Effect of Ketogenic Diet on Cognitive Dysfunction associated with Alzheimer's disease

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Abstract

Background: Alzheimer's disease (AD) represents the most prevalent type of dementia, characterized by neurodegenerative and neurobehavioral changes, memory loss and cognitive difficulties. Previous studies have indicated that ketogenic diet (KD) has neuroprotective effects. However, the impact of KD on AD – associated pathology and its protective mechanism remains unclear.

Objectives: The present study aims to investigate the efficacy of KD in improving cognitive dysfunction and neurodegenerative pathology of AD, as well as the possible underlying mechanisms.

Materials and methods: This study was an experimental randomized control trial, 30 male albino Sprague Dowely rats were included; rats were divided into three groups; sham control, AD model rats and AD-KD fed group. AD was induced by intraperitoneal injection of aluminum chloride ($AlCl_3$) for 60 days in AD model rats and AD-KD fed group then AD-KD fed group continue on KD with a fat to carbohydrate and protein ratio of 3.1:1 for another 8 weeks. We investigated the effect of KD on cognitive dysfunction associated with AD. For this study, T-maze test, acetylcholinesterase (AChE) enzyme, malondialdehyde (MDA), and superoxide dismutase (SOD) enzyme assessment using ELISA, and histological assessment of the hippocampus using H&E and toluidine blue staining were performed.

Results: Compared with sham control group, results showed significantly decreased alteration score percentage in forced alteration T-maze test ($p<0,001$), increased levels of AChE enzyme ($p<0,001$) and oxidative stress biomarkers indicated by increased level of MDA ($p<0,001$) and decreased level of SOD enzyme ($p<0,001$), with declined neuronal survival in the hippocampus of AD model rats. Feeding on KD for eight weeks ameliorate AD- associated cognitive impairments by decreasing oxidative stress, increasing neuronal survival and reducing neural apoptosis in the hippocampus.

Conclusion: The resulting data have the potential to provide ketogenic diet as a new type of non-pharmacological therapy for AD.

Keywords: Alzheimer's disease; Cognition; Apoptosis; Ketogenic diet; Oxidative stress; Acetylcholinesterase enzyme.

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Introduction

Alzheimer's disease (AD) represents the most prevalent type of dementia, which is estimated to impact approximately 24 million people globally, with predictions indicating a near-doubling of it`s prevalence every two decades **(Broom et al., 2019)**. It is characterized by neurodegenerative and neurobehavioral changes, as well as memory loss and cognitive difficulties **(Albadrani et al., 2024)**. Research suggests that the disease progression in Alzheimer's is driven by an overabundance of toxic amyloid beta (Aβ) plaques and tangles, accumulating in the mitochondria causing its dysfunction which causes excessive production of reactive oxygen species (ROS) **(Kumar et al., 2024).** Lipoperoxidation caused by ROS, disrupts the membrane`s organization, affecting its fluidity and permeability, and impairs the function of enzymes and second messenger systems bound to the membrane causing brain damage **(Anwar et al., 2024; Malard et al., 2021).**

The central nervous system (CNS) is highly dependent on adequate blood flow because of it`s high oxygen metabolism and strict aerobic glucose needs, leading to cell damage or death upon even brief disruptions **(Malard et al., 2021).** With AD there is cerebral amyloid angiopathy **(Leitner et al., 2024),** leading to some degree of brain ischemia which predispose to increased production of ROS resulting from low adenosine triphosphate (ATP) levels, loss of Ca^2 homeostasis, excitotoxicity, arachidonic acid disruptions, mitochondrial dysfunction, acidosis and edema **(Malard et al., 2021)**. Formation of amyloid angiopathy triggered by ROS leads to escalated ischemia, additional free radicals, and a vicious cycle of increasing oxidative stress **(Liu et al., 2023).**

AChE is a crucial component of membranes that helps maintains its structure and regulate permeability during synaptic transmission, it catalytically breaks down cholinergic neurotransmitters, effectively ending transmission at cholinergic synapses **(Xia et al., 2024).** Previous researches have shown that AChE is involved in apoptosis, where cells having higher AChE expression are more prone to apoptosis **(Xia et al., 2024).** Increased Ca^2 entry triggers activity of AChE, linking elevated AChE expression near amyloid plaques to imbalance in Ca^2 homeostasis. This imbalance may also be a result of excessive oxidative stress and lipoperoxidation. The amyloid β- AChE complex is more neurotoxic and accelerates Aβ deposition **(Walczak-Nowicka & Herbet, 2021)**. Imbalance in Ca^2 homeostasis decreases cell membrane fluidity eventually exposing more active sites **(Chib et al., 2024).**

Under normal circumstances, glucose is the primary energy substrate for the brain **(López-Ojeda & Hurley, 2023)**. However, when glucose levels are low, the liver produces ketone bodies that can provide energy to extrahepatic tissues, including the brain **(Jang et al., 2023).** Research reports that ketones as β-hydroxybutyrate, acetoacetate, and acetone, as mitochondrial energy substrates has reduced amyloid neurotoxicity and it`s associated diseases, protected neurons and enhanced memory while the mechanisms behind this remain unclear **(Wei et al., 2022)**. So, it's important to explore non-pharmacological therapies in AD, to improve symptoms and to decrease progression of the disease.

In our study we investigated the efficacy of KD to improve cognitive dysfunction and neurodegenerative pathology of AD, and the possible mechanisms involved.

Materials and methods

This study was an experimental randomized control trial, conducted between August to November 2024. The study was

conducted at the animal house of Qena Faculty of medicine, South Valley University. All experimental procedures were approved by the Animal Ethical Committee of the Faculty of Medicine, south valley University.

Ethical approval code: SVU-MED-PHY003 -2-24-8-916.

Materials and devices used

- Aluminum chloride powder was obtained from Al-Gomhouria Company for Pharmaceuticals, Chemicals, Medical Devices and Supplies (Assuit, Egypt).
- Diethyl ether ≥99.5% (GC) inhalant from Sigma-Aldrich Corp., St. Louis, MO, USA.
- Rat MDA(Malondialdehyde) ELISA Kit Cat. No. MBS268427 from My BioSource company, USA.
- Rat SOD1(Superoxide Dismutase 1, Soluble) ELISA Kit Cat. No. E-EL-R1424 from Elabscience , USA.
- Rat AChE(Acetylcholinesterase) ELISA Kit Cat. No. E-EL-R0355 from Elabscience , USA.
- Readwell Touch Microplate Elisa Reader, Robonik, India.

Experimental animal

In this study thirty male albino Sprague Dowely rats were involved, aged from 6 months to one year and weighing between 150 to 200 gm. Rats were acquired from the Egyptian Company for the Production of Antisera, Vaccines, and Drugs Helwan, Egypt. They were housed in polypropylene cages with stainless steel covers (41 × 34 × 16 cm) up to 4 per cage, in a standard environment of room temperature at 22±2 °C and 12/12- hour day/night cycle. Rats were given free access to water and a standard pellet diet. All rats were adapted for a one-week before the start of the experimental study for acclimatization **(Ahmed et al., 202**3). All rats performed the T. maze and only rats with a score of 100% were included in the experiment.

Animal grouping and study design:

Rats were randomly divided into three groups, with 10 rats in each group. **Group I (Sham control):** rats in this group received intraperitoneal (IP) saline injections for 60 days and were fed standard pellet diet until the study concluded. **Group II (Alzheimer's disease (AD) model):** rats were injected with 0.2 ml aluminum chloride ($AlCl_3$) dissolved in distilled water, at a dose of 100 mg/kg body weight for 60 days, and to enhance absorption, the injections were administrated over three consecutive days with one-day intervals **(Ogunlade et al., 2022).** They were fed on a standard pellet diet till the end of the study. **Group III (AD model fed on a ketogenic diet):** AD was induced in this group as in Group II. However, they were fed on a ketogenic diet with a fat to carbohydrate plus protein ratio of 3.1:1 for a duration of 8 weeks. The specific nutrient composition for every 100 mg is detailed in (**Table.1) (Utami et al., 2021).**

Table 1. Basic nutrient content of both standard and ketogenic diet.

Component	Energy (kj)	Protein (gm)	Fat (gm)	Carbohydrates (gm)	Dietary fibers	Calcium (mg)	Phosphorus (mg)	Vitamin D (µg)
Standard diet	1338	14.5	4	55.5	4.5	720	600	2.5
Ketogenic diet	2804	18.2	65.1	2.7	7.4	500	300	2.5

Assessment methods

1. Body weight assessment: All rats were weighed at the beginning (baseline) and at the end of the experiment using analytical digital scale (A&D, USA), and body weight gain percentage (BWG%) was calculated using the following formula **(Ibrahem et al., 2022).**

$$BWG = \frac{Final\ body\ weight\ (FBW) - initial\ body\ weight(IBW)}{initial\ body\ weight(IBW)} \times 100$$

2. Behavioral assessment: Before animal scarification, the learning and memory of rats were assessed by the T Maze Forced Alternation test. It was performed according to the method described previously by **(Beppe et al., 2024)**

3. Brain weight assessment: After scarification, all rats` brains were weighed using analytical digital scale (A&D, USA).

Sampling

At the end of the study, the rats were fasted for 12 hours before being anesthetized by diethyl Ether Inhalation and sacrificed by decapitation **(Jiang et al., 2024)**. The brain was then retrieved, and the hippocampus was quickly isolated. One side of the hippocampus was immersed in liquid nitrogen and stored at -80 °C for the measurement of acetylcholinesterase (AChE) enzyme, malondialdehyde (MDA), and superoxide dismutase (SOD) levels. A specimen of the hippocampus from the other side was placed in 10% paraformaldehyde for histological examination **(Sylvestre et al., 2022).**

1. **Enzyme-Linked Immunosorbent Assay (ELISA) for measuring the level of AChE, MDA and SOD enzymes** Each hippocampal tissue sample was placed in 0.3-2.5 ml of ice-cold buffer (pH 7.4 at 4 °C) containing 25mM Hepes, 0.1%CHAPS,5 mM MgCl2, 2 mM AEBSF, 1 mM EDTA,130 µM Bestatin, 14 µM E64, 1mM leupeptin, and 0.3 µM Aprotinin, and was then homogenized. The hippocampal homogenates were centrifuged at 16000 g for 20 minutes at 4 °C. The total protein content was measured in each supernatant, which was subsequently used for ELISA **(Vanneau et al., 2024).** Quantitative determination of AChE,
2. MDA, and SOD enzyme concentrations in rat tissue homogenate samples was performed using ELISA kit, according to the manufacturer`s instructions. A sandwich ELISA detection method was used, with absorbance from each sample measured induplicate using a microplate reader at a wavelength of 450 nm. For tissue homogenate samples, data were reported as nmol/mg protein for MDA, U/MG protein for SOD **(Tchoubou et al., 2023),** and µmol/mg protein for AChE **(Saliu et al., 2021).**
3. **Histological assessment :** Half of the brain from all experimental groups was fixed using Wrobel-Mostafa-fixative **(Abd-Elhafeez et al., 2023).** This fixative contains 25% freshly produced paraformaldehyde, 40 ml of phosphate buffer (0.2M, pH 7.4), 125ml of saturated picric acid, 37.5 ml of calcium chloride, and 25 % glutaraldehyde. Distilled water is added to bring the total volume up to 250 ml. The fixation process lasted for 24 hours, after which the samples were dehydrated in an ascending series of ethanol concentrations (50%, 70%, 80%, 90%, 100% and 100%II) for 30 minutes at each concentration, except for the 100% ethanol, which was only used for 15 minutes at each change. The samples were then cleaned in xylene I, II, and III, with each change lasting for 1 hour. Subsequently, they were embedded in

paraplast I, II, and III (Sigma Aldrich) for one hour at each stage before being placed in paraffin blocks and sectioned. For light microscopic examination, paraffin sections were cut at thickness of 5-7 µM using a Richert Leica RM 2125 microtome (Germany). The sections were stained with hematoxylin and eosin (HE) and toluidine blue stains, following the procedures outlined in Bancroft`s "Theory and Practice of Histological Techniques" (Soliman et al., 2022)

Statistical analysis

Data were verified and coded by the researcher. Statistical analysis was performed using SPSS version 27. Descriptive statistics, including means and standard deviations (SD), were calculated. Tests of significance: For continuous variables with two related categories, a paired samples T-test was conducted to compare means of normally distributed data. For continuous variables with two unrelated categories, an unpaired samples T-test was conducted to compare means of normally distributed data. For continuous variables with more than two categories, an ANOVA test was used to assess mean differences, followed by a post-hoc test with Tukey corrections. Additionally, the correlation between AChE, MDA, SOD enzymes was examined using Pearson correlation tests, also the correlation between cognition and (AChE) activity. A P- value of less than 0.05 was considered significant.

Results

Effect of ketogenic diet on body weight

As shown in **Table 2**, there was no significant difference in the initial body weight among the groups (P=0.8488). However, at the end of the study, a significant difference was observed in the final body weight between the groups (P<0.001). Additionally, all groups exhibited a significant difference between initial and final body weights (P<0.001) as shown in (**Table.2)**. The sham control group experienced an increase in body weight of 11.7 ± 2.7%, while the AD model group had a decrease of 6.8 ± 3.4%, and the AD + KD group decreased by 15.4 ± 1.9%.

The mean body weight difference in the AD model group was significantly lower than that of the sham control group (P<0.001), and the mean body weight difference in the AD + KD group was significantly lower than that of the AD model group (P<0.001). Furthermore, the sham control group showed significant weight gain (P<0.001), whereas the AD model and AD + KD groups demonstrated significant weight loss (P<0.001).

Table 2. Comparison of different parameters between experimental groups

Parameters	Sham control group N =10	AD model group N =10	AD + KD group N =10	P- value (ANOVA test)
Body weight				
Initial body weight (gm)	180.2 ±2	180.7 ±1.4	180.2 ± 2.1	0.8488
Final body weight	201.1 ± 3.7	168.6 ±5.9***	152.4± 3.4###	P<0.001
Body weight difference	20.9 ± 4.95	-12.1 ± 6.12**	-27.8 ± 3.49##	P<0.001
Percentages of body weight change	11.7 ± 2.7%	-6.8 ± 3.4%**	-15.4 ± 1.9%##	P<0.001
P- value [b]	P<0.001	P<0.001	P<0.001	

Brain weight (gm)				
	1.83± 0.11	1.66±0.1**	1.31±0.35#	P<0.001

Data: Mean± SD. *P value < 0.05, **P value < 0.01, ***P value < 0.001, vs. sham control group. #P value < 0.05, ##P value < 0.01, ###P value < 0.001, vs. AD model group. P- value [a]: ANOVA test was used to compare the mean difference between groups with Post-hoc test used for pairwise comparison with Tukey correction, P- value [b]: Paired -Samples T-test was used to compare the proportion difference between initial and final body weights, P value < 0.05 is statistically significant.

Effect of ketogenic diet on Brain weight

As shown in (**Table.2),** there was a significant difference (P < 0.001) in the mean brain weight among the experimental groups. The mean brain weight in the AD model group was significantly lower (P < 0.001) than that of the sham control group, measuring 1.66 ± 0.1 grams compared to 1.83 ± 0.11 grams, respectively. Additionally, the mean brain weight in the AD + KD group was significantly lower (P < 0.05) than that of the AD model group, with values of 1.31 ± 0.35 grams versus 1.66 ± 0.1 grams, respectively.

Effect of ketogenic diet on neurobehavioral performance (T-maze forced Alternation test)

The results presented in (**Fig.1)** indicate a significant difference (P < 0.001) in the mean percentage alternation scores among the different groups. In the AD model group, rats demonstrated memory deficits in the T-Maze compared to the sham control group, as shown by a significant reduction (P < 0.001) in the mean percentage alternation score (100% in the sham control group versus 36 ±16% in the AD model group). Conversely, the rats fed a ketogenic diet (KD) showed a significant improvement (P < 0.001) in their mean percentage alternation scores compared to the AD model group (76 ± 21% versus 36 ± 13%, respectively). Although the KD treated group showed a decrease in brain weight, the cognitive function improved, and this was evident in **(Fig.2)** which presents the results from Pearson's correlation test indicating a non-significant correlation between brain weight and cognition indicated by the mean percentage alternation score in T-maze, (r= 0.091, N=30, p= 0.63).

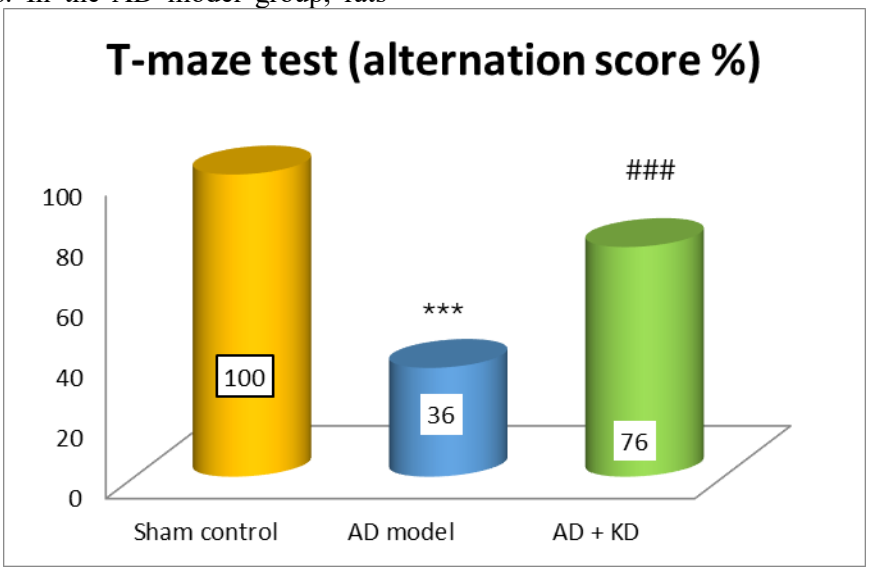

Fig.1. The mean value of alternation score (%) of T. maze in different experimental groups.
Data was represented as mean ±SD. Data was analyzed by one way ANOVA test, Post hoc Tukey test and independent samples T. test (N=10 rats per group). ***P < 0.001 when compared to sham control; ### P < 0.001 when compared to AD model group. P value < 0.05 is statistically significant.

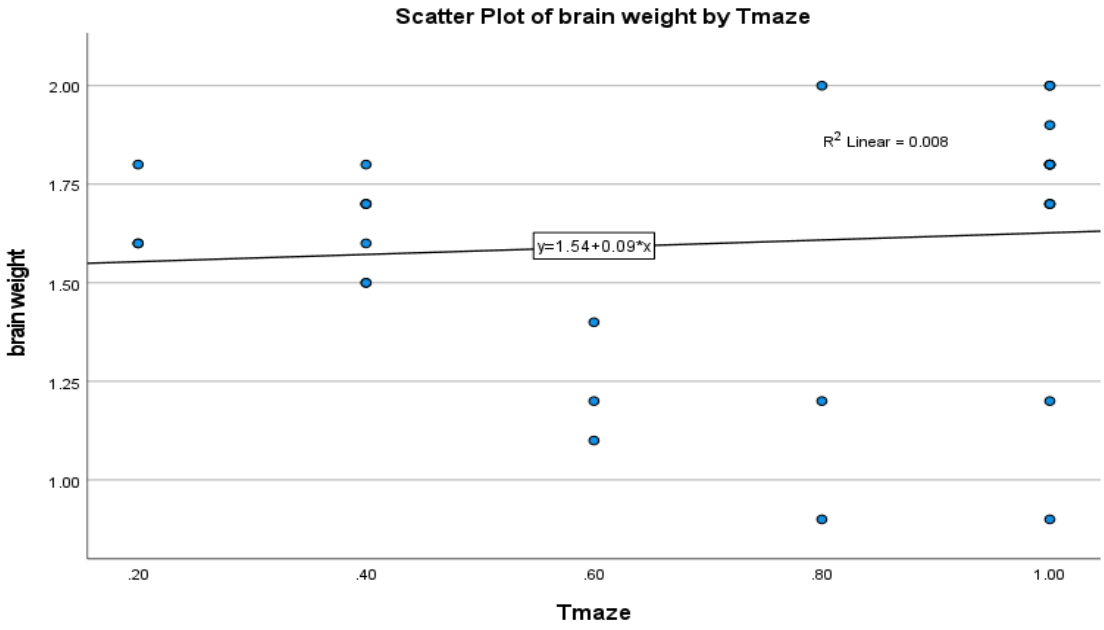

Fig.2.Scatter plot of T- maze by brain weight

Biochemical results

In our study, we employed various chemicals to explore the underlying mechanisms associated with cognitive deficits linked to AD and to understand the beneficial effects of the KD. MDA was utilized as a lipid peroxidation marker, while SOD served as an enzymatic antioxidant. Additionally, we assessed the activity of AChE as an apoptotic marker.

a. Effect of ketogenic diet on AChE levels in the hippocampus: The results presented in **(Fig.3)**, indicate a significant increase ($P < 0.05$) in the mean level of AChE in the hippocampal tissue of the AD model group compared to the sham control group (1.5 ± 0.03 versus 0.36 ± 0.08, respectively). Furthermore, the AChE levels were significantly decreased ($P < 0.001$) in the AD + KD group when compared to the AD model group (0.82 ± 0.03 versus 1.5 ± 0.03, respectively).

(Fig.4) presented the results from Pearson's correlation test showing a significant negative correlation between AChE and cognition indicated by the mean percentage alternation score in T-maze, (r= -0.894, N=30, p< 0. 001). This suggests that the upregulation of AChE enzyme is contributed to cognitive impairment from AD.

b. Effect of ketogenic diet on MDA and SOD levels in the hippocampus: (Fig.3) showed a significant increase ($P<0.05$) in the mean level of MDA in the hippocampal tissue of the AD model group compared to the sham control group, with values of 0.96±0.08 and 0.28±0.11, respectively. This increase was significantly reduced ($P<0.001$) in the AD + KD group when compared to the AD model group, with levels of 0.63±0.09 versus 0.96±0.08, respectively.

Additionally, **(Fig.3),** indicated a significant decrease ($P<0.05$) in the mean level of SOD in the hippocampal tissue of the AD model group compared to the sham control group (0.44±0.07 versus 0.84±0.07, respectively). Moreover, this decrease was significantly reduced ($P<0.001$) in the AD + KD group compared to the AD model group, with levels of 0.58±0.05 versus 0.44±0.07, respectively.

(Fig.5), and **(Fig.6)** presented the results from Pearson's correlation test. The findings indicated a significant positive correlation between AChE and MDA levels, with a correlation coefficient of (r=0.947**, N=30, p< 0.001). Conversely, there was a significant negative correlation between AChE and SOD levels, with a correlation coefficient of

(r= -0.899, N=30, p< 0.001). This suggests that higher levels of the AChE enzyme are associated with increased levels of MDA and decreased levels of SOD.

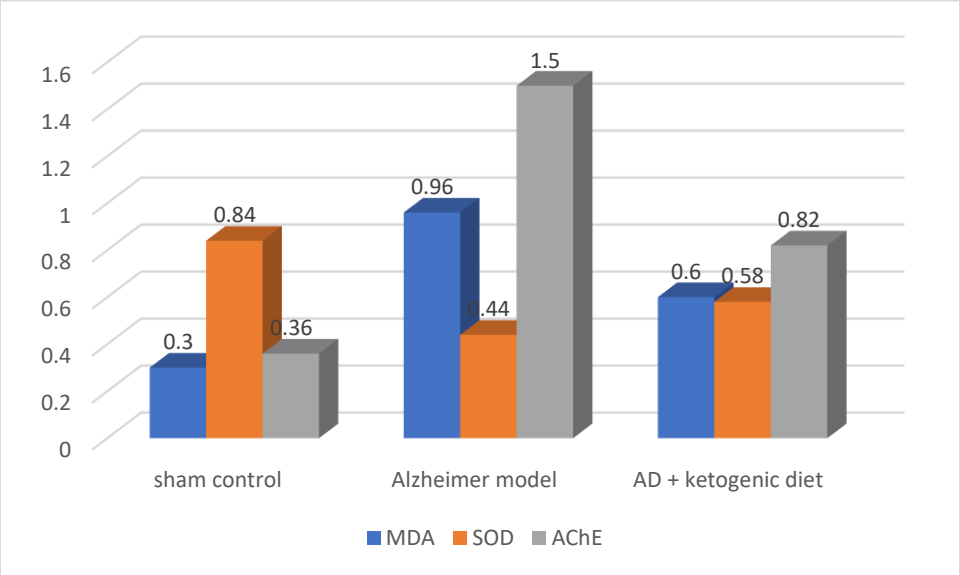

Fig. 3. Comparison of different biochemical parameters between experimental groups.

Data was represented as mean ±SD. Data was analyzed by one way ANOVA test, Post hoc Tukey test and independent samples T. test (N=10 rats per group). ***P < 0.001 when compared to sham control; ### P < 0.001 when compared to AD model group. P value < 0.05 is statistically significant.

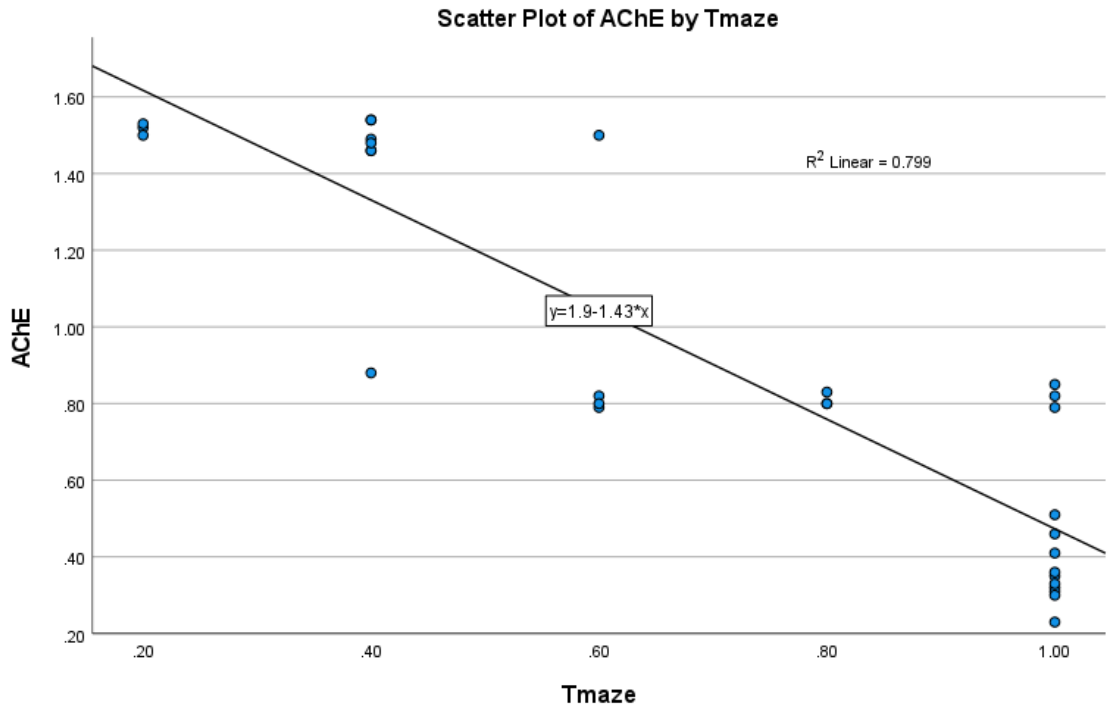

Fig.4. Scatter plot of T- maze by AChE enzyme activity

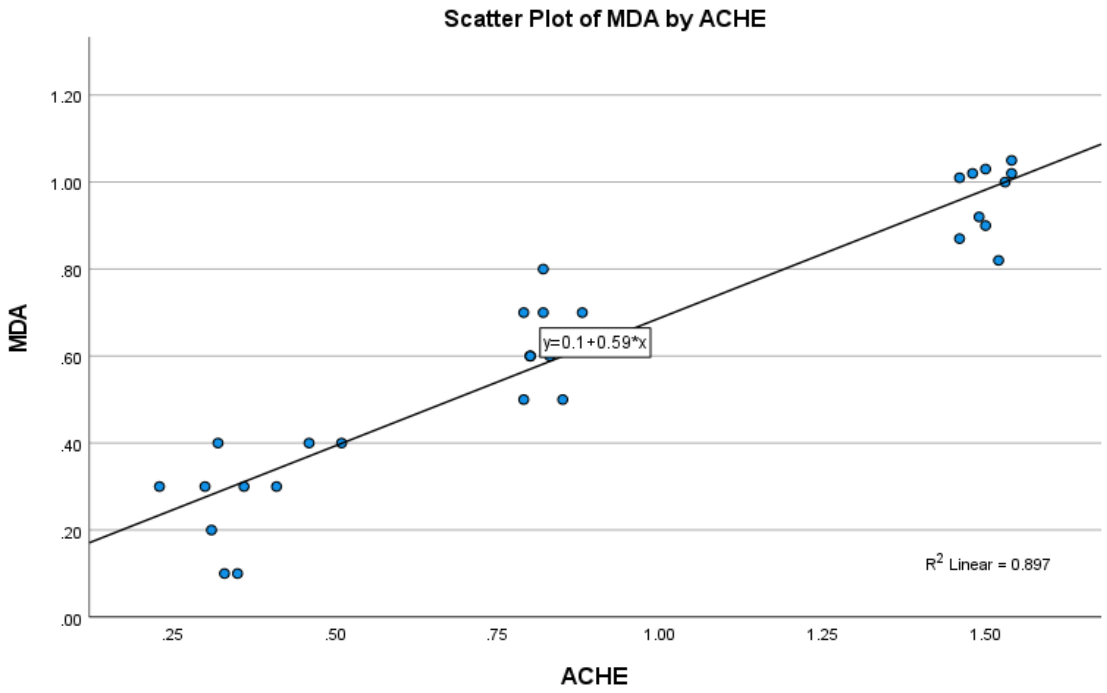

Fig.5.Scatter plot of MDA by AChE enzyme activity

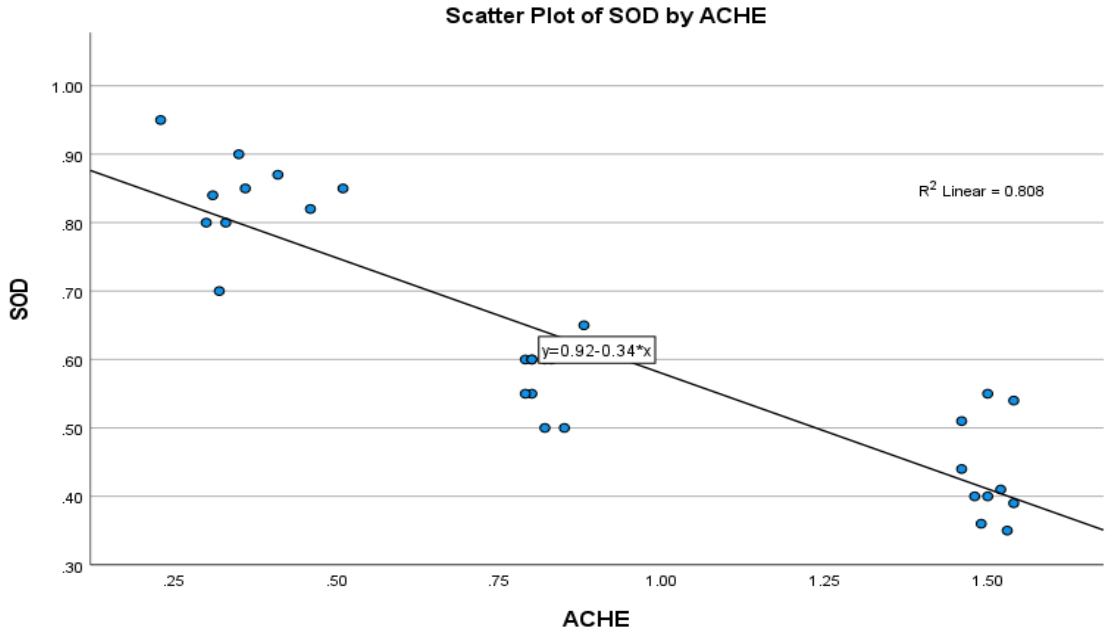

Fig.6. Scatter plot of SOD by AChE enzyme activity

Histological investigation

The hematoxylin and eosin staining of the hippocampal tissue sections revealed that the sham control group displayed the typical histological arrangement in distinct regions known as cornu ammonis (CA) and dentate gyrus (DG). The cornu ammonis (CA) area divided into 4 subdivisions: CA1, CA2, CA3 & CA4 regions. CA4 projects into concavity of dentate gyrus that is formed of

small granule cells. (**Fig.7 A, B**). Each of these regions consisted of five layers: stratum alveolus, stratum oriens, stratum pyramidale, stratum radiatum, and stratum lacunosum-moleculare (Figure 8). Our study results indicated the presence of amyloid plaques and amyloid angiopathy in the vascular walls, along with a decrease in the pyramidal layer of neuron cells in the CA1 area of the AD model group, as shown in **Fig.7** (**C, D**). In contrast, the AD + KD group exhibited a high concentration of pyramidal neurons arranged normally, highlighting a difference from the AD model group. Furthermore, the AD + KD group showed healthy blood vessels, as illustrated in **Fig.7 (E, F, G).** (**Fig. 8)** shows the five layers of CA1, CA2, CA3, and CA4 regions in the hippocampus (Part).

When toluidine blue staining was applied, the vesicular nuclei of neurons in the sham control group were surrounded by Nissl blue granules, as illustrated in (**Fig. 9A)**. Both the AD + KD group (**Fig.8B**) and the sham control group (**Fig.9A**) exhibited prominent bluish Nissl granules around the vesicular nuclei of neurons, in contrast to the AD model group (**Fig.9C**), which displayed a different pattern.

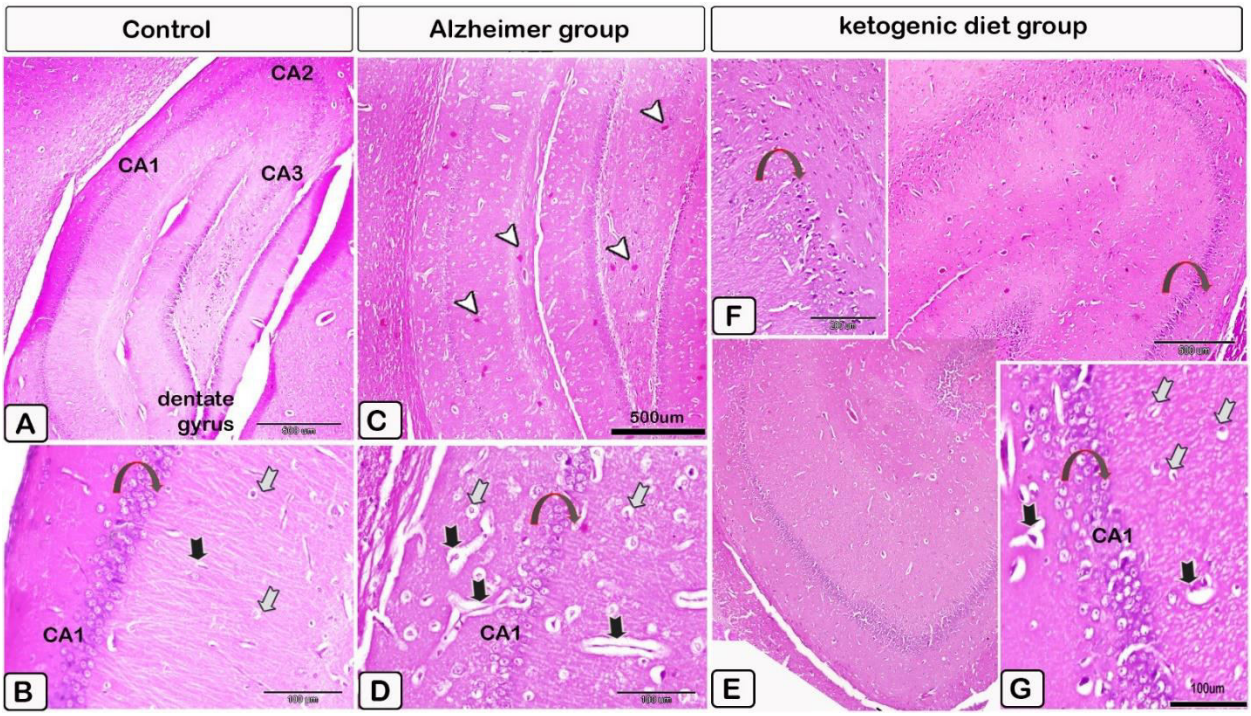

Fig.7. Photomicrograph of paraffin sections of the hippocampus of different experimental groups. Using the H&E A: A photomicrograph of paraffin sections of the sham control group's hippocampus taken with H&E reveals a typical structure of the brain, filled with numerous neuron cells in different areas. **B:** Sections of the CA1 hippocampal area stained with H&E in the sham control group show that the vascular wall does not show any signs of amyloid angiopathy (black arrow). **C:** Amyloid plaques (**white** arrowheads) are visible in this photomicrograph of paraffin sections of the hippocampus taken from the AD model group and examined with H&E. **D:** A photograph of the AD model group's hippocampi taken from paraffin sections. The use of H&E demonstrates the presence of amyloid angiopathy in the vascular wall (black arrow) and a decrease in the middle pyramidal (curved arrow) layer of neuron cells in the CA1 area. **E, F:** H&E-stained photomicrographs of paraffin slices from the hippocampi of rats following a ketogenic diet. After being restored to their original architectural pattern, the treated

group shows tightly packed pyramidal neurons (curved arrows), in contrast to the AD model group. The arrows indicated in **G** indicate a decrease in amyloid angiopathy within the blood vessel walls when compared to the AD model group. The curved arrow in the pictures indicates the thickness of the layer of pyramidal neurons. On figure B, D, and G the white arrow pointed to glia cells.

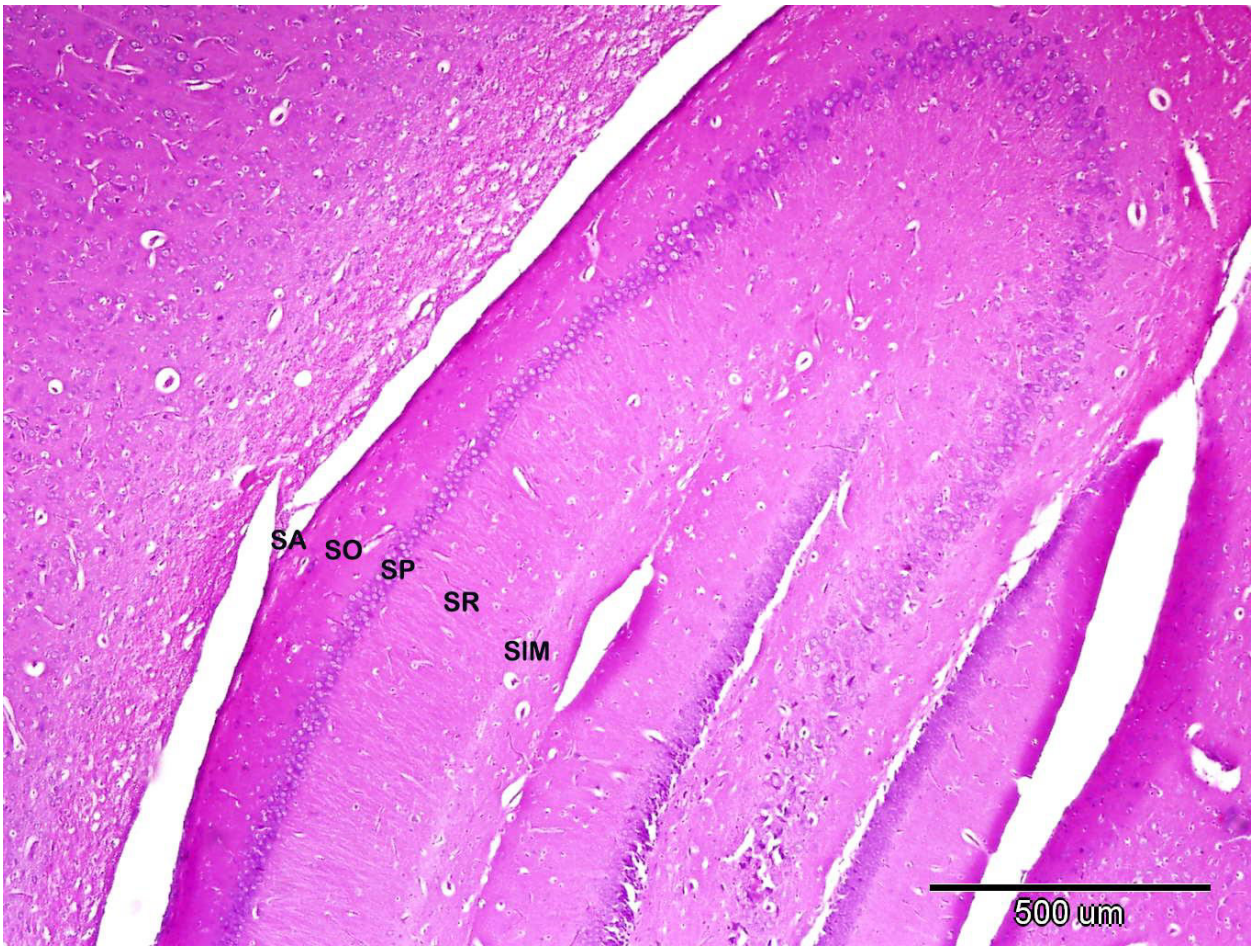

Fig.8. Hippocampus proper contains five strata. SA = stratum alveolus, SO = Stratum Oriens, SP=Stratum Pyramidale, SR=Stratum Radiatum, SLM=Stratum Lacunosum-moleculare.

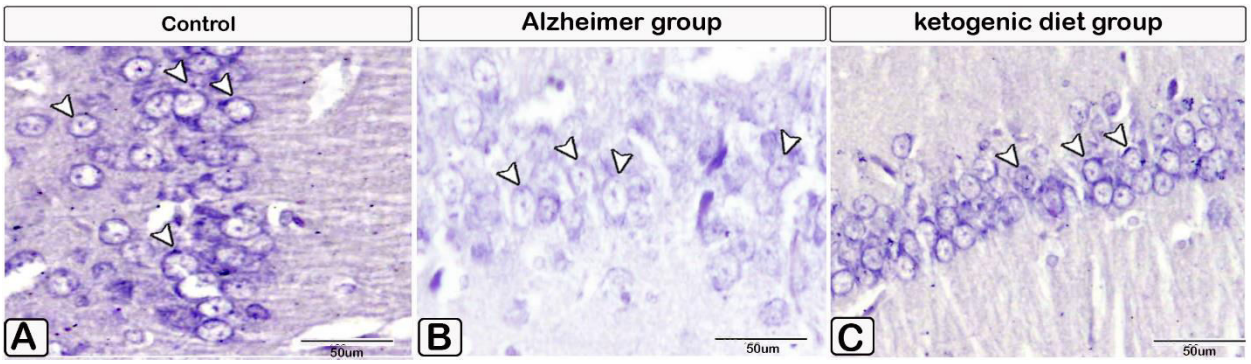

Fig.9. A photomicrograph of paraffin sections of the hippocampus stained with toluidine blue of different experimental groups: the sham control group (A), the AD's group (B), and the

AD + KD group (C). The image shows the blue-stained Nissl granules on the cytoplasm of neurons in the sham control group and the AD + KD group (represented by arrowheads). On the cytoplasm of neurons in the AD's group are weak, blue-stained Nissl granules, as seen in the photograph.

Discussion

In Upper Egypt the prevalence of Alzheimer's disease (AD) is 1% among 50 years old patients and older, this increases substantially to 9.7% for 80 years old patients. Interestingly, early-onset AD is observed at a notably high rate of 7.9% amongst individuals below the age of 65 **(El-Azzab et al., 2023)**.

AD gradually deteriorates cognitive abilities, starting with recent memory loss and impacting all intellectual functions overtime. The prevalence of comorbid cardiovascular conditions such as hypertension, diabetic vasculopathy, and cerebrovascular events has been increasing. Subsequently, deteriorated vascular integrity may occur, particularly through disruptions to the blood brain barrier`s specialized transport proteins. This eventually enables the accumulation of the amyloid beta (Aβ) protein in cerebral tissues, leading to inflammatory responses which potentially exacerbate the pathological transformations occurring within brain **(Badji et al., 2023).**

Methods to induce AD-like symptoms in rats have included the use of chemicals, enabling researchers to screen therapeutic drugs targeting cognitive dysfunctions. Among the chemicals utilized for this purpose are heavy metals, scopolamine, ethanol, colchicine, streptozotocin, lipopolysaccharide, and okadaic acid. This research aimed at enhancing our understanding of the pathogenesis of this devastating disease, particularly using heavy metals, specifically aluminum chloride ($AlCl_3$) **(Prajapat et al., 2023).**

Previous researches demonstrated that environmental heavy metals exhibit neurotoxic properties, hindering brain development and exhibiting associations with neurodegenerative disorders such as AD **(Pamphlett et al., 2023).** Notably, aluminum (Al) has been found to contribute to the formation and accumulation of senile plaques and neurofibrillary tangles, thereby precipitating progressive neurodegenerative processes and cellular degeneration. Experimental investigations have further revealed that Al administration can precipitate oxidative stress and cholinergic impairments in rats **(Ogunlade et al., 2022)**. An important route of exposure to Al is the use of Al pots which can leach the metal into foods during cooking and lead to it`s accumulation in the body organs including the brain **(Abu-Elfotuh et al., 2023).**

In this study, significant body weight loss was observed in the AD model group compared to sham control group, as unexplained weight loss and cachexia are key symptoms of AD **(Farsani et al., 2024).** AD may be linked to disrupted body weight regulation **processes (López-Gambero et al., 2022)**, or decreased independence in self-feeding **(Walters, 2023),** which aligns with our findings. Furthermore, our research has indicated that following a KD led to a significant reduction in body weight compared to both the sham control group and the AD model group. This result is consistent with previous studies suggesting this weight loss results from increased lipolysis caused by lower insulin levels, and enhanced fatty acid oxidation, which reduces fat storage in adipose tissue **(Diana & Atmaka, 2020)**. Also, ketones can subpress appetite causing reduced overall calorie intake. Additionally low-carbohydrate KD promotes gluconeogenesis **(Zhu et al., 2022)**. Moreover, the thermogenic effects of protein and fat

accentuate weight reduction **(Basolo et al., 2022; Zhu et al., 2022).**

In our study, significant brain atrophy was observed in the AD model group compared to sham control group, this is in line with **(Niikura, 2022)** who stated that brain atrophy caused by neuronal loss, is a major characteristic of AD. Excessive Aβ-protein is considered to play a significant role in neuronal cell death through multiple cytotoxic mechanisms, involving increase of the intracellular Ca^2 level, oxidative stress, and cellular apoptosis **(Ghosh et al., 2024).** In our study, we found that following KD resulted in a significant decrease in brain weight compared to both the sham control group and AD model group. Potential mechanisms underlying this decrease may be attributed to diminished inflammatory responses, decreased edema **(Xu et al., 2022)**, and diminished tissue damage, including amyloid plaque reduction **(Zhu et al., 2022)**, without compromising neural network functionality, or indeed enhancing it. Furthermore, Activation of autophagy, induced by KD, could concurrently contribute to reduced mass while promoting functional improvement **(Jiang et al., 2022).**

Significant deterioration in spatial working memory, as measured by behavioral parameters in the T-maze, was observed in AD model group. Memory and spatial navigation impairments have been proposed as cognitive biomarkers for AD **(O'Leary & Brown, 2022). (Ziontz et al., 2021)** linked cognitive decline to the accumulation of tau in areas responsible for spatial processing and memory, including the hippocampus, para- hippocampal gyrus, parietal lobe, retro-splenial cortex, and prefrontal cortex. Moreover, significant improvements in spatial working memory, as observed following feeding on KD (AD+KD group). These improvements indicate that the cognitive decline associated with AD might be mitigated by ketones which enhance mitochondrial activity and biosynthesis, reduce inflammation and oxidative stress (Tao et al., 2022). Additionally, **(Williams et al., 2024)** suggested that ketones have anti-apoptotic effects, as evidenced by reduced expression of clusterin and caspase-3, as well as increased calbindin.

Our study revealed that the AD model group exhibited higher levels of AChE. To our knowledge, this is the first study to investigate the effect of KD on AChE enzyme activity. The Aβ-protein enhances AChE activity by increasing calcium influx through L-type voltage dependent calcium channels this process decreases cell membrane integrity, exposing more active sites of the enzyme and resulting in increased AChE expression around amyloid plaques. Furthermore, the rise in oxidative stress and lipid peroxidation may also contribute to this increase **(Chib et al., 2024).** Several studies suggest that AChE not only aids in the aggregation of Aβ into fibrils but also promotes neuronal apoptosis, making it an apoptotic marker **(Xia et al., 2024).** Our findings indicated that AChE levels increased in the AD + KD group compared to AD model group, suggesting that KD may influence AChE activity by decreasing Aβ levels, oxidative stress, lipid peroxidation, or cellular apoptosis. Also, the upregulation of AChE in AD model group contributed to cognitive impairment associated with AD by correlation analysis. As AChE was downregulated in the hippocampus tissue after feeding on KD, KD has an anti-cognitive impairment-like effect in the process of AD.

The increased Malondialdehyde (MDA) level, a marker of lipid peroxidation **(Hassanpour et al., 2025)**, together with a decreased Superoxide dismutase (SOD) level, among the most potent antioxidants **(Nie et al., 2023)** was observed in our study

in the AD model group suggesting the presence of increased oxidative stress. Oxidative stress is a key factor and risk element in the neurodegenerative processes associated with AD **(Amidfar et al., 2020).** The accumulation of Aβ leads to mitochondrial dysfunction, resulting in excessive production of reactive oxygen species (ROS) **(Kumar et al., 2024)**. As individuals age, there is an increased risk of cerebrovascular conditions such as atherosclerosis and cerebral amyloid angiopathy **(Stelmashook et al., 2023).** These pathophysiological conditions can induce varying degrees of cerebral ischemia, leading to decline in ATP levels, disruption in calcium homeostasis, excitotoxicity, alteration in arachidonic acid metabolism, mitochondrial dysfunction, acidification, and edema, and so predispose brain tissue to increased ROS production **(Mormone et al., 2023).** The generation of ROS contributes to the development of amyloid angiopathy, creating a vicious cycle of increased ischemia and further free radical production. MDA level is increased because of increased lipid peroxidation resulting from ROS generation which also causes damage to both proteins and DNA contributing to brain damage which also occurs due to disruptions to neurotransmission and gene expression **(Liu et al., 2023)**. Our research indicated furthermore, that a KD could reduce oxidative stress marked by significant decrease in MDA level, and increase in SOD level in AD+ KD group compared to AD model group, which is consistent with **(Jiang et al., 2022)**. They explained that KD protect against ROS production by improving mitochondrial respiration and increasing NAD+/NADH ratio and so improving redox reactions, mitochondrial biogenesis, and cellular respiration, which stabilizes neurotransmission and improves neuroprotective action **(Jiang et al., 2022).**

The neuropathologic hallmark of AD are Aβ protein, and neurofibrillary tangles (NFTs), leading to progressive neuronal degeneration and cell death **(Falode et al., 2022),** which is obvious in the histological investigation of AD model group revealing a reduction in the middle pyramidal (P) layer of neuron cells in all layers of the hippocampus compared to sham control group with numerous necrotic cells and areas of neuronal loss, amyloid angiopathy, and neurofibrillary tangles. In addition, these histopathological changes were ameliorated by KD which is evident by restoration of the normal architectural pattern of hippocampal neurons which exhibit densely packed healthy pyramidal neurons with vesicular nuclei and a few others with darkly stained nuclei. These results agree with **(Zhu et al., 2022)** who related these changes to hypoglycemia associated with KD. It suppresses insulin/IGF-1 signaling resulting in reduced protein synthesis and increased degradation, which can lead to clearance of degradation-sensitive proteins as amyloidic peptides, affecting the Aβ levels **(Zhu et al., 2022).**

So, KD may perform its anti-cognitive impairment action either indirectly through decreasing cellular apoptosis by reducing the oxidative stress and AChE, or directly through clearing Aβ plaques thus decreasing brain ischemia.

Conclusion

Our study revealed the effectiveness of KD as a new non-medical treatment for AD as it could ameliorate the neurodegenerative pathology in the hippocampus of AD model rats.Feeding on KD for 8 weeks wasn`t sufficient to show the long-term effect of KD on cognition, so we recommend increasing the period of feeding on KD.

References

- **Abd-Elhafeez H H, Rutland C S, Soliman S A. (2023)**. Morphology of migrating telocytes and their potential role in stem cell

differentiation during cartilage development in catfish (Clarias gariepinus). Microscopy Research and Technique, 86(9): 1108-1121.

- **Abu-Elfotuh K, Selim H M R M, Riad O K M, Hamdan A M, Hassanin S O, Sharif A F, et al. (2023).** The protective effects of sesamol and/or the probiotic, Lactobacillus rhamnosus, against aluminum chloride-induced neurotoxicity and hepatotoxicity in rats: Modulation of Wnt/β-catenin/GSK-3β, JAK-2/STAT-3, PPAR-γ, inflammatory, and apoptotic pathways. Frontiers in Pharmacology, 14: 1208252.
- **Ahmed O G, Eliwa K A A-S, Toghan R, Fadel S A M, Zaki S M. (2023).** Vitamin D mitigates hippocampus apoptosis induced by diabetes. SVU-International Journal of Medical Sciences, 6(2): 586-596.
- **Albadrani H M, Chauhan P, Ashique S, Babu M A, Iqbal D, Almutary A G, et al. (2024).** Mechanistic insights into the potential role of dietary polyphenols and their nanoformulation in the management of Alzheimer's disease. Biomedicine & pharmacotherapy, 174: 116376.
- **Amidfar M, de Oliveira J, Kucharska E, Budni J, Kim Y-K. (2020).** The role of CREB and BDNF in neurobiology and treatment of Alzheimer's disease. Life sciences, 257: 118020.
- **Anwar S, Alrumaihi F, Sarwar T, Babiker A Y, Khan A A, Prabhu S V, et al. (2024).** Exploring Therapeutic Potential of Catalase: Strategies in Disease Prevention and Management. Biomolecules, 14(6): 697.
- **Badji A, Youwakim J, Cooper A, Westman E, Marseglia A. (2023).** Vascular cognitive impairment–Past, present, and future challenges. Ageing Research Reviews: 102042.
- **Basolo A, Magno S, Santini F, Ceccarini G. (2022).** Ketogenic diet and weight loss: is there an effect on energy expenditure? Nutrients, 14(9): 1814.
- **Beppe G J, REBE R N, MAÏTEMWA M-I, FOLEFACK A I, BARGA B P, ALLAH-DOUM N G, et al. (2024).** Neuroprotective impact of Ximenia americana aqueous bark extract on Diazepam-induced memory impairment in mice via its antioxidant potential. Notulae Scientia Biologicae, 16(1): 11713-11713.
- **Broom G M, Shaw I C, Rucklidge J J. (2019).** The ketogenic diet as a potential treatment and prevention strategy for Alzheimer's disease. Nutrition, 60: 118-121.
- **Chib S, Dutta B J, Chalotra R, Abubakar M, Kumar P, Singh T G, et al. (2024).** Role of Flavonoids in Mitigating the Pathological Complexities and Treatment Hurdles in Alzheimer's Disease. Phytotherapy Research.
- **Diana R, Atmaka D R. (2020).** Ketogenic diet for weight loss and its implication on health: A literature study. Media Gizi Indonesia, 15(3): 184-193.
- **El-Azzab S I, Alam F H, I Hassan N I H. (2023).** Effects of Psycho-educational Program Alone and Combined with Reminiscence Therapy on Depression, Cognitive and Non-cognitive Behaviors among Patients with Dementia. Port Said Scientific Journal of Nursing, 10(1): 320-344.
- **Falode J A, Akinmoladun A C, Olaleye M T, Akindahunsi A A. (2022).** Kigelia africana (Lam.) Benth leaf extract inhibits rat brain and liver mitochondrial membrane permeability transition pore opening. Drug and chemical toxicology, 45(4): 1614-1624.
- **Farsani M S, Fathi M, Farsani Z H, Karaji Z G. (2024).** Swimming alters some proteins of skeletal muscle tissue in rats with Alzheimer-like phenotype. Archives of Gerontology and Geriatrics, 117: 105260.
- **Ghosh P, Narang K, Iyer P K. (2024).** Role of Amyloid Beta in Neurodegeneration and Therapeutic Strategies for Neuroprotection. In Neuroprotection: Method and Protocols: 337-354. Springer.
- **Hassanpour H, Mojtahed M, Nasiri L, Vaez-Mahdavi M-R, Fallah A A. (2025).** Association of sulfur mustard toxicity with oxidant/antioxidant system in veterans: A meta-analysis of case-control studies. International Immunopharmacology, 147: 114007.
- **Ibrahem E S, Mohamed G H, El-Gazar A F. (2022).** Therapeutic Effect of Equisetum arvense L. on Bone and Scale Biomarkers in

Female Rats with Induced Osteoporosis. Egyptian Journal of Chemistry, 65(12): 457-466.

- **Jang J, Kim S R, Lee J E, Lee S, Son H J, Choe W, et al. (2023).** Molecular Mechanisms of Neuroprotection by Ketone Bodies and Ketogenic Diet in Cerebral Ischemia and Neurodegenerative Diseases. International journal of molecular sciences, 25(1): 124.
- **Jiang W, Zhang M, Cao R, Wang X, Zuo Y. (2024).** Different ethanol exposure durations affect cytochrome P450 2E1-mediated sevoflurane metabolism in rat liver. BMC anesthesiology, 24(1): 321.
- **Jiang Z, Yin X, Wang M, Chen T, Wang Y, Gao Z, et al. (2022).** Effects of ketogenic diet on neuroinflammation in neurodegenerative diseases. Aging and disease, 13(4): 1146.
- **Kumar S, Shukla A K, Yadav V K, Srivastava A, Dwivedi D, Singh S P. (2024).** Immunopathogenesis of Alzheimer's disease, Parkinson's Disease, and other Neurodegenerative Diseases. Advances in Diagnostics and Immunotherapeutics for Neurodegenerative Diseases: 32-64.
- **Leitner D, Kavanagh T, Kanshin E, Balcomb K, Pires G, Thierry M, et al. (2024).** Differences in the cerebral amyloid angiopathy proteome in Alzheimer's disease and mild cognitive impairment. Acta Neuropathologica, 148(1): 9.
- **Liu G, Yang C, Wang X, Chen X, Wang Y, Le W. (2023).** Oxygen metabolism abnormality and Alzheimer's disease: An update. Redox Biology: 102955.
- **López-Gambero A J, Pacheco-Sánchez B, Rosell-Valle C, Medina-Vera D, Navarro J A, del Mar Fernández-Arjona M, et al. (2022).** Dietary administration of D-chiro-inositol attenuates sex-specific metabolic imbalances in the 5xFAD mouse model of Alzheimer's disease. Biomedicine & pharmacotherapy, 150: 112994.
- **López-Ojeda W, Hurley R A. (2023).** Ketone bodies and brain metabolism: new insights and perspectives for neurological diseases. The Journal of Neuropsychiatry and Clinical Neurosciences, 35(2): 104-109.
- **Malard E, Valable S, Bernaudin M, Pérès E, Chatre L. (2021).** The reactive species interactome in the brain. Antioxidants & Redox Signaling, 35(14): 1176-1206.
- **Mormone E, Iorio E L, Abate L, Rodolfo C. (2023).** Sirtuins and redox signaling interplay in neurogenesis, neurodegenerative diseases, and neural cell reprogramming. Frontiers in Neuroscience, 17: 1073689.
- **Nie P-C, Yang R-L, Zhou J-J, Dewer Y, Shang S-Q. (2023).** Elucidating the effect of temperature stress on the protein content, total antioxidant capacity, and antioxidant enzyme activities in Tetranychus urticae (Acari: Tetranychidae). Insects, 14(5): 429.
- **Niikura T. (2022).** Humanin and Alzheimer's disease: The beginning of a new field. Biochimica et Biophysica Acta (BBA)-General Subjects, 1866(1): 130024.
- **O'Leary T P, Brown R E. (2022).** Visuo-spatial learning and memory impairments in the 5xFAD mouse model of Alzheimer's disease: Effects of age, sex, albinism, and motor impairments. Genes, Brain and Behavior, 21(4): e12794.
- **Ogunlade B, Adelakun S, Agie J. (2022).** Nutritional supplementation of gallic acid ameliorates Alzheimer-type hippocampal neurodegeneration and cognitive impairment induced by aluminum chloride exposure in adult Wistar rats. Drug and chemical toxicology, 45(2): 651-662.
- **Pamphlett R, Buckland M E, Bishop D P. (2023).** Potentially toxic elements in the brains of people with multiple sclerosis. Scientific Reports, 13(1): 655.
- **Prajapat M, Kaur G, Choudhary G, Pahwa P, Bansal S, Joshi R, et al. (2023).** A systematic review for the development of Alzheimer's disease in in vitro models: a focus on different inducing agents. Frontiers in Aging Neuroscience, 15: 1296919.
- **Saliu I O, Amoo Z A, Khan M F, Olaleye M T, Rema V, Akinmoladun A C. (2021).** Abatement of neurobehavioral and neurochemical dysfunctions in cerebral ischemia/reperfusion injury by Tetrapleura tetraptera fruit extract. Journal of ethnopharmacology, 264: 113284.

- **Soliman S A, Emeish W F, Abdel-Hafeez H H. (2022).** Lactoferrin improves the immune response and resistance of silver carp, a hematological, light (histochemical and immunohistochemical), fluorescent, and scanning electron microscopic study. Microscopy Research and Technique, 85(11): 3565-3581.
- **Stelmashook E V, Voronkov D N, Stavrovskaya A V, Novikova S V, Yamshikova N G, Olshanskij A S, et al. (2023).** Neuroprotective effects of methylene blue in streptozotocin-induced model of Alzheimer's disease. Brain Research, 1805: 148290.
- **Sylvestre D A, Otoki Y, Metherel A H, Bazinet R P, Slupsky C M, Taha A Y. (2022).** Effects of hypercapnia/ischemia and dissection on the rat brain metabolome. Neurochemistry International, 156: 105294.
- T**ao Y, Leng S X, Zhang H. (2022).** Ketogenic diet: an effective treatment approach for neurodegenerative diseases. Current neuropharmacology, 20(12): 2303.
- **Tchoubou Z, Koubala B B, Ndjonka D. (2023).** Influence of Sterculia setigera Delile extract on oxidative stress enzyme activity and treatment of impaired glucose homeostasis in Streptozotocin-induced diabetic Wistar rats. Pharmacological Research-Modern Chinese Medicine, 7: 100253.
- **Utami D M, Herawati L, I'tishom R, Al-Ari M A, Miftahussurur M, Rejeki P S. (2021).** Ketogenic diet slows down weight gain in juvenile mus musculus with benzopyrene as cancer inducer. Indian Journal of Forensic Medicine & Toxicology, 15(1): 2268-2274.
- **Vanneau T, Quiquempoix M, Erkel M-C, Drogou C, Trignol A, Sauvet F, et al. (2024).** Beneficial effects of photoperiod lengthening on sleep characteristics and mechanical hyperalgesia in injured rats. eneuro, 11(3).
- **Walczak-Nowicka Ł J, Herbet M. (2021).** Acetylcholinesterase inhibitors in the treatment of neurodegenerative diseases and the role of acetylcholinesterase in their pathogenesis. International journal of molecular sciences, 22(17): 9290.
- **Walters E R. (2023).** "It's more than just eating"-Family carer lived experiences of eating and appetite in people with dementia University of Southampton].
- **Wei P, Li X, Wang S, Dong Y, Yin H, Gu Z, et al. (2022).** Silibinin ameliorates formaldehyde-induced cognitive impairment by inhibiting oxidative stress. Oxidative Medicine and Cellular Longevity, 2022(1): 5981353.
- **Williams R S, Boison D, Masino S A, Rho J M. (2024).** Mechanisms of Ketogenic Diet Action. Jasper's Basic Mechanisms of the Epilepsies.
- **Xia J, Dong S, Yang L, Wang F, Xing S, Du J, et al. (2024).** Design, synthesis, and biological evaluation of novel tryptanthrin derivatives as selective acetylcholinesterase inhibitors for the treatment of Alzheimer's disease. Bioorganic Chemistry, 143: 106980.
- **Xu Y, Jiang C, Wu J, Liu P, Deng X, Zhang Y, et al. (2022).** Ketogenic diet ameliorates cognitive impairment and neuroinflammation in a mouse model of Alzheimer's disease. CNS Neuroscience & Therapeutics, 28(4): 580-592.
- **Zhu H, Bi D, Zhang Y, Kong C, Du J, Wu X, et al. (2022).** Ketogenic diet for human diseases: the underlying mechanisms and potential for clinical implementations. Signal transduction and targeted therapy, 7(1): 11.
- **Ziontz J, Adams J N, Harrison T M, Baker S L, Jagust W J. (2021).** Hippocampal connectivity with retrosplenial cortex is linked to neocortical tau accumulation and memory function. Journal of Neuroscience, 41(42): 8839-8847.