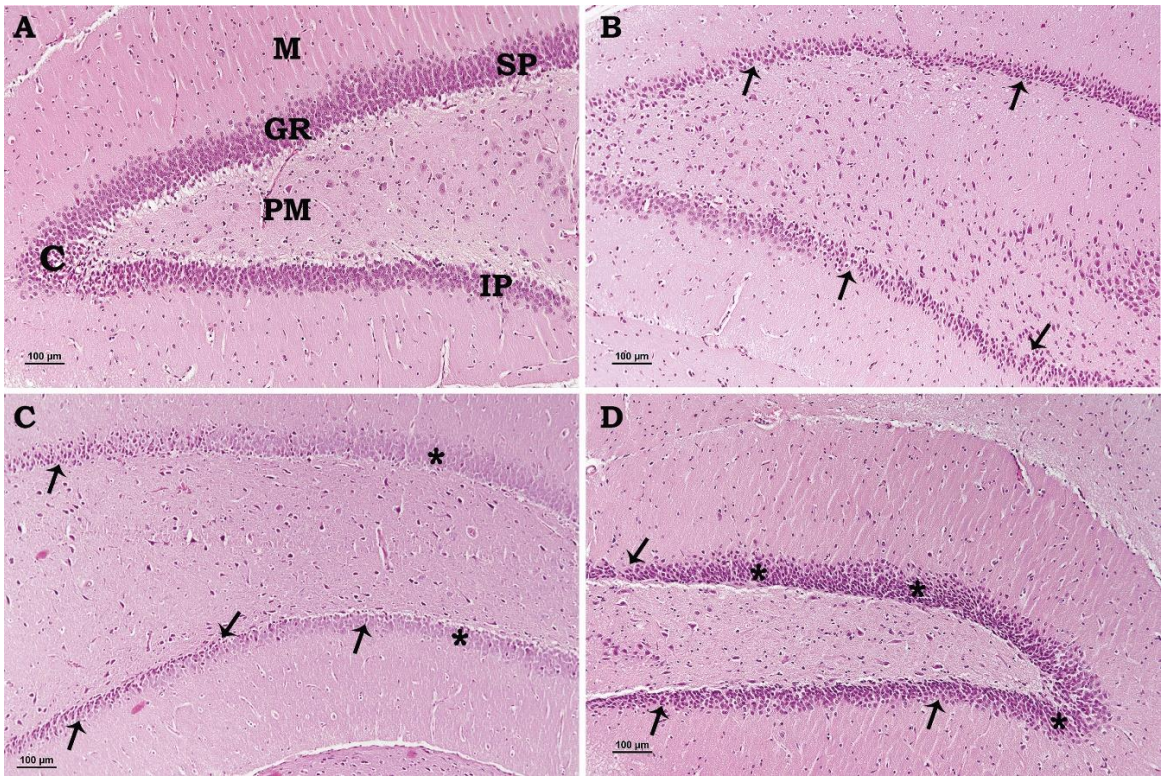

Figure 2. *Loading* ***A: Control Group.*** *Suprapiramidal wing (SP), Infrapiramidal wing (IP), Molecular layer (M), Granular layer (GR), Polymorphic layer (PM), Crest (C).* ***B: Den Group.*** *Marked dissociation and reduced number of granular layer cells (arrows).* ***C: LPE Group.*** *Less morphologic changes in granular layer cells (dissociation and reduction in granular layer cells) (arrows) and partially smooth cells (*) compared to the DEN group.* ***D: NPE Group.*** *Very few dissociations and decreases in granular layer cells (arrows), cells similar to the control group (*). H&E, (Bar=100 µm).*

Discussion

DEN is a nitrosamine that has been identified as carcinogenic to humans and animals [3]. Therefore, it has been used in various experimental studies on cancer and toxicities in different years [3,35,36]. DEN has also been used in neurotoxicity studies in the brain and hippocampus [3,4,36]. The hippocampus is one of the most sensitive brain regions in terms of toxicity and aging-induced pathological changes [37].

Some histopathologically evaluated studies of hippocampal damage induced by different stimuli have reported neuronal degenerations, vacuoles, irregular ghost nucleated cells, decreased thickness of the pyramidal cell layer and significant decreases in the thickness of the granular layer [37,38,39,40,]. However, our study's findings were incompatible with any findings except for substantial reductions in granular layer thickness.

In a study testing the hypothesis that exposure to N-nitrosodiethylamine (NDEA) causes AD-type molecular and biochemical abnormalities in central nervous system (CNS) neurons, it was reported that NDEA increases DNA damage, causes insulin, IGF resistance and IGF-II deficiency in CNS neurons, and causes changes in the ratio of AD-related proteins [4]. In our study, degenerations and changes in the cerebral cortex are due to DNA damage and toxicity. In this respect, our findings were consistent with the study of de la Monte and Tong [4]. However, no significant morphologic changes other than dissociation and a decrease in cell number were observed in the CA region of DEN-treated rats.

In a study investigating the effect of streptozotocin (STZ) on some functional, histological and molecular aspects of the hippocampus in streptozotocin-induced diabetic rats. It was reported that neuronal disorganisation increased and neural-microglial density decreased in the hippocampus of diabetic rats. In contrast, neural disorganisation decreased, neural-microglial density increased, and the thickness of the pyramidal-molecular layer in the hippocampus increased in the groups given the active substance [41]. Our study observed neural dissociation and decreased cell density in DEN-treated rats. It was revealed that the thickness of the pyramidal-molecular layer increased with the reduction of these effects in the group's given plant extract. Our findings were consistent with the above study in terms of both damage groups and treatment groups.

Shanab et al. investigated the possible neuroprotective role of the CopperII-albumin complex against DEN-induced neurotoxicity in mice. They reported that severe hemorrhagic changes with mononuclear cell infiltration and focal microglia cell aggregations were observed in the brain tissues of the DEN-treated group. In addition, they also noticed variable thinning in granular and Purkinje cell layers [36]. In our study, degenerations in the cerebral cortex were observed in DEN-treated groups. However, only reduction and dissociation of granular layer cells were observed in the hippocampus region.

Our findings showed remarkable reduction and dissociation in the granular layer cells in the CA regions of DEN group rats. On the other hand, granular layer cells in the CA regions of LPE group rats showed less dissociation and partially smooth cells in some regions compared to the DEN group. However, granular layer cells in the CA regions of NPE group rats were similar to the normal arrangement tendency in the control group and were significantly more numerous.

Conclusion

E. spectabilis M. Bieb, due to its antioxidant effects, may have a protective effect on the CA, which plays an important role in DEN-induced neurotoxicity and AD and PD diseases and is one of the most sensitive regions affected by toxicity and damage. Our study has contributed to the literature on DEN-induced neurotoxicity and is among the first articles to histopathologically examine DEN toxicity in CA, which is important in this context. In this regard, we support further studies on DEN-induced neurodegeneration and the investigation of various protective and therapeutic agents. Our article will lead to further studies on DEN-induced neurotoxicity and Cornu Ammonis damage.

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Conflicts of interest

The authors report no conflicts of interest.

Ethics committee approval

This study was conducted with the permission of Van Yüzüncü Yil University Animal Experiments Local Ethics Committee on 25.04.2024 with the number 2024/04-01.

References

1. Lijinsky W. N-Nitroso compounds in the diet. Mutat Res. 1999;(443):129–38. https://doi.org/10.1016/s1383-5742(99)00015-0
2. Sotty J, Bablon P, Weiss PH, Soussan P. Diethylnitrosamine Induction of Hepatocarcinogenesis in Mice. Methods Mol Biol. 2024;(2769):15-25. https://doi.org/10.1007/978-1-0716-3694-7_2
3. Lopez TP, Tostado JAGA, Dias F, Maltez KHM. Metformin Prevents NDEA-Induced Memory Impairments Associated with Attenuating Beta-Amyloid, Tumor Necrosis Factor-Alpha, and Interleukin-6 Levels in the

Hippocampus of Rats. Biomolecules 2023;(13):1289. https://doi.org/10.3390/biom13091289

4. de la Monte SM, Tong M. Mechanisms of nitrosamine-mediated neurodegeneration: potential relevance to sporadic Alzheimer's disease. J Alzheimers Dis 2009;17(4):817-25. https://doi.org/10.3233/JAD-2009-1098.
5. Sheweita AS, El-Bendery HA, Mostafa HM. Novel Study on N-Nitrosamines as Risk Factors of Cardiovascular Diseases. Biomedical Research International. 2014;817019. https://doi.org/10.1155/2014/817019
6. Vitaglione P, Morisco F, Caporaso N, Fogliano V. Dietary antioxidant compounds and liver health. Critical Review Food Science and Nutrition. 2004;44(7-8):575-86. https://doi.org/10.1080/10408690490911701
7. Chuang CH, Hu ML. Synergistic DNA damage and lipid peroxidation in cultured human white blood cells exposed to 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone and ultraviolet A. Environ Mol Mutagen. 2006;(47):73–81. https://doi.org/10.1002/em.20168
8. Yadav AS, Bhatnagar D. Chemo-preventive effect of Star anise in N-nitrosodiethylamine initiated and phenobarbital promoted hepato-carcinogenesis. Chem Biol Interact. 2007;(169):207–14. https://doi.org/10.1016/j.cbi.2007.06.032
9. Espey MG, Miranda KM, Thomas DD, Xavier S, Citrin D, Vitek MP, et al. A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen oxide species. Ann N Y Acad Sci. 2002;(962):195–206. https://doi.org/10.1111/j.1749-6632.2002.tb04068.x
10. de la Monte SM, Wands JR. Alzheimer's Disease is Type 3 Diabetes: Evidence Reviewed. J Diabetes Science Tech. 2008;(2):1101–13. https://doi.org/10.1177/193229680800200619
11. Lester-Coll N, Rivera EJ, Soscia SJ, Doiron K, Wands JR, de la Monte SM. Intracerebral streptozotocin model of type 3 diabetes: relevance to sporadic Alzheimer's disease. J Alzheimers Dis. 2006;(9):13–33. https://doi.org/10.3233/jad-2006-9102
12. Salkovic-Petrisic M, Tribl F, Schmidt M, Hoyer S, Riederer P. Alzheimer-like changes in protein kinase B and glycogen synthase kinase-3 in rat frontal cortex and hippocampus after damage to the insulin signalling pathway. J Neurochem. 2006;(96):1005–15. https://doi.org/10.1111/j.1471-4159.2005.03637.x
13. Verna L, Whysner J, Williams GM. N-nitrosodiethylamine mechanistic data and risk asseessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. Pharmacology and Therapeutics. 1996;(71):57-81. https://doi.org/10.1016/0163-7258(96)00062-9
14. Park EJ. Lee JH. Yu GY. He G. Ali SR. Holzer RG. et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell. 2010;140(2):197-208. https://doi.org/10.1016/j.cell.2009.12.052
15. Kimura M, Fujii Y, Yamamoto R, Yafune A, Hayashi S, Suzuki K, et al. Involvement of multiple cell cycle aberrations in early preneoplastic liver cell lesions by tumor promotion with thioacetamide in a two-stage rat hepatocarcinogenesis model. Experimental and Toxicologic Pathology. 2013;(65):979-88. https://doi.org/10.1016/j.etp.2013.01.012
16. Pizzino G. Irrera N. Cucinotta M. Pallio G. Mannino F. Arcoraci, V. Squadrito F. et al. Oxidative Stress: Harms and Benefits for Human Health. Oxid. Med. Cell. Longev. 2017;2017:8416763. https://doi.org/10.1155/2017/8416763
17. Singh A. Kukreti R. Saso L. Kukreti S. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. Molecules. 2019;(24):1583. https://doi.org/10.3390/molecules24081583
18. G Spiteller. Enzymic lipid peroxidation-α consequence of cell injury?. Free Radic. Biol. Med. 1996;(21):1003–9. https://doi.org/10.1016/S0891-5849(96)00268-7
19. Wang X. Wang W. Li L. Perry G. Lee HG. Zhu X. Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochim. Biophys. Acta. 2014;(1842):1240–47. https://doi.org/10.1016/j.bbadis.2013.10.015
20. Jantas, D. Laso´n W. Preclinical Evidence for the Interplay between Oxidative Stress and RIP1-Dependent Cell Death in Neurodegeneration: State of the Art and Possible Therapeutic Implications. Antioxidants (Basel). 2021;(10):1518. https://doi.org/10.3390/antiox10101518
21. Barr ML, Klernam JA: The human nervous system. 5th ed. Philadelphia: JB Lippincott Comp, 1988; 266-269.
22. Augustinack JC, van der Kouwe AJ, Salat DH, Benner T, Stevens AA, Annese J, et al. H.M.'s contributions to neuroscience: A review and autopsy studies. Hippocampus. 2014;24(11):1267-86. https://doi.org/10.1002/hipo.22354
23. Rowland DC, Moser MB. Time finds its place in the hippocampus. Neuron. 2013;78(6):953-4. https://doi.org/10.1016/j.neuron.2013.05.039
24. Schmidt B, Marrone DF, Markus EJ. Disambiguating the similar: The dentate gyrus and pattern separation. Behav Brain Res. 2012;226(1):56-65. https://doi.org/10.1016/j.bbr.2011.08.039
25. Sendrowski K, Sobaniec W. Hippocampus, hippocampal sclerosis and epilepsy. Pharmacol Rep. 2013;65(3):555-65. https://doi.org/10.1016/S1734-1140(13)71033-8
26. Izci Y, Erbaş C. Hippocampus: Its Structure and Functions. Türk Nöroşir Derg. 2015;25(3):287-95. http://norosirurji.dergisi.org/pdf.php?id=1095
27. Wixted JT, Squire LR, Jang Y, Papesh MH, Goldinger SD, Kuhn JR, et al. Sparse and distributed coding of episodic memory in neurons of the human hippocampus. Proc Natl Acad Sci USA. 2014;111(26):9621-6. https://doi.org/10.1073/pnas.1408365111
28. Hashish HA. Histopathologic effect of prenatal topiramate ex posure on rat cerebral cortex and hippocampus. J Interdiscipl Histopathol. 2014;(2):61-8. https://doi.org/10.5455/JIHP.20140130125547
29. Huang TT, Zou Y, Corniola R. Oxidative stress and adult neurogenesis--effects of radiation and superoxide dismutase deficiency. Semin Cell Dev Biol. 2012;(23):738-44. https://doi.org/10.1016/j.semcdb.2012.04.003
30. Mükemre M, Behçet L, Çakilcioğlu U. Ethnobotanical study on medicinal plants in villages of Çatak (VanTurkey). J Ethnopharmacol. 2015;166:361–374. https://doi.org/10.1016/j.jep.2015.03.040
31. Amiri MS, Joharchi MR. Ethnobotanical investigation of traditional medicinal plants commercialized in the markets of Mashhad, Iran. Avicenna J Phytomed. 2013;(3):254-71.
32. Abubaker SR, Hidayat HJ. Anti-tumor potential of local aslerk (Eremurus spectabilis) leaf extracts by HPLC and applying on cancer cell lines in vitro. Iraqi J Cancer Med Genet. 2015;8(2):123-8. https://doi.org/10.29409/ijcmg.v8i2.155
33. Güzel E, Boğa R, Bursal E. Determınatıon of Antıoxıdant Actıvıtıes of Asphodelus aestivus. Muş Alparslan University Journal of Science. 2013;1(1):17-25.
34. Mushtaq A, Masoodi MH, Wali AF, Ganai BA. Total phenolic content, total flavonoid content, in vitro antioxidant activity and antimicrobial activity against human pathogenic bacteria of Eremurus himalaicus–An edible herb of North Western Himalayas. Free Radic Antioxid. 2017;7(1): 90-4. https://doi.org/10.5530/fra.2017.1.14
35. Bansal AK, Bansal M, Soni G, Bhatnagar D. Protective role of Vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chemico-Biological Interactions. 2005;(156):101–11. https://doi.org/10.1016/j.cbi.2005.08.001
36. Shanab O, Mostafa L, Abdeen A, Atiac R, Nassar AY, Youssef M, et al. Modulatory mechanisms of copperII-albumin complex toward N-

nitrosodiethylamine-induced neurotoxicity in mice via regulating oxidative damage, inflammatory, and apoptotic signaling pathways. Ecotoxicology and Environmental Safety. 2024;(270):115841. https://doi.org/10.1016/j.ecoenv.2023.115841

37. Lin JY, Kuo WW, Baskaran R, Kuo CH, Chen YA, Chen WST, et al. Swimming exercise stimulates IGF1/ PI3K/Akt and AMPK/SIRT1/ PGC1α survival signaling to suppress apoptosis and inflammation in aging hippocampus. AGING. 2020;(12):8. https://doi.org/10.18632/aging.103046
38. Kamar SA, Abdel Malak HW, Saad SA. Effect of caffeinated energy drinks on the structure of hippocampal cornu ammonis 1 and dentate gyrus of adult male albino rats. Anat Cell Biol. 2020;(53):330-41. https://doi.org/10.5115/acb.20.136
39. Owoeye O, Onwuka SK. Lead Toxicity: Effect of Launaea taraxacifolia on the Histological and Oxidative alterations in Rat Regio III Cornu ammonis and Cerebellum. Anatomy Journal of Africa. 2016;5(1):783-794.
40. Bekheet EA, Amin MA. Role of Panax Ginseng extract in ameliorating the effects of intermittent restraint stress on the hippocampus of adult male albino rats: Histological and Immunohistochemical study. The Egyptian Journal of Anatomy, 2020;43(1):162-73. https://doi.org/10.21608/EJANA.2019.16777.1042
41. Keshvari M, Rahmati M, Mirnasouri R, Chehelcheraghi F. Effects of endurance exercise and Urtica dioica on the functional, histological and molecular aspects of the hippocampus in STZ-Induced diabetic rats. Journal of Ethnopharmacology. 2020;(256):112801. https://doi.org/10.1016/j.jep.2020.112801