Egypt. J. Vet. Sci. **Vol. 56,** No. 3, pp. 559-573 (2025)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

A comparative Study of the Effects of Virgin Coconut Oil and Vitamin D on Alzheimer's Disease Induced by Aluminium Chloride in Male Albino Rats Hippocampus

Ohoud Aldemh*, Fatma A. Al-Nefeiy and Ebtesam Bawazir
Department of Biology, Faculty of Science, University of Jeddah, Jeddah, Saudi Arabia

Abstract

ALZHEIMER'S DISEASE (AD) is a neurological disorder that is leading to dementia worldwide. This study aims to determine the effects of virgin coconut oil and vitamin D on AD induced by aluminum chloride in rats. Thirty adult male Albino Wister rats were divided into five groups (six rats for each group).The first group served as a control group; the second group was given an oral dose of 200 mg/kg b.w. $AlCl_3$ every day for 6 weeks; and the third group was supplemented with VD orally daily at a dose of 500 IU/kg b.w. for 6 weeks and an oral dose of 200 mg/kg b.w. $AlCl_3$ after one hour. The fourth group was given an oral dose of 5 ml of VCO and a dose of 200 mg/kg b.w. after one hour of $AlCl_3$ daily. The last group received an oral dose of 1.5 mg/kg b.w. of Rivastigmine (RT) daily and an oral dose of 200 mg/kg b.w. of $AlCl_3$ after one hour. After six weeks, the behavioral test was performed in rats to assess learning and memory, and physiological and histological studies was made in the hippocampus and blood plasma to determine the effect of VCO and VD on $AlCl_3$-induced AD in rats. $AlCl_3$ led to a significant decrease in the memory and learning maze test. Further, a significant increase in AchE, LPO, and NFL, along with a significant decrease in DA, reduced GSH and SOD. In the hippocampus histological examination, we observed an increase in neuronal loss in $AlCl_3$-treated rats. While VCO and VD-treated rats showed a reduction in the damage caused by $AlCl_3$ associated with improvements in behavioral and physiological alterations. According to this study, a daily oral dosage of VCO and VD improves learning and memory in AD rats.

Keywords: Alzheimer's disease, Aluminum chloride, Vitamin D, Virgin coconut oil, Hippocampus

Introduction

Alzheimer's disease AD is a neurological disorder that is the leading cause of dementia worldwide [1] AD is a neurodegenerative condition characterised by the gradual degradation of hippocampal and cortical neurons that leads to impairment of memory and cognitive capacity. It is one of society's most financially demanding diseases. Although short-term memory loss is typically the first clinical sign, distant memory retrieval remains quite intact throughout the disease [2]. Low acetylcholine levels, increased oxidative stress, and antioxidant enzyme disruption produce learning and memory problems in Alzheimer's disease, which is considered as irreversible, progressive neurological condition. Also, reduced brain size, degeneration and death of hippocampal neurons, and aggregations of amyloid and tau proteins are all pathological features [3].

Aluminium AL is a neurotoxin that has been linked to the development of AD [4]. It enters the human body through antacids, water, food, deodorants, and medicines. AL accumulates inside

*Corresponding author: Ohoud Aldemh, E-mail: o.aldemh@gmail.com, Tel.: +966555347479
(Received 24/02/2024, accepted 29/04/2024)
DOI: 10.21608/EJVS.2024.271293.1872

the hippocampus and brain cerebral cortex , that known to be susceptible to AD [5,6]. Aluminium chloride $AlCl_3$ is neurotoxic, extremely physiologically reactive, and capable of causing harm to critical brain biochemistry. It can cause neuronal dysfunction and cell death by a series of disruptive events ranging from apoptosis to rapid, severe necrosis [7].

Rivastigmine RT is a cholinesterase inhibitor prescribed to treat AD dementia. On April 21, 2000, the FDA authorised it for the treatment of AD under the brand name "Exelon"[8]. This drug increases cholinergic activity by suppressing the enzyme acetylcholinesterase, which catalyzes the breakdown acetylcholine quite rapidly [9].

It is used to treat the symptoms of moderate to severe Alzheimer's disease, for greater solubility, it is employed in the form of a tartrate salt. Rivastigmine belongs to a class of chemicals known as carbamates, which cause carbamoylation of the enzyme's active site and inhibit its action [10].

Virgin coconut oil VCO is a useful food oil [11]. VCO is a natural plant extract that is studied for its ability to protect against the symptoms of AD. VCO demonstrated the potential to prevent brain cell death and enhance cognitive function in AD patients [12].VCO is commonly derived from fresh coconut using a low-heat method with no additives to guarantee that bioactive constituents such as polyphenols, essential fat, and glycolipids are retained in the oil. VCO has been shown to have several potential beneficial properties as an antioxidant, anti-inflammatory, and anti-hypercholesterol agent. Many studies have been conducted to study various treatment strategies for avoiding the progression of AD, and VCO has been widely researched as a natural neuroprotectant against AD [13].

Vitamin D VD is a steroid hormone with classical roles in calcium metabolism and bone health regulation [14]. It has important roles in proliferation and differentiation, calcium signaling within the brain, and neurotrophic and neuroprotective actions; it may also alter neurotransmission and synaptic plasticity[15]. It penetrates the blood-brain barrier and binds to receptors present in neurons throughout the central nervous system [16].Also, it inhibits neuronal necrosis via regulating voltage-gated calcium channels [17].Moreover, it has antioxidant properties, that help to reduce oxidative stress induced by glutamate and dopaminergic toxins [18].The current research is suggested to examine the effect of VCO as well as VD on Alzheimer's disease in rats induced by aluminium chloride, as assessed by, behavioral aspects such as locomotors activity and exploratory, biochemical studies, acetylcholinesterase AchE, dopamine DA, glutathione GSH, superoxide dismutase SOD lipid and peroxidation LPO in the hippocampus include measuring, Neurofilament light chain NFL in blood. Also, histopathological examination of all rats hippocampus in the experimental study groups.

Material and Methods

Animals

Thirty adult male albino rats weighing 170 - 200 g were obtained from Mansour Scientific Foundation for Research and Development, Jeddah, Saudi Arabia. The animals were divided randomly into groups and housed in cages with relative humidity of 30-70%, in the temperature range of 20-24°C and light-controlled room on an alternating 12:12 h light/dark cycle with given free access to food and water. The animals were adapted to the laboratory conditions for two weeks before the beginning of the experiments. All experiments were approved and made regarding the rules of the Animal Care and Use Committee of King Abdul-Aziz University, Faculty of Pharmacology, Jeddah, Saudi Arabia (Reference NO. 784.20 animal study).

Chemicals

Aluminium chloride $AlCl_3$ was purchased from Alpha Chemika Mumbai, Maharashtra, India supplied as white powder. Rivastigmine (Exelon®) 1.5 mg capsule was obtained from (Novartis Company, Basel, Switzerland). Sodium chloride (NaCl) and sodium hydroxide NaOH were obtained from (Panreac, Barcelona, Spain). Potassium chloride KCl, disodium phosphate Na_2HPO_4 and formaldehyde were obtained from (Riedel-dehaen, Sleeze, Germany). AchE Enzyme Linked Immunosorbent Assay (ELISA) kit, DA ELISA kits, GSH ELISA kit, SOD ELISA Kit and LPO ELISA Kit were purchased from (My BioSource, San Diego, U.S.A.).

Preparation of chemicals

$AlCl_3$ was used to induce Alzheimer's like disease in animals. Animals were administered $AlCl_3$ orally with 200 mg/kg b.w. for six weeks[19]. 2 grams of $AlCl_3$ dissolve in 40 ml of distilled water mix very well and store in the

fridge in the dark until use. Rivastigmine tartrate RT administered orally at a dose of 1.5 mg/kg b.w. [20]. 29 mg RT dissolved in 29 ml of distilled water. The doses are prepared daily to maintain the drug's effectiveness.

Supplement

VCO was purchased from iherb, brand name California Gold Nutrition. Was given at a dose of 5 ml/kg b.w [21]. VD Vitamin D3 (Cholecalciferol $C_{27}H_{44}O$), oral drops were purchased from Alnahdi pharmacy, brand name vidrop, VD diet corresponds to a daily dose of 500 IU/kg/day[22] 1 ml of VD dissolve in 4.6 ml of corn oil and store in the fridge in the dark until use.

Study groups

The rats were divided in to 5 experimental groups. The first group (control group) was given normal saline (0.9% NaCl) orally each day for 6 weeks, the second group was administered an oral dose of 0.4 ml. $AlCl_3$ every day for 6 weeks, the third group was supplemented with VD orally daily at a dose of 0.2 ml and $AlCl_3$ after 1 hour at a dose of 0.4 ml for 6 weeks, the fourth group was supplemented with VCO orally daily at a dose of 1 ml and $AlCl_3$ after 1 hour at a dose of 0.4 ml for 6 weeks. The last group received 0.3 ml RT orally daily for 6 weeks, followed by $AlCl_3$ after 1 hour at the same dosage as the second group.

Behaviour Study (Y Maze learning test)

Is used to assess disruptive behavior, natural spontaneous alternation spatial memory, short term memory, and cognitive decline in rodents [23].

Tissue preparations

After the behavioral test, the animals were euthanized by decapitation following the rules and regulations of King Abdul-Aziz University, Faculty of Pharmacology, Jeddah, Saudi Arabia. After dissection, their whole brains were removed carefully and washed in saline and divided in a sagittal plane into two halves. The right sided cerebral hemisphere for histopathological examination and the hippocampus of the left side was dissected for homogenate preparation. The tissue was immediately frozen in -80 °C for preparation of homogenate. For biochemical studies the frozen hippocampus are homogenized by using digital high-speed homogenizer (Homogenizer revolution per minute 15,000 rpm w) (Category: Emulsifier/Homogenizers Model: KRH-I).Then, centrifuged for 15 minutes. at 3000 revolutions per min by using (Fristaden Lab Digital Centrifuge 0-4000RPM | LCD Display | Bench-Top). After that supernatant was separated, aliquot and stored in Eppendorf tube at -80 °C for the biochemical assay.

Biochemical assay

ELISA kits were used for the measurement of hippocampus and cortex tissues homogenate for AchE (Cat. # MBS725468), SOD ELISA Kit (Cat. # MBS036924),DA Elisa kit (Cat. # MBS725908),GSH ELISA Kit (Cat. # MBS265966), LPO ELISA Kit (Cat. # MBS2515688) and NFL ELISA Kit (Cat. #MBS9399608)

Histopathological studies

After fixation of brain tissues in formalin saline (10%) for 24 hours, brain tissues were subjected to serial dilution of alcohol for dehydration, sectioned in to 5 µm thickness and stained with Hematoxyline and Eosine (H & E) and Congo red stain to be examined by a light microscope**.**

Statistical analysis

The statistical investigation was conducted utilizing the one-way analysis of variance (ANOVA) technique which is used to revealthe differences between groups in the different variables (biochemical measurements, blood analysis, and behavioral experiments) and Scheffe's test was used for homogeneous groups and Dunnett'sC for heterogeneous groups.

Results

Behaviour study (Maze learning test)

According to Fig. 1, the rats were all less relative average than the control group (0.30), with the lowest percentage being when treated with $AlCl_3$ after three weeks with a percent of (0.14), followed by (VCO+$AlCl_3$) and (VD+$AlCl_3$) with a ratio of (0.19) for each. After six weeks revealed that the effect of $AlCl_3$ had the least percentage of (0.00), followed by (RT+$AlCl_3$), (VCO+$AlCl_3$), and (VD+$AlCl_3$), each having a ratio of (0.14-0.15-0.14).

The amounts of AchE in the hippocampus homogenate was substantially increased in $AlCl_3$ group (3.63) compared to the control group (0.35) ($P<0.01$). Hippocampus AchE levels were reduced significantly in (VCO+ $AlCl_3$), (VD+$AlCl_3$) groups, and (RT+$AlCl_3$) group respectively (2.62-2.63) and (1.66) compared with $AlCl_3$ group (0.35) ($P<0.01$) (Fig. 2).

Hippocampus homogenate levels of DA

Figure 3 provide a comparison of the

hippocampal homogenate concentrations of DA in various examined groups with the control and $AlCl_3$ groups. The concentrations of DA in the hippocampal homogenate were considerably lower in the $AlCl_3$ group (1.80) compared to the control group (11.23)($P<0.01$). While DA levels were significantly increased in (VCO+ $AlCl_3$),($VD+AlCl_3$) groups ,and ($RT+AlCl_3$) group, respectively (12.23-11.72) and (6.03) compared with rats treated with $AlCl_3$ (1.80) ($P<0.01$).

Hippocampus homogenate levels of GSH

GSH concentrations in the hippocampal homogenate were substantially lower in the $AlCl_3$ group (2.23) compared to the control (18.37) ($P<0.01$). Furthermore,GSH levels in rats treated with $AlCl_3$ were substantially lower than in the (VCO+ $AlCl_3$), (VD+ $AlCl_3$) groups and (RT+ $AlCl_3$) group respectively (13.83- 13,17-9) compared with rats treated with $AlCl_3$ (2.23) ($P<0.01$) (Fig. 4).

Hippocampus homogenate levels of SOD

Figure 5, shows a comparison of the hippocampus homogenate amounts of SOD in various studied groups with control and $AlCl_3$ groups. The levels of SOD in the hippocampus homogenate were significantly decreased in $AlCl_3$ and ($RT+AlCl_3$) groups respectively (96.17-113) compared with the control group (178)($P<0.01$). While hippocampus SOD levels were significantly increased in (VCO+ $AlCl_3$), (VD+ $AlCl_3$), groups and ($RT+AlCl_3$) groups respectively (181- 177) and (113) compared with rats treated with $AlCl_3$ (96.17) ($P<0.01$).

Hippocampus homogenate levels of LPO

The levels of LPO in the hippocampus homogenate was significantly increased in $AlCl_3$ and (RT+ $AlCl_3$) groups (2.67 - 1.69) compared to control group (0.63) ($P<0.01$) Meanwhile, hippocampus LPO levels were significantly decreased in ($VCO+AlCl_3$), ($VD+AlCl_3$) groups and ($RT+AlCl_3$) group respectively (0.68- 0.55) and (1.69) compared with rats treated with $AlCl_3$ (2.76) ($P<0.01$)(Fig. 6)

Levels of NFL in plasma

Figure 7, indicate the comparison of NFL plasma levels in the examined groups with the control and $AlCl_3$ groups. The levels of NFL in plasma was significantly increased in $AlCl_3$ and (RT+ $AlCl_3$) groups respectively (42.83-29.64) compared to control group (21.77) ($P<0.01$). Meanwhile, plasma NFL levels were significantly decreased in ($VCO+AlCl_3$), ($VD+AlCl_3$), groups and ($RT+AlCl_3$) group respectively (21.32- 21.52) and (29.64) compared with rats treated with $AlCl_3$ (42.83) ($P<0.01$).

Histopathological studies

Histopathological investigation of the hippocampus confirmed the current findings. After six weeks, light microscopic inspection of the control group sections showed its normal shape and structure, which included Cornu Ammonis CA and dentate gyrus DG. CA had a C-shaped body with the following regions: CA1, CA2, CA3, and CA4 (Fig. 8). Both CA1 and CA3 (Figs. 9 A,B) were composed of well-defined 3 layers: outer molecular ML, middle pyramidal cell PCL and inner polymorphic layers PmL. ThePCL was the main one and was formed of several layers of densely packed small pyramidal cells PC in CA1 and less densely packed large PC in CA3. The PCs were triangular in shape with vesicular nuclei and prominent processes. Both ML and PmL consisted of a pinkish background "neuropil" that contained axons and dendrites, few scattered glial cells gl some blood capillaries bc (Figs. 9 B, C).The DG (Fig. 9 C) had a V-shaped structure enclosing CA4 region by upper and lower limbs. Each limb was formed of 3 layers; outer ML, intermediate granular GCL and inner PmL. The GCL consisted of closely packed small granular cells with rounded nuclei. The ML and PmL contained few gl and bc. Congo red-stained brain sections of the control group showed no detection of any amyloid plaque deposition in different regions of the hippocampus (Figs. 9 D, E, F).While examination of the hippocampus from $AlCl_3$-treated group revealed the presence of variable changes in different regions of the hippocampus. The main changes were seen in the PCL of CA1 and CA3, which appeared disorganized as compared to the control group (Figs. 10 A, B). Many PC showed degenerative changes where they lost their shape and appeared shrunk with dark cytoplasm and condensed nuclei. Both ML and PmL showed increased gl and dilated bc. Regarding DG (Fig. 10 C), the main changes occurred in GCL that appeared disorganized with many GC appeareddegenerated and shrinked with vacuolated cytoplasm and condensed nucleus. ML and PmL layers of DG showed increased gl with pericellular haloes, dilated bc .Congo red staining of the hippocampus (Figs. 10 D, E, F) showed multifocal, moderate deposition of amyloid plaques in ML and PmL of the hippocampus compared to the control group.

While the investigation of the hippocampus from $AlCl_3$ and VD group revealed improved histopathological changes as compared with $AlCl_3$ group with relatively normal thickness of the PCL in CA1 and CA3. Most of the PC were preserved in shape and showing vesicular nuclei but few cells appeared shrinked with dark condensed nuclei. ML and PmL showed increased gl and some dilated (bc) (Figs. 11A, B). Regarding DG (Fig. 11 C), VD administration resulted in improved architecture with the main changes occurred in GCL. However, some granular cells appeared shrinked with condensed nuclei. Congo red staining of hippocampus (Figs. 11 D, E, F) showed no deposition of amyloid plaques in ML and PmL of the hippocampus as compared with $AlCl_3$ group. Furthermore, the hippocampus of $AlCl_3$ and VCO group showed some improvement of the histopathological changes as compared with $AlCl_3$ group with relatively more or less normal thickness of the PCL in both CA1 and CA3. Although many PC were normal in shape, some cells appeared shrinked with dark condensed nuclei. Also, the ML and PmL showed increased gl and some dilated bc (Figs. 12 A, B). Regarding DG (Fig. 12 C), VCO administration resulted in moderately improved architecture with the main changes occurred in GCL as compared with $AlCl_3$ group. However, some GC still appeared shrinked with condensed nuclei. Congo red staining (Figs. 12 D, E, F) showed mild deposition of amyloid plaques in the ML and PmL of different hippocampal regions as compared with $AlCl_3$ group. On the other hand, examination of the hippocampus from $AlCl_3$ and RT group revealed better histopathological changes as compared with $AlCl_3$ group with relatively normal thickness of the PCL in CA1 and CA3. Most of the PC were conserved in shape and showing vesicular nuclei. However, some cells appeared shrinked with dark condensed nuclei. Also, the ML and PmL showed increased gl and some dilated bc (Figs. 13 A, B). Regarding DG (Fig. 13 C), VD administration resulted in improved architecture with the main changes occurred in GCL. However, some GC appeared shrinked with condensed nuclei. Congo red staining of hippocampus(Figs. 13 D, E, F) showed no deposition of amyloid plaques in ML and PmL layers of the hippocampus as compared with $AlCl_3$ group.

Discussion

Alzheimer's disease is a common neurodegenerative disease that impairs memory, cognitive abilities, and, subsequently, the ability to accomplish everyday tasks [24]. AD has been identified as a severe medical and societal issue that is aggravated by the growing number of elderly people. The approach for currently available Alzheimer's medications are based on inhibiting brain AchE and increasing the effectiveness of certain neuroprotective treatments [25]. Natural products provide several choices for slowing the progression and symptoms of many diseases, including Alzheimer's [26].The present study attempted to evaluate whether the administration of VCO and VD would have preventive effects on AD induced by $AlCl_3$ with behavioral, physiological disturbances and histological damages in male albino Wiste rats. The finding of the study revealed that six weeks oral administration of $AlCl_3$ (200mg/kg b.w) in rats leads to behavioral, physiological, and histological changes. The study finding was consistent with [27]. $AlCl_3$ in AD considered cognitive deficits to be the most important clinical indicator and a severe health concern. The results suggested that oral dose of VCO and VD for six weeks reduced significantly AchE activity as well as increased GHS,SOD and NFL in (VCO+$AlCl_3$) and (VD+$AlCl_3$) groups compared to $AlCl_3$ group, the present results were in agreement with [28,29,30,31]. CVO has protective effects due to the prevalence of phenolic compounds and a diversity of substances capable of inhibiting AchE, resulting in increased AchE amount. Furthermore, the current investigation found that six weeks of oral administration of RT with $AlCl_3$ resulted in a considerable reduction in AchE activities of the homogenate hippocampus [32]. It has been reported that $AlCl_3$ administration caused loss of neurons in the hippocampus but the results also revealed a neuroprotective potential of VCO and VD by reducing degeneration of the brain tissue, many earlier research discovered that polyphenols in VCO might stimulate the brain by lowering cell death in the hippocampus due to the antioxidant impact, VD is also an antioxidant. It enhances cellular activities that reduce oxidative stress induced by neurotransmitter toxicity. As a result, it prevents neuronal apoptosis [33,34].

Conclusions

In conclusion, the current study revealed that model AD induced by $AlCl_3$ caused behavioral, physiological, and histological changes in Albino Wister rats and that $AlCl_3$ caused increases in the levels of NFL in blood and AchE, LPO in

hippocampus homogenate, resulting in oxidative stress and a significant decline in antioxidant enzymes GSH as well as SOD compared to the control group. In contrast, the present research discovered that VCO and VD has a strong preventative influence in the hippocampus, as indicated by behavioral, biochemical, and histological improvements in rats. The preventive effect of VCO and VD reversed the memory impairment caused by AD induced by $AlCl_3$. It also reduced nerve impairment in AD rats by reducing oxidative activity and increasing antioxidant enzymes. According to the findings of the study, the usage of VCO and VD can slow the progression of AD, help to reduce symptoms associated with the disease, as well as improve memory deficits by decreasing behavioral disturbances as proven in this study. In my opinion, we should support the natural alternatives treatment for AD and enhance people to maintain VD levels which improves cognitive abilities and memory according to the results and provides an effective treatment that helps AD patients avoid the side effects of chemical drugs and encourages further research and studies on the treatment of AD.

Acknowledgements

The authors would like to extend special thanks to Professor Jamal Saied (Anatomy Department, Faculty of Medicine, KAU, Jeddah, Saudi Arabia)" Histology technical unit" for his generous advice and support.

Conflict of interest

The authors has no conflict of interest to report.

Ethical approve

This work was supported by King Abdul-Aziz University, Faculty of Pharmacology, Jeddah, Saudi Arabia (ethical approval no.784.20).

Funding statement

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author`s contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ohoud Aldemh, Fatima Alnefaei and Ebtesam Bawazir. The first draft of the manuscript was written by Ohoud Aldemh and co-authors commented on previous versions of the manuscript. Co-authors read and approved the final manuscript.

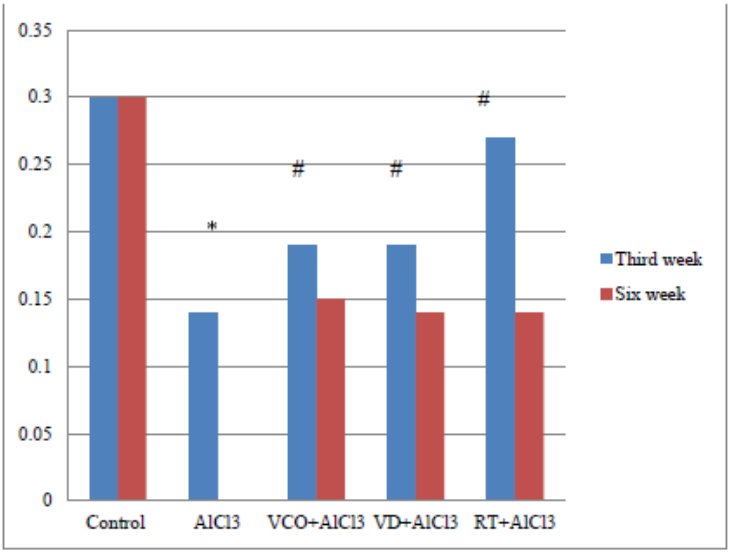

Fig.1. Evaluation of preventive impact of virgin coconut oil and vitamin D on $AlCl_3$ induced Alzheimer's disease in rats by using maze learning test
Data was expressed as mean +/- standard error
***: significance versus control (P<0.01)**
#: significance versus AlCl3 (P<0.01)
Significance was made using One way ANOVA test (Scheffe's test)

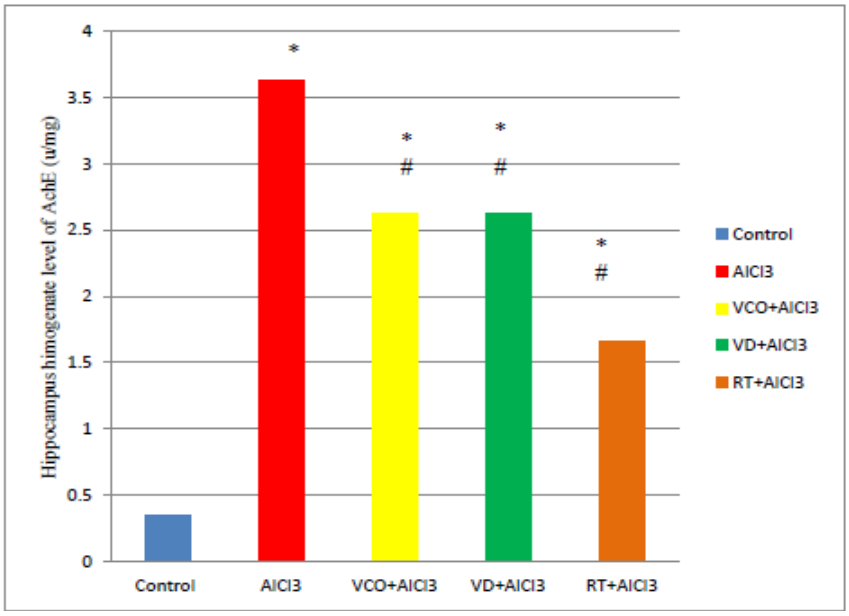

Fig. 2. Impact of virgin coconut oil and vitamin D on acetylcholinesterase levels in hippocampus homogenate (u/mg) for protection from AlCl3 induced Alzheimer's disease in rats.
Data was expressed as mean +/- standard error
***: relevance compared to the control (P<0.01).**
#: relevance compared to AlCl3 (P<0.01).

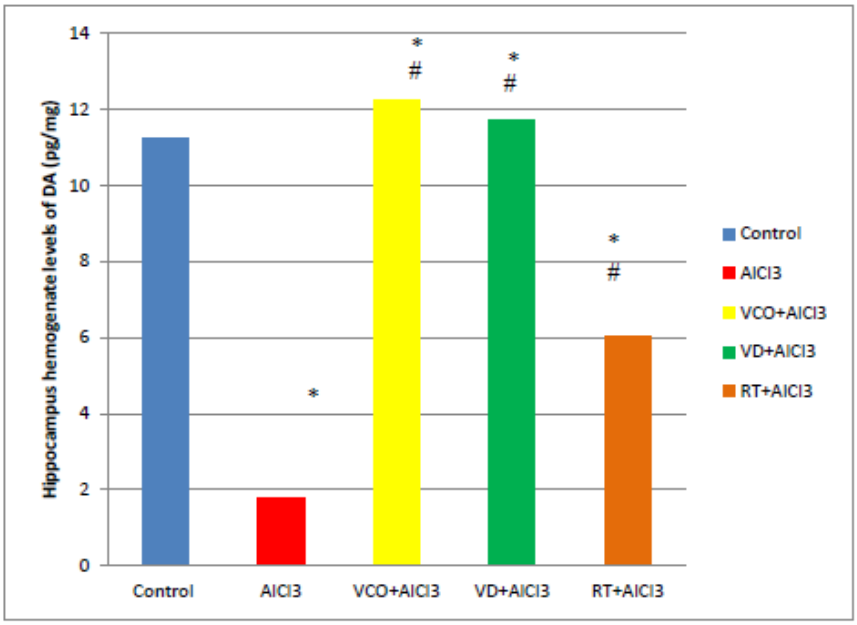

Fig. 3. Effect of virgin coconut oil and vitamin D on dopamine levels in hippocampus homogenate (pg/mg) for protection from AlCl3 induced Alzheimer's disease in rats.
Data was expressed as mean +/- standard error
***: significance versus control (P<0.01)**
#: significance versus AlCl3 (P<0.01)
Significance was made using One Way ANOVA test (Scheffe's test)

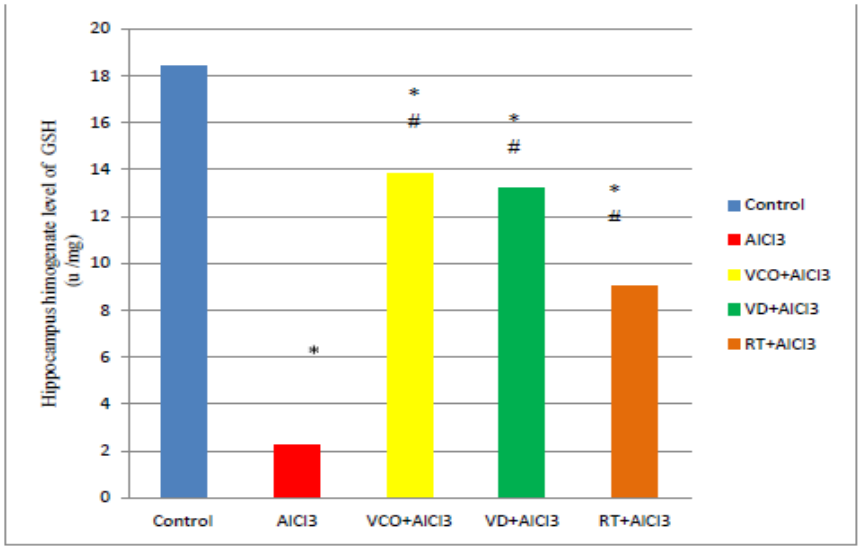

Fig. 4. Influence of virgin coconut oil and vitamin D on glutathione levels in hippocampus homogenate (u/mg) for protection from AlCl3 induced Alzheimer's disease in rats.

Data was expressed as mean +/- standard error
***: relevance against control (P<0.01)**
#: relevance versus AlCl3 (P<0.01)
The one-way ANOVA test (Scheffe's test) was used to evaluate relevance.

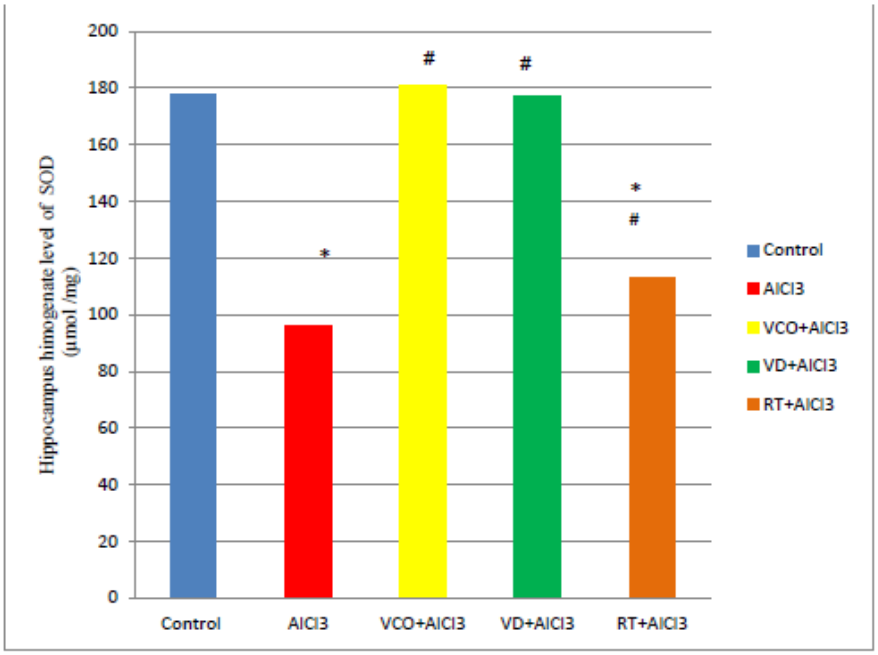

Fig. 5. The influence of virgin coconut oil and vitamin D on superoxide dismutase concentrations in hippocampal homogenates (µmol/mg) for protection against AlCl3-induced Alzheimer's disease in rats.
Data was recorded as mean +/- standard error
***: significance versus control (P<0.01)**
#: significance versus AlCl3 (P<0.01)
Significance was made using One way ANOVA test (Scheffe's test)

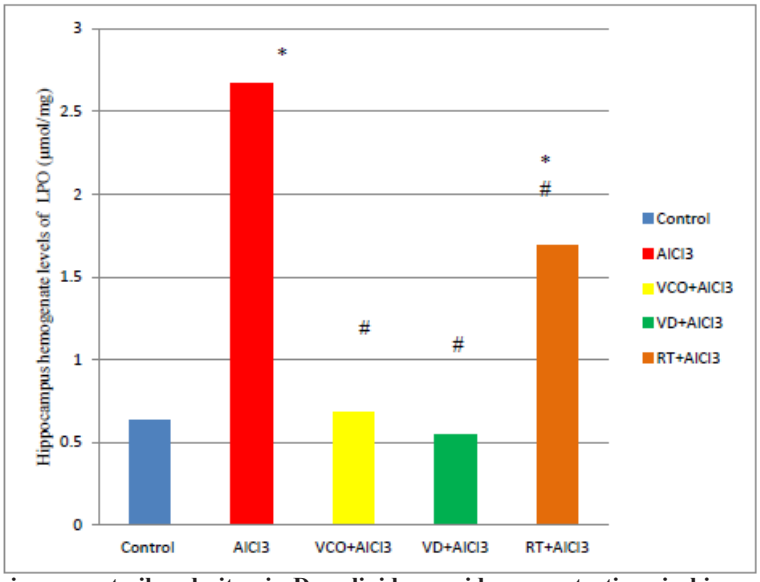

Fig. 6. Effect of virgin coconut oil and vitamin D on lipid peroxide concentrations in hippocampal homogenate (µmol/mg) for AlCl3-induced Alzheimer's disease prevention in rats.

Data was recorded as mean +/- standard error
***: significance versus control (P<0.01)**
#: significance versus AlCl3 (P<0.01)
Significance was made using One way ANOVA test (Scheffe's test)

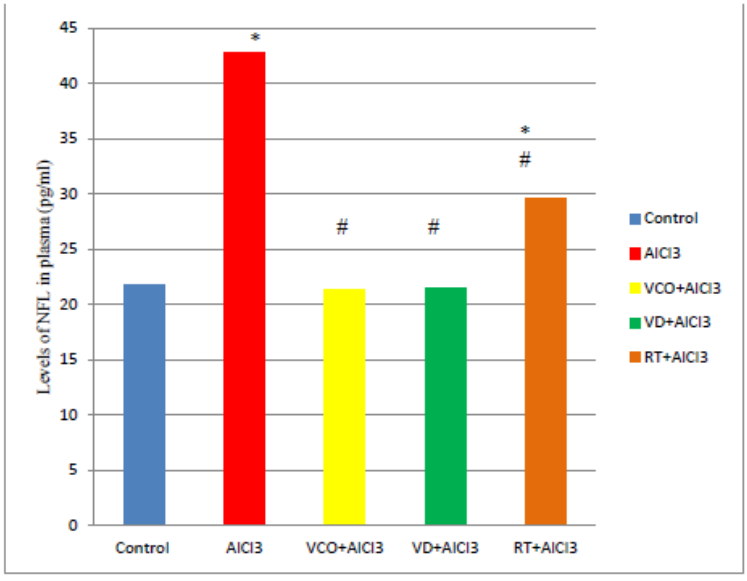

Fig. 7.Virgin coconut oil and vitamin D reaction of Neurofilament Light Chain amounts in plasma (pg/ml) were shown to be preventive versus AlCl3 induced Alzheimer's disease in rats.

The findings were recorded as mean +/- standard error
***: significance versus control (P<0.01).**
#: significance relative to AlCl3 (P<0.01).
Significance was made using One way ANOVA test (Scheffe's test)

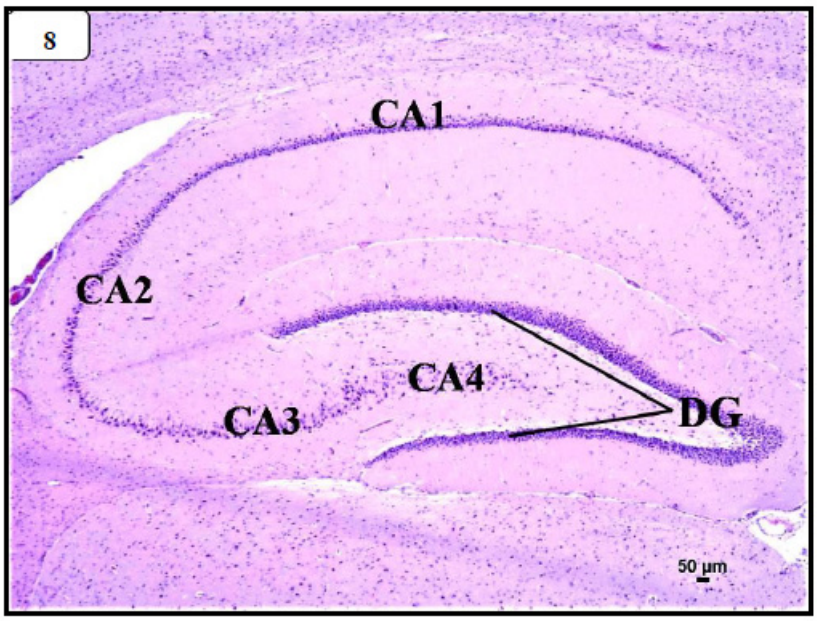

Fig. 8. A photomicrographs of H &E stained brain sections from control group showing C-shaped CA and DG. Notice the different parts of CA: CA1, CA2, CA3, and CA4. [H&E x 40].

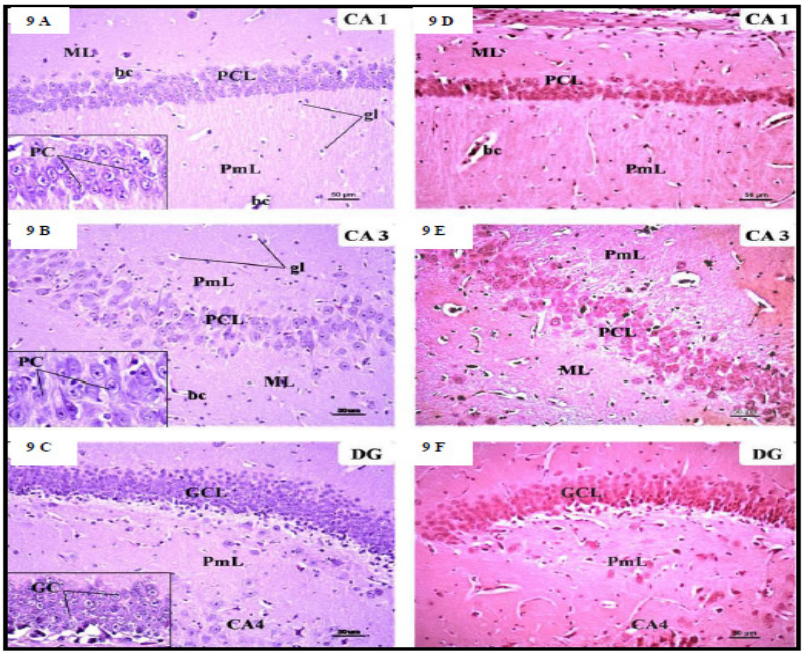

Fig. 9. Representative photomicrographs of the hippocampal regions from control group showing: A) 3 layers of CA1 ML, PCL and PmL. PCL was formed of well-organized compact layers of small PC Both ML and PmL contained some gl with small dark nuclei and perinuclear halos and few bc inside the pinkish neuropil matrix. [H&E x 200, Inset x 400]. B) CA3 was formed of 3 layers; ML, PCL, and PmL. PCL was formed of less packed large PC with large vesicular nuclei with prominent nucleoli and pale basophilic cytoplasm (Inset). Both ML and PmL contained some gl with small dark nucleiand perinuclear halosand few bc inside the pinkish neuropil matrix. [H&E x 200, Inset x 400] C) DG contained 3layers: ML, GCL and PmL. GCL was formed of densely packed rounded to oval GC. [H&E x 200, Inset x 400](D, E, F) showed Congo red-stained sections of CA1, CA3 and DG from control group with no detection of any amyloid plaques deposition [Congo red x 200].

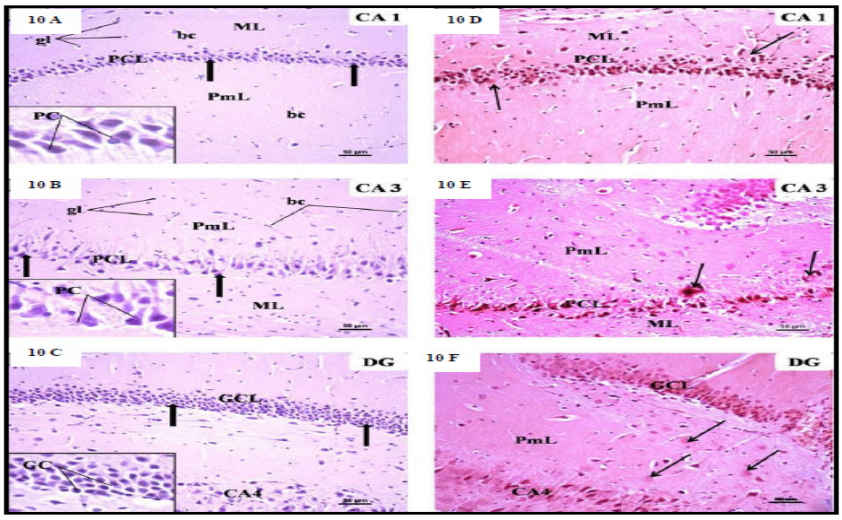

Fig. 10. Representative photomicrographs of the hippocampal regions from $AlCl_3$-treated group showing: A) CA1 with thinner and disorganized PCL as compared to the control group. Most PC appeared shrinked with loss of their vesicular shape and dark cytoplasm (thick arrow). Both ML and PmL layers contained more gl and dilated bc. [H&E x 200,Inset x 400] B) CA3 with thinner or loose PCL as compared to the control group. Most PC appeared shrinked and lost their shape with dark cytoplasm (thick arrow). ML and PmL layers contained more gl and dilated bc. [H&E x 200, Inset x 400] C) DG with dense GCL and darcondensed GC. [H&E x 200, Inset x 400] (D, E, F) showed Congo red-stained sections of CA1, CA3 and DG with multifocal, moderate deposition of amyloid plaques in ML and PmL layers as compared to the control group (arrow head).

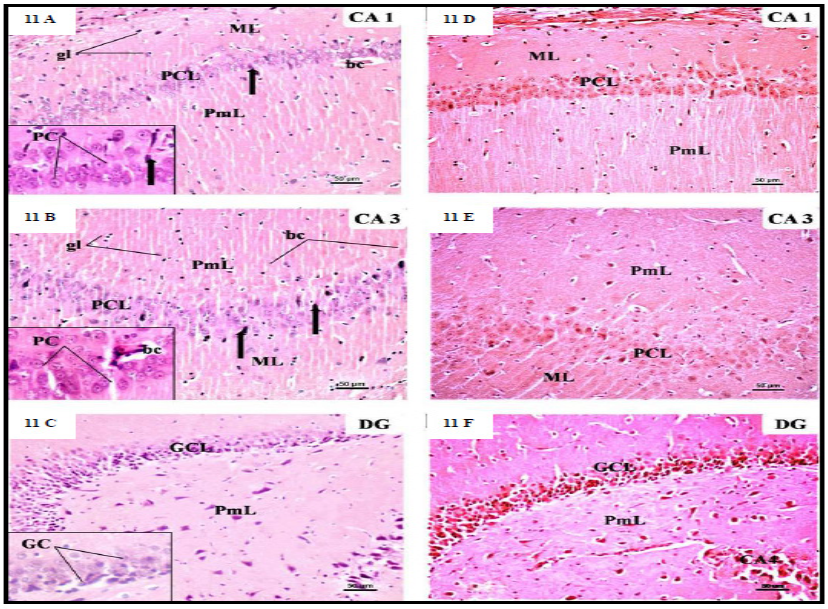

Fig. 11. Representative photomicrographs of the hippocampal regions from AlCl3 and VD treated group showing: A) CA1 was formed of three layers; ML, PCL and PmL. PCL was formed of multiple compact layers of small PC containing large vesicular nuclei and pale basophilic cytoplasm (Inset). Few cells appeared shrinked with dark condensed nuclei (thick arrow) Both ML and PmL contained more gl and more dilated bc. [H&E x 200, Inset x 400] B) CA3 was formed of three layers; the ML, PCL and PmL. PCL was formed of less packed large PC with large vesicular nuclei and pale basophilic cytoplasm (Inset). Both ML and PmL contained some gl and bc. Few cells appeared shrinked with dark condensed nuclei (thick arrow) [H&E x 200, Inset x 400].C) DG was formed of three layers: ML, GCL and PmL. The inset of GCL contained densely packed rounded to oval GC. [H&E x 200, Inset x 400](D, E, F) showed Congo red-stained sections of CA1, CA3 and DG with no detection of any amyloid plaques deposition [Congo red x 200].

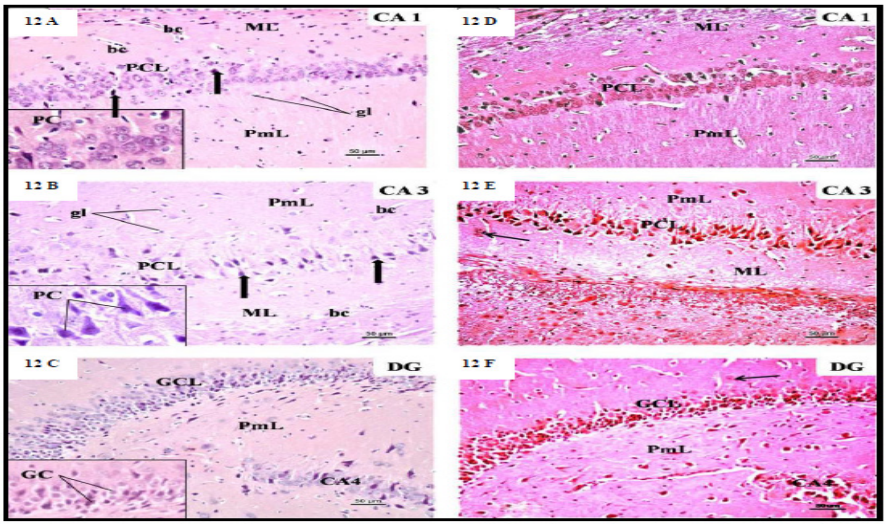

Fig. 12. Representative photomicrographs of the hippocampal regions from AlCl3 and CVO treated group showing: A) CA1 was formed of three layers; ML, PCL and PmL. PCL was formed of multiple compact layers of small PC containing large vesicular nuclei and pale basophilic cytoplasm (Inset). Some cells appeared shrinked with dark condensed nuclei (thick arrow) Both ML and PmL contained more gl and more dilated bc. [H&E x 200, Inset x 400] B) CA3 was formed of three layers; the ML, PCL and PmL. PCL was formed of less packed large PC with large vesicular nuclei and pale basophilic cytoplasm (Inset). Notice the presence of some shrinked cells with dark condensed nuclei (thick arrow). Both ML and PmL contained some gl and bc. [H&E x 200, Inset x 400] C) DG was formed of three layers: ML, GCL and PmL. The inset of GCL contained densely packed rounded to oval GC. [H&E x 200, Inset x 400] (D, E, F) showed Congo red-stained sections of CA1, CA3 and DG with mild detection of few amyloid plaques deposition (arrow) [Congo red x 200].

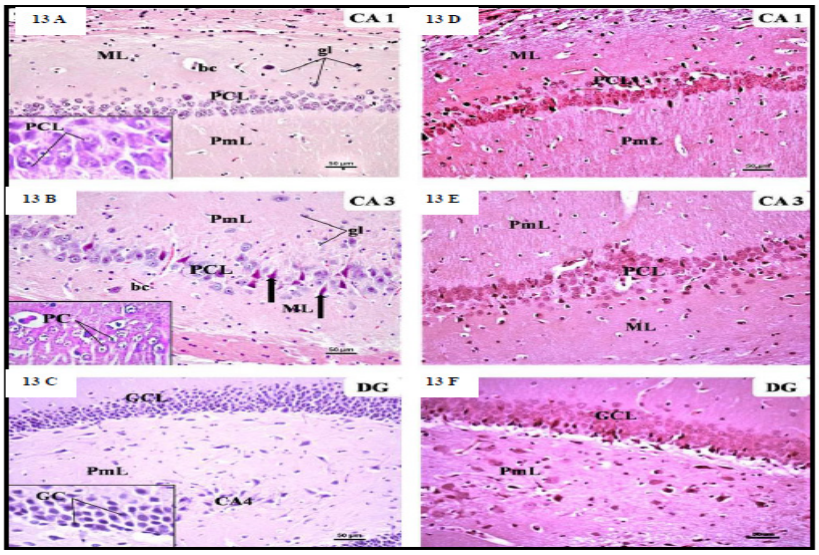

Fig. 13. Representative photomicrographs of the hippocampal regions from AlCl3 and Riva treated group showing: A) CA1 was formed of three layers; outer ML, middle PCL, inner PmL. PCL was formed of multiple compact layers of small PC containing large vesicular nuclei and pale basophilic cytoplasm (Inset). Both ML and PmL contained more gl and more dilated bc. [H&E x 200, Inset x 400] B) CA3 was formed of three layers; the ML, PCL, PmL. PCL was formed of less packed large PC with large vesicular nuclei and pale basophilic cytoplasm (Inset). Some cells appeared shrinked with dark condensed nuclei (thick arrow) Both ML and PmL contained some gl and bc. Few cells appeared shrinked with dark condensed nuclei (thick arrow) [H&E x 200, Inset x 400].C) DG was formed of three layers: ML, GCL and PmL. The inset of GCL contained densely packed rounded to oval GC. [H&E x 200, Inset x 400].(D, E, F) showed Congo red-stained sections of CA1, CA3 and DG with no detection of any amyloid plaque deposition [Congo red x 200].

References

1. Wilson, R.S., Segawa, E., Boyle, P.A., Anagnos, S.E., Hizel, L.P. and Bennett, D.A. The natural history of cognitive decline in Alzheimer's disease. *Psychology and Aging*, **27**(4), 1008–1017 (2012). https://doi.org/10.1037/a0029857

2. Lakshmi, B.V.S., Sudhakar, M. and Prakash, K.S. Protective Effect of Selenium Against Aluminum Chloride-Induced Alzheimer's Disease: Behavioral and Biochemical Alterations in Rats. *Biol Trace Elem. Res.,* **165**, 67–74 (2015). https://doi.org/10.1007/s12011-015-0229-3

3. Sun, Z. Z., Chen, Z. B., Jiang, H., Li, L. L., Li, E. G. and Xu, Y. Alteration of Aβ metabolism related molecules in pre-dementia induced by $AlCl_3$ and d-galactose. *Age,* **31**, 277–284 (2009). https://doi.org/10.1007/s11357-009-9099-y

4. Flaten, T. P. Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. *Brain Res. Bull*., **55**, 187–196 (2001). https://doi.org/10.1016/S0361-9230(01)00459-2

5. Campbell, A. The potential role of aluminium in Alzheimer's disease. *Nephrol. Dial. Transplant.,* **17**, 17–20 (2002). https://doi.org/10.1093/ndt/17.suppl_2.17

6. Exley, C. What is the risk of aluminum as a neurotoxin?. *Expert Review of Neurotherapeutics*, **14**(6), 589-591 (2014). https://doi.org/10.1586/14737175.2014.915745

7. Nampoothiri, M., John, J., Kumar, N., Mudgal, J., Nampurath, G. K. and Chamallamudi, M. R. Modulatory role of simvastatin against aluminium chloride-induced behavioural and biochemical changes in rats. *Behavioural Neurology*, **2015**, 210169 (2015).

8. Jann, M. W. Rivastigmine, a new-generation cholinesterase inhibitor for the treatment of Alzheimer's disease. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, **20**(1), 1-12 (2000).

9. Spencer, C.M. and Noble, S.Rivastigmine a review of its use in Alzheimer's disease. *Drug Aging*., **13**, 391–411(1998).

10. Williams, B.R., Nazarians, A. and Gill, M.A. A review of rivastigmine: a reversible cholinesterase inhibitor. *National Center for Biotechnology Information*, **25**(6),1634-1653 (2003).

11. Marina, A., Che Man, Y.and Amin, I. Virgin coconut oil: emerging functional food oil. *Trends in Food Science & Technology*, **20**, 481–487 (2009).

12. De la Rubia Orti, J.E. How does coconut oil affect cognitive performance in Alzheimer patients? *Nutr. Hosp*., **34** (2), 352-356 (2017). https://doi.org/10.20960/nh.780

13. Alghamdi, B.S. Possible prophylactic anti-excitotoxic and anti-oxidant effects of virgin coconut oil on aluminium chloride-induced Alzheimer's in rat models. *Journal of Integrative. Neuroscience,* **17**, 593–607 (2018).

14. Hossein-Nezhad, A. and Holick, M.F. Vitamin D for health: a global perspective. *Mayo. Clinic. Proceedings*, **88**, 720–755(2013). https://doi.org/10.1016/j.mayocp.2013.05.011

15. Groves, N. J., McGrath, J. J. and Burne, T. H. Vitamin D as a neurosteroid affecting the developing and adult brain. *Annual Review of Nutrition*, **34**, 117-141 (2014).

16. Annweiler, C., Allali, G., Allain, P., Bridenbaugh, S., Schott, A. M., Kressig, R. W. and Beauchet, O. Vitamin D and cognitive performance in adults: a systematic review. *European Journal of Neurology*, **16**(10), 1083-1089 (2009).

17. Brewer, L.D.; Thibault, V. and Chen, K.C.Vitamin D hormone confers neuroprotein in parallel with down regulation of L-type calcium channel expression in hippocampal neurons. *Journal of Neuroscience*, **21**, 98–108 (2001). https://doi.org/10.1523/JNEUROSCI.21-01-00098.2001

18. Ibi, M.; Sawada, H. and Nakanishi, M.Protective effects of 1 alpha, 25-(OH) (2) D(3) against the neurotoxicity of glutamate and reactive oxygen species in mesencephalic culture. *Neuropharmacology*, **40**, 761–771 (2001). https://doi.org/10.1016/S0028-3908(01)00009-0

19. Olanrewaju, J.A. ; Akinola, O.B.; Olatunji, S.Y.; Owolabi, J.O.; Fabiyi, O.S.; Ukonne, U.E. and Desalu, A.B.O. Effects of Virgin Coconut Oil on Aluminium Chloride-Induced Alzheimer-Like Dementia in the Prefrontal Cortex. *Journal of Advances in Medical and Pharmaceutical Sciences*, **18**(1), 1-12 (2018).

20. Abdel Aal, R.; Assi, A. and Kostandy, B.Rivastigmine reverses aluminum-induced behavioral changes in rats. *European Journal of Pharmacology,* **659**(2-3), 169-176 (2011).

21. Alghamdi, B.S. Possible prophylactic anti-excitotoxic and anti-oxidant effects of virgin coconut oil on aluminium chloride-induced Alzheimer's in rat models. *Journal of Integrative. Neuroscience,* **17**, 593–607(2018).

22. Morello, M.; Landel, V.; Lacassagne, E. and Baranger, k. Vitamin D Improves Neurogenesis and Cognition in a Mouse Model of Alzheimer's Disease. *Molecular Neurobiology*, **55**, 6463–6479 (2018).

23. Prieur, E. and Jadajavi, N. Assessing Spatial Working Memory Using the Spontaneous Alternation Y-maze Test in Aged Male Mice. *Bio. Protocol*., **9** (3), e3162 (2019).

24. Apostolova, L. G. Alzheimer disease. Continuum: *Lifelong Learning in Neurology,* **22** (2), 419–434 (2016).

25. Lane, R. M., Potkin, S. G. and Enz, A. Targeting acetyl cholinesterase& butyrylcholinesterase in dementia. *International Journal of Neuropsychopharmacology*, **9**(1), 101-124 (2006).

26. Bui, T. T. and Nguyen, T. H. Natural Product for the Treatment of Alzheimer's disease. *Journal of Basic and Clinical Physiology and Pharmacology*, **28**(5), 413-423 (2017).

27. Bhattacharjee, S., Zhao, Y., Hill, J. M., Percy, M. & Lukiw, J. Aluminum and its potential contribution to Alzheimer's disease (AD). *Frontiers in Aging Neuroscience*, **6**, 62, 1-3 (2014).

28. Alghamdi, B.S. Possible prophylactic anti-excitotoxic and anti-oxidant effects of virgin coconut oil on aluminium chloride-induced Alzheimer's in rat models. *Journal of Integrative. Neuroscience,* **17**, 593–607 (2018).

29. Olanrewaju, J.A. ; Akinola, O.B.; Olatunji, S.Y.; Owolabi, J.O.; Fabiyi, O.S.; Ukonne, U.E. and Desalu, A.B.O. Effects of Virgin Coconut Oil on Aluminium Chloride-Induced Alzheimer-Like Dementia in the Prefrontal Cortex. *Journal of Advances in Medical and Pharmaceutical Sciences*, **18**(1), 1-12(2018).

30. Annweiler, C.; Schott, A.M. and Berrut, G.Vitamin D and aging: neurological issues. *Neuropsychobiology*, **62**,139–150 (2010).

31. Lewczuk, P., Ermann, N., Andreasson, U., Schultheis, C., Podhorna, J., Spitzer, P. and Zetterberg, H. Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. *Alzheimer's Research &Therapy*, **10**(1), 1-10 (2018).

32. Rombouts, S. A. R. B., Barkhof, F., Van Meel, C. S. and Scheltens, P. Alterations in brain activation during cholinergic enhancement with rivastigmine in Alzheimer's disease. *Journal of Neurology, Neurosurgery & Psychiatry*, **73**(6), 665-671(2002).

33. Salat, D. and Mehta, V. Effectiveness of vitamin D supplements in the patients suffering from Alzheimer disease. *International Journal of Advanced Research in Medicine,* **3**(2), 95-97 (2021). https://doi.org/10.22271/27069567.2021.v3.i2b.223

34. Ibi, M.; Sawada, H.; Nakanishi, M.; Kume, T.; Katsuki, H.; Kaneko,S.; Shimohama, S. and Akaike, A.Protective effects of 1 alpha, 25-(OH) (2) D(3) against the neurotoxicity of glutamate and reactive oxygen species in mesencephalic culture. *Neuropharmacology,* **40**, 761–771 (2001). https://doi.org/10.1016/S0028-3908(01)00009-0

دراسة مقارنة لتأثير زيت جوز الهند البكر وفيتامين د على مرض الزهايمر المستحدث بواسطة كلوريد الألومنيوم في قرن آمون في ذكور الجرذان البيضاء

عهود طالب الدمح ، فاطمة عبد الرحمن النفيعي و ابتسام عبد الله باوزير
قسم العلوم البيولوجيه - كلية العلوم - جامعة جدة - جدة - المملكة العربية السعودية.

الملخص

مرض الزهايمر هو اضطراب عصبي يؤدي إلى الخرف في جميع أنحاء العالم. تهدف هذه الدراسة إلى تحديد آثار زيت جوز الهند البكر وفيتامين دال على مرض الزهايمر المستحدث بواسطة كلوريد الألومنيوم في الجرذان. تم تقسيم ثلاثين ذكر بالغ من الجرذان البيضاء إلى خمس مجموعات (ستة جرذان لكل مجموعة) المجموعة الأولى استخدمت كمجموعة ضابطة ،المجموعة الثانية أعطيت جرعة فموية قدرها ٢٠٠ ملغم لكل كجم من وزن الجسم من كلوريد الأمونيوم يوميا لمدة ٦ أسابيع؛ تم اعطاء المجموعة الثالثة فيتامين دال عن طريق الفم يومياً بجرعة ٥٠٠ وحدة دولية لكل كجم من وزن الجسم. لمدة ٦ أسابيع وجرعة فموية قدرها ٢٠٠ ملغم لكل كجم من وزن الجسم كلوريد الألمونيوم بعد ساعة واحدة. المجموعة الرابعة أعطيت جرعة فموية قدرها ٥ مل من زيت جوز الهند البكر وجرعة ٢٠٠ ملغم لكل كجم من وزن الجسم بعد ساعة واحدة من تناول كلوريد الألمونيوم يومياً لمدة ٦ أسابيع كما تلقت المجموعة الأخيرة جرعة فموية قدرها ١.٥ ملغم لكل كجم من وزن الجسم من عقار الريفاستيجمين يومياً لمدة ٦ أسابيع وجرعة فموية قدرها ٢٠٠ ملغم لكل كجم من وزن الجسم كلوريد الألمونيوم بعد ساعة واحدة. بعد انتهاء المدة المحدده، تم إجراء الاختبار السلوكي على الجرذان لتقييم التعلم والذاكرة، وتم إجراء دراسات فسيولوجية ونسيجية في منطقة قرن آمون وبلازما الدم لتحديد تأثير زيت جوز الهند البكر و فيتامين دال على مرض الزهايمر المستحدث بواسطة كلوريد الألمونيوم. أدى كلوريد الألمونيوم إلى انخفاض كبير في الذاكرة واختبار متاهة التعلم. علاوة على ذلك، هناك زيادة كبيرة في مستوى انزيم اسيتيل كولين استريز و بيروكسيد الدهون و سلسلة بروتين الخيوط العصبية ، إلى جانب انخفاض كبير في مستوى الدوبامين، و الجلوتاثيون و فوق اكسيد الدسموتاز. في الفحص النسيجي لقرن آمون، لاحظنا زيادة في فقدان الخلايا العصبية في الجرذان المعالجة بكلوريد الألمونيوم بينما أظهرت الجرذان المعالجة بزيت جوز الهند البكر و فيتامين دال انخفاضًا في الأضرار الناجمة عن كلوريد الألمونيوم والمرتبطة بالتحسينات في التغيرات السلوكية والفسيولوجية. وفقا لهذه الدراسة، فإن جرعة يومية عن طريق الفم من زيت جوز الهند البكر و فيتامين دال تعمل على تحسين التعلم والذاكرة في ذكور الجرذان البيضاء.

الكلمات الدالة: مرض الزهايمر، كلوريد الألومنيوم، فيتامين دال، زيت جوز الهند البكر، قرن آمون.