DOES KETOGENIC DIET HAVE BENEFICIAL EFFECTS ON ALZHEIMER'S BIOMARKERS?

Hoda S. Ibrahim, Aml F. Elgazar*, Amr A. Rezq and Mariam H. Mahmoud

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder that impairs mental development ability and interrupts neurocognitive function. The ketogenic diet (KD) is a low-carbohydrate with high-fat and adequate-protein. The present study was conducted to investigate the effect of KD on Aluminum chloride ($AlCl_3$) - induced Alzheimer's disease in rats. Forty male albino rats were split into four groups: group 1 and group 2 were kept as negative and positive control rats ($AlCl_3$-treated rats), respectively, and were feeding on the normal basal diet. While the other remaining two groups treated with $AlCl_3$ were fed on ketogenic diets (62% fat, 14% protein and 14% carbohydrate) and (67% fat, 14% protein and 10% carbohydrate), respectively, for 8 weeks. At the end of the experiment period, Amyloid beta $_{1\text{-}42}$ ($A\beta_{1\text{-}42}$), Phosphorylated tau protein (p-tau), Acetylcholinesterase (ACHE), Beta-hydroxybutyrate (β-HB), malondialdehyde (MDA), Superoxide dismutase (SOD), glutathione (GSH) and total antioxidant capacity (TAC) were determined for all studied groups. Results revealed that $AlCl_3$-treated rats and fed on the two different KDs with the different concentrations of fats and carbohydrates had a significant ($p< 0.05$) decrease in plasma and brain tissue of ($A\beta_{1\text{-}42}$) and p-tau in brain tissue, as well as the decrease in serum concentrations of AChE and MDA. On the other hand, there was a significant increase in serum β-HB concentrations and the activity of antioxidant enzymes (SOD and GSH), and TAC. In addition, histopathological inspection of brain tissues confirmed the improvements in Alzheimer's biomarkers. Finally, the obtained results prove that KDs with the two different proportions of fats and carbohydrates leads to an improvement in brain function as an indicator of preventing deterioration resulting from Alzheimer's disease.

Keywords*:* Ketogenic Diet; Neurodegenerative Disorder; Amyloid beta$_{1\text{-}42}$($A\beta_{1\text{-}42}$); Mental Development; Aluminum chloride.

Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Egypt.
***Corresponding author:** Aml Fawzy Elgazar **Email:** dr_aml_fawzy@yahoo.com

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1. INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia, which is estimated to affect as many as 24 million people worldwide, a prevalence that is expected to double every 20 years **(Reitz *et al.*, 2011).** It is also a heterogeneous and multifactorial disorder, characterized by cognitive impairment with a progressive decline in memory, disorientation, impaired self-care, and personality changes (**Rusek *et al.*,2019**). The most common symptom present at the beginning of AD is associated with short-term memory deficits, which affect daily activities (**Lange *et al.*, 2017**). In addition, cognitive deficits, resulting from the loss of neurons, are susceptible to neurofibrillary degeneration located in the limbic system, subcortical structures, archicortex and neocortex, and progressive synaptic dysfunction (**Serrano-Pozo *et al.*, 2011**). The initial phase of AD is involved in a short term memory loss and progressive other disease signs like alterations in the mood and behavior, aggressions, confusions, avoiding of peoples and social connections, and long term memory loss (**Livingston *et al.*, 2020**). AD affects the patients in a different way, as their experience in signs and progression of disease is diverse (**Weller and Budson, 2018**) because of the variations in the factors like age and genetics (**Fan *et al.*, 2020**). The prime cause of mortality in AD patients is not typically because of these alterations in the brain tissues but because of their related difficulties like pneumonia, immobility, and malnutrition because of the trouble in food consumption (**Scott *et al.*, 2020**).

Pathologically, AD involves progressive deposition of amyloid β-peptide (Aβ) as amyloid plaques, hyperphosphorylated tau protein intracellularly as neurofibrillary tangles (NFTs) and neuronal loss in

the hippocampus (**Kelley and Petersen, 2007**). Moreover, patients with AD present mitochondrial dysfunction and metabolic changes, such as impaired glucose utilization in the brain (glucose hypometabolism) (**Swerdlow, 2011**).

The ketogenic diet is a very high-fat and low-carbohydrate diet. This restriction triggers a systemic shift from glucose metabolism toward the metabolism of fatty acids (FAs) yielding ketone bodies (KBs), such as acetoacetate (AcAc) and β-hydroxybutyrate (BHB) as substrates for energy (**Taylor *et al.*, 2019**). Approximately 20% of basal metabolism in the adult brain is provided by the oxidation of 100–120 g of glucose over 24 hrs. The KD provides sufficient protein for growth and development, but insufficient amounts of carbohydrates for the metabolic requirements. Thus, energy is mostly derived from fat delivered in the diet and by the utilization of body fat (**Gasior *et al.*, 2006**).

The ketogenic diet was initially established in to be used in refractory epilepsy therapy (**Pinto *et al.*, 2018**). To date, there are pieces of evidence showing that it has gained interest as a potential therapy for neurodegenerative disorders (**Reger *et al.*, 2004** and **Van der Auwera *et al.*, 2005**), Parkinson's disease (**VanItallie *et al.*, 2005**), amyotrophic lateral sclerosis (**Zhao *et al.*, 2006**) and insulin resistance in type 2 diabetes (**Augustin *et al.*, 2018**). Moreover, because of altered glucose metabolism, it may have anti-tumor effects (**Zarnowski *et al.*, 2012**), and gastric cancer (**Otto *et al.*, 2008**). The present study was conducted to investigate the effect of KD on Aluminum chloride ($AlCl_3$) - induced Alzheimer's disease in rats.

2. MATERIALS AND METHODS:

- **Materials**

Rats: Forty adult male albino rats of the Sprague Dawley strain weighing (190 ± 10 g) were purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Egypt.

Constituents of Basal and Ketogenic Diets: All the nutrient ingredients needed for the preparation of the basal diet (AIN 93-M) according to the nutritional requirements of rats and to the preparation of ketogenic diets were purchased from the El-Gomhorya Company for Trading Drugs and Chemicals, Cairo, Egypt. Sucrose, soybean oil, margarine and starch were purchased from the local market.

Chemicals and Kits for Biochemical Analysis: Almonium chloride ($AlCl_3$), diethyl ether and other chemicals used in this study were acquired from the El-Gomhoriya Company for Trading Drugs and Chemicals, Cairo, Egypt. Kits for biochemical assay were purchased from the Gamma Trade Co., for Pharmaceutical and Chemical, Dokki, Egypt.

- **Methods:**

Preparation of Basal Diet (AIN-93M): All components of the basal diet as presented in **Table 1** were mixed together to fulfil the desirable adequate dietary intake for keeping the health state of rats as confirmed by **Reeves *et al.*, (1993)**. Concisely, each 1 kg diet consists of 10g vitamin mixture, 35g mineral mixture, 2.5g choline chloride, 1.8 g L-cysteine and 0.008g Tertbutyl-hydroquinone.

Preparation of Ketogenic Diets: Two different types of KDs were prepared as described by **Iacovides *et al.*, (2022)** with some modifications. Fat in the diet was composed of margarine and soy oil, the only protein source used was casein (85% protein) and the only carbohydrate source was starch and dextrinized cornstarch. In brief, the first type consisted of 62% fat, 14% protein, and 14% carbohydrate. While the second type consisted of 67% fat, 14% protein, and 10% carbohydrate **Table 1** describes the constituents of the two different models of KDs.

Table 1: Constituents of normal basal diet and the two different types of KDs per 1 kg of diet.

Constituents \ Diets		Normal Basal Diet	Ketogenic Diets	
		AIN-93M diet	Type 1	Type 2
Protein	Casein (85% protein)	140 g	140 g	140 g
	L-cysteine	1.8 g	1.8 g	1.8 g
Carbohydrates	Cornstarch	465.70	104.7	75
	Dextrinized cornstarch	155 g	36	25
	Sucrose	100g	0	0
Fiber		50g	50g	50g
Fats	Soybean oil	40g	50g	50g
	Margarine	-	570	620
Mineral mixture		35g	35g	35g
Vitamin mixture		10g	10g	10g

Choline chloride	2.5g	2.5g	2.5g
Tert-butylhydroquinone	0.008g	0.008g	0.008g

Induction of Alzheimer's Disease: Aluminum chloride ($AlCl_3$) was used to induce Alzheimer's disease in rats as stated by **Bitra *et al.*, (2014).** In brief, AlCl3 was dissolved in distilled water and intraperitoneal (i.p.) injected at a dose of 4.2 mg/kg body weight for 28 days.

Experimental Design*:* The experiment included forty adult male albino rats of Sprague Dawley strain weighting (190 ± 10). All the rats were housed in a good health state and kept in wire cages at the animal house of the Faculty of Home Economics under standard condition of the light/dark cycle (12 hr/12 hr), temperature (22-4°C) and relative humidity (45%-50%). Prior to the study period, water and basal diet were freely available to the rats for one week for acclimatization. After the acclimatization period (one week), all animals have been randomly divided into two main groups. The first main group (n= 10 rats) was kept as normal rats, while the second main group (n=30 rats) was intraperitoneal (i.p.) injected by 4.2 mg/kg body weight for 28 days. Following 28 d of $AlCl_3$ injection, Alzheimer's disease was confirmed by measuring the estimation of Amyloid beta $_{1-42}$ in plasma and brain tissues of rats. The results were showed increasing in the concentrations of the Amyloid beta $_{1-42}$ in $Alcl_3$ treated rats as compared to normal rats. After that, the second main group was divided into three groups (n=10 rats of each) as follows:

Group I: Negative control group (normal rats), rats were fed on the normal basal diet only.

Group II: Positive control group (treated rats with $AlCl_3$), rats were fed on the normal basal diet.

Groups III: Treated rats with $AlCl_3$ and fed on ketogenic diet type 1.

Groups IV: Treated rats with $AlCl_3$ and fed on ketogenic diet type 2.

Blood Collection for Plasma and Serum Separation: At the end of the experimental period (12 weeks), rats in all groups were fasted for 12 hours, anesthetized with diethyl ether. Part of portal vein whole blood samples were collected into commercially available anticoagulant-treated tubes (EDTA-treated). Then, the whole blood was centrifuged using refrigerated centrifuge at 2000 xg for 15 minutes to obtain plasma. Then, clear plasma samples were taken into the Eppendorf's tubes (1.5 mL) and stored at -20°C until they were used in biochemical analysis. While, the other part of blood samples was collected in clean, dry centrifuge tubes and left to coagulate at room temperature. The clotted blood was centrifuged at 3000 rpm for 15 minutes to obtain serum. Then, clear serum samples were taken into the Eppendorf's tubes (1.5 mL) and stored at -20°C until they were used in biochemical analysis.

The whole brain of each animal was rapidly carefully dissected, and sagittal divided into two halves. The first half was immersed in neutral buffered formalin 10% for histopathology examination. The second half was immediately homogenized to give 10% (w/v) homogenate in ice-cold medium containing phosphate buffer (pH 7.4). The homogenate was centrifuged at 1800xg for 10 min in cooling centrifuge at 4°C. The supernatant (10%) was separated and kept at -80°C until being assayed for the biochemical analysis.

Biochemical Analysis:

Estimation of Serum Levels of TC, TG, TL, HDL-c and LDL-c: Serum levels of total cholesterol (TC), triglycerides (TG), total lipid (TL), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) were estimated using commercial reagent kits (Biomed diagnostic, Egypt) as described by **Zollner and kirsch (1962), Vassault *et al.*, (1986)**, **Hostmark *et al.*, (1991), Friedwald *et al.*, (1972)** and **Young, (2001),** respectively.

Estimation of Aβ $_{1-42}$ and p-tau: Amyloid beta$_{1-42}$ concentrations in plasma and brain tissue of rats were measured as described previously with an anti- Aβ $_{1-42}$ antibody ELISA (**Lemere *et al.*, 2002**). Phosphorylated tau protein (p-tau) concentration in brain tissue was determined according to **(Hunter *et al.*, 2004).**

Estimation of AChE and β-HB: Acetylcholinesterase (AChE) in serum was estimated according to **(Colovic *et al.*, 2013).** Beta-hydroxybutyrate (β-HB) in serum was determined according to (**Thomas, 1998).**

Estimation of Malondialdehyde and Activities of Antioxidant Enzymes and TAC: The serum concentration of MDA and the activity of superoxide dismutase (SOD) and glutathione (GSH) enzymes were determined using commercial assaying kits (Cayman Practice ELISA Kits).

The principal method for the determination of oxidative stress depends on colorimetric by quantifying thiobarbituric acid (TBA) reactivity as malondialdehyde (MDA) in a spectrophotometer adjusted at 532 nm according to the described method by **Draper and Hadley, (1990).**

The serum activity of SOD was assayed according to the kit's instructions as described by **Wheeler *et al.*, (1990)**. The color change is measured

spectrophotometrically at 450 nm. The serum activity of GSH was assayed according to the kit's instructions as described by **Beutler *et al.*, (1963)** using spectrophotometrically at 340nm.
OxiSelect™ (STA-360) kit was used to measure total antioxidant capacity (TAC) based on a reduction of copper II (Cu^{+2}) to copper I (Cu^{+}) by antioxidants like uric acid at 490 nm as described by **Trachootham *et al.*, (2008)**.

Cerebrum Histopathological Screening: The histopathological screening process for the cerebrums of all rats was carried out as referred procedures by **Kier (1990)**. Briefly, cerebrum samples were carefully washed in an isotonic solution, dried on a filter paper and immersed in buffered formalin (10%). Afterwards, the fixed cerebrum specimens were dehydrated in a graded ethyl alcohol from 50 to 100%. Subsequently, specimens were cleared by Xylol, immersed in paraffin bulk, sliced to 6 µm thickness and colored with Hematoxylin (HX) and eosin (E) for the inspection.

STATISTICAL ANALYSIS:

Data was evaluated statistically using computerized SPSS package program (*SPSS 22.00 software for Windows*) by one-way analysis of variance (ANOVA). The obtained data was expressed as Mean ± SD and the significant difference among means was estimated at $p<0.05$ (**Artimage and Berry, 1987).**

3. RESULTS AND DISCUSSION

Effect of ketogenic diets on serum lipid profile:

The delimited results in **Table 2** summarized that normal rats (negative control group) have a significant ($p <0.05$) decrease in the serum concentrations of TC, TG, TL and LDL-c, and increase in HDL-c level as compared to that of the $Alcl_3$-treated rats and fed on the normal basal diet alone (positive control rats). Alongside, feeding rats on the two types of Ketogenic diets (KD), combined with IP injection by $Alcl_3$ brought about a significant ($p<0.05$) increase in the serum TC, TG, TL and LDL-c, and decrease in HDL-c levels, in comparison to treated rats with $Alcl_3$ and fed on the normal basal diet alone. The most get better in the serum lipid profile test results were observed in the $Alcl_3$-treated rats and fed on type one of KD, compared to those treated with type two of KD.

Table 2: Comparison the effect of KDs on serum concentrations of TC, TG, TL, HDL-c and LDL-c levels in $Alcl_3$-treated rats.

Groups \ Parameters		TC (mg/dl)	TG (mg/dl)	TL (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Negative Control (-ve)		90.1±0.70^{d}	75.9±0.70^{d}	403.71±4.82^{e}	42.2±0.54^{a}	40.8±0.54^{d}
Positive Control (+ve)		95.0±0.75^{c}	87.7±0.79^{c}	440.71±13.97^{b}	40.8±0.54^{b}	47.4±0.61^{b}
$Alcl_3$-treated groups + KDs	**Type 1 of KD**	97.7±0.63^{b}	90.0±0.75^{b}	508.0±11.8^{c}	29.3±0.79^{c}	55.5±0.47^{c}
	Type 2 of KD	100.8±0.79^{a}	94.3±0.79^{a}	575.0±39.37^{a}	26.6±0.61^{d}	61.4±0.91^{a}

Values are expressed as Mean ± Standard Error (M±SE), Means with different letters in each column are significantly differs at $p< 0.05$; **- ve**: Negative Control Group; **+ve**: Positive Control Group; **KD**: Ketogenic diet; **TC:** Total Cholesterol**; TG:** Triglycerides; **TL**: Total Lipids; **HDL-C:** high density lipoprotein cholesterol; **LDL-C:** low density lipoprotein cholesterol.

The current results were compatible with **Abdolhaleem *et al.*, (2019)** who revealed that administration of $AlCl_3$ induced significant increases in the serum levels of TC, TG, TL and LDL-c, and decreases in HDL-c. Additionally, **Aita, (2014)** recorded that administration of $AlCl_3$ caused a marked elevation in serum TG, TC and LDL-c levels with a significant decline in HDL-c levels, compared to the control group. **Yousef, (2004)** indicated that the accumulation of $AlCl_3$ in the liver resulted in increased lipid peroxidation and loss of membrane integrity, which might be important determinants of altered lipid metabolism and increased serum levels of TC, TG, TL and LDL-c. Furthermore, **Abdolhaleem *et al.*, (2019)** demonstrated that the accumulation of $AlCl_3$ in the liver leads to a disturbance in lipid metabolism and, in turn, to the elevation in lipid profile.

With regard to the effect of feeding treated rats with $AlCl_3$ on the two different types of KDs, the obtained results were agreed with **Kwiterovich *et al.*, (2003)** who reported that the high-fat ketogenic diet significantly increased the mean plasma levels of TG, TC, LDL, and VLDL, and non-HDL cholesterol in children. Also, **Rezq and El-Khamisy**, **(2011)** who showed that high-fat diet results in dyslipidaemic changes by increase serum TG, VLDL, TC and LDL-c and decrease serum HDL-c levels. On the other contrary, our results disagreed with (**Jornayvaz et al., 2010)** who demonstrated that mice fed a KD during 6 weeks had lower total cholesterol and triglycerides levels than with other diets. However, **Noain et al., (2020)** have reported increased levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C) as a result of KDs. It has been postulated that this elevation in LDL-C would not

likely increase cardiovascular complications due to the large LDL-C particle size. In this case report, we present a case of a rapid increase, followed by a rapid correction of LDL-C, in a patient following a ketogenic diet.

Effect of ketogenic diets on the concentrations of $A\beta_{1-42}$ in plasma and brain tissue and p-tau levels in brain tissues:

Results in **Table 3** exhibit the effect of feeding $Alcl_3$-treated rats on KDs on plasma and brain tissue levels of $A\beta_{1-42,}$ and brain tissue concentrations of p-tau levels. The results showed a significant ($p<0.05$) increase in the plasma and brain tissue levels of $A\beta_{1-42}$, compared to the negative control group. In contrast, $Alcl_3$-treated groups and fed on the two types of KDs had a significant ($p<0.05$) decrease of the plasma and brain tissue levels of Aβ1-42 as compared to the positive control rats fed on the normal basal diet alone. The better concentrations of plasma and brain tissue levels of Aβ1-42 were observed in $Alcl_3$-treated rats feeding on the type two of KD, followed by those fed on the type one of KD.

Concerning phosphorylated Tau protein (p-tau) levels in brain tissue, results showed that there was a significant ($P< 0.05$) increase in the positive control group ($Alcl_3$-treated rats) fed on the normal basal diet, compared to the negative control group (normal rat). However, $Alcl_3$-treated rats feeding on the different two types of ketogenic diets had a significant ($P<0.05$) decrease in the brain tissue concentrations of p-tau, compared to the $Alcl_3$-treated rats feeding on a normal basal diet. As exhibited, there is a good improvement in brain tissue concentrations of p-tau in $Alcl_3$-treated rats feeding on type two of KD, compared to $Alcl_3$-treated rats fed on the basal diet and type one of KD.

Table 3: Comparison the effect of KDs on plasma and brain tissue concentrations of $A\beta_{1-42}$ and brain tissue concentrations of p-tau in $Alcl_3$-treated rats.

Parameters / Groups		$A\beta_{1-42}$ Plasma (Pg/mL)	$A\beta_{1-42}$ Brain tissue (ng/ mg tissue)	Brain tissues p-tau (ng/ mg tissue)
Negative Control (-ve)		155.00±0.79^{b}	88.20 ±1.23^{d}	6.60 ±0.36^{d}
Positive Control (+ve)		236.00±1.49^{a}	135.00 ±0.94^{a}	18.00 ±0.27^{a}
$Alcl_3$-treated groups + KDs	**Type 1 of KD**	154.00±0.84^{c}	106.00 ±0.79^{b}	11.00 ±0.16^{b}
	Type 2 of KD	152.00±0.79^{d}	98.6 0±1.07^{c}	9.30 ±0.19^{c}

Values are expressed as Mean ± Standard Error (M±SE), Means with different letters in each column are significantly differs at $p< 0.05$; **- ve**: Negative Control Group; **+ve**: Positive Control Group; **KD**: Ketogenic diet; **$A\beta_{1-42}$:** Amyloid beta$_{1-42}$; **p-tau:** Phosphorylated tau protein

The obtained results were in accordance with **Abdel-Salam *et al.*, (2016)** who recorded that the treated rats with $AlCl_3$ had significant amyloid deposited in brain tissues. **Xiao *et al.*, (2021)** reported that brain contents Aβ1-42 was drastically elevated in the $AlCl_3$-triggered AD animals as compared with normal control rats. Additionally, growing evidence unveiled that metal toxicity like Al are connected to neurological ailments and Al is the most potent neurotoxicant (**Huat *et al.*, 2019**). The brain is a potential target for Al toxicity and it could easily cross the blood brain barrier (BBB) through its high affinity for the receptors and eventually accumulate into the brain (**Chiroma *et al.*, 2019**). Furthermore, **Haass and Selkoe, (2007)** recorded that the accumulation of Aβ deposits in senile plaques, which is caused by the abnormal processing of the amyloid precursor protein, is one of the most significant neuropathological findings in the brains of patients with AD. These amyloid deposits initiate a cascade of oxidative and inflammatory events, ultimately leading to neuronal cell death.

With regard to the effect of KDs on the levels of $A\beta_{1-42}$ in $AlCl_3$-treated rats, the present results agreed with **Kashiwaya *et al.*, (2000)**, who demonstrated that KDs reduces the accumulation of Aβ and protects against Aβ neurotoxicity. Also, **Xu *et al.*, (2022)** indicated that KD attenuated Aβ deposition, and reduced neuroinflammation. It is capable of reducing the pathology associated with AD and enhancing learning and memory, likely by protecting neurons and synapses by lowering neuroinflammation and neurotoxic Aβ accumulation. In addition, **Neth *et al.*, (2020)** showed that KD has a beneficial effect on Aβ in both AD rats as well as in humans at risk for AD. **Taylor *et al.*, (2022)** demonstrated that ketotherapies (KTs) potentially modulate amyloid-β through various direct and indirect mechanisms targeting poor mitochondrial bioenergetics, increased ROS, and increased inflammation. KTs, especially the ketogenic diet, reduce systemic insulin and potentially improve peripheral metabolic status, which may improve systemic inflammation and reduce Aβ. The ketone body, β-hydroxybutyrate, serves as an energy substrate for mitochondrial metabolism, upregulates the astrocyte-neuron lactate shuttle, activates hydrocarboxylic acid receptor 2 to regulate inflammation and may directly scavenge

ROS. Through bioenergetic effects in the mitochondria, KTs stimulate genesis of new mitochondria, increase uncoupling of the electron transport chain to increase ATP production, generate less ROS than glucose metabolism, and reduce mitochondrial import of amyloid precursor protein and Aβ. KTs also activate nuclear factor-E2 related factor 2 to upregulate synthesis of ROS-scavenging antioxidants and AMP-activated protein kinase to regulate transcription of pro-inflammatory cytokines. With regard to the effect of $AlCl_3$ on the levels of p-tau in brain tissue, the present study was agreed with **Alves *et al*., (2012)** who reported that $AlCl_3$ exhibited a significant increase in p-tau levels, as the etiology of AD. Also, **Mohamed *et al*., (2020)** showed that the mean value of tau protein in the brain tissue in AD group was statistically significant higher ($p<0.05$) than the corresponding value of the normal control group. In the normal brain of rats, tau binds to microtubules to stabilize them and accelerate axonal transport (**Kontaxi *et al*., 2017**). Additionally, **Henry *et al*., (2013)** demonstrated that AD exhibits two hallmark brain lesions, the Neurofibrillary Tangles (NFTs) and senile plaques. NFTs are formed by intraneuronal accumulation of paired helical filaments composed of abnormally hyperphosphorylated tau protein and senile plaques contain Aβ. Also, tau is hyperphosphorylated in AD, which causes it to separate from microtubules and assemble in the paired helical filaments and dystrophies neuritis (**Spillantini and Goedert, 2013**). Remarkably, the administration of KDs improved AD by lowering tau hyper-phosphorylation. The obtained result was inconsistent with **Kashiwaya *et al*., (2013**) who showed that KDs reduced abnormal phosphorylated Tau protein functional performance and protect the hippocampus from attack by amyloid precursor protein (APP). Therefore, ketogenic diet has properties to reduce the pathology of the amyloid and tau protein in AD rats.

Effect of KDs on the serum concentrations of AChE and β-HB in $Alcl_3$-treated rats:

The effect of KD on serum levels of Acetylcholinesterase (AChE**)** and Beta-hydroxybutyrate (β-HB**)** in $Alcl_3$-treated rats are recorded in Table **4.** The results revealed that the positive control group ($Alcl_3$-treated rats) fed on a normal basal diet had a significant ($p<0.05$) increase in serum concentrations of AChE and a non-significant increase in the serum BHB level, compared to the negative control group (normal rats). In contrast, feeding $Alcl_3$-treated rats on the two different types of KDs caused significant ($p<0.05$) decreases in serum concentrations of AChE and increases in serum levels of β-HB as compared to the positive control rats feeding on the normal basal diet.

The highest improvement in serum levels of AChE and β-HB was presented in $Alcl_3$-treated rats feeding on type two of KD, compared to feeding on type one.

Table 4: Comparison the effect of KDs on the serum concentrations of AChE and β-HB in $Alcl_3$-treated rats.

Parameters / Groups		**AChE** U/L	**β-HB** mmol/l
Negative Control (-ve)		99.00 ± 1.49^{c}	32.80 ± 2.44^{c}
Positive Control (+ve)		182.00 ± 0.52^{a}	34.60 ± 1.96^{c}
$Alcl_3$-treated groups + KDs	**Type 1 of KD**	116.00 ± 0.94^{b}	46.00 ± 2.00^{b}
	Type 2 of KD	99.00 ± 0.79^{c}	49.80 ± 2.04^{a}

Values are expressed as Mean ± Standard Error (M±SE), Means with different letters in each column are significantly differs at $p< 0.05$; **- ve**: Negative Control Group; **+ve**: Positive Control Group; **KD**: Ketogenic diet; **AChE:** Acetylcholinesterase; **β-HB:** Beta-hydroxybutyrate

The current results were in accordance with **Kakkar and Kaur, (2011)** who revealed that there was elevated activity of AChE in $AlCl_3$-treated rats. Likewise, **Xiao *et al*., (2021)** showed a significant elevation in the brain contents of AChE in $AlCl_3$-triggered AD animals as compared with normal control animals. The contemporary effect may be attributed to the direct effect of Al, where Al interacts with the peripheral sites of AChE and modifies its secondary structure and eventually enhances its activity. Furthermore, **Thenmozhi *et al*., (2015)** demonstrated that Al causes a disorder in cholinergic neurotransmission in animals that had been exposed to it, leading to memory alterations. Likewise, **Auti and Kulkarni, (2019)** revealed that ACh is a cholinergic neurotransmitter with an imperative role in the neuronal signal transmission between neurons and it was tightly related to the upholding of learning and memory in the brain. AChE is the enzyme that participates in the hydrolyzing of ACh into choline and acetate. The commencement of AD starts with the ACh absence and thus reducing the AChE activity that improves the ACh status has a positive influence on cognitive function.

With regard to the effect of KDs on the serum β-HB levels, our results were in the same line with **Van-der-Auwera *et al*., (2005)** who indicated that

$AlCl_3$-treated rats and the fed on the two types of KDs have a significant increase in serum β-HB levels, and suggested that cause of the metabolic shift toward fat utilization in groups fed on KDs. Also, **Martin *et al.*, (2006)** referred to that a high-fat, low-carbohydrate diet or a form of calorie restriction resulting in ketone body metabolism and increased β-hydroxybutyrate (β-HB) levels in the blood. Thus, a ketosis-like condition is generated under the circumstance of low blood glucose or glycogen depletion in which the liver particularly provides fatty acid-derived β-HB (**Paoli *et al.*, 2015**). Additionally, **Abbasi *et al.*, (2021)** revealed that under the circumstance of carbohydrate (CHO) restriction, fatty acids break down to β-HB, which turns out to be a compensatory energy fuel in the brain, heart, kidneys, and muscles.

Effect of KDs on serum levels of MDA, SOD, GSH and TAC in $Alcl_3$-treated rats:

The results of the effect of the two different types of KDs on the serum level of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD) and reduced glutathione (GSH) enzymes as well as total antioxidant capacity (TAC) in the $Alcl_3$-treated rats are recorded in Table **5**. Results showed that $Alcl_3$-treated rats who were fed on the normal basal diet had a significant increase ($p<0.05$) in serum levels of MDA, and decrease in serum activities of the antioxidant enzymes (SOD and GSH) and TAC, compared to the normal rats fed on the same diet. However, feeding $Alcl_3$-treated rats on KDs with the two different levels of fats and carbohydrates has a significant decrease at $p<0.05$ in serum levels of MDA, and increase in the activities of SOD and GSH, and TAC, compared with that of $Alcl_3$-treated rats and fed on the normal basal diet only.

Table 5: Comparison the effect of KDs on the serum concentrations of MDA, SOD, GSH and TAC in $Alcl_3$-treated rats.

Groups \ Parameters		MDA µmol/L	SOD µ/L	GSH mmol/L	TAC mM/L
Negative Control (-ve)		34.60±0.61^{d}	7.60±0.39^{a}	4.26±0.55^{a}	1.95±0.14^{a}
Positive Control (+ve)		80.30±0.54^{a}	3.10±0.23^{d}	1.26±0.05^{d}	0.84±0.1^{d}
$Alcl_3$-treated groups + KDs	**Type 1 of KD**	57.60±1.58^{b}	4.70±0.26^{b}	3.03±0.24^{b}	1.75±0.11^{b}
	Type 2 of KD	35.50±0.67^{c}	4.30±0.26^{c}	2.67±0.23^{c}	1.02±0.03^{c}

Values are expressed as Mean ± Standard Error (M±SE), Means with different letters in each column are significantly differs at $p< 0.05$; **- ve**: Negative Control Group; **+ve**: Positive Control Group; **KD**: Ketogenic diet; **MDA:** Malondialdehyde; **SOD:** Superoxide dismutase; **GSH:** Glutathione; **TAC:** Total Antioxidant Capacity

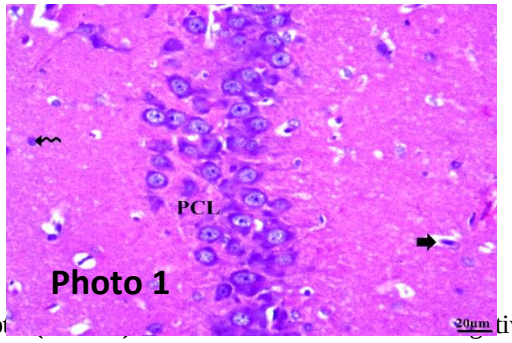
The present study provides a perfect correlation between serum lipid peroxidation products as indicated by MDA and the activity of antioxidant enzymes, which play an important role in the antioxidant system. The increase in serum MDA and the decrease in serum activity of antioxidant enzymes, as seen in serum of $AlCl_3$-treated rats and fed on the basal diet, can lead to the excessive availability of superoxide and peroxyl radicals, which in turn generate hydroxyl radicals, resulting in the initiation and propagation of more lipid peroxidation products. Our results agreed with **Khalil *et al.*, (2020)** who reported that there was a significant increase in lipid peroxidation, expressed as malondialdehyde (MDA) and a significant decrease in total antioxidant capacity (TAC) in the $AlCl_3$-treated group, compared with the normal group. Also, **Maksoud *et al.*, (2020)** revealed that injection of $AlCl_3$ affected oxidative stress markers. There, levels of MDA, was significantly increased and $AlCl_3$ also reduced SOD and GSH. It can be concluded that $AlCl_3$ caused brain oxidative damage. The other evidence shows that oxidative stress plays a pathogenic action in chronic inflammatory ailments and is an imperative cause that could influence the instigation and pathological progression of AD (**Wang *et al.*, 2014**). Oxidative stress. As well, **Zhao and Zhao, (2013)** revealed that oxidative stress often influences neurological modifications, which include neurofibrillary tangles, neural apoptosis, amyloid deposits, and mitochondrial dysfunction that was often implicated in the pathological progression of AD. These results also, were nearly similar to the reported studies of (**Wahby *et al.*, 2017**) who reported that brain homogenate SOD, GPxas and CAT were significantly decreased after injection of $AlCl_3$ at a dose of 34 mg/kg compared with the control group. Oxidative stress has long been implicated with various neurodegenerative disorders, including AD. Metals are well-known causative agents for oxidative stress-induced neurodegeneration, and recently, aluminum chloride ($AlCl_3$) has been frequently associated with neuronal disorders, such as AD (**Bolognin *et al.*, 2011**).

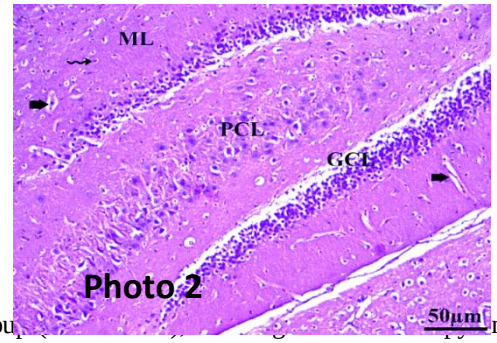
Thenmozhi *et al*., (2015) reported that $AlCl_3$ is absorbed into the blood-brain barrier (BBB) and accumulated in the brain, primarily in the hippocampus, responsible for memory and learning. Prolonged accumulation of Al causes neurotoxicity by the development of neurofibrillary tangles and amyloid aggregates.

In contrast, the present study documented that the two different types of KDs have significant effects on decreasing levels of MDA and increasing activities of antioxidant enzymes (SOD and GSH) and TAC in $AlCl_3$-treated rats fed on KDs, compared to that of $AlCl_3$-treated rats fed on normal diets. Accordingly, our study can demonstrate that KD reduced $AlCl_3$-induced oxidative stress in rats. It was in agreement with **Rhyu *et al*., (2014)** who showed an increase in HDL level and lower in MDA level of Taekwondo athletes after a ketogenic diet, by 3 weeks. In a study concerning healthy women, **Nazarewicz *et al*., (2007)** showed that 14 days of KD resulted in improved total antioxidative status as well as increased uric acid and HDL levels. They interpreted this result as demonstrating the effect of a KD on antioxidative capacity. Furthermore, there is evidence that ketone bodies potentially decreased ROS production decrease as mentioned by **Bough and Rho, (2007**) who revealed that KD leads to the production of ketone bodies, such as β-hydroxybutyrate and acetoacetate, which can be used as an alternative energy source and reduced lipid peroxidation. Also, **Lu *et al*., (2018)** found that KD reduces oxidative stress by suppressing some signal pathways after spinal cord injury. As well, **Pinto *et al*., (2018)** showed that KD improves mitochondrial function and reduces oxidative stress, and improves mitochondrial respiration by reducing the production of reactive oxygen species of β-hydroxybutyrate. **Parry *et al*., (2018)** reported that liver SOD and CAT levels were higher in the KD group compared to the normal diet group. Additionally, KD increases the activity of glutathione peroxides, an enzyme that reduces lipid peroxidation, in the rat hippocampus (**Ziegler *et al*., 2003**). The KD also increases production of specific mitochondrial uncoupling proteins (UCPs). UCPs function to dissipate mitochondrial membrane potential, reducing the formation of reactive oxygen species (**Sullivan *et al*., 2004**). The KD likely induces UCP production through the action of fatty acids (**Rho, 2008**). Ketone bodies have been reported to scavenge diverse reactive oxygen species, including hydroxyl radicals (**Haces *et al*., 2008**). Although, the mechanisms behind oxidative stress in brain still not completely understood, many studies suggested the role of nuclear factor E2-related factor 2 (Nrf2); a transcription factor; in response to stimuli including oxidative stress. Nrf2 translocate to the nucleus and binds to the antioxidant response element (ARE) (**Hichor *et al*., 2018**). Nrf2/ARE signaling is responsible for regulation of cellular redox status and modulation of antioxidant defense genes including heme oxygenase 1 (HO-1), CAT, SOD, and GSH (**Sun *et al*., 2017**).

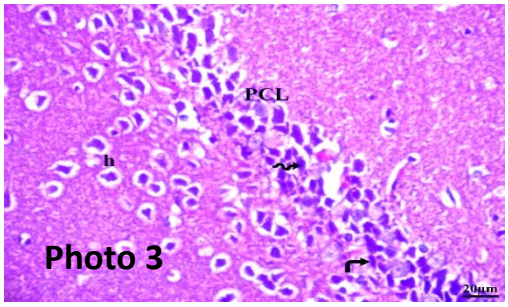
Histopathological inspections result of brain

Microscopically, the brain tissues of rats from control negative group (normal rats) revealed that both the pyramidal cell layer (PCL) and the molecular layer (ML) of the hippocampus are in healthy condition. Pyramidal neurons in the PCL are tightly packed and organized, and their cell bodies are relatively small. They also have vesicular nuclei and very little cytoplasm. Glial cells, denoted by a wavy arrow, can be found coexisting with normal blood capillaries (BV) in the molecular layer (ML). There is a clear delineation of the granule cell layer in the dentate gyrus (GCL). Granule cell bodies, which can take on a variety of shapes ranging from spherical to oval, can be seen grouped together in the GCL (**Photo 1 and 2**). In contrast, as shown in **Photo 3 and 4**, brain tissues of rats from control positive group (AlCl3-treated rats) fed the normal basal diet, the cell bodies of pyramidal neurons in the hippocampus are disordered and loosely packed. These neurons appear black, shrunken (curved arrow), and have pyknotic nuclei (zigzag arrow) with pericellular haloes (h). Additionally, the granular cell layer (GCL) of the dentate gyrus (DG) exhibited a structure that was disorganized. Meanwhile, the brain tissues of rats from AlCl3- treated group and feeding on the type one KD showed the hippocampus showed only a moderate degree of improvement and exhibited only a slight degree of disorganization in between the pyramidal (PCL) and granular cell layers (GCL). Only a few neurons exhibited pyknotic changes, and pericellular haloes were present (h) as shown in **Photo 5 and 6**. Brain tissues of rats from treated rats with AlCl3 and feeding on the type two of KD exhibited both the pyramidal cell layer (PCL) and the molecular layer of the hippocampus appear to have a conventional structure and organization (**Photo 7**). The cell bodies of pyramidal neurons in the PCL are relatively small, and their nuclei was vesicular. In the molecular layer, there were some glial cells been shown (indicated by the zigzag arrow) (ML). Within the dentate gyrus (DG), the granule cell layer, also known as the GCL, is very distinguishable (**Photo 8**).





Pho tive grou midal cell layer (PCL) and the molecular layer (ML) of the hippocampus are in healthy condition with no histological changes.



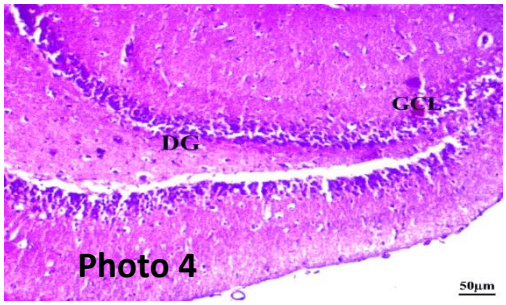
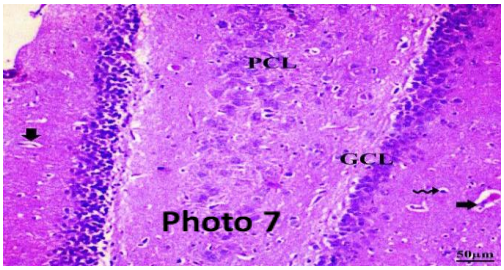


Photo 3 and 4: Brain tissues of rats from control positive group (AlCl3-treated rats) fed on the normal basal diet, showing the cell bodies of pyramidal neurons in the hippocampus are disordered and loosely packed.



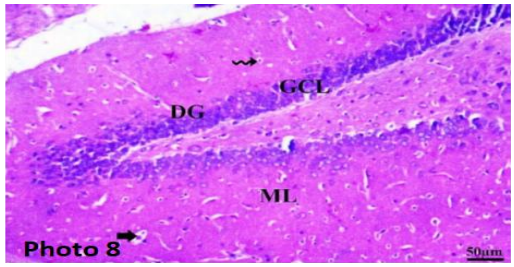
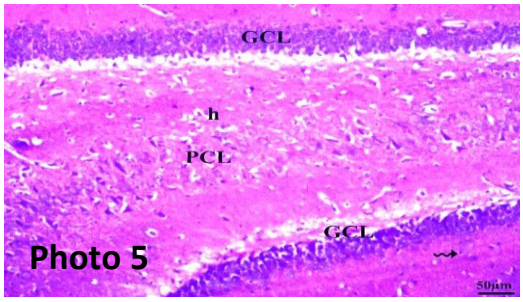


Photo 5 and 6: Brain tissues of rats from treated rats with AlCl3 and feeding on the type one of KD showing the hippocampus showed only a moderate degree of improvement and exhibited only a slight degree of disorganization in between the pyramidal (PCL) and granular cell layers (GCL). Only a few neurons exhibited pyknotic changes (the zigzag arrow), and pericellular haloes were present (h).



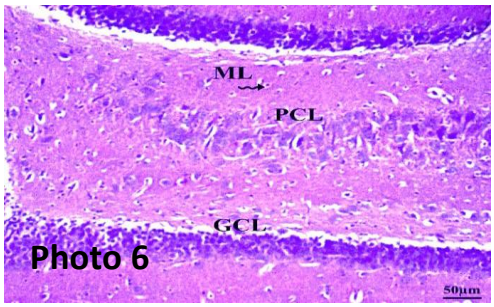


Photo 7 and 8: Brain tissues of rats from treated rats with AlCl3 and feeding on the type two of KD showing both the pyramidal cell layer (PCL) and the molecular layer of the hippocampus appear to have a conventional structure and organization.

The present study displayed that Alcl3-induced Alzheimer's could be rescued by the treatment with ketogenic diets as indicated by biochemical analysis and histopathological examenation. Likewise, a concomitant reduction in neurotoxic effects after ketogenic diet treatment. In the current study our results in the same line with that reported by (**Anwar *et al.*, 2021**) who observed that the brain sections from Alzheimer's rats revealed loss of normal architecture, indicated by the distortion of pyramidal layers in the hippocampus and loosely packed, pyramidal cells appeared shrunken and had degenerative changes, along with the appearance of pyknotic nuclei with pericellular haloes. Also, the granular cell layer of the dentate gyrus structure was disorganized. In addition, **Xiao et al., (2021)** revealed that the AlCl3-triggered AD animals displayed the various degenerating cells within the dentate gyrus and cornus ammonis portions with occurrence of microglia cells and the areas of reduced cell density. The hippocampus of the rivestigmine administered animals demonstrated the almost normal hippocampus structures.

Regarding the ketogenic diet-treated groups, a histological structure of the hippocampus layers was observed with a moderate improvement in the pyramidal cells and granular cell layers, and a slight degree of disorganization compared to AlCl3-treated rats and fed on normal basal diet. Although only a few neurons exhibited pyknotic changes in Alcl3-treated rats, feeding on KD consisted of 62% fat and 14% carbohydrate. However, with increasing fat and lowering carbohydrates as in type two of KD (67% fat and 10% carbohydrates), a normal histological structure of the two layers (pyramidal cells and granular cell layers) was observed and the granule cell layer was clearly visible within the dentate gyrus.

4. CONCLUSION

The results of our study showed that both the two different types of KDs enhanced lipid profile by decreasing TC, TG, TL, LDL-c and increasing HDL-c. Also, there were improving levels of Amyloid beta $_{1-42}$, Phosphorylated tau protein, acetylcholinesterase, Beta-hydroxybutyrate, and, preventing oxidative damage by decreasing MDA, and increasing SOD, GSH and TAC.

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