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ORIGINAL ARTICLE

Role of Intermittent Fasting on Improvement of Cognitive Functions in Rat Model of Type 2 Diabetes

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ABSTRACT

Background: The link between diabetes mellitus and cognitive impairment becomes evident, making it important to find means to maintain cognitive performance in diabetic patients. Keeping normal cognitive functions is crucial for maintaining an active, independent lifestyle. Intermittent fasting (IF) is an eating regimen in which a period of eating alternates with fasting period each day. During fasting, diet may include low amount or non-caloric food. IF could promote neurogenesis and cognition.

Aim: Evaluation of the role of IF on cognitive functions in adult male type 2 diabetic albino rats.

Methods: The study included 24 adult male albino rats weighing 120-190 gm. Rats assigned into three groups 8 rats each: control group (C) had free access to chow and water; Diabetic group (DM) type 2 diabetes mellitus (T2DM) was induced with high fat diet for two weeks then intraperitoneal injection with 35 mg/kg of streptozotocin; Intermittent fasting group (IF) after T2DM induction, rats were subjected to 3 months of 18 hours/day fasting. At the end of the experiment, memory and mood were assessed using modified T Maze test and modified forced swimming test respectively. Then rats were sacrificed, and hippocampal samples were collected for superoxide dismutase (SOD), and malondialdehyde (MDA) assay.

Results: Three months IF caused a significant increase in spontaneous alternation in modified T Maze test results; IF group ($P<0.001$) versus DM group. A significant decrease in immobility time (seconds) in IF group versus DM rats ($P<0.001$). IF group had significantly lower MDA levels than DM group ($P<0.001$).

Conclusions: IF has improved memory and mood in T2DM rats. This may be attributed partially to oxidative stress alleviation.

Keywords: Type 2 diabetes mellitus, working memory, depression, Intermittent fasting, rats.

NTRODUCTION

Global diabetes diagnoses showed that type 2 diabetes mellitus (T2DM) has a proportion of 98% of all diagnosed diabetes cases [1]. Concerns are considerably increased that diabetes prevalence will continue to rise markedly. Global data showed that the incidence rate of T2DM among adolescents and young adults increased in the last two decades [2].

Cognitive impairment in young adult with T2DM is high up to 19.5% of all diagnosed cases; although in the USA, it represents less than 4% of the healthy adults below 65 years [3]

Cognitive profiles in patients with T2DM showed deteriorations in cognition, affecting information-processing speed, verbal memory, and executive functions [4]. Also, the duration of T2DM revealed a

negative relationship with cognitive function scores [5].

Cognitive dysfunction in diabetic individuals can be attributed to interactions between metabolic abnormalities, micro- and macrovascular complications, in addition to the coexisting morbidities [6-10].

Diabetes affects mental capacity and neuronal function, either due to hypoperfusion of the brain tissues due to cerebrovascular disease, or changes in glucose transporters leading to abnormal neuronal glucose uptake and metabolism, or disturbed brain metabolism due to insulin resistance, or recurrent incidence of hypoglycemic attacks [11]. Previous studies documented decreased expression of GLUT1 and GLUT3, predominantly expressed by the neurons, in the different brain areas in T2DM subjects [12] Hyperglycemia induces abnormal endothelial cell proliferation, narrowing the blood vessels, and decline in brain perfusion affecting blood-brain barrier integrity and brain homeostasis [13].

Recently, intermittent fasting (IF) became a wide spread regimen, yet it is a controversial therapeutic adjuvant. IF possesses advantageous effects regarding lipid profile, weight loss, glycemic control, and the fat distribution in obese and T2DM subjects. It is considered a safe and feasible intervention [14]. A large body of evidence indicates the significant effect of nutrition on brain structure and function. Hippocampus-dependent cognition and adult hippocampal neurogenesis were enhanced on daily energy restriction and IF [15].

We hypothesized that cognitive dysfunction and mood changes in T2DM could be ameliorated by IF, with the possible involvement of oxidative stress.

METHODS

I-Animals:

The current study included 24 mature male albino rats weighing 120-190 grams. They were retrieved from the Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. Animals were kept at the Animal House of Faculty of Medicine, Zagazig University, Zagazig, Egypt in steel wire cages. Animals were housed in standard conditions for two weeks prior to the commencement of the trials, fed standard food, and given free access to water. The Zagazig University's Institutional Animal Care and Use Committee approved the research protocol (ZU-IACUC /2/F/19/2022).

II-Groups:

After accommodation, animals were assigned to three groups. Control group (n=8), rats were bred on a normal diet [16]. Type 2 diabetic group (n=8), rats received a diet enriched in fat for 2weeks and then were injected (IP) with Streptozotocin (STZ) (35 mg /kg / BW). Intermittent fasting group (n=8), after induction of diabetes rats were exposed to daily fasting from 14:00 to 08:00 for 3 months (18 hours/day) [17].

III-Induction of Type 2 diabetes mellitus (T2DM)

Rats received a diet rich in fat (25% fat, 15% protein, 51% starch, and 5% fiber) [18] Two weeks later, rats were injected (I.P) by STZ (35 mg /kg / BW) dissolved in citrate buffer (PH 4.5**).** Diabetes was confirmed when random blood glucose level > 300 mg/dl [19].

IV- Preparation of Brain Tissue: Soon after sacrifaction, the brain was excised and rinsed with saline. The hippocampus was dissected and then homogenized. Subsequent centrifugation at 4°C for 15 min was performed. The resultant supernatant storage at was performed at -80°C.

V-Laboratory Measurements:

Estimation of SOD and MDA in the hippocampal homogenates was performed colorimetrically using the kits provided by Bio-diagnostic, Egypt by the means of Sunostik, China.

VI- Modified T-maze test:

The T-shaped platform of the maze (for rats) was built with a 600 mm x 165 mm start arm and 400 mm x 100 mm goal arms at the upper apex of the "T". The thickness of the walls was 5.5 mm x 8 mm (floor thickness). A central partition was at the junction of the start arm to the goal arm, where this partition extended from the back wall of the T-maze and 100 mm into the start arm dividing the goal arms. Animals at the bottom of the T select one of the arms at the end of the stem. When two successive trials were given, the second trial shows rodent tendency to choose the previously non- visited arm. A proportion of correct choices (alternations) per rat was estimated as follows:

$$\frac{\text{Number of correct choices (Alternations)}}{\text{Total possible alternations}}$$ [20].

VII- Modified forced swim test: Evaluation of the depressive behavior is assessed by the modified forced swim test. Each rat was put in a glass cylinder (100 cm diameter X 60 cm height) full of water 23 to 25°C. Water level doesn't permit the rat to touch the floor nor climb over the edge. The animal was observed for 5 minutes, and the immobility time was measured [21].

Statistical analysis: Collected data were analyzed by SPSS for Windows version 16. One-way analysis of variance (ANOVA) followed by LSD test were used for comparison between the studied groups. P values <0.05 were considered to be statistically significant.

RESULTS

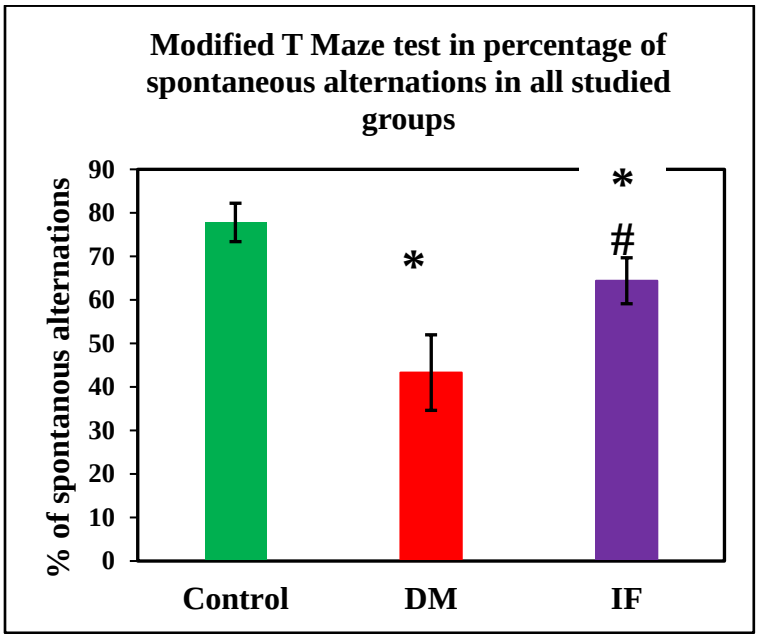
The results show a significant increase in the spontaneous alternations in the modified T Maze test in the IF group versus DM group while still significantly less than control rats (P<0.001) **Figure (1).**

The results indicate a significant increase in the mean value of immobility time in the DM group versus control rats (P<0.001) while the IF group had a significant decrease in time of immobility in seconds versus DM group (P<0.001), meanwhile, it showed no significant difference from control group (P>0.05) **Figure (2).**

The results demonstrated a significant decrease in SOD levels in hippocampal homogenates (Umol /g tissue) in DM and IF groups versus control group **Figure (3).**

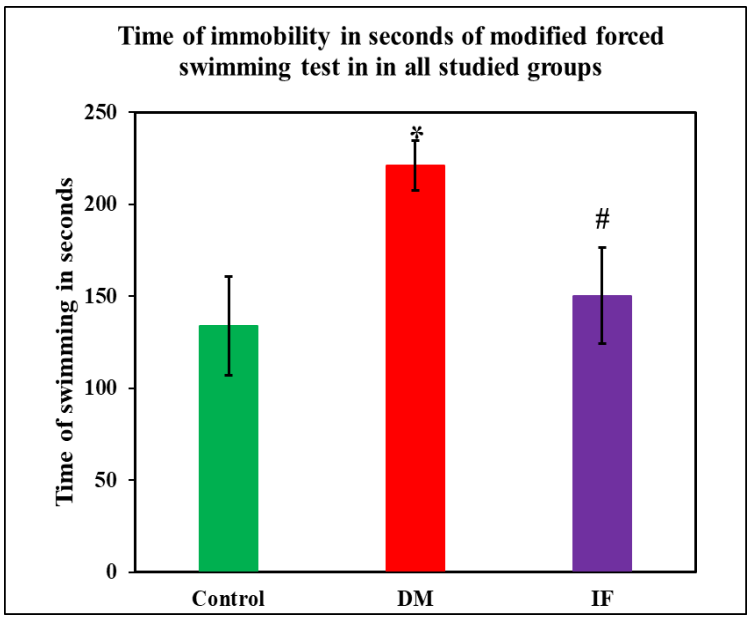
Our results demonstrated a significant decline in the mean value of MDA levels in hippocampal homogenate (nmol /g tissue) in the IF group when compared with the DM rats (P<0.001). Meanwhile, it was significantly more than the control rats (P<0.001) **Figure (4).**

Figure (1) shows the results of the modified T Maze test in the percentage of spontaneous alternations in all studied groups.



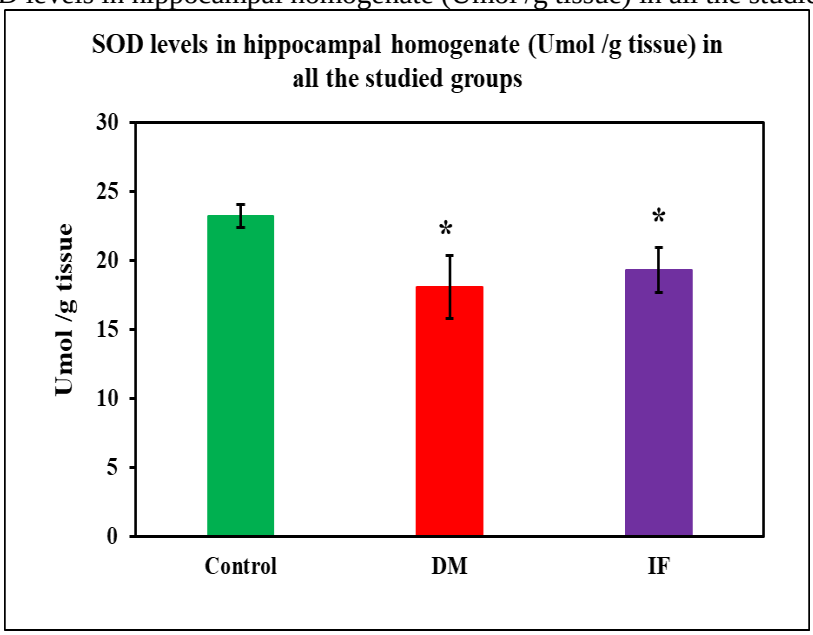
* VS control group
\# VS DM group

Figure (2) shows the time of immobility in seconds of modified forced swimming test in all studied groups.



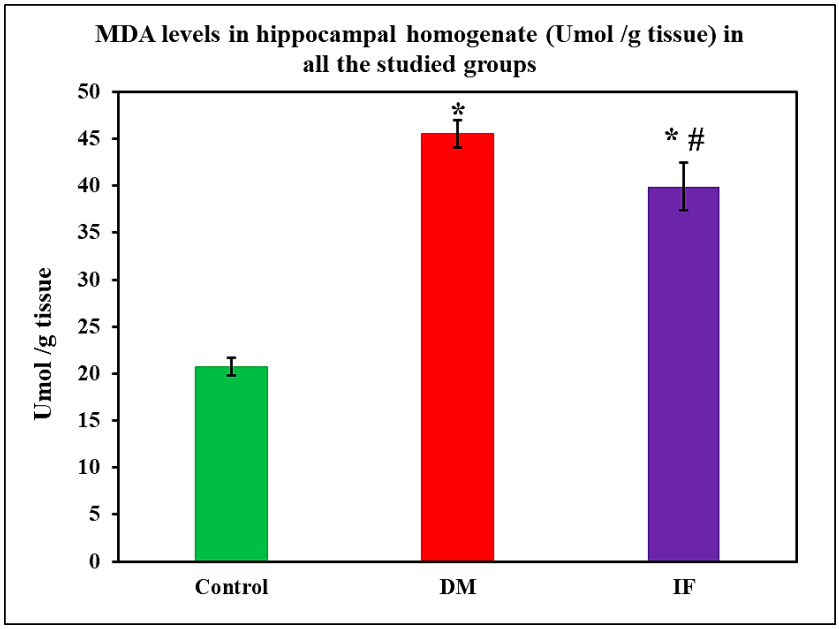
* VS control group
# VS DM group

Figure (3) Shows SOD levels in hippocampal homogenate (Umol /g tissue) in all the studied groups.



* VS control group

Figure (4) Shows MDA levels in hippocampal homogenate (Umol /g tissue) in all the studied groups.



* VS control group
# VS DM group

DISCUSSION

Diabetes mellitus (DM) is a chronic condition marked by altered insulin secretion and utilization, which results in hyperglycemia. Multiple organ systems are affected by this multisystemic disease [22]. DM complications might be macrovascular or microvascular in nature. Microvascular problems have an impact on the neurological system. Owing to the dependance of brain and neural tissues on glucose as a fuel, Changes in the metabolism of carbohydrates have a direct impact on the cerebral processes such as cognition, decision-making ability, and memory [23]. DM is a risk factor for Alzheimer's disease, vascular-based dementia, and progressive cognitive impairment. [24]. Cognitive impairment comprises memory loss, mood, and behavioral changes [25]. Cognitive impairment ranges from mild cognitive deficit that slightly affect the self-dependance and behavior to severe dementia which hinders the daily life activities [26].

IF is a form of interrupted dietary restriction which can increase life expectancy, improve energy metabolism, and lower the risk of various age-related illnesses. IF may also help with insulin resistance, protect the functionality of the central nervous system, and alleviate complications related to diabetes. [27].

The present study showed that T2DM resulted in less spontaneous alternation in the T-maze test compared to the control rats leading to impaired cognitive function in learning ability and memory. Similarly, previous reports showed that T2DM had lower percentages of correct response and spontaneous alternation in the T-maze test, noting that DM caused spatial learning and memory deficits [28, 29].

The forced swimming test is a widely used behavioral tests for studying mood. The duration of immobility during the test period shows depressive-like behavior in rats [30. 31]. In our study, the results of the forced swimming test showed an increase in the immobility time in diabetic rats compared to control similar to a previous report [32].

The study of the antioxidant activity in hippocampal tissue revealed decreased levels of SOD and increased MDA levels in the diabetic group which was also reported in previous studies [29, 32]. It seems plausible that oxidative stress is involved in diabetic cognitive changes due to the high lipid content, high oxygen consumption rate, and low antioxidant enzymes in brain which make it sensitive to oxidative stress [33]. Oxidative stress contributes to mitochondrial dysfunction and accelerated apoptosis and leads to neuronal impairment, and synapse loss [17, 34]

Insulin resistance in the brain may also decrease antioxidant enzymes and increase free radical content, which chemically modifies the lipids disturbing the structure and function of the nerve cells [35].

The result of our study revealed that IF could ameliorate the cognitive impairment caused by

T2DM where it caused an increase in the spontaneous alternation in the T-maze test. In line with our results, IF possessed an improving effect on spatial working memory impairments in mice [36].

In addition, the forced swimming test showed a decline in the immobility time, indicating improved depressive-like behavior due to IF. Reduction in the time spent immobile in the forced swimming test was reported in fasted mice [37].

It was explained that certain signaling pathways in the prefrontal cortex and hippocampus were activated by fasting, and the hippocampus' synaptic transmission was changed. [38].

Also, IF was able to reduce the oxidative stress in the hippocampal tissues in the present study as shown by increased SOD and decreased MDA levels. Oxidative stress is one of the factors controlling neurogenesis. By producing new neurons in response to oxidative stress, IF boosts hippocampal neurogenesis and lessens brain damage.[17].

Conclusions: Intermittent fasting was able to improve cognitive function -memory and mood- in Type 2 diabetes mellitus (T2DM) in albino rats. This could be partially attributed to alleviation of oxidative stress.

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Conflicts of interest: None

REFERENCES

1. Green, A., Hede, S. M., Patterson, C. C., Wild, S. H., Imperatore, G., Roglic, G., et al. Type 1 diabetes in 2017: global estimates of incident and prevalent cases in children and adults. *Diabetologia*. 2021; *64*, 2741-2750.
2. Xie, J., Wang, M., Long, Z., Ning, H., Li, J., Cao, Y& Pan, A. Global burden of type 2 diabetes in adolescents and young adults, 1990-2019: systematic analysis of the Global Burden of Disease Study 2019. 2022; *bmj*, *379*.
3. Alzheimer's Association. (2014). Alzheimer's Disease Facts and Figures. Chicago, IL: Alzheimer's Association (2014).
4. Awad, N., Gagnon, M., & Messier, C. The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. *Journal of clinical and experimental neuropsychology*. 2004; *26*(8), 1044-1080.
5. Roy, S., Kim, N., Desai, A., Komaragiri, M., Baxi, N., Jassil, N., et al. Cognitive function and control of type 2 diabetes mellitus in young adults. *North American journal of medical sciences*. 2015; *7*(5), 220.
6. Longo, M., Bellastella, G., Maiorino, M. I., Meier, J. J., Esposito, K., & Giugliano, D. Diabetes and aging: from treatment goals to pharmacologic therapy. *Frontiers in Endocrinology*. 2019; *10*, 45.
7. He, C., Gao, P., Cui, Y., Li, Q., Li, Y., Lu, Z., et al. Low-glucose-sensitive TRPC6 dysfunction drives hypoglycemia-induced cognitive impairment in diabetes. *Clinical and Translational Medicine*. 2020; *10*(6), e205.
8. Mimenza-Alvarado, A. J., Jiménez-Castillo, G. A., Yeverino-Castro, S. G., Barragán-Berlanga, A. J., Pérez-Zepeda, M. U., Ávila-Funes, J. A., et al. Effect of poor glycemic control in cognitive performance in the elderly with type 2 diabetes mellitus: The Mexican Health and Aging Study. *BMC geriatrics* .2020;*20* (1), 1-8.
9. Wong, C. W., O, W. T. W., Wong, K. W. S., Ma, R., Hui, E., & Kwok, C. Y. T. Randomized trial of a patient empowerment and cognitive training program for older people with diabetes mellitus and cognitive impairment. *Geriatrics & Gerontology International*.2020; *20*(12), 1164-1170.
10. Yang, X., Chen, Y., Zhang, W., Zhang, Z., Yang, X., Wang, P., et al. Association between inflammatory biomarkers and cognitive dysfunction analyzed by MRI in diabetes patients. *Diabetes, Metabolic Syndrome and Obesity*. 2020; 4059-4065.
11. Sebastian, M. J., Khan, S. K., Pappachan, J. M., & Jeeyavudeen, M. S. Diabetes and cognitive function: An evidence-based current perspective. *World Journal of Diabetes*. 202*314*(2), 92.
12. Shah, K., DeSilva, S., & Abbruscato, T. (2012). The role of glucose transporters in brain disease: diabetes and Alzheimer's disease. *International journal of molecular sciences*, *13*(10), 12629-12655.
13. Gorelick, P. B., Scuteri, A., Black, S. E., DeCarli, C., Greenberg, S. M., Iadecola, C., et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*.2011; *42*(9), 2672-2713.
14. Morales-Suarez-Varela, M., Collado Sanchez, E., Peraita-Costa, I., Llopis-Morales, A., & Soriano, J. M. Intermittent fasting and the

possible benefits in obesity, diabetes, and multiple sclerosis: a systematic review of randomized clinical trials. *Nutrients*. 2021; *13*(9), 3179.

15. Kim, C., Pinto, A. M., Bordoli, C., Buckner, L. P., Kaplan, P. C., Del Arenal, I. M., et al. Energy restriction enhances adult hippocampal neurogenesis-associated memory after four weeks in an adult human population with central obesity; a randomized controlled trial. *Nutrients*. 2020; *12*(3), 638.
16. Ahrén, B., & Scheurink, A. J. Marked hyperleptinemia after high-fat diet associated with severe glucose intolerance in mice. *European journal of endocrinology*. 1998; *139*(4), 461-467.
17. Elesawy, B. H., Raafat, B. M., Muqbali, A. A., Abbas, A. M., & Sakr, H. F. The impact of intermittent fasting on brain-derived neurotrophic factor, neurotrophin 3, and rat behavior in a rat model of type 2 diabetes mellitus. *Brain Sciences*. 2021;*11*(2), 242.
18. Xiang, X., Wang, Z., Zhu, Y., Bian, L., & Yang, Y. Dosage of streptozocin in inducing rat model of type 2 diabetes mellitus. *Wei sheng yan jiu= Journal of hygiene research*. 2010; *39*(2), 138-142.
19. Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C. L., & Ramarao, P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacological research*. 2005; *52*(4), 313-320.
20. Deacon, R. M., & Rawlins, J. N. P. T-maze alternation in the rodent. *Nature protocols* .2006;*1*(1), 7-12.
21. Porsolt, R. D., Le Pichon, M., & Jalfre, M. L. Depression: a new animal model sensitive to antidepressant treatments. *Nature*. 1977; *266*(5604), 730-732.
22. Biessels, G. J., Staekenborg, S., Brunner, E., Brayne, C., & Scheltens, P. Risk of dementia in diabetes mellitus: a systematic review. *The Lancet Neurology*. 2006; *5*(1), 64-74.
23. Silva, J. L. D., Ribeiro, L. T. C., Santos, N. R. P. D., Beserra, V. C. A. D. S., & Fragoso, Y. D. The influence of diabetes mellitus II on cognitive performance. *Dementia & neuropsychologia*. 2012; *6*, 80-84.
24. Sebastian, M. J., Khan, S. K., Pappachan, J. M., & Jeeyavudeen, M. S. Diabetes and cognitive function: An evidence-based current perspective. *World Journal of Diabetes*. 2023;*14*(2), 92.
25. Palomo-Osuna, J., Dueñas, M., Naranjo, C., De Sola, H., Salazar, A., & Failde, I. Factors related to cognitive function in type-2 diabetes and neuropathic pain patients, the role of mood and sleep disorders in this relationship. *Scientific Reports*. 2022; *12*(1), 15442.
26. Li, W., Huang, E., & Gao, S. Type 1 diabetes mellitus and cognitive impairments: a systematic review. *Journal of Alzheimer's disease*. 2017; *57*(1), 29-36.
27. Anson, R. M., Guo, Z., de Cabo, R., Iyun, T., Rios, M., Hagepanos, A., et al. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. *Proceedings of the National Academy of Sciences*. 2003; *100*(10), 6216-6220.
28. Ekong, M. B., Odinukaeze, F. N., Nwonu, A. C., Mbadugha, C. C., & Nwakanma, A. A. Brain activities of streptozotocin-induced diabetic Wistar rats treated with gliclazide: Behavioural, biochemical and histomorphology studies. *IBRO Neuroscience Reports*. 2022; *12*, 271-279.
29. Ismail, T. R., Yap, C. G., Naidu, R., & Pamidi, N. Environmental Enrichment and Metformin Improve Metabolic Functions, Hippocampal Neuron Survival, and Hippocampal-Dependent Memory in High-Fat/High-Sucrose Diet-Induced Type 2 Diabetic Rats. *Biology*. 2023; *12*(3), 480.
30. Castagné, V., Moser, P., Roux, S., & Porsolt, R. D. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Current protocols in pharmacology*. 2010; *49*(1), 5-8.
31. Chen, L., Chen, M., Wang, F., Sun, Z., Quanzhi, H., Geng, M., et al. Antidepressant-like effects of shuyusan in rats exposed to chronic stress: effects on hypothalamic-pituitary-adrenal function. *Evidence-based complementary and alternative medicine*. *2012*.
32. Shivavedi, N., Tej, G. N. V. C., Neogi, K., & Nayak, P. K. Ascorbic acid therapy: a potential strategy against comorbid depression-like behavior in streptozotocin-nicotinamide-induced diabetic rats. *Biomedicine & Pharmacotherapy*. 2019; *109*, 351-359.
33. Pistell, P. J., Morrison, C. D., Gupta, S., Knight, A. G., Keller, J. N., Ingram, D. K., et al. Cognitive impairment following high fat diet

consumption is associated with brain inflammation. *Journal of neuroimmunology*. 2010; *219*(1-2), 25-32.

34. Butterfield, D. A., Di Domenico, F., & Barone, E. Elevated risk of type 2 diabetes for development of Alzheimer disease: a key role for oxidative stress in brain. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. 2014; *1842*(9), 1693-1706.
35. Lobo, V., Patil, A., Phatak, A., & Chandra, N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*. 2010; *4*(8), 118.
36. Hassanpour, A., Hassanpour, A., Rezvani, M. E., Sharifabad, M. H., & Basiri, M. (2019). The Effect of Intermittent Fasting Diet on the Hippocampus of Adult Male Mouse After Inducing Demyelination by Ethidium Bromide Injection. *International Journal of Morphology*, *37*(3).
37. Andika, F. R., Yoon, J. H., Kim, G. S., & Jeong, Y. Intermittent fasting alleviates cognitive impairments and hippocampal neuronal loss but enhances astrocytosis in mice with subcortical vascular dementia. *The Journal of Nutrition*. 2021; *151*(3), 722-730.
38. Cheng, Z. Q., Fan, J., Zhao, F. Y., Su, J. Y., Sun, Q. H., Cui, R. J., et al. Fasting produces antidepressant-like effects via activating mammalian target of rapamycin complex 1 signaling pathway in ovariectomized mice. *Neural Regeneration Research*. 2023; *18*(9), 2075.

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