Coconut oil ameliorates behavioral alterations in rat model of Alzheimer's Disease via attenuating oxidative damage and cholinergic impairment and upregulating synaptic transmission

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Research Article

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Abstract

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative condition marked by cognitive impairment. Although coconut oil has been shown to be potentially beneficial in reducing AD-related cognitive deficits, information on its mechanism of action is limited. Thus, we investigated the effects of coconut oil on spatial cognitive ability and non-cognitive functions in a rat model of AD induced by G-galactose (D-GAL) and aluminum chloride ($AlCl_3$), and examined the changes in synaptic transmission, cholinergic activity, neurotrophic factors and oxidative stress in this process. The AD model was established by administering D-GAL and $AlCl_3$ for 90 days, while also supplementing with coconut oil during this time. Cognitive and non-cognitive abilities of the rats were evaluated at the end of the 90-day supplementation period. In addition, biochemical markers related to the pathogenesis of the AD were measures in the hippocampus tissue. Exposure to D-GAL/$AlCl_3$ resulted in a reduction in locomotor activity and exploratory behavior, an elevation in anxiety-like behavior, and an impairment of spatial learning and memory. The aforementioned behavioral disturbances were observed to coincide with increased oxidative stress and cholinergic impairment, as well as reduced synaptic transmission and levels of neurotrophins in the hippocampus. Interestingly, treatment with coconut oil attenuated all the neuropathological changes mentioned above. These findings suggest that coconut oil shows protective effects against cognitive and non-cognitive impairment, AD pathology markers, oxidative stress, synaptic transmission, and cholinergic function in a D-GAL/$AlCl_3$-induced AD rat model.

1. INTRODUCTION

Alzheimer's disease (AD), a chronic, progressive neurological condition that accounts for 60 to 80% of all dementia cases [1]. Cognitive decline, memory loss, and mood changes are its defining traits. Eventually, total memory and personality loss follow [2]. This clinical picture is due to the extensive neuron and synapse loss in the hippocampal and cortical brain regions [3,4].

The etiology of AD is quite intricate, varied, and not entirely certain. The loss of the acetylcholine (ACh), oxidative damage, mitochondrial dysfunction, neuroinflammation, and amyloid plaques accumulation with hyperphosphorylated tau protein in the brain are just a few of the many explanations that have been put up [5]. The underlying etiology of AD, however, has also been linked to metabolic diseases including brain insulin resistance, which results in neuronal death [6]. More importantly, such pathologies may progress gradually over decades before the first signs of the disease appear [7]. Interestingly, hippocampal neurogenesis is also impaired before typical AD symptoms appear [8].

Despite extensive research, there is still no effective medication that either blocks the advancement of AD pathology or delays the onset of AD. The search for alternate AD prevention and treatment methods has resulted from this. As a result, many natural substances have been suggested to be effective in the treatment and prevention of AD [9,10]. One of the prominent foods that is strongly linked to healthy brain function is coconut oil [11,12]. Medium chain triglycerides are abundant in coconut oil. These are taken in by the liver as medium chain fatty acids, where they are then converted into ketone bodies [13]. Since the

dysregulation of glucose metabolism is one of the key characteristics of AD, the brain can use ketone bodies as a substitute for glucose, its primary energy source, when glucose is unavailable [14]. It has been also shown that the usage of coconut oil may improve blood pressure, and cholesterol levels which are risk factors for the AD [15].

There is little and conflicting scientific evidence that coconut oil can attenuate cognitive functions and AD-related biochemical markers in AD [16–20]. Some of the experimental animal studies [17–19], have reported beneficial in reducing AD-related cognitive deficits by improving mitochondrial function and reducing amyloid precursor protein (APP) and phosphorylated tau. On the other hand, detrimental effects on hippocampal morphology and behavior have been reported [16,20]. Furthermore, in vitro studies have shown that coconut oil can reduce the toxic effect of amyloid peptides on cortical neurons [21,22]. Therefore, coconut oil can be used as a preventive or therapeutic intervention in AD.

In this study, the rat model of AD was established by consolidating D-(+)-galactose (D-GAL) with $AlCl_3$. This combination causes AD-like symptoms such as amyloid β (Aβ) accumulation and impaired learning and memory ability [23,24]. To date, the potential neuroprotective effect of coconut oil against experimental AD has not been fully demonstrated. This investigation was designed to demonstrate the effect of coconut oil supplementation on cognitive and other behavioral functions using the open field (OF), elevated plus maze (EPM) and Morris water maze (MWM) tests. Amyloid beta 1–42 ($A\beta_{1-42}$) and microtubule associated protein tau (MAP_T), as markers of AD pathology, acetylcholinesterase (AChE), as a marker of cholinergic activity, synaptophysin (SYP), as a marker of synaptic function, BDNF, as a neurotrophic factor and malondialdehyde (MDA), protein carbonyl (PC), glutathione (GSH) and superoxide dismutase (SOD) as markers of oxidative/antioxidative system were measured in the hippocampus.

2. METHODS

2.1. Chemicals and reagents

D-GAL (Cat no: G0625) and $AlCl_3$ (Cat no: 294713) were purchased from Sigma Aldrich, Co. (St. Louis, MO, USA). Coconut oil was kindly donated by Zade Vital (Zade Global Inc. Konya, Turkey). It was extracted by using the cold pressed method. Fatty acid composition of coconut oil is given in Table 1. Commercial ELISA kits were obtained from Elabscience Biotechnology Co. (Wuhan, China) for $A\beta_{1-42}$, MAP_T, AChE, SYP and BDNF, from Cayman Chemical (Ann Arbor, MI, USA) for GSH and SOD, from Oxis International Inc. (Foster City, California, USA) for MDA, and from Sunred Biological Technology (Shanghai, China) for PC.

Table 1
Fatty acid composition of coconut oil

Fatty acids	Name	% Composition
C6:0	Caproic acid	0.53
C8:0	Caprylic acid	7.19
C10:0	Capric acid	5.98
C12:0	Lauric acid	49.40
C14:0	Myristic acid	19.01
C16:0	Palmitic acid	8.41
C18:0	Stearic acid	3.28
C18:1	Oleic acid	5.02
C18:2	Linoleic acid	1.10
C18:3	Linolenic acid	0.02
C20:0	Arachidic acid	0.06

2.2. Animals and experimental design

Twenty-eight Wistar female rats (20 months old) weighing 350–400 g were used in this study. The animals were kept in standard housing circumstances, which included a 12-hour light and dark cycle, room temperature of 23 ± 1°C, and 50% humidity. Animals were given unlimited access to tap water and standard rat pellets. The Institutional Animal Care and Use Committee gave its approval to all experimental methods.

The rats were compartmentalized into four groups of seven in each: Control (C), Coconut oil (CO), AD model (AD), and AD model + coconut oil (AD + CO). In our lab, the average lifetime of 20-month-old female Wistar rats is over 75%. Aged female rats were used in this study, since estrogen deficiency is a risk factor for the AD [25]. In addition, between the ages of 15 and 20 months, female rats are estimated to go through menopause [26]. The timing of behavioral tests and interventions is depicted in Fig. 1.

2.3. Experimental model and treatments

According to our previous study, the D-GAL/$AlCl_3$-induced AD rat model was established [24]. Rats in the AD model groups (AD and AD + CO groups) were given 60 mg kg^{-1} D-GAL dissolved in physiological saline intraperitoneally, and 5 mg kg^{-1} $AlCl_3$ dissolved in distilled water intragastrically once daily for 90 days. To the control groups, the same amount of physiological saline was administered. 2 ml kg^{-1} of coconut oil was given to the rats in the treatment groups (CO and AD + CO groups) beginning with the D-GAL/$AlCl_3$ administration and continued for 90 days [27,28]

2.4. Behavioral studies

Animals from each experimental group underwent the OF, EPM, and MWM tests to determine the impact of coconut oil supplementation. The Ethovision XT 9.0 system (Noldus Information Technology, Wageningen, The Netherlands) was used to collect and analyze data. All behavioral tests were performed in a sound- and light-isolated room and were observed throughout the study by the same treatment-blind person at the same hours of the day and. The OF and EPM devices were cleaned with 70% ethanol between tests to omit the olfactory clues.

2.4.1. Open field

The OF test was performed in a black opaque plexiglass box (80 × 80 × 40 cm) left open at the top. The animal was put in the middle of the box, and over the course of five minutes, the animals' horizontal and vertical movements were recorded.

2.4.2. Elevated plus maze

The EPM has four arms that cross at right angles and has a height of 50 cm above the floor and a length of 50 x 10 cm. It is built of a wooden floor and a black plexiglass wall. A 40 cm high wall was constructed to enclose the opposite arm, sometimes frequently known as the closed arm. A center platform (5 x 5 cm) was formed at the intersection of the four arms. Each trial began with the animal being placed in the neutral space where the four arms cross, facing one of the open arms, and was given five minutes to investigate.

2.4.3. Morris water maze

In the MWM test, a specially designed pool was used in which the temperature of the water was maintained at 23 ± 1°C. The pool was imaginatively subdivided into geographic NW, SW, NE, and SE quadrants. A square platform was placed in the center of the target quadrant (SE), 1–2 cm below the surface of the water. Visual cues were put at various sets around the maze walls to help with platform positioning. Training trial and probe trial were the two main tasks carried out during the MWM experiment.

The training trial was made up of a single session and four blocks of trials, each lasting 60 seconds over the course of four days. Each block contained four trials that began at various locations. Throughout the training experiment, the platform's location remained the same. During the training phase, each animal was dropped into the water facing the maze wall from a quadrant that the researcher had chosen at random. The animal had a maximum of 60 seconds in each block of the training phase to use the spatial signals throughout the maze to locate the concealed platform beneath the water's surface and take a break there. If the animal was unable to locate the platform during the initial experiment, the researcher manually led it there. In any way, the animal rested for 30 seconds on the platform.

A 90-second probe trial was conducted after the platform was removed (24 hours had passed since the last training session). The rat was let loose into the water during the probe trial from the quarter across from the target quarter, and it was free to swim around.

2.5. Tissue collection

Animals were anesthetized with ether and decapitated twenty-four hours after the final behavioral test. Hippocampal tissues were quickly separated from the brain on an ice pack, frozen in liquid nitrogen, and maintained at − 80°C for biochemical analysis.

2.6. Biochemical parameters assay

2.6.1. Preparation of hippocampal tissue homogenate for biochemical estimations

Hippocampus tissues were homogenized with 20 volumes of ice-cold phosphate buffered saline (pH: 7.4). After homogenization, the samples were centrifuged at 12,000 g for 10 min at 4°C to obtain supernatants. Protein concentrations were determined using the Lowry method [29].

2.6.2. Enzyme-Linked Immunosorbent Assays (ELISA)

Commercially available ELISA kits were used to determine the hippocampal levels of the $A\beta_{1-42}$ (Cat No: E-EL-R1402), MAP_T (Cat No: E-EL-R0943), AChE (Cat No: E-EL-R0355), SYP (Cat No: E-EL-R0932), BDNF (Cat No: E-EL-R1235), MDA (Cat. No: 21044), PC (Cat. No: 201-11-1700), SOD (Cat.no: 706002), and GSH (Cat. No: 703002). The manufacturer's instructions were strictly followed during every assay.

2.7. Data and statistical analysis

Using SPSS 25.0 (IBM-SPSS Inc., Chicago, IL, USA), the findings were examined. Shapiro-Wilk's normality test was used to determine the normal distribution of all data sets. A two-way analysis of variance (ANOVA), a two-way ANOVA with repeated measures, and a Bonferroni post hoc multiple comparison were used to correctly examine differences between groups. The standard error of the mean (SEM) was used to express data. All statistical tests were considered significant if P 0.05. The GraphPad Prism 9.5.1 (GraphPad Software, LLC, San Diego, CA, USA) was used to draw each plot.

3. RESULTS

3.1. Body weight changes

Because the baseline body weight was similar in all four groups (mean values 368–386 g), the body weight changes was compared (Fig. 2). Every group's body weight increased over the course of the study ($F_{(3,22)}$ = 185.836; P = 0.000). Additionally, body weight gains of the coconut oil supplemented groups (CO and AD + CO) were higher than the non-supplemented groups (C and AD) ($F_{(3,22)}$ = 4.855; P = 0.010).

3.2. Behavioral Analysis

3.2.1. Locomotor activity

Total distance traveled and average velocity are measures of locomotor activity in the OF and EPM tests. In the OF test, locomotor activity is also measured by the time spent as a mobile. On the total distance traveled in the OF and EPM tests, the effect of AD was observed ($F_{(1,24)}$ = 4.553; p = 0.043 and $F_{(1,24)}$ = 7.048; p = 0.014, respectively), but the effect of coconut oil ($F_{(1,24)}$ = 0.328; p = 0.572 and $F_{(1,24)}$ = 0.058; p = 0.811, respectively) and the interaction of AD x coconut oil ($F_{(1,24)}$ = 0.488; p = 0.491 and $F_{(1,24)}$ = 0.520; p = 0.478, respectively) was not observed. However, the post hoc analysis result in the OF and EPM tests did not reveal any difference between groups ($F_{(3,27)}$ = 1.790; p = 0.176 and $F_{(3,27)}$ = 2.542; p = 0.080, respectively) (Fig. 3D and 4D). In the OF test, average velocity did reveal an effect of AD ($F_{(1,24)}$ = 39.954, p = 0.000), but not an effect of a coconut oil ($F_{(1,24)}$ = 0.019, p = 0.890) and AD x coconut oil interaction ($F_{(1,24)}$ = 0.535, p = 0.471). The post hoc test showed that it was lower in the AD and AD + CO groups than in the C and CO groups ($F_{(3,27)}$ = 13.503; p = 0.000) (Fig. 3F). In the EPM test, did not reveal a significant effects of AD ($F_{(1,24)}$ = 3.957, p = 0.058), coconut oil ($F_{(1,24)}$ = 0.071, p = 0.793) and AD x coconut oil interaction ($F_{(1,24)}$ = 0.374, p = 0.545) at average velocity (Fig. 4A). In the time spent as a mobile variable, effects of AD ($F_{(1,24)}$ = 4.398, p = 0.047) and AD x coconut oil interaction ($F_{(1,24)}$ = 4.498; p = 0.044) were observed, while the effect of coconut oil was not observed ($F_{(1,24)}$ = 0.228; p = 0.637). The post hoc test showed that the AD group spent less time being mobile compared to the C group ($F_{(3,27)}$ = 3.041; p = 0.048) (Fig. 3E). These findings indicate that locomotor activity is decreased with D-GAL/$AlCl_3$ administration particularly in the OF test, but coconut oil supplementation did not reverse this impairment.

3.2.2. Exploratory behavior

The rearing counts and zone transitions are indicators of exploratory behavior in the OF test. The rearing counts did not reveal a significant effect of AD ($F_{(1,24)}$ = 0.194, p = 0.663) and coconut oil ($F_{(1,24)}$ = 2.809, p = 0.107), but reveal a significant AD x coconut oil interaction ($F_{(1,24)}$ = 17.168, p = 0.000). The post hoc test showed that it was lower in the AD group than the C and AD + CO groups ($F_{(3,27)}$ = 6.723; p = 0.002) (Fig. 3C). The main effects of AD ($F_{(1,24)}$ = 51.130; p = 0.000), coconut oil ($F_{(1,24)}$ = 6.552; p = 0.017) and AD x coconut oil interaction ($F_{(1,24)}$ = 26.087; p = 0.000) was observed in the zone transition counts parameter. The post hoc test showed that the zone transition counts were lower in the AD group than in the C, CO and AD + CO groups. Additionally, it was lower in the AD + CO group than in the CO group ($F_{(3,27)}$ = 27.913; p = 0.000) (Fig. 3G). These data indicate that exploratory behavior is negatively affected by the D-GAL/$AlCl_3$ administration, however coconut oil supplementation has been found to reverse this decline.

3.2.3. Anxiety-like behavior

The defecation and grooming counts during the OF test are indices of anxiety-like behavior. In the EPM test, the number of entries into closed and open arms is another indicator of anxiety-like behavior. There was a significant main effects of AD ($F_{(1,24)}$ = 12.623; p = 0.002), coconut oil ($F_{(1,24)}$ = 30.545; p = 0.000) and AD x coconut oil interaction ($F_{(1,24)}$ = 62.338; p = 0.000) on the defecation counts. The post hoc analysis showed that the defecation counts were higher in the AD group than in the C, CO and AD + CO groups. Additionally, it was lower in the AD + CO group than in the CO group ($F_{(3,27)}$ = 35.169; p = 0.000) (Fig. 3A). In the OF test, the grooming counts revealed a significant effect of AD ($F_{(1,24)}$ = 6.618, p = 0.017), but not coconut oil ($F_{(1,24)}$ = 0.265, p = 0.612) and AD × coconut oil interaction ($F_{(1,24)}$ = 1.441, p = 0.242). However, there was no difference between groups according to the post hoc analysis result ($F_{(3,27)}$ = 2.775; p = 0.063) (Fig. 3B). In the EPM test, AD ($F_{(1,24)}$ = 10.345; p = 0.004), coconut oil ($F_{(1,24)}$ = 22.253; p = 0.000) and AD x coconut oil interaction ($F_{(1,24)}$ = 9.011; p = 0.006) had a significant main effect on the number of entries in the closed arms. The post hoc test showed that it was higher in the AD group than in the C, CO and AD + CO groups ($F_{(3,27)}$ = 13.870; p = 0.000) (Fig. 4B). The number of entries in the open arms, AD had a significant effect ($F_{(1,24)}$ = 15.034, p = 0.001), but not coconut oil ($F_{(1,24)}$ = 4.125, p = 0.053) and AD x coconut oil interaction ($F_{(1,24)}$ = 1.670, p = 0.209). The post hoc test showed that it was lower in the AD group than the C and CO groups ($F_{(3,27)}$ = 6.943; p = 0.002) (Fig. 4C). These findings suggest that coconut oil supplementation reduces the degree of anxiety-like behavior while D-GAL/$AlCl_3$ treatment increases anxiety levels.

3.2.4. Spatial learning and memory

Using the MWM test, we examined spatial learning and memory in order to determine whether coconut oil supplementation affects D-GAL/$AlCl_3$-induced cognitive impairment. According to a repeated measures ANOVA test, all groups experienced a reduction in total distance traveled ($F_{(3,22)}$ = 82.465; P = 0.000), latency ($F_{(3,22)}$ = 107.246; P = 0.000), thigmotactic behavior ($F_{(3,22)}$ = 161.884; P = 0.000), and average velocity ($F_{(3,22)}$ = 16.381; P = 0.000) over the course of the MWM's training trial. Additionally, the thigmotactic behavior on the first day of the training trial was higher in the AD group than in the C, CO, and AD + CO groups (P < 0.05), despite the fact that the total distance traveled, latency, and average swimming speed did not change across groups (P > 0.05) (Fig. 5A–D). These findings suggest that across training trails, rats' spatial learning capacity increased across all groups.

Twenty-four hours after the last training trial, the probe trial was performed. A separate two-way ANOVAs of time spent in target quadrat, thigmotactic behavior, and time spent in platform zone revealed the significant main effect of coconut oil ($F_{(1,24)}$ = 20.698, p = 0.000; $F_{(1,24)}$ = 12.310, p = 0.002; $F_{(1,24)}$ = 35.260, p = 0.000, respectively), but not the main effects of AD ($F_{(1,24)}$ = 2.944, p = 0.099; $F_{(1,24)}$ = 1.130, p = 0.298; $F_{(1,24)}$ = 0.858, p = 0.363, respectively) and AD x coconut oil interaction ($F_{(1,24)}$ = 0.165, p = 0.688; $F_{(1,24)}$ = 3.883, p = 0.060; $F_{(1,24)}$ = 2.173, p = 0.153, respectively). The post hoc analysis showed that time spent in target quadrant was lower in the AD group than in the CO and AD + CO groups. Additionally, it was higher in the CO group than in the C group ($F_{(3,27)}$ = 7.936; p = 0.001) (Fig. 6B). Thigmotactic behavior was higher

in the AD group than in the CO and AD + CO groups ($F_{(3,27)}$ = 5.775; p = 0.004) (Fig. 6D). Total time spent in platform zone was lower in the C and AD groups than in the CO and AD + CO groups. ($F_{(3,27)}$ = 12.704; p = 0.000) (Fig. 6E). In the total distance traveled and swimming speed, AD ($F_{(1,24)}$ = 15.373, p = 0.000 and $F_{(1,24)}$ = 6.636, p = 0.017, respectively) and AD x coconut oil interaction ($F_{(1,24)}$ = 12.799, p = 0.002 and $F_{(1,24)}$ = 15.722, p = 0.001, respectively) had significant main effects, whereas coconut oil did not ($F_{(1,24)}$ = 3.206, p = 0.086 and $F_{(1,24)}$ = 1.940, p = 0.176, respectively). Post hoc analysis indicated that total distance traveled and average swimming speed were lower in the AD group than in the C, CO and AD + CO groups. ($F_{(3,27)}$ = 10.460; p = 0.000 and $F_{(3,27)}$ = 8.100; p = 0.001, respectively) (Fig. 6A and 6C). In the MWM probe test, the platform crossing counts revealed a significant effect of coconut oil ($F_{(1,24)}$ = 35.260, p = 0.000), but AD ($F_{(1,24)}$ = 0.858, p = 0.363) and AD x coconut oil interaction ($F_{(1,24)}$ = 2.173, p = 0.153) had no significant effect. The post hoc test showed that it was lower in the AD group than in the C, CO and AD + CO groups ($F_{(3,27)}$ = 12.764; p = 0.000) (Fig. 6F). These data suggest that D-GAL/$AlCl_3$ administration impairs the learning ability of the rats and coconut oil supplementation protects against this impairment.

3.3. Biochemical analysis

3.3.1. AD pathology markers

In this study, we evaluated the accumulation of Aβ and tau phosphorylation, two pathological features observed in AD, by measuring $Aβ_{1-42}$ and MAP_T levels in the hippocampus. A two-way ANOVA of $Aβ_{1-42}$ indicated significant main effects of AD ($F_{(1,24)}$ = 16.388, p = 0.000), coconut oil ($F_{(1,24)}$ = 25.839, p = 0.000) and AD x coconut oil interaction ($F_{(1,24)}$ = 10.001, p = 0.004). The post hoc analysis showed that $Aβ_{1-42}$ level was higher in the AD group than in the C, CO and AD + CO groups ($F_{(3,27)}$ = 17.393; p = 0.000) (Fig. 7A). MAP_T levels did reveal a significant effects of AD ($F_{(1,24)}$ = 4.808, p = 0.038) and coconut oil ($F_{(1,24)}$ = 6.729, p = 0.016), but not AD × coconut oil interaction ($F_{(1,24)}$ = 1.269, p = 0.271). The post hoc test showed that it was higher in the AD group than in the CO group ($F_{(3,27)}$ = 4.268; p = 0.015) (Fig. 7B). These findings suggest that coconut oil supplementation decreases the severity of AD pathology.

3.3.2. Neurotrophic factors, synaptic transmission and cholinergic activity

Since neurotrophic factors, synaptic transmission and cholinergic activity play important roles in the pathogenesis of AD, we evaluated the BDNF, SYP and AChE in the hippocampus. In the SYP and AChE, main effects of AD ($F_{(1,24)}$ = 4.360, p = 0.048 and $F_{(1,24)}$ = 45.309, p = 0.000, respectively), coconut oil ($F_{(1,24)}$ = 14.707, p = 0.001 and $F_{(1,24)}$ = 37.971, p = 0.000, respectively) and AD x coconut oil interaction ($F_{(1,24)}$ = 6.497, p = 0.018 and $F_{(1,24)}$ = 37.394, p = 0.000, respectively) were observed. According to the post hoc analysis results, in the AD group, SYP levels were lower than in the C, CO and AD + CO groups, while AChE concentrations were higher than in the C, CO and AD + CO groups ($F_{(3.27)}$ = 8,522, p = 0.000 and

$F_{(3.27)}$ = 40,224; p = 0.000, respectively) (Figs. 8A and 8B). BDNF levels revealed a significant effect of AD ($F_{(1,24)}$ = 14.133, p = 0.001) but not the coconut oil ($F_{(1,24)}$ = 1.785, p = 0.194) and AD x coconut oil interaction ($F_{(1,24)}$ = 3.174, p = 0.088). The post hoc test showed that it was lower in the AD group than in the C and CO groups ($F_{(3,27)}$ = 6.364; p = 0.003) (Fig. 8C). These data indicate that D-GAL/$AlCl_3$ administration impairs neurotrophic factors, synaptic transmission and cholinergic activity, but coconut oil supplementation prevents this impairment.

3.3.3. Oxidative stress and antioxidant defense

We measured the levels of MDA, PC, GSH, and SOD in the hippocampus tissues because oxidative stress and the antioxidant defense system are crucial in the pathophysiology of AD. There were significant main effects of the AD ($F_{(1,24)}$ = 27.980, p = 0.000), coconut oil ($F_{(1,24)}$ = 21.500, p = 0.000) and AD x coconut oil interaction ($F_{(1,24)}$ = 38.983, p = 0.000) on MDA concentration according to the two-way ANOVA test. Post hoc analysis showed that it was higher in the AD group than in the C, CO and AD + CO groups ($F_{(3.27)}$ = 28,488, p = 0.000) (Fig. 9A). On the PC levels, coconut oil ($F_{(1,24)}$ = 9.541; p = 0.000) and AD x coconut oil interaction ($F_{(1,24)}$ = 26.319; p = 0.000) had a significant main effect, AD had no effect ($F_{(1,24)}$ = 1.379; p = 0.252). The post hoc test showed that it was higher in the AD group than in the C, CO and AD + CO groups. Additionally it was higher in the AD + CO group than in the CO group ($F_{(3,27)}$ = 12.413; p = 0.000) (Fig. 9B). SOD activity revealed a significant effect of coconut oil ($F_{(1,24)}$ = 6.541, p = 0.017), but not an AD ($F_{(1,24)}$ = 1.771, p = 0.196) and AD x coconut oil interaction ($F_{(1,24)}$ = 2.763, p = 0.109). The post hoc test showed that SOD activity was lower in the AD group than in the AD + CO group ($F_{(3,27)}$ = 3.691; p = 0.026) (Fig. 9C). GSH level did not reveal significant effects of AD ($F_{(1,24)}$ = 0.027, p = 0.872), coconut oil ($F_{(1,24)}$ = 2.341, p = 0.139) and AD x coconut oil interaction ($F_{(1,24)}$ = 0.081, p = 0.778) (Fig. 9D). Collectively, our results show that supplementing with coconut oil rescues the impairment in the hippocampal oxidative stress and antioxidant system caused by D-GAL/$AlCl_3$ treatment.

4. DISCUSSION

The current study was performed to explore the effect of coconut oil supplementation on cognitive and non-cognitive functions and levels of the AD pathology markers, neurotrophic factors, cholinergic activity, synaptic transmission and oxidative stress in the D-GAL/$AlCl_3$ administration-induced experimental AD model. Our results show that coconut oil (2 ml kg^{-1}) supplementation did not affect locomotor activity, but led to improvement of exploratory and anxiety-like behaviors and spatial learning and memory functions. Moreover, it is noteworthy that in the experimental AD model, coconut oil supplementation suppressed AD pathology markers and cholinergic activity, while improving synaptic transmission, neurotrophic factors, and oxidative stress.

Different animal models are employed to examine multiple aspects of AD. Transgenic animals are not the best study subjects for the more prevalent sporadic type of AD, because the familial type accounts for

less than 1% of all AD cases [30]. Although it requires numerous invasive administrations of the chemical substance over a lengthy period of time, the use of some compounds, such as Aβ protein, D-GAL, and $AlCl_3$, gives superior models to research sporadic AD type [31]. It has been claimed that the cognitive impairment and pathological abnormalities of clinical AD patients can be more accurately simulated by the AD model caused by D-GAL/$AlCl_3$ [32]. In light of these considerations, a combination of D-GAL/$AlCl_3$ was chosen in this study to induce a sporadic pattern of AD.

Regarding the body weight change, our data show that body weights of all groups increased over the study period and this increase was greater in the coconut oil supplemented groups. Coconut oil supplementation has been shown to increase, decrease, or not affect body weight. Consistent with our findings, Ströher et al. [28] reported that 21 days of coconut oil supplementation caused an increase in body weight of young rats. However, according to the Alves et al. [33] body weight decreased in spontaneously hypertensive rats after 4 weeks of coconut oil supplementation. Additionally, some studies [16,27,34] have reported that coconut oil does not cause any changes in body weight. We think that the saturated fatty acids in the coconut oil may increase food consumption, which in turn may raise body weight.

Consistent with previous studies showing that non-cognitive functions such as locomotor activity and exploratory behavior are negatively affected by AD [35–38], non-cognitive functions were decreased in the D-GAL/$AlCl_3$ administered rats in this study as well. There can be two explanations for this. Firstly, it may be due to reduced movements of the animal in the test box, and secondly, it may be the result of decreased interest in objects. Contrary to our results, there are also studies [39–41] showing that locomotor activity and exploratory behavior are not different from healthy controls. These differences in outcomes can be related to variations in animal species, ages, and sexes, as well as the period between behavioral testing and variations in AD induction methods. Our current findings revealed that coconut oil produced a positive effect on our AD model by improving exploratory behavior but not locomotor activity. Consistent with our findings, coconut oil has been shown to improve non-cognitive symptoms [42,43].

Rats given D-GAL/$AlCl_3$ treatment in this study spent more time in the closed arms and less time in the open arms of the EPM, and they defecated more frequently during the OF test, showing that they were more "anxious" than the healthy controls. It has been shown in both clinical and experimental studies that AD causes anxiety-like behaviors [24,37,44,45]. In the early stages of AD, anxiety may occur as a psychological response to the illness and because of difficulties in adapting to AD. In the late stages of AD, severe cognitive decline limits emotional reactions and expression. Finally, due to the severity of AD's effects on the person, anxiety is worse in early-onset AD [46]. The molecular mechanisms linking AD to anxiety are not fully understood. However, the increase in anxiety levels in this study can be attributed to Aβ protein, and tau accumulation and oxidative damage. The present findings showed that coconut oil supplementation resulted in reduction in anxiety levels measured in both OF and EPM tests. In line with our findings, it has been reported in various studies that coconut oil reduces the level of anxiety and this effect is due to the medium chain fatty acids in its content [42,43,47].

The MWM test, one of the most frequently used tests in behavioral research, measures spatial memory and learning capacity [48]. AD-induced cognitive decline has been reported in different experimental models such as transgenic animals [49], icv-STZ injection [50], hippocampal Aβ injection [40], and administration of D-GAL/$AlCl_3$ [23,51], and in different behavioral testing devices such as novel object recognition [52], MWM [40,51,52] and Y maze [53], which is consistent with our findings. Our group has previously shown that D-GAL/$AlCl_3$ exposure causes cognitive impairment at the same dose and exposure time as in the current investigation [24]. In the current study, a considerably high number of platform crossings, time spent in the platform zone, and time spent in the target quadrant during the MWM probe trial clearly demonstrated the neuroprotective impact of coconut oil. These findings are in line with those of other studies [17–19], which demonstrate that coconut oil can restore memory and spatial learning deficits brought on by experimental AD. In this study, in addition to cognitive functions, non-cognitive functions such as locomotor activity (total distance traveled and velocity) and anxiety level (thigmotactic behavior) were also evaluated in the MWM probe trail test. Consistent with the findings from the EPM and OF tests, coconut oil supplementation improved non-cognitive disturbances in addition to cognitive functions.

As one of the biochemical results of this study, there was an increase in hippocampal $A\beta_{1-42}$ and MAP_T levels in rats treated with D-GAL/$AlCl_3$. It is well known that accumulation of $A\beta_{1-42}$ and MAP_T in brain regions serving memory and cognition such as the hippocampus strongly contributes to the development of AD [3]. Consistent with our results, numerous studies [23,24] have shown that administration of D-GAL/$AlCl_3$ causes an increase in Aβ and tau protein levels. These two pathogenic characteristics of AD may be related. Aβ may trigger aberrant protein kinase activation, phosphorylate tau abnormally, and result in neuronal death [54,55]. Additionally, it has been established that phosphorylated tau can be used as a therapeutic target in AD and that this protein is inversely correlated with the severity of the disease [56]. In this study, administration of coconut oil reduced the hippocampal levels of $A\beta_{1-42}$ and MAP_T in rats treated with D-GAL/$AlCl_3$. The beneficial effects of coconut oil on hippocampal Aβ and tau levels have been demonstrated in both in vivo [17–19] and in vitro [21,22,57] studies. The therapeutic impact of coconut oil in AD is demonstrated by a decrease in the hippocampal levels of Aβ and tau. The hydrogen of Aβ can be held by the hydroxyl group of phenolic substances, reducing the buildup of Aβ [58]. Ferulic acid, a phenolic substance found in coconut oil, prevents Aβ accumulation by reducing and binding to Aβ fibers, inhibiting the prolongation process [11].

ACh is a cholinergic neurotransmitter important for learning and memory [59]. According to the cholinergic theory of AD, cholinergic neuron and reduced ACh-mediated neurotransmission degeneration in the hippocampus are both factors in cognitive loss in AD [60]. Since AChE is the main enzyme that breaks down ACh in the synaptic cleft, it serves as a marker for the loss of cholinergic neurons in the brain [61]. Consistent with previous studies [23,39,52], in this study, a compromised cholinergic system was observed in rats given D-GAL/$AlCl_3$, as indicated by increased AChE activity. In the current work, rats' hippocampal AChE activity was dramatically decreased by co-administration of coconut oil and D-GAL/$AlCl_3$. In line with our findings, Attia et al. [19] showed that coconut oil consumption reduced the rise

in hippocampal AChE activity induced by Al in young rats. Similarly, Rahim et al. [62] showed that supplementing young, healthy rats with coconut oil reduced AChE activity, which in turn improved cognitive skills. This is due to both the active polyphenol compounds in coconut oil, which can enhance cholinergic neurotransmission by modulating AChE activity, and the cytokinins (phytohormones) present, which are thought to have anti-aging properties [63–65].

Since increased AChE activity in AD terminates synaptic transmission by hydrolyzing ACh to acetate and choline [66], in this study we analyzed SYP levels, a marker of synaptic transmission. In the present study, D-GAL/$AlCl_3$ administration induced a reduction in the hippocampal SYP levels. Consistent with our findings, both animal [67–69] and human [70] studies have showed that hippocampal SYP levels are decreased in AD disease. Furthermore, a relationship was found between decreased SYP in the hippocampus and the severity of cognitive impairment in AD [70,71]. In the present study, coconut oil administration restored hippocampal SYP levels. Consistent with our findings, an in vitro study [22] showed that coconut oil activates Akt and ERK, reducing the number of cells in which caspase is cleaved, thereby recovering SYP loss. Therefore, one of the molecular mechanisms of the positive effects of coconut oil on learning and memory impairment in AD may be its increase in SYP levels.

In the current investigation, treatment of D-GAL/$AlCl_3$ led to an increase in hippocampal oxidative stress with higher levels of MDA and PC and lower SOD activity. According to the available data, oxidative stress contributes to the etiopathology of AD by generating mitochondrial dysfunction, increasing tau hyperphosphorylation and neurofibrillary tangle formation, boosting Aβ-mediated neurotoxicity, and promoting neuronal death and synaptic dysfunction [72–74]. According to some reports, the development of AD is a direct result of the brain's increased oxidative stress [75]. By boosting SOD activity and reducing the rise in MDA and PC levels, coconut oil supplementation changed the oxidative/antioxidative state of D-GAL/$AlCl_3$-treated rats, demonstrating its capacity to scavenge free radicals and antioxidative activity. Consistent with our findings, coconut oil has been shown to reduce reactive oxygen species production in vitro [22], increase GSH levels in both the hippocampus and prefrontal cortex, and decrease MDA levels in the cortex, in vivo [17]. The presence of phenolic acids such as caffeic acid, syringic acid, p-coumaric acid, vanillic acid, and ferulic acid, which play a significant role in antioxidation, can be used to explain the antioxidant mechanism of action of coconut oil [76]. It is reported that the production of adenosine triphosphate in AD is inhibited due to oxidative damage to enzymes involved in glucose metabolism, which reduces the amount of energy available to the brain [77]. Also, the decreased level of the antioxidant enzymes reported in AD also inhibit the activity of the appropriate detoxification mechanism [78]. Since coconut oil is converted into ketone bodies by the liver and provides an important alternative energy source to the brain [76], this function may also contribute to its antioxidant effect. Thus, alleviation of D-GAL/$AlCl_3$-induced behavioral alterations by coconut oil may be related to its antioxidant effect.

In this study, experimental AD model resulted in a reduction in hippocampal BDNF levels, while coconut oil supplementation tended to increase BDNF levels. BDNF is a member of the neurotrophin family, which is involved in processes such as synaptic plasticity, cell survival and energy metabolism [79]. Many

studies have shown that BDNF levels are reduced in postmortem specimens from patients with AD [80] and in experimental animal models of AD [81]. In AD, Aβ accumulation attenuates cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), resulting in a decrease in BDNF levels [82]. As in this study, in addition to Aβ accumulation, increased AChE activity and oxidative stress may also cause decreased BDNF level. Consistent with our findings, decreased BDNF signaling causes impairment in spatial learning and memory [83]. Due to the medium chain triglycerides in coconut oil content, it modulates the energy metabolism of the brain and may provide neuroprotection through increased BDNF expression, reduced neuroinflammation, and enhanced neurotransmission [84]. However, to our knowledge, there are no studies investigating the effect of coconut oil supplementation on hippocampal BDNF levels in AD. Our study is the first research on this subject. Our findings showed that coconut oil tended to increase hippocampal BNDF levels in the experimental AD model. This increase in BDNF levels may have been mediated by decreased AChE activity and oxidative stress. Consistent with our findings, Attia et al. [19] reported that coconut oil increased serum BDNF levels in an experimental AD model. Taken together, these findings indicate that coconut oil administration stimulates upregulation of BDNF levels, followed by improvement in cognitive function and subsequent neuroprotection.

5. CONCLUSION

In conclusion, our findings from this study showed that coconut oil supplementation could reduce cognitive and non-cognitive dysfunctions in the experimental AD model induced by administration of D-GAL/$AlCl_3$ in rats. These effects of coconut oil are mediated by increased synaptic transmission, neurotrophic factor synthesis and inhibition of oxidative stress and AChE. According to the results of this investigation, coconut oil may be a suitable candidate for use in clinical settings as an adjuvant treatment agent for cognitive and non-cognitive impairments linked to AD. To clarify the precise mechanism underlying the impact of coconut oil in attenuating AD, more research is required.

Declarations

Author Contributions

M.B. and N.O. performed the experiments, statistical analysis and wrote the manuscript. All authors read and approved the final version of the manuscript.

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Data availability

The datasets used and/or analysis during the current study are availability from the corresponding author on reasonable request.

Declarations

Ethics Approval

All of the experimental procedures were performed in line with the principles and guidelines of the institutional animal care and use committee of Selçuk University for the use of laboratory animals.

Consent to Participate

Not applicable.

Consent to Publication

Not applicable.

Competing Interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figures

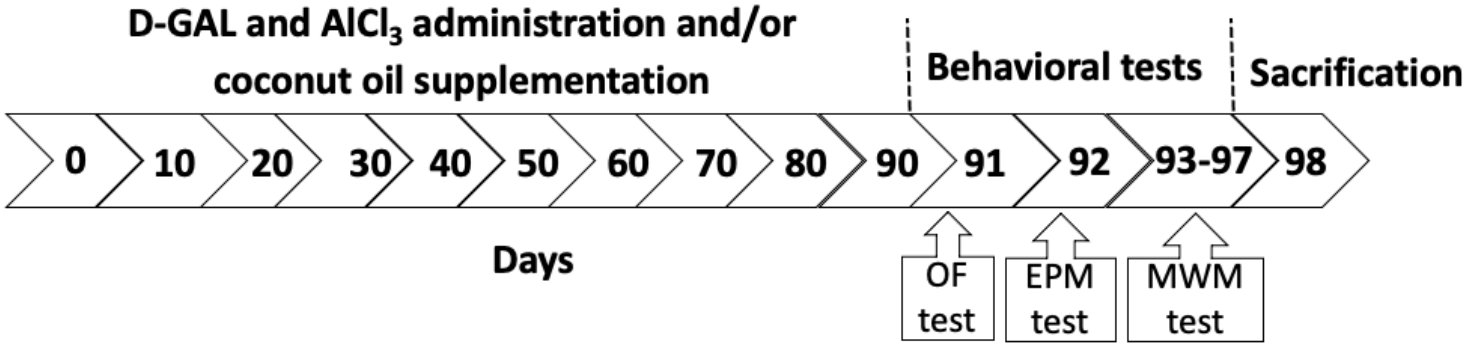

Figure 1

Study design

OF: Open field, EPM: Elevated plus maze, MWM: Morris water maze.

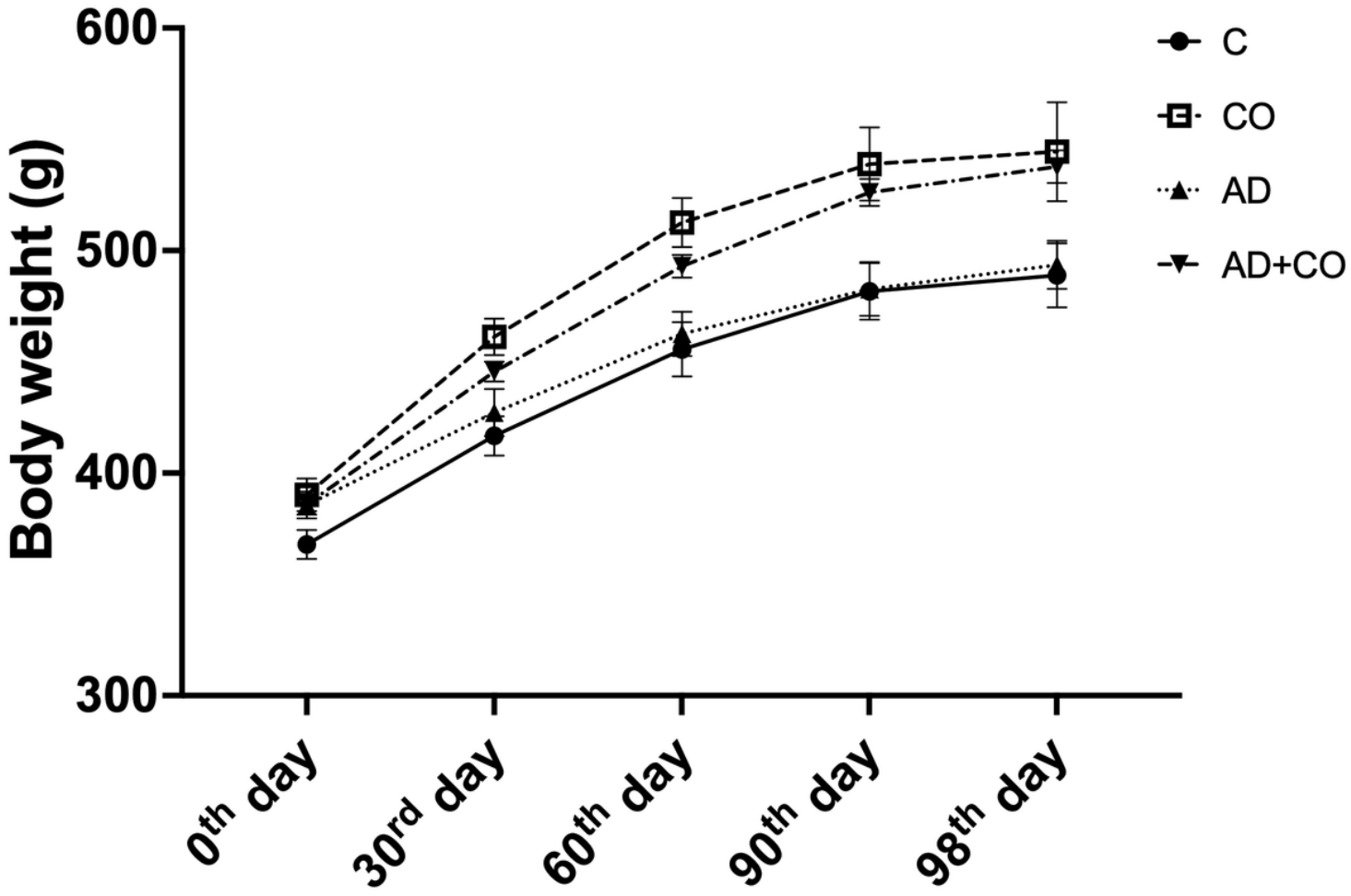

Figure 2

Body weights of the groups (n = 7 per group). Data are expressed as mean ± SEM. C: Control group, CO: Coconut oil group, AD: Alzheimer's Disease model group, and AD + CO: Alzheimer's Disease model + coconut oil group.

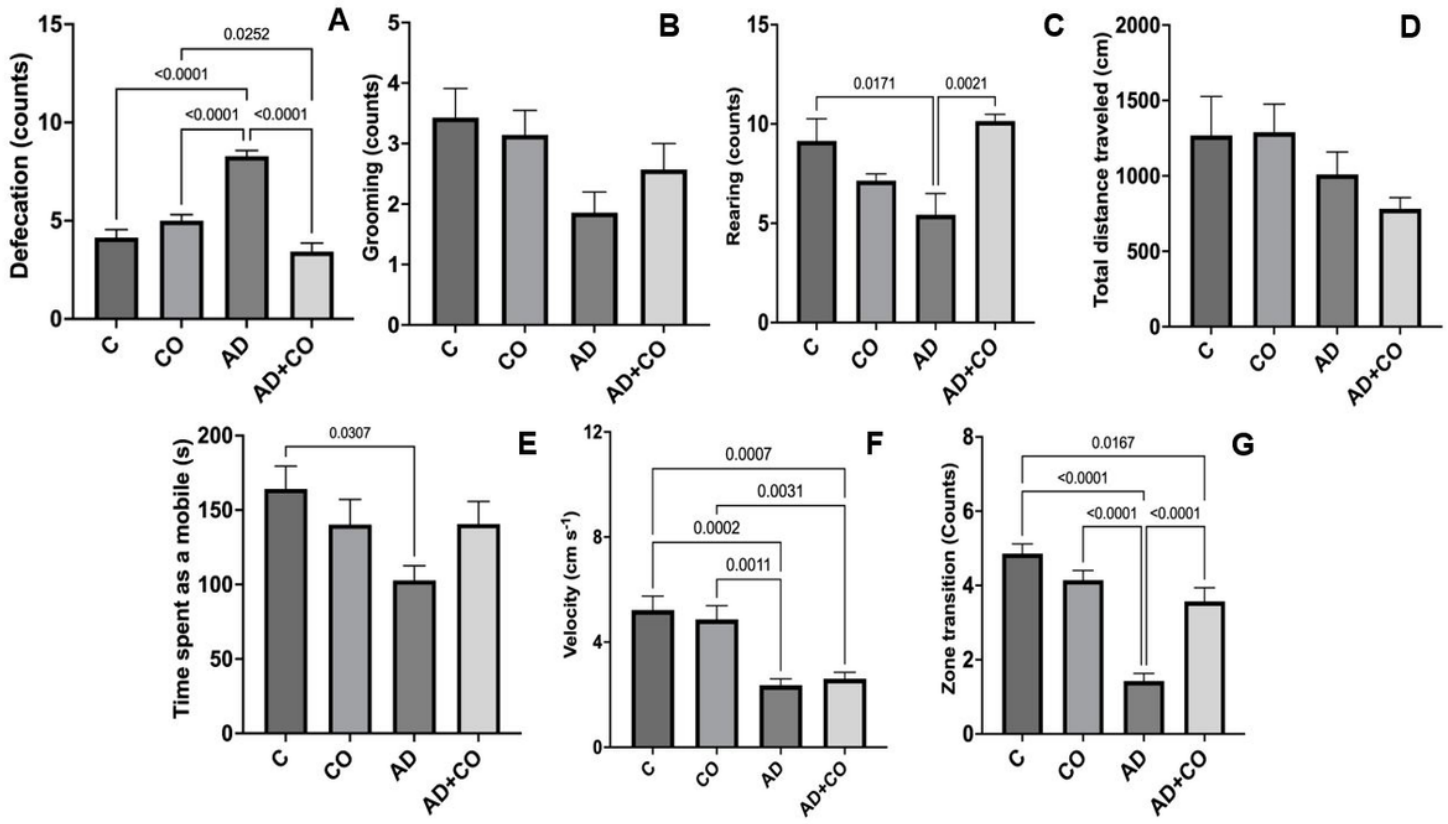

Figure 3

Effects of coconut oil supplementation on the defecation (A), grooming (B), and rearing (C) counts, total distance traveled (D), time spent as a mobile (E), velocity (F) and zone transition counts (G) during the open field test in the experimental AD model (n = 7 per group). Data are expressed as mean ± SEM. C: Control group, CO: Coconut oil group, AD: Alzheimer's Disease model group, and AD + CO: Alzheimer's Disease model + coconut oil group.

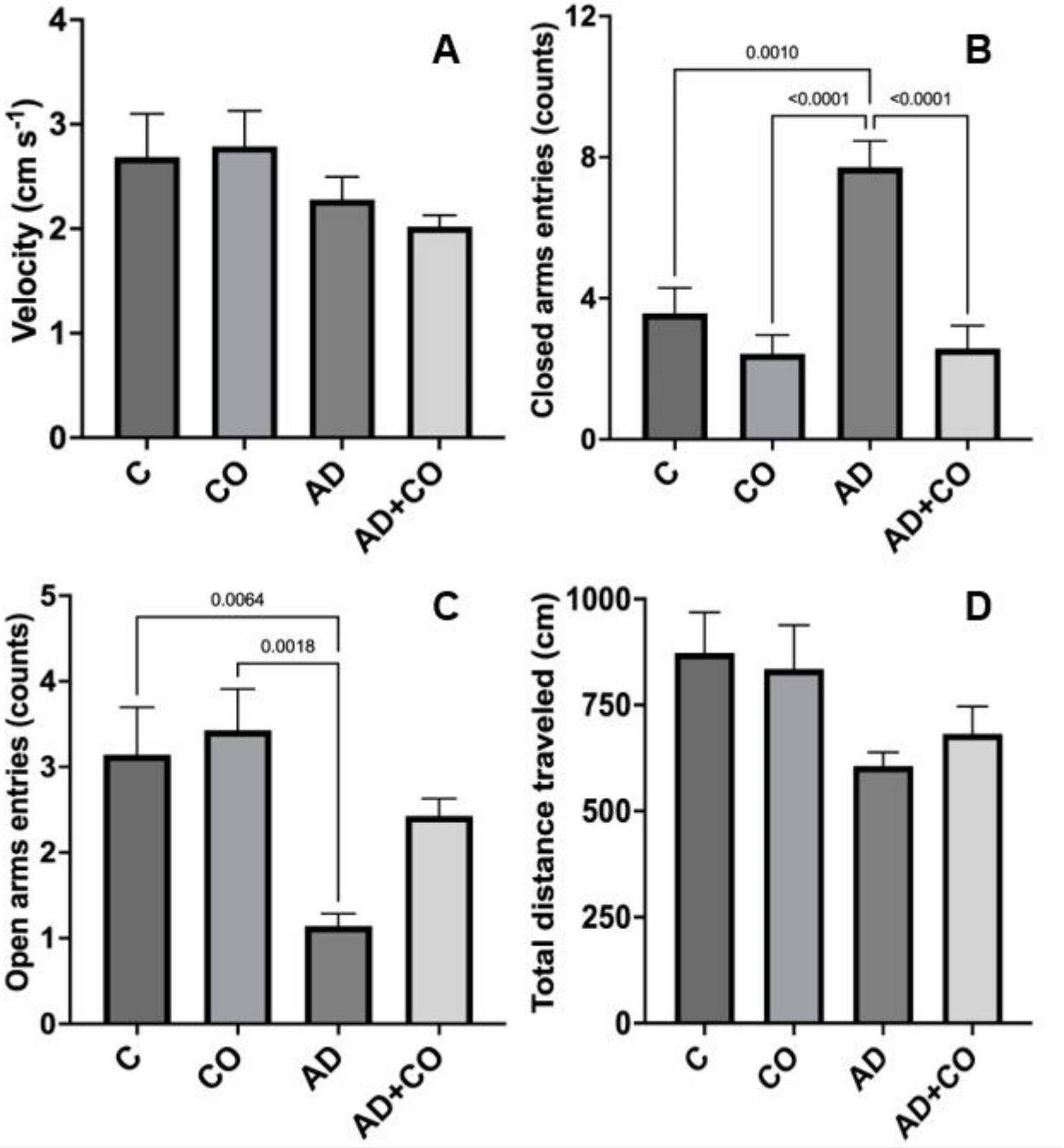

Figure 4

Effects of coconut oil supplementation on mean velocity (A), number of entries in closed arms (B), and open arms (C), and total distance traveled (D) during the elevated plus maze test of rats which were exposed to D-GAL/$AlCl_3$ (n = 7 per group). Data are expressed as mean ± SEM. C: Control group, CO: Coconut oil group, AD: Alzheimer's Disease model group, and AD + CO: Alzheimer's Disease model + coconut oil group.

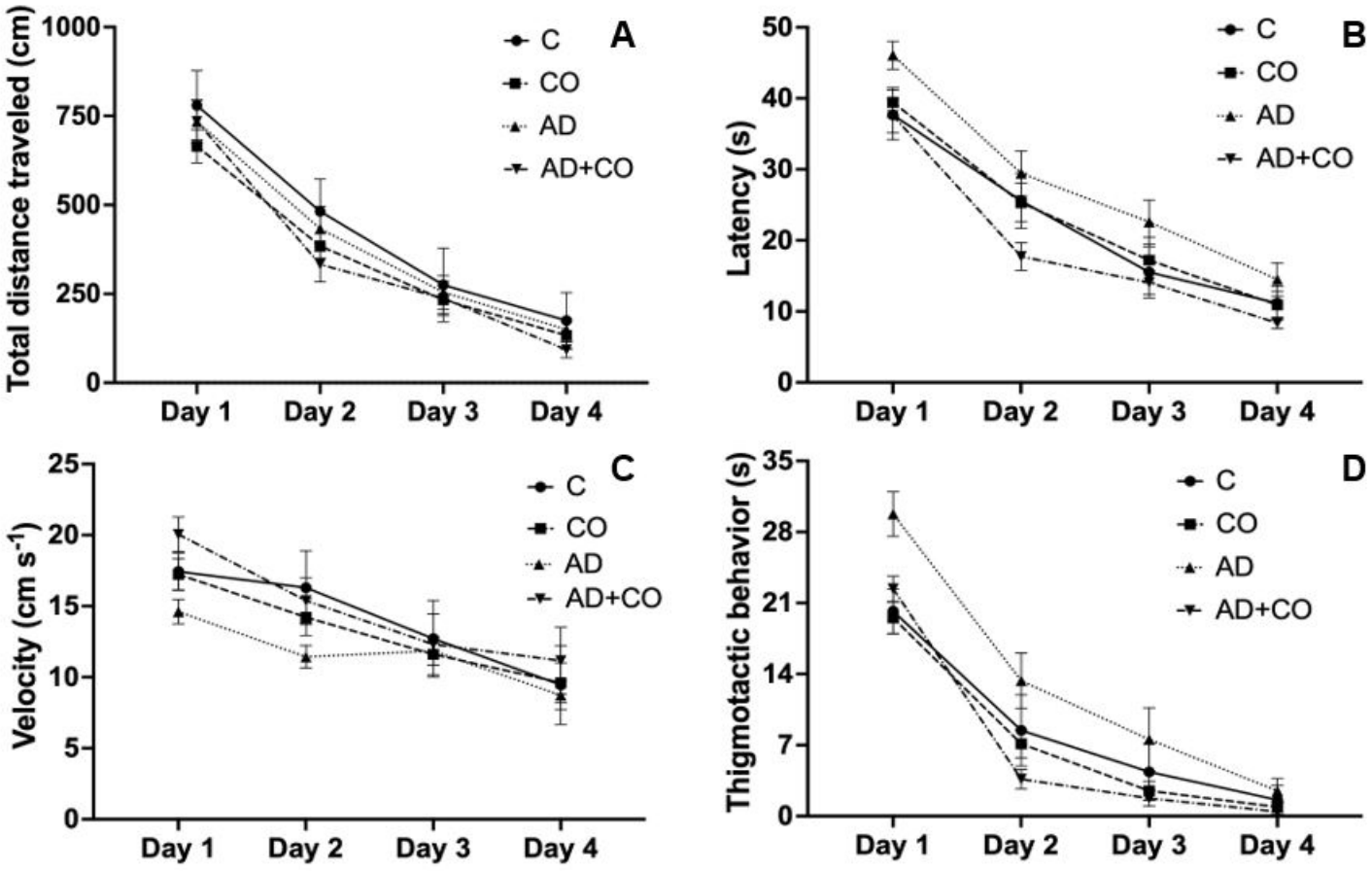

Figure 5

Effects of coconut oil supplementation on total distance traveled (A), latency to find the platform (B), average velocity (C), and thigmotactic behavior (D) performance during Morris water maze training trial in experimental AD-induces rats (n = 7 per group). Data are expressed as mean ± SEM. C: Control group, CO: Coconut oil group, AD: Alzheimer's Disease model group, and AD + CO: Alzheimer's Disease model + coconut oil group.

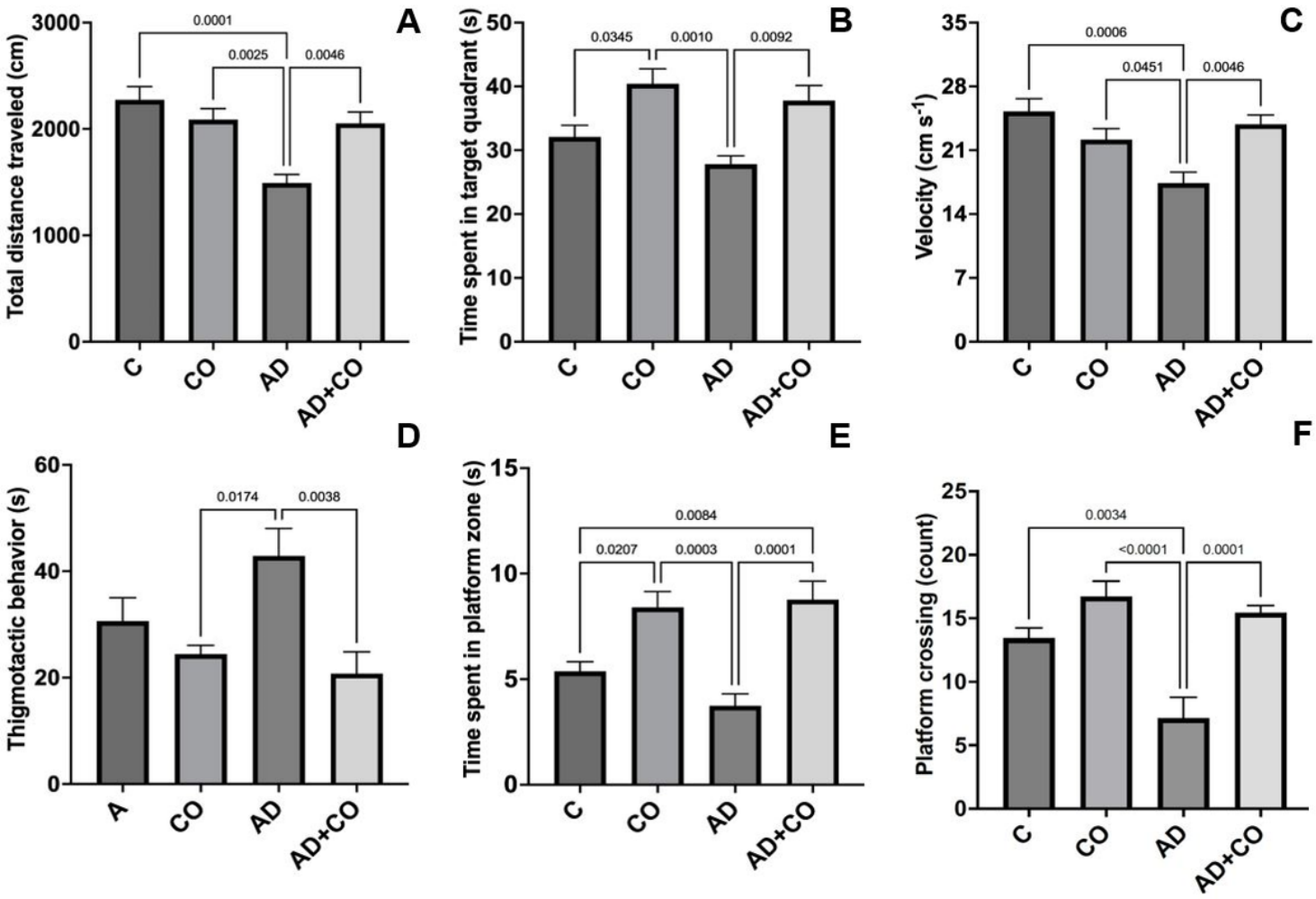

Figure 6

Effects of coconut oil supplementation on Morris water maze probe trial performance in experimental AD-induced rats. Total distance traveled (A) time spent in target quadrant (B), average swimming speed (C), thigmotactic behavior (D), time spent in platform zone (E), and platform crossing counts (F) (n = 7 per group). Data are expressed as mean ± SEM. C: Control group, CO: Coconut oil group, AD: Alzheimer's Disease model group, and AD + CO: Alzheimer's Disease model + coconut oil group.

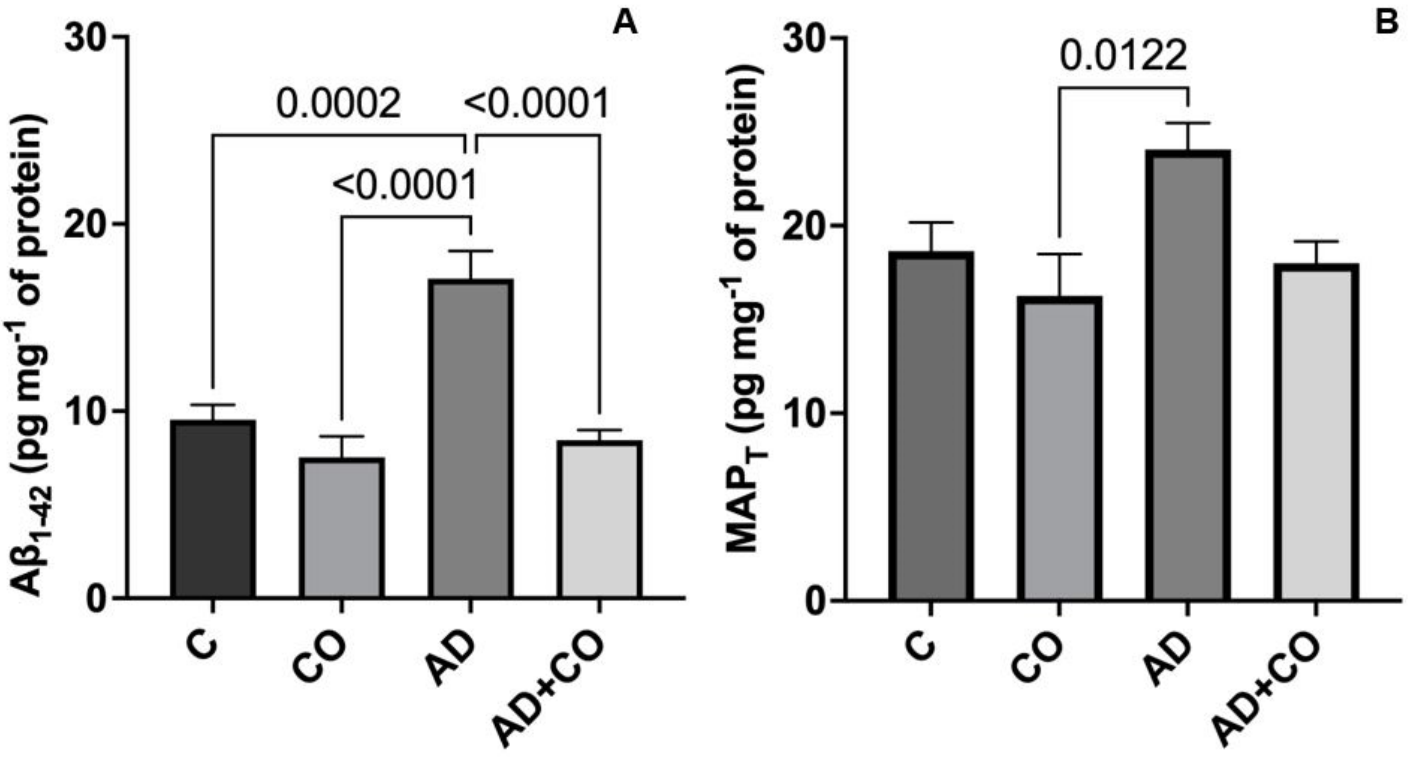

Figure 7

Effect of coconut oil supplementation on hippocampal amyloid beta 1–42 ($A\beta_{1-42}$) (A) and microtubule associated protein Tau (MAP_T) (B) levels in the experimental AD-induced rats (n = 7 per group). Data are expressed as mean ± SEM. C: Control group, CO: Coconut oil group, AD: Alzheimer's Disease model group, and AD + CO: Alzheimer's Disease model + coconut oil group.

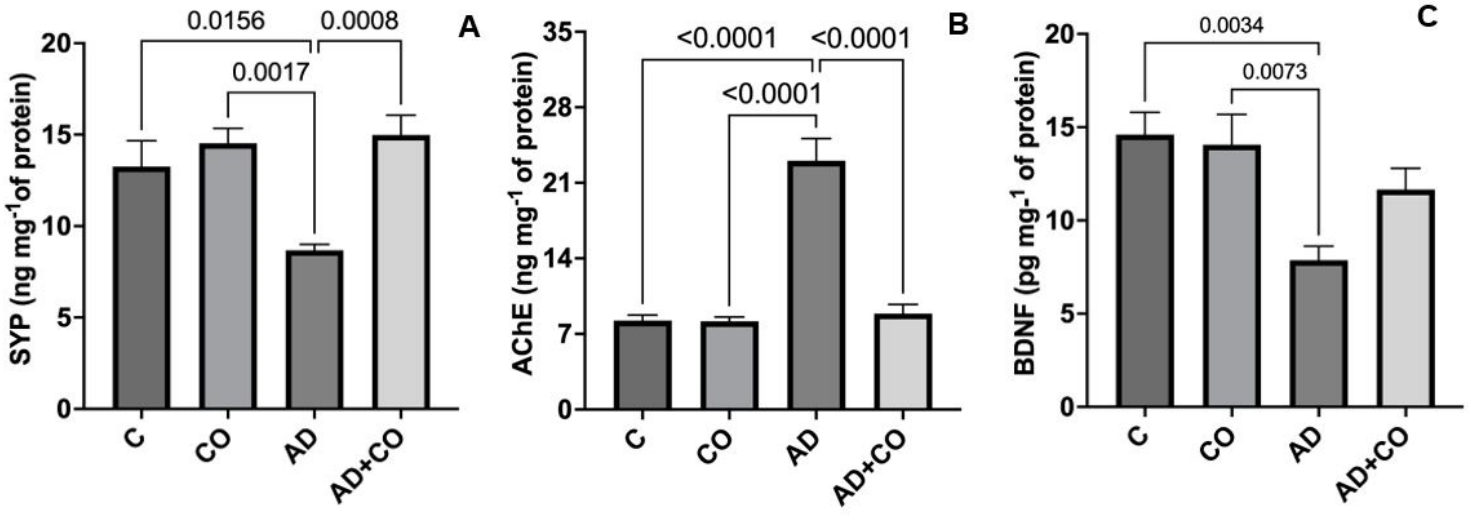

Figure 8

Effects of coconut oil supplementation on hippocampal synaptophysin (SYP) (A), acetylcholinesterase (AChE) (B), and brain-derived neurotrophic factor (BDNF) (C) in the experimental AD-induced rats (n = 7 per group). Data are expressed as mean ± SEM. C: Control group, CO: Coconut oil group, AD: Alzheimer's Disease model group, and AD + CO: Alzheimer's Disease model + coconut oil group.

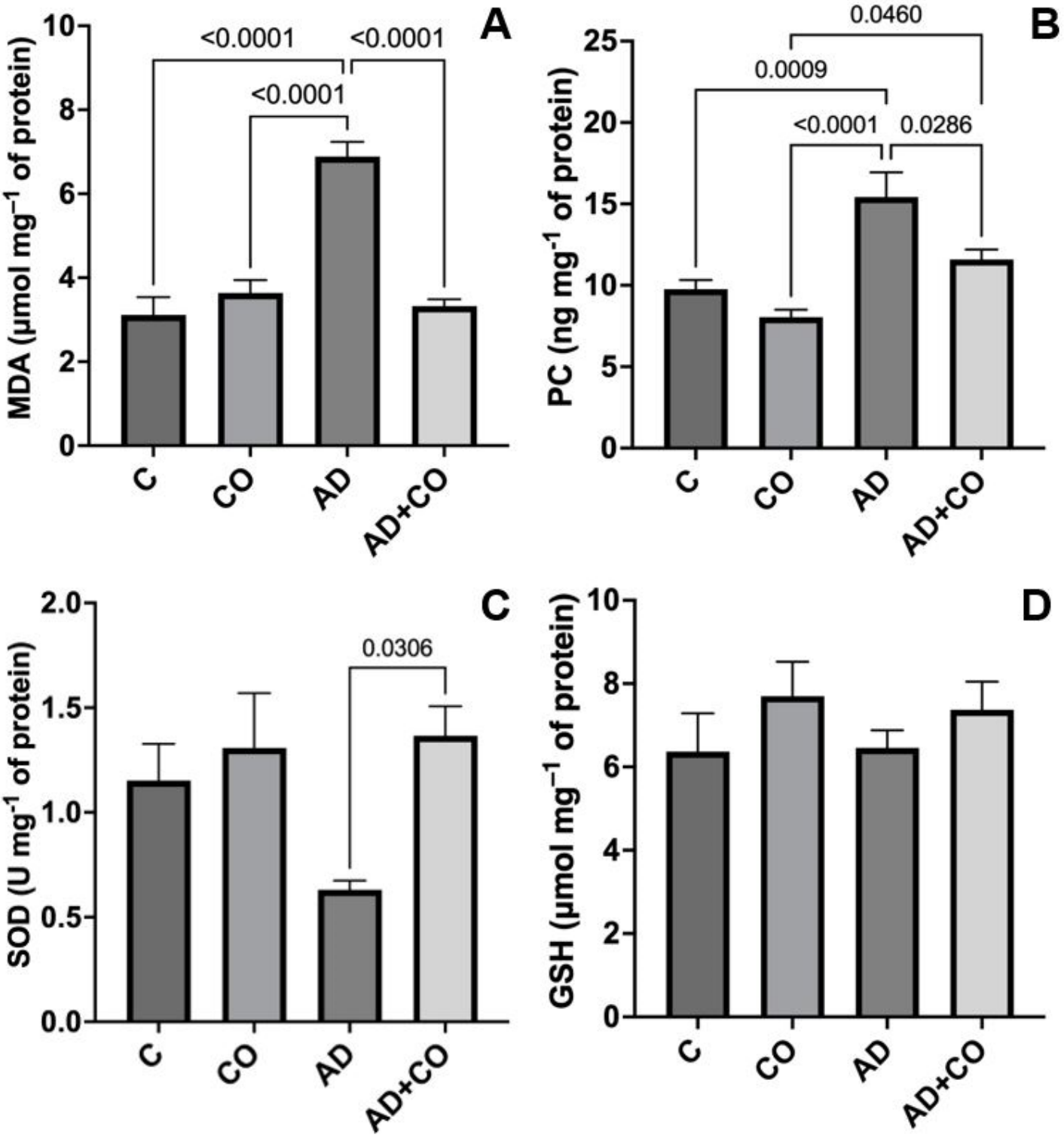

Figure 9

Effects of coconut oil supplementation on hippocampal malondialdehyde (MDA) (A), protein carbonyl (PC) (B), superoxide dismutase (SOD) (C), and glutathione (GSH) (D) concentrations experimental AD-induced rats (n = 7 per group). Data are expressed as mean ± SEM. C: Control group, CO: Coconut oil group, AD: Alzheimer's Disease model group, and AD + CO: Alzheimer's Disease model + coconut oil group.