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Carvedilol Protects against Cognitive Dysfunction through Inhibition of Amyloid Beta (1-42) and Inflammation in Aluminum Chloride Induced Alzheimer's Disease Compared to Donepezil as a Reference Drug in Wistar Rats

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Abstract

The current study aimed to assess and compare the preventive effects of carvedilol (CAR) and donepezil (DONP) on aluminum chloride ($AlCl_3$)-induced Alzheimer's Disease (AD). A total of 40 male adult Wistar rats were allocated into four groups at random (10 rats each). Saline was given to the control group intraperitoneally (i.p) for six weeks. The second group received saline for two successive weeks followed by 70 mg/kg/day $AlCl_3$ i.p. for four successive weeks. The third group received 1 mg/kg CAR i.p. for two sequential weeks, followed by CAR and $AlCl_3$ concurrently for four weeks. The fourth group received DONP 0.75 mg/kg i.p. alone for two successive weeks, followed by DONP and $AlCl_3$ simultaneously for a further four weeks. $AlCl_3$ administration demonstrated a marked discrepancy in learning and memory indicated by a deficit in behavioral tests, elevation of serum and brain nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), aluminum (Al), acetylcholinesterase (AChE), and amyloid-beta (Aβ)$_{(1-42)}$ protein levels. On the other hand, $AlCl_3$ administration significantly reduced superoxide dismutase (SOD) and catalase (CAT) activities compared to the control group. However, treating rats with CAR or DONP concurrently with $AlCl_3$ produced significant amelioration of behavioral test measurements, accompanied by a significant improvement in biochemical parameters. In addition, histopathological changes confirmed the behavioral and biochemical results. Moreover, CAR produced significant effects compared to DONP. The present study suggests that CAR protects against $AlCl_3$-induced cognitive dysfunction through its anti-inflammatory, antioxidant, and protective effects against Aβ in a rat model of Alzheimer's disease.

Keywords: Alzheimer's disease; Amyloid Beta; Carvedilol; Donepezil; Oxidative Stress; Behavioral tests.

1. Introduction

Aluminum (Al) is a copious metal on the earth, and a cumulative toxic heavy metal in the body, moreover, it can reach the body through medicine, cooking materials, and water leading to toxic effects in many organs **(Willhite et al. 2014)**. The brain is a possible target for aluminum toxicity, it accumulates mainly in the hippocampus and frontal cortex producing abnormal accumulation of amyloid-beta (Aβ), neuroinflammation, and neuronal necrosis **(Cheng et al. 2019; Chiroma et al. 2019)**.

Among the elderly, Alzheimer's disease (AD) is a widespread neurodegenerative disease with a gradual decline in cognitive function significantly affecting the quality of life, which is its major feature (**Rossini et al. 2020**). The pathological marks of AD involve extracellular aggregation of Aβ protein in the brain and hyperphosphorylation of tau protein inside neurons (**Hardy and Selkoe 2002; Ahmad et al. 2020**), as well as, neuroinflammation, mitochondrial damage, oxidative stress which are associated with synaptic and neuronal loss (**Henstridge et al. 2016**). Many types of research revealed that atrophy of cholinergic neurons in the basal forebrain in the early AD is supposed to mediate short-term memory loss (**Maurer and Williams 2017**). However, it was detected that neuronal cell survival was affected by N-methyl-D-aspartic acid receptor (NMDAR) signaling, where its over-stimulation has an important role in the AD-related progressive neuronal loss (**Wang and Reddy 2017**).

Based on the previous facts, some drugs are currently used for the treatment of AD. Some of them are acetylcholinesterase inhibitors (AChEi) such as donepezil (DONP), rivastigmine, and galantamine. Others are glutamate antagonists such as memantine (**Anand and Singh 2013; Andrieu et al. 2015**). After decades of research, long-term clinical uses demonstrate that these agents have played a significant role in the management of the early stage of AD, but they cannot prohibit or reverse the progression of the disease (**Mossello and Ballini 2012**).

Mid-life elevation of blood pressure has been predictable as a risk factor for AD, and its relationship with AD has been recognized (**Law and Yeong 2020**). Carvedilol (CAR), a nonselective β-adrenergic receptor blocker, is vastly used in congestive heart failure, hypertension, and ischemic heart disease (**Packer et al., 1996; Kumar et al., 2011**). Moreover, it has an antioxidant activity attributed to its carbazole moiety, and it is around 10-fold more effective than vitamin E as an antioxidant (**Savitz et al. 2000**). In addition, CAR has anti-inflammatory and antiapoptotic effects through its modulating property on caspase-3 and nuclear factor-erythroid factor 2-related factor 2 (Nrf2) pathways (**Ahmed and Mohammed 2021**). Considering this, we evaluated the possible protective effect of CAR on cognitive dysfunction induced by AlCl₃ and compared its effect to DONP as a standard drug. In addition, we investigated the mechanisms by which CAR can produce this effect.

2. Materials and Methods

2.1. Animals

Adult male Wistar rats (n=40) weighing 200-250 grams were used. Animals were got from the animal residence, Faculty of Medicine, Sohag University, with room temperature kept at 22-24ºC. Animals were kept in the normal light/dark cycle and were fed an ordinary animal diet with free access to water. Following the criteria of the EU Directive 2010/63/EU for animal studies, the experimental procedure was carried out and approved by the Scientific Research Ethical Committee of the Faculty of Medicine, Sohag University, Egypt (Approval No. IACUC 5/13/2021/02).

2.2. Drugs and chemicals

Alpha Chemika provided aluminum chloride (AlCl₃) (Mumbai, India). AK Scientific, Inc. was the seller of CAR (USA). DONP and acetylcholinesterase (AChE) kits (UK) were purchased from Sigma Aldrich Company. Bio-diagnostic Co. Egypt provided superoxide dismutase (SOD), catalase (CAT), and nitric oxide (NO) kits. Wuhan EIAab Science Co. Ltd provided a kit for measuring tumor necrosis factor-alpha (TNF-α) (China). Kit for measuring total protein levels was purchased from the Egyptian Company for Biotechnology, Egypt. Kit for measuring Aβ was obtained from SinoGeneClon Co., Ltd, (China).

2.3. Experimental design

Rats were randomly classified into four groups (10 animals each). The control group was daily treated with normal saline for the entire period of the experiment. AlCl₃ treated group was daily injected with normal saline for two successive weeks followed by daily administration of AlCl₃ at a dose of 70 mg/kg/day for four successive weeks (**Ali et al. 2016**). The CAR-treated group was daily injected with CAR 1mg/kg (**Veerendra Kumar and Gupta 2003**) only for two consecutive weeks, followed by AlCl₃ associated with CAR for four successive weeks. DONP treated group was daily administered 0.75 mg/kg DONP alone (**Cutuli et al. 2013**) for two consecutive weeks followed by administration of AlCl₃ concomitant with DONP for four successive weeks. The method of administration of all drugs in all groups was

through the intraperitoneal route.

2.4. Behavioral tests

2.4.1. Morris water maze (MWM) test

It is used to evaluate the spatial learning and memory abilities of a rat. The test is made up of a big rotund puddle placed in an illuminated room, (180 cm in diameter and 60 cm in height) filled with water to 40 cm depth at $28 \pm 1°C$; non-toxic dye was used to make the water opaque. The puddle is equally chopped into four quadrants with letters N, S, E, W placed on its edge. 9 cm diameter rounded platform was put in one quadrant. The platform was 2.5 cm above the water level throughout the acquisition phase, while 2.5 cm beneath the water level during the retrieval phase. In the acquisition phase, the rat was dropped gently in the water facing the puddle wall and allowed 120 sec to reach the platform; if the rat failed, it was guided. The rat was let stay on the platform for 20 sec. The initial acquisition latency (IAL) was measured by the time it took the rat to reach the platform. Each rat was imperiled to 4 successive trials, 5 min between each.

Following 24 h after the IAL, each rat was randomly freed in the puddle at one of the edges facing the wall of the puddle to examine the retrieval of the learned task termed retrieval latency (RL) (**Veerendra Kumar and Gupta 2003**).

2.4.2. Novel object recognition (NOR) test

The test is used to measure memory alterations based on rodents' natural need to investigate new objects (looking at, sniffing, touching) over familiar ones. The familiarization phase was made 24 h before the test phase. Familiarization was done by placing each animal in a black-painted wood open-field box (60×60×40 cm) containing two identical objects in two adjoining corners, 9 cm from the walls, with his head positioned opposite the objects. The time allowed is 15 sec for exploring these objects. Exploration was defined as steering the nose at an interval of ≤ 2 cm away from the object with the nose or paws touching it. The action of turning around or sitting on the object was not included in the list. A rat was excluded from the experiment when failing to examine the objects for 15 sec with a cut-off time of 4 min. Between trials, the test box and objects were cleaned with 70% ethyl alcohol. In the test phase, animals were subjected to the same object previously seen during the familiarization phase and a novel object (the two objects are different in shape and color). The time

spent examining each object by the animals was recorded using a stopwatch. Memory preference was evaluated using the recognition index (**Farhat et al. 2017**).

$$Recognition\ index = Tn/(Tn + Tf)\ x\ 100$$

Tn = time spent exploring the novel object.
Tf = time spent exploring the familiar object.

2.4.3. Passive avoidance test

The test was performed in the apparatus model consisting of two chambers (20 ×25 ×30 cm each) separated by a wall containing a communicating hole of 8 cm diameter. One chamber was maintained lighted. On the first day, acquisition trials were conducted, rats were located individually in the lighted room, and once they entered the dark chamber, painful stimuli (electric shock 40V, 0.6 mA for 2 sec) was delivered to their foot. The rats were promptly returned to their cages. In the retention trial, performed 24 h later, the rats were located again in the lighted room, and the period between placement in the lighted room and entry to the dark one was recorded (step-through latency). If the rats did not enter the dark chamber within the 5 min test period, the test was ended, and the step-through latency was recorded as 300 sec (**Abdel-Aal et al. 2011**).

2.5. Sample collection

2.5.1. Blood samples

Twenty-four hours after the last behavioral test, rats were anesthetized by inhaled diethyl ether before decapitation, blood samples were collected in labeled centrifuge tubes. The serum was separated following centrifugation of blood at 3500 rpm for 10 min and stored quickly at –20ºC until the analysis time.

2.5.2. Tissue samples

The brain of each animal was rapidly dissected, washed with cold isotonic saline, and weighed. The brain was divided into three portions. The first one was homogenized in 10 ml ice-cold sodium phosphate buffer (50mM, pH 7.4) per gram tissue (v/w) and centrifuged at 4000 rpm for 15 min at 4ºC; the supernatant was separated for biochemical analysis. The second part was used to estimate the Al concentration, the samples were weighed and baked in an oven at 80°C. The containing part was

fixed in 10% formalin solution and prepared for histopathological studies.

2.6. Biochemical analysis

2.6.1. Determination of superoxide dismutase (SOD) activity

Serum and brain SOD levels were measured by the colorimetric method. Kit was purchased from Bio-diagnostic Co. Egypt CAT. No. SD 2521 The assay depends on the capability of the SOD enzyme to prevent the phenazine methosulphate-mediated reduction of nitro blue tetrazolium (NBT) dye. The changes in absorbance were evaluated at 560 nm. SOD activity in serum and brain tissues was represented as U/ml and U/mg of protein, respectively (**Nishikimi et al. 1972**).

2.6.2. Determination of catalase (CAT) activity

Colorimetric technique was used to quantify serum and brain CAT activity. Kit was obtained from Bio-diagnostic Co. Egypt with CAT. No. CA 2517. The assay is based on CAT reacting with a known amount of H_2O_2; the reaction is terminated with a CAT inhibitor in the presence of peroxidase, and the remaining H_2O_2 reacts with 3,5-Dichloro-2-hydroxybenzene sulfonic acid and 4- amino-phenazone to synthesize a chromophore. The absorbance alteration was estimated at 510 nm. The activity of CAT in serum and brain tissues was measured in U/L and U/mg protein, respectively (**Aebi 1984**).

2.6.3. Determination of nitric oxide (NO) level

According to Montgomery and Dymock, serum and brain NO levels were measured using a colorimetric method. Kits were obtained from Bio-diagnostic Co. Egypt with CAT. No. NO 2533. The method depends on the formation of nitrous acid diazotize sulphanilamide coupled with N-(1-naphthyl) ethylenediamine in the presence of nitrite and acid environment. The changes in absorbance were estimated at 540 nm. The Nitrite levels in the serum and brain samples were represented in µmol/L and µmol/mg protein, respectively (**Montgomery and Dymock 1961**).

2.6.4. Determination of tumor necrosis factor-alpha (TNF-α) level

An ELISA kit; purchased from Wuhan EIAab Science Co. Ltd (China); was used to determine the serum and brain levels of TNF-α according to the

hippocampus manufacturer's instructions CAT. No: E0133r. The Stat fax 2600 microplate reader (Awareness Technologies, Palm City, USA) was used to determine the results. TNF-α levels in the blood and the brain were measured in pg/ml and pg/mg protein, respectively.

2.6.5. Determination of aluminum (Al) concentration

Al levels in serum or brain tissues were estimated by PerkinElmer Atomic Absorption spectrometer (Analyst 4000, USA) with a hollow cathode lamp at 309.3 nm. 200 ul of serum or 1gm of brain tissue was dried at 80°C. 2.5 ml of a mixture of perchloric acid ($HCLO_4$) and 30% nitric acid HNO_3 4:1 was added to the samples at 100°C for 5 min for digestion. Standard Al solution was prepared using Al stock solution (1000 ug/ml) (CPI International, USA). The serum and brain tissue Al levels were calculated in µg/ml and µg/g tissue, respectively (**Zhu et al. 2014**).

2.6.6. Determination of acetylcholinesterase (AChE) activity

Brain levels of AChE were measured with kits obtained from sigma Aldrich Company (UK) with CAT. No MAK119 using a spectrophotometric method according to **Ellman (1961)**, using a microtiter plate reader (Awareness Stat Fax- 2200, USA). 10 µl of brain homogenate was added to dithionitrobenzoic acid (DTNB) buffer for a final volume of 195µl, then incubated for 10 min at 26°C. Five µl of the substrate acetylthiocholine was added, where yellow ions of thionitrobenzoic acid (TNB) were formed. The absorbance changes were measured at 412 nm, and the level of AChE was expressed as µmol/min/mg protein (**Nostrandt et al. 1993**).

2.6.7. Determination of amyloid-beta (Aβ1-42) level

Brain levels of Aβ1-42 were measured using sandwich enzyme-linked immunosorbent assay (ELISA) kits obtained from SinoGeneClon Co., Ltd, (China) CAT. No SG-21230. 50 µL of standard solution and 40 µL of sample diluent were added to standard and sample wells respectively, then 10 µL of brain tissue homogenate was added to sample wells, mixed gently, and incubated for 30 min at 37℃. The washing solution was added and drained, then 50 µL of HRP-conjugate reagent was added to each well. 50 µL of chromogen solution A and B followed by a stop solution were added. After 15 min, absorbance was measured at 450 nm using an ELISA microplate reader (Awareness Stat

Fax- 2200, USA). The concentration of $A\beta_{1-42}$ in the samples was determined by comparing the optical density (OD) of the samples to the standard curve. Brain $A\beta_{1-42}$ levels were expressed in ng/mg protein **(Xia et al. 2009)**.

2.6.8. Determination of total protein

Total protein content in brain tissue was measured by the Biuret method **(Gornall et al. 1949)** and was used to demonstrate the concentration of different brain parameters per mg protein.

2.7. Histopathological Examination

After fixation of hippocampal tissue in 10% neutral formalin and dehydration in ascending concentrations of alcohol, it was then instilled in Paraffin. Micro-Teck microtome (Germany) was used to prepare slices of 5 μm thickness. After 5 minutes of hematoxylin staining, the slides were rinsed in tap water for 3 minutes. Then, they were stained in 1% aqueous eosin solution for 1 min, then washed in the tap water for 3 min, and finally dehydrated alcohol. Slides were cleared in xylene and studied by an Olympus (model CX21) Japan light microscope.

2.8. Statistical analysis

The SPSS program, version 25 software package, was used to conduct all statistical analyses. The values were described as mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was used to statistically examine the data. Furthermore, to assess differences among individual groups, a Tukey post hoc test was used. When $P < 0.05$, the difference was considered significant.

3. Results

3.1. Morris water maze (MWM) test

Table 1 revealed that i.p. administration of $AlCl_3$ (70 mg/kg/day) to rats for four successive weeks produced a significant increase ($p< 0.01$) in IAL and RL of the MWM test compared to the control group. However, daily i.p. administration of CAR or DONP alone for two weeks and concomitant with $AlCl_3$ for four successive weeks produced significant improvement ($p<0.01$) in the IAL and RL of the MWM test compared to the $AlCl_3$ group. The CAR-treated group revealed more reduction in IAL and RL than the DONP-treated group but with no statistically significant alterations (p=0.28 and

p=0.08), respectively.

3.2. Novel object recognition (NOR) test

Administration of $AlCl_3$ (70 mg/kg/day) to rats for four successive weeks produced a significant decrease ($p< 0.01$) in the recognition index of the NOR test compared to the control group. However, daily i.p. treatment with CAR or DONP for two successive weeks and simultaneously with $AlCl_3$ for four successive weeks led to a significant increase ($p< 0.01$) in the recognition index of NOR test compared to the $AlCl_3$ treated group with no statistically significant difference between CAR and DONP treated groups **(Table 2)**.

3.3. Passive avoidance test

Step through latency duration of the passive avoidance test showed a significant decrease ($p< 0.01$) in $AlCl_3$ treated group compared to the control group. However, treatments of the rats with either CAR or DONP alone and then simultaneously with $AlCl_3$ showed a significant elevation ($p< 0.01$) in step-through latency of the Passive avoidance test compared to the $AlCl_3$ treated group with no significant variance between CAR and DONP treated groups **(Table 3)**.

3.4. Effects of CAR and DONP on body weight

Fig. 1 demonstrates that $AlCl_3$ administration to experimental animals produced a significant reduction ($p<0.01$) in rat's body weight compared to the control group. Whilst, administration of CAR or DONP concomitant with $AlCl_3$ produced significant restoration ($p<0.01$) of body weight near to control values compared to $AlCl_3$ treated group with no significant alteration (p=0.92) between CAR and DONP treated groups.

3.5. Effects of CAR and DONP on SOD activity

Serum and brain activities of SOD were significantly decreased ($p< 0.01$) in $AlCl_3$ treated group compared to the control group. However, there was a significant elevation ($p< 0.01$) in serum and brain levels of SOD in both CAR and DONP compared to the $AlCl_3$ treated group, where CAR produced a significant increase ($p< 0.05$) compared to the DONP treated group **(Fig. 2)**.

Table 1. Effect of carvedilol (1mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on IAL and RL in Morris Water Maze test, in AlCl₃ -induced Alzheimer's disease in rats.

Groups	IAL (sec)	RL (sec)
Control	39.50 ± 1.01	14.60 ± 0.95
AlCl₃	55.60 ± 3.47 **	52.20 ± 3.20 **
AlCl₃ + CAR	26.30 ± 2.10••	13.60 ± 0.91••
AlCl₃ + DONP	32.00 ± 1.43••	20.3 ± 1.54••

All data are expressed as the mean $\pm$ SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at ($p < 0.01$) vs. control group. •• Significant change at ($p < 0.01$) vs. AlCl₃-treated group. IAL= initial acquisition latency, RL= retrieval latency. AlCl₃=aluminum chloride, CAR=carvedilol, DONP =donepezil.

Table 2. Effect of carvedilol (1mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on recognition index in Novel object recognition test in AlCl₃-induced Alzheimer's disease in rats.

Groups	Recognition index
Control	68.30 ± 1.00
AlCl₃	47.65 ± 1.42**
AlCl₃ + CAR	61.85 ± 3.02••
AlCl₃ + DONP	57.38 ± 1.10 ••

All data are expressed as the mean $\pm$ SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at ($p < 0.01$) vs. control group. •• Significant change at ($p < 0.01$) vs. AlCl₃-treated group. AlCl₃=aluminum chloride, CAR=carvedilol, DONP =donepezil.

Table 3. Effect of carvedilol (1 mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on step-through latency of Passive avoidance test in AlCl₃ -induced Alzheimer's disease in rats.

Groups	Step-through latency (sec)
Control	238.00 ± 21.85
AlCl₃	122.10 ± 7.21**
AlCl₃ + CAR	254.30 ± 9.66••
AlCl₃ + DONP	212.20 ± 11.53••

All data are expressed as the mean $\pm$ SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at ($p < 0.01$) vs. control group. •• Significant change at ($p < 0.01$) vs. AlCl₃-treated group. AlCl₃=aluminum chloride, CAR=carvedilol, DONP =donepezil.
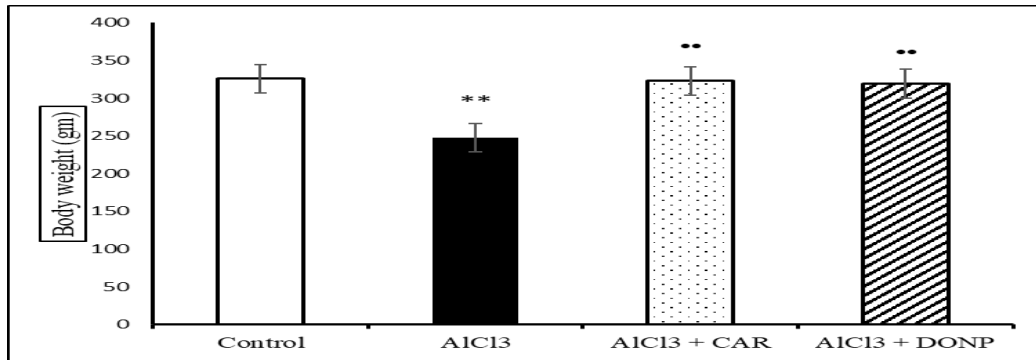
Figure 1. Effect of carvedilol (1 mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on body weight in AlCl$_3$-induced Alzheimer's disease in rats.
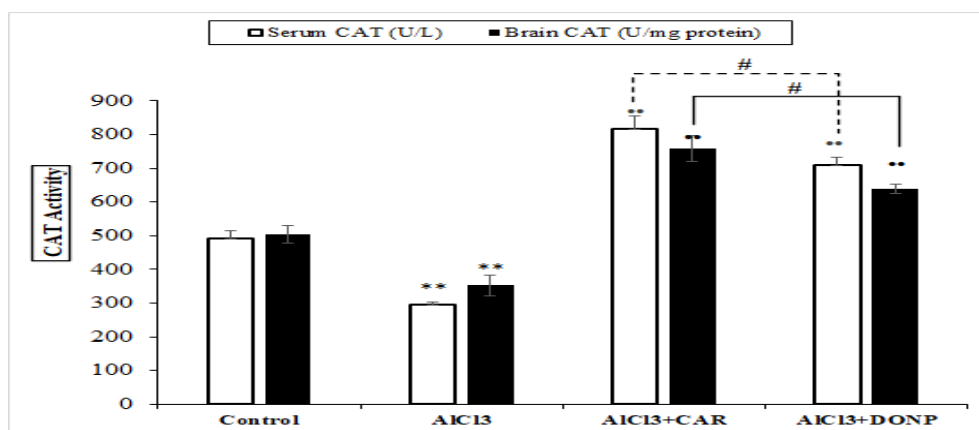
All data are expressed as the mean ± SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at (p < 0.01) vs. control group. •• Significant change at (p < 0.01) vs. AlCl$_3$-treated group. AlCl$_3$=aluminum chloride, CAR=carvedilol, DONP =donepezil.
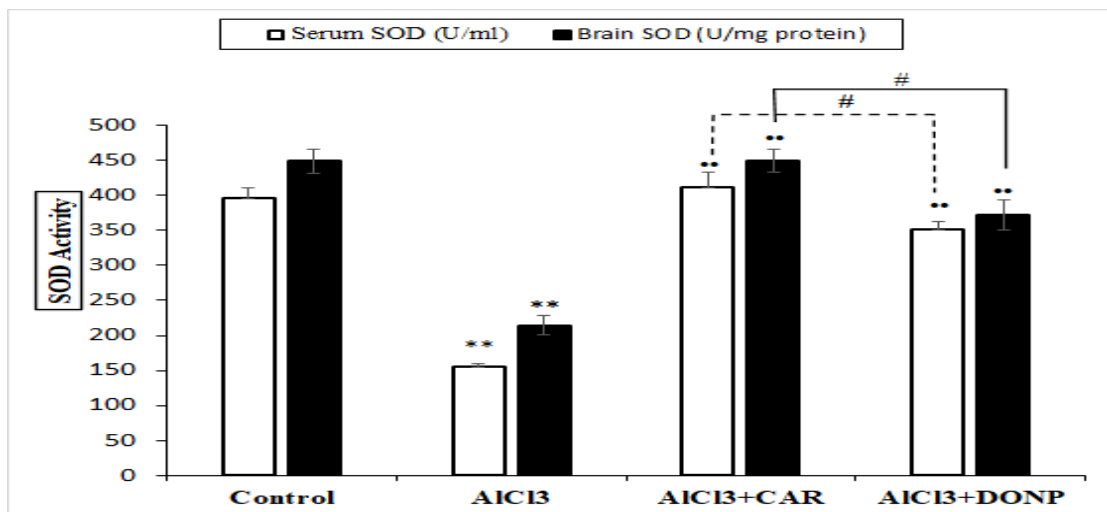
Figure 2. Effect of carvedilol (1 mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on serum and brain SOD activity in AlCl$_3$-induced Alzheimer's disease in rats.

All data are expressed as the mean ± SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at (p < 0.01) vs. control group. •• Significant change at (p < 0.01) vs. AlCl$_3$-treated group. # Significant change at (p < 0.05) vs. AlCl$_3$+CAR treated group. SOD=superoxide dismutase, AlCl$_3$=aluminum chloride, CAR=carvedilol, DONP =donepezil.

3.6. Effects of CAR and DONP on CAT activity

As shown in Fig. 3, daily i.p. administration of 70 mg/kg/day AlCl$_3$ for four successive weeks resulted in a significant decrease (p< 0.01) in serum and brain CAT activity compared to the control group. Furthermore, compared to AlCl$_3$ treated group, administration of CAR and DONP before and concomitant with AlCl$_3$ for four successive weeks resulted in a significant increase (p< 0.01) in serum and brain CAT activity. However, there was a significant discrepancy (p< 0.05) between the CAR and DONP treated groups (**Fig. 3**).

3.7. Effects of CAR and DONP on NO level

Treatment of experimental animals with AlCl$_3$ led to a significant elevation in both serum and brain levels of NO compared to the control group. However, treatments of the rats with either CAR or DONP concurrently with AlCl$_3$ led to a significant decrease (p< 0.01) in both serum and brain NO levels compared to AlCl$_3$ treated group. However, CAR produced a significant decline (p< 0.05) compared to DONP treated group (**Fig. 4**).

Figure 3. Effect of carvedilol (1 mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on serum and brain CAT in AlCl$_3$-induced Alzheimer's disease in rats. All data are expressed as the mean ± SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at ($p < 0.01$) vs. control group. •• Significant change at ($p < 0.01$) vs. AlCl$_3$-treated group. # Significant change at ($p < 0.05$) vs. AlCl$_3$+CAR treated group. CAT=catalase, AlCl$_3$=aluminum chloride, CAR=carvedilol, DONP =donepezil.
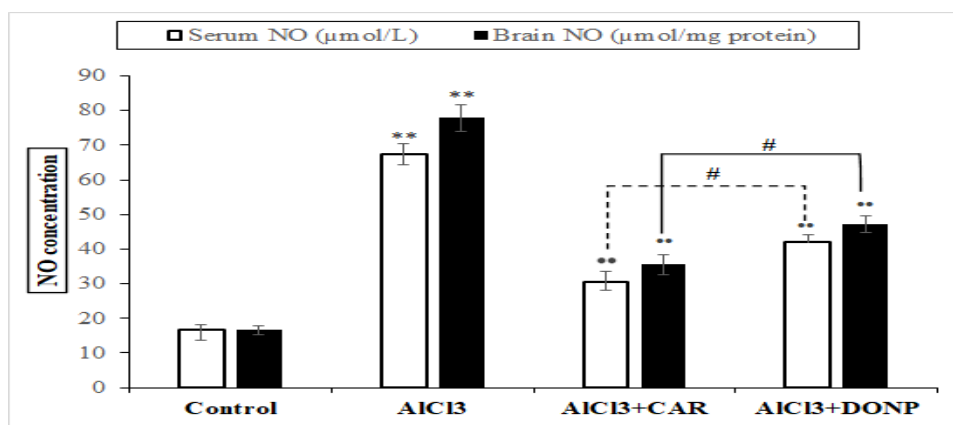
Figure 4. Effect of carvedilol (1 mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on serum and brain NO in AlCl$_3$-induced Alzheimer's disease in rats. All data are expressed as the mean ± SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at ($p < 0.01$) vs. control group. •• Significant change at ($p < 0.01$) vs. AlCl$_3$-treated group. # Significant change at ($p < 0.05$) vs. AlCl$_3$+CAR treated group. NO= nitric oxide, AlCl$_3$=aluminum chloride, CAR=carvedilol, DONP =donepezil.

3.8. Effects of CAR and DONP on TNF-α level

In comparison to the control group, administration of AlCl$_3$ to experimental animals revealed a significant increase ($p< 0.01$) of serum and brain TNF-α. However, CAR or DONP administration concurrently with AlCl$_3$ produced a significant reduction in TNF-α compared to AlCl$_3$ treated group, while there was a significant decrease ($p< 0.01$) and ($p< 0.05$) in its serum and brain levels simultaneously with AlCl$_3$ resulted in a significant reduction ($p< 0.01$) in serum and brain Al levels compared to AlCl$_3$ treated group. However, treatment with CAR led to no significant difference

respectively after CAR treatment compared to DONP treated group (**Fig. 5**).

3.9. Effects of CAR and DONP on Al concentration

Compared to the control group, there was a significant elevation ($p< 0.01$) in serum and brain Al levels in the group treated with 70 mg/kg/day AlCl$_3$ for four successive weeks. Besides, daily i.p. treatment of the rats with CAR or DONP treatment in Al serum and brain levels ($p= 0.46$) and ($p= 0.88$) respectively compared to DONP treated group (**Fig. 6**).
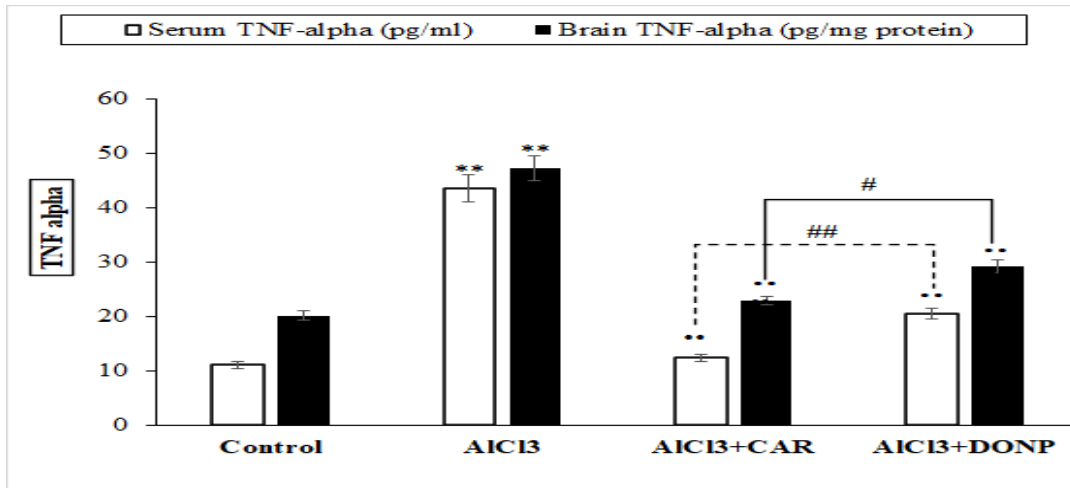
Figure 5. Effect of carvedilol (1 mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on serum and brain TNF-α in AlCl$_3$-induced Alzheimer's disease in rats.
All data are expressed as the mean ± SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at (p < 0.01) vs. control group. •• Significant change at (p < 0.01) vs. AlCl$_3$-treated group. # Significant change at (p < 0.05) vs. AlCl$_3$+CAR treated group. ## Significant change at (p < 0.01) vs. AlCl$_3$+CAR treated group. TNF-alpha = tumor necrosis alpha, AlCl$_3$=aluminum chloride, CAR=carvedilol, DONP =donepezil.
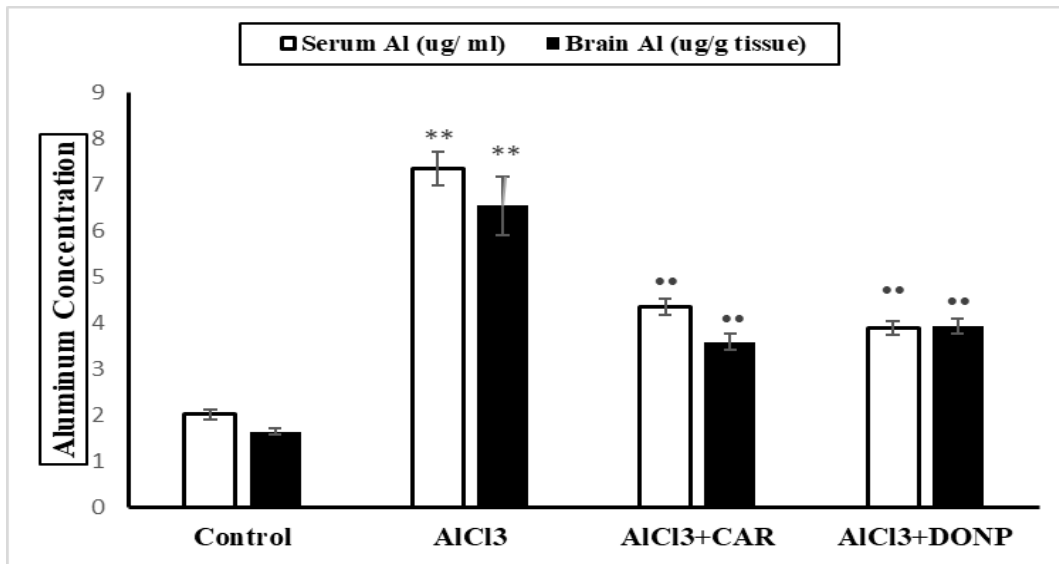
Figure 6. Effect of carvedilol (1 mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on serum and brain Al in AlCl$_3$-induced Alzheimer's disease in rats.
All data are expressed as the mean ± SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at (p < 0.01) vs. control group. •• Significant change at (p < 0.01) vs. AlCl$_3$-treated group. Al= aluminum, AlCl$_3$=aluminum chloride, CAR=carvedilol, DONP =donepezil.

3.10. Effects of CAR and DONP on AChE activity

Treatment of the rats with 70 mg/kg/day AlCl$_3$ for four successive weeks produced a significant (p< 0.01) increase in brain AChE activities compared to the control group. However, daily administration of

activity in the DONP treated group compared to the CAR treated group (**Fig. 7**).

3.11. Effects of CAR and DONP on Aβ$_{1-42}$ level

As shown in **Fig. 8**, i.p. administration of 70 mg/kg/day AlCl$_3$ to rats for four successive weeks produced a significant (p< 0.01) increase in brain CAR or DONP for two successive weeks before administration of AlCl$_3$ and for four successive weeks concomitant with it produced a significant decrease (p< 0.01) in brain AChE activities

compared to AlCl₃ treated group. On the other hand, there was a significant reduction in AChE

Aβ₁₋₄₂ protein compared to the control group. Daily i.p. administration of CAR or DONP before and

concomitant with AlCl₃ produced a significant decrease (p< 0.01) in brain Aβ₁₋₄₂ protein levels compared to AlCl₃ treated group, and there was no significant alteration (p= 0.06) between CAR and DONP treated groups.
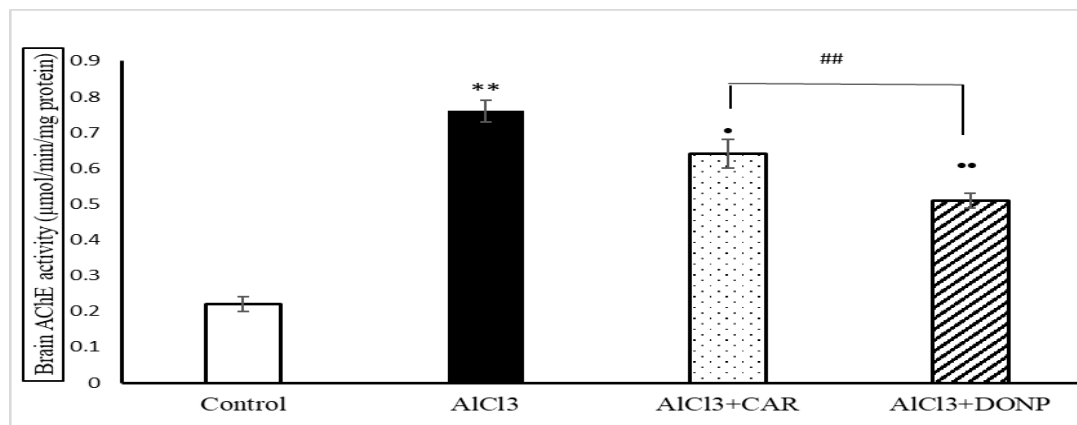
Figure 7. Effect of carvedilol (1 mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on brain AChE in AlCl₃-induced Alzheimer's disease in rats.
All data are expressed as the mean ± SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at (p < 0.01) vs. control group. •• Significant change at (p < 0.01) vs. AlCl₃-treated group. ## Significant change at (p < 0.01) vs. AlCl₃+CAR treated group. AChE= acetylcholinesterase, AlCl₃=aluminum chloride, CAR=carvedilol, DONP =donepezil.
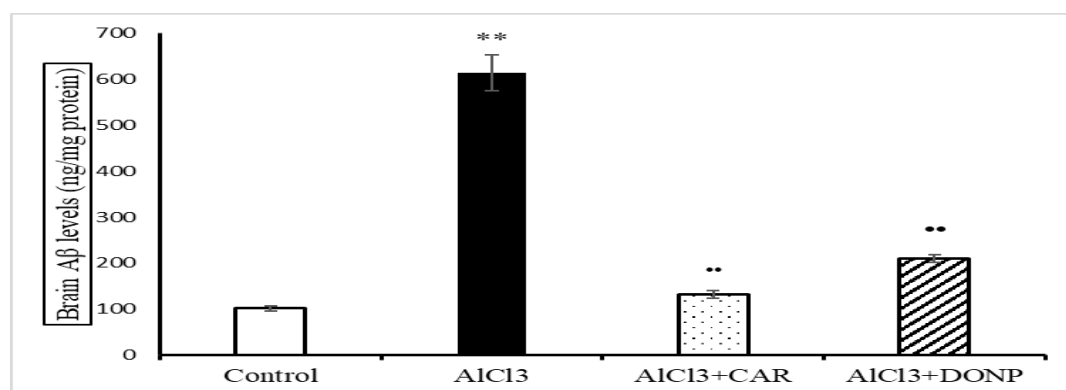

Figure 8. Effect of carvedilol (1 mg/kg/d i.p.) or donepezil (0.75 mg/kg/d i.p.) on brain Aβ₁₋₄₂ in AlCl₃-induced Alzheimer's disease in rats.
All data are expressed as the mean ± SEM (n = 10). Data were analyzed by one-way ANOVA followed by a post hoc Tukey's test. ** Significant change at (p < 0.01) vs. control group. •• Significant change at (p < 0.01) vs. AlCl₃-treated group. Aβ= Amyloid beta, AlCl₃=aluminum chloride, CAR=carvedilol, DONP =donepezil.

3.12. Histopathological changes

Examination of H&E-stained sections of the hippocampal tissue of the control group declared normal brain architecture contained neurons with rounded vesicular nuclei, glial cells, and nerve fibers **(Fig. 9a)**. Sections of hippocampal tissue of rats treated with AlCl₃ revealed that mostly degenerated nerve cells with wide perineural space, congested blood vessels with wide perivascular

space. Glial cells showed numerous empty spaces (Fig. 9b). However, sections of CAR and DONP -treated brain tissue showed that most of the cortical neurons, glial cells, and blood vessels appeared more or less like the control group. Some degenerated nerve cells and spaces of the neutrophil were observed (Fig. 9c & d).
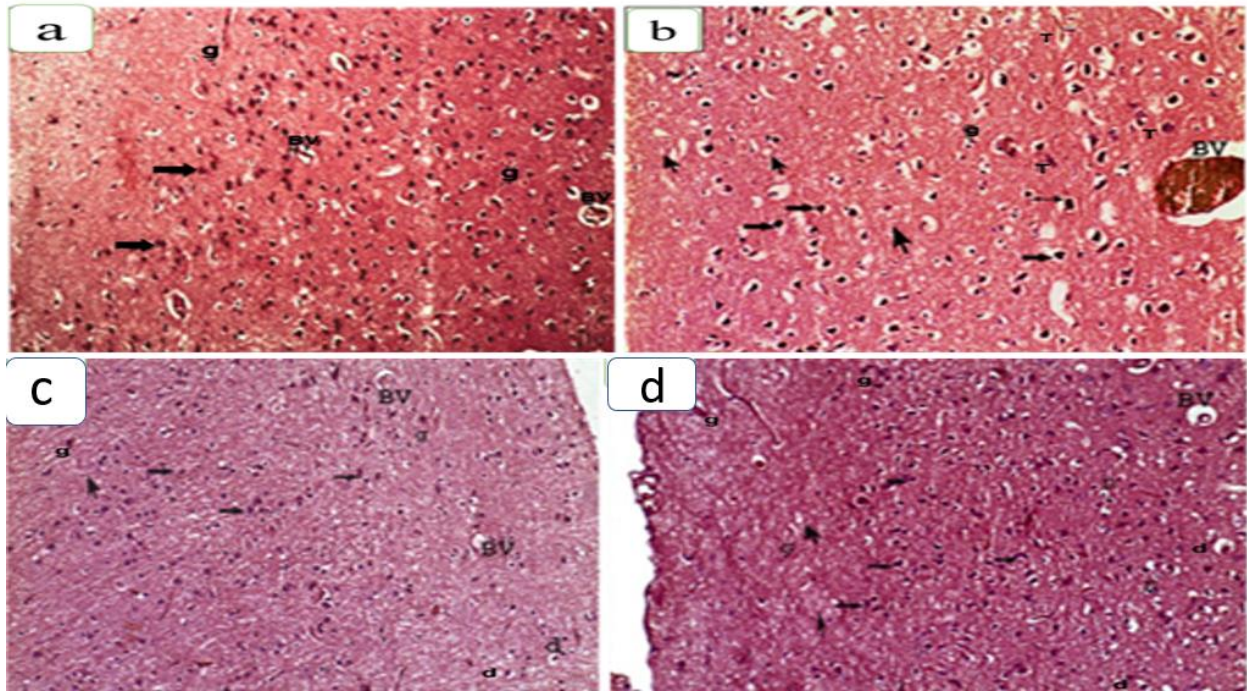
Figure 9. (a) Photomicrograph of a section of hippocampal tissue of the control group showing nerve cells (arrows), glial cells (g), and blood vessels (BV). (**b**) Photomicrograph of a section of the brain of $AlCl_3$ treated group showing degenerated nerve cells (horizontal arrows) with ring shape nucleus, gliosis (g), congested blood vessel (BV), neurofibrillary tangles (T), spongy appearance with numerous empty spaces (upside arrows). **(c)** Photomicrograph of a section of the brain of carvedilol treated group showing nerve cells (horizontal arrows), glial cell (g), and blood vessel (BV). Residual empty spaces in the neuropil (upside arrow) and degenerated nerve cells (d). (**d**): Photomicrograph of a section of the brain of donepezil treated group showing nerve cells (horizontal arrow), glial cell (g), and blood vessel (BV), with residual empty spaces in the neutrophil (upside arrows) and degenerated nerve cells (d). (H&E X200).

4. Discussion

The purpose of this study was to assess the neuroprotective effect of CAR in $AlCl_3$- induced AD, using DONP as a reference drug.

This work indicated that administration of $AlCl_3$ for four successive weeks resulted in significant impairment in memory and cognitive function as demonstrated by behavioral analysis associated with a reduction in body weight. The current results agree with the results of **(Abdel-Aal et al. 2011; Nampoothiri et al. 2015; Justin Thenmozhi et al. 2016)** where they explained these effects by an accumulation of Al ions mainly in the hippocampus and cortex regions, which are responsible for learning and memory. In addition, Al led to the destruction of cholinergic neurons and reduced acetylcholine release **(Kaur et al. 2021)**. Also, these effects could be attributed to the ability of Al to interfere with cyclic GMP, which is involved in long-term potentiation in the cerebellum leading to

cognitive dysfunction **(Canales et al. 2001)**. Bodyweight reduction was attributed to the less desire for food intake that occurs with cognitive dysfunction **(Justin Thenmozhi et al. 2015)**. In addition, $AlCl_3$ administration decreased water intake and induced transient diarrhea **(Kowalczyk et al. 2004)**.

On the other hand, our result revealed that CAR or DONP treatment led to significant improvement in cognitive function and body weight compared to $AlCl_3$ treated group. These findings are in harmony with **(Wang et al. 2011; Ahmed and Mohammed 2021)**, where CAR treatment significantly attenuated cognitive deterioration in TgCRD8 and Tg2576 AD mice models, evaluated by both MWM and NOR tests **(Wang et al. 2011)**. Furthermore, **Yahaya et al. (2013)** studied the effectiveness of CAR in ameliorating scopolamine-induced memory deficit in mice and attributed its effectiveness to the β-adrenergic blocking effect **(Yahaya et al., 2013)**. Also, **Arrieta-Cruz et al.**

between Al^{3+} and Fe^{3+} ions then enter throughout the blood-brain barrier (BBB) to the brain (**Day et al. 1994; Oshiro et al. 1998**).

The present study revealed that CAR and DONP produced significant improvement in the previous parameters where CAR produced a significant effect compared to the DONP -treated group. This agrees with (**Sahu et al. 2014; Oboh et al. 2017; Eid et al. 2019**). The powerful antioxidant action of CAR was ascribed to its free radical scavenging ability and restriction of the formation of hydroxyl radicals (**Flesch et al. 1999**).

Moreover, CAR was reported to upregulate the Nrf2, a transcriptional factor that induces the expression of antioxidant-dependent genes (**Ma 2013; Ahmed and Mohammed 2021**), and to reduce NO levels by reduction of the expression of the inducible nitric oxide synthase (iNOS) enzyme (**Al-Olayan et al. 2015**). The anti-inflammatory effect of CAR could be elucidated by reducing protein expression of NF-κB during the inflammatory process (**Sahu et al. 2014**). Also, CAR inhibited the mRNA expression of inflammatory cytokines and IL-1β protein expression in myocarditis (**Yuan et al. 2004**).

Our current data detected that $AlCl_3$ administration significantly elevated both brain AChE enzyme activity and $A\beta_{1-42}$ levels. However, CAR or DONP treated groups showed significant amelioration of both measures. Moreover, the present findings are strongly supported by the histopathological abnormalities in the hippocampal tissue of the $AlCl_3$ treated group.

The previously mentioned results agree with the study of (**Yang et al. 2014**), where they interpreted their findings by the influence of Al on neurofibrillary tangles, neuronal apoptosis, amyloid deposition, and mitochondrial dysfunction, all of which have been linked to the progression of AD (**Zhao and Zhao, 2013**). Also, the effect of Al on AChE activity could be clarified by the effect of Al on AChE synthesis or choline uptake in synaptosomes (**Moshtaghie et al. 1999**).

Moreover, **Kakkar and Kaur 2011** reported that Al was found to be a strong cholinotoxic agent, which was attributed to a reaction between aluminum and AChE peripheral sites, which modified AChE's secondary structure and increased its activity (**Kakkar and Kaur 2011**).

Amyloid-beta accumulation in the brain is the prominent feature of AD; it is implicated in neuronal loss and cognitive dysfunction during the disease progression. The imbalance between Aβ production and Aβ clearance is the main cause of abnormal aggregation of Aβ in AD (**Haass and Selkoe 2007**).

In the amyloidosis pathway, β and γ secretase enzymes sequentially cleave the amyloid precursor protein (APP), forming $A\beta_{1-42}$. Otherwise, α and γ secretases act in the non-amyloid pathway, preventing Aβ generation and seems to be a protective pathway (**Vekrellis et al. 2000**).

Our result revealed that Al increased the levels of $A\beta_{1-42}$ in the hippocampus, which is in harmony with (**Yang et al. 2019; McDonald et al. 2021**). Moreover, Al builds up in the hippocampus and frontal brain, causing elevated APP expression and Aβ deposition (**Abdel-Aal et al. 2011**). Other research provided evidence that Al could change Aβ structure and β sheet structure content suggesting Al simplifies the aggregation of Aβ peptides (**Zhang et al. 2019**). In addition, the injection of Al increased the expression of $A\beta_{1-42}$ through the elevation of β and γ secretase enzymes in the hippocampus, which is responsible for the induction of the amyloidosis pathway (**Vekrellis et al. 2000**). Also, Al inhibited protein kinase C (PKC) activity, increasing β amyloidogenesis in cultured neuroblastoma cells (**Kawahara and Kato-Negishi 2011**).

However, our study revealed that CAR produced an amelioration effect of AChE activity, which agrees with (**Veerendra Kumar and Gupta 2003**). The mechanism might be attributed to its beta-blocking activity (**Kumar et al. 2011**). CAR is a lipophilic compound and easily passes through the BBB where it produces powerful central antioxidant effects (**Savitz et al. 2000**). Also, the downregulation of AChE could be speculated to CAR's anti-inflammatory effect (**Yahaya et al., 2013**).

Also, CAR protected against $A\beta_{1-42}$ accumulation where this result agrees with (**Howlett et al. 1999; Rosenberg et al. 2008; Wang et al. 2011; Ghosh and Verma 2021**). They explained this result by mentioning that CAR had a specific 3-dimensional pharmacophore confirmation that allowed it to interact with Aβ and prevent oligomeric fibrils formation (**Howlett et al. 1999**). Also, CAR interfered with Aβ aggregation by inhibiting Aβ peptide-protein interactions (**Wang et al. 2011**).

DONP as an AChE inhibitor is a proven pharmacological therapy for the symptomatic treatment of AD (**Anand and Singh 2013**). So, DONP was used as a positive reference drug to treat cognitive deficits and evaluate the efficacy of CAR in protection against AD. DONP protected against cognitive dysfunction through improvement in cholinergic transmission (**Oboh et al. 2017**). Moreover, it prevented memory loss by affecting the ADA (adenosine deaminase) enzyme responsible for depleting extracellular adenosine, an important neuromodulator in synaptic plasticity (**Burnstock et al. 2011**). In addition, DONP produced an antioxidant effect due to its free radicals DPPH and OH and Fe^{2+} chelating capabilities (**Oboh et al. 2017**).

5. Conclusion

The present study confirms that $AlCl_3$ significantly impairs memory and increases the oxidative stress and inflammation associated with the elevation of AChE enzyme activity and hippocampal Aβ protein levels. The beneficial effect of CAR is more significant than DONP, which is used as a standard drug in AD, so it may be recommended in patients who have cardiovascular disease concomitant with dementia and related conditions. CAR exerts beneficial effects on memory deficit through decreasing oxidative stress, inflammation, Al chelating effect, and reduction of $Aβ_{1-42}$ protein. Additional work is needed to investigate the molecular mechanisms underlying these effects.

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