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The Neuroprotective Effects of Policosanol on Learning and Memory Impairment in a Male Rat Model of Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) as a neurodegenerative disease is recognized with progressive cognitive function failure, which is determined by beta-amyloid (Aβ) accumulation in extracellular space and hyperphosphorylation of intracellular Tau protein. Aβ stimulates some kinds of active oxygen and causes oxidative stresses and apoptosis. Policosanol (PCO) is a reducing lipid complement, which has antioxidant and anti-inflammatory activities. In the current research, the PCO effects on learning and memory impairment were investigated in a rat model of AD. Healthy adult male Wistar rats (230–250g) were divided randomly into 7 groups (n=6-7): Control, Sham (5 μL of phosphate-buffered saline, intracerebroventricular (ICV) microinjection), AD model (5 μL, ICV injection of Aβ), acacia gum (50 mg/kg, 8 weeks, gavage), PCO (50 mg/kg, 8 weeks, gavage), AD + acacia gum (50 mg/kg, 8 weeks, gavage), and AD + PCO (50 mg/kg, 8 weeks, gavage). Passive avoidance learning (PAL) and memory were assessed by shuttle box, cognitive memory by novel object recognition (NOR), and spatial memory by the Morris water maze (MWM) test. The oxidant and antioxidant parameters were examined at the end of the experiments. According to our results, ICV injection of Aβ caused reduced cognitive memory in NOR, spatial memory in MWM, and passive avoidance in PAL tests. PCO caused a recovery in cognitive memory, spatial memory, and PAL memory. Aβ plaques increased in the AD group, while PCO decreased it. Aβ increased total oxidant status and decreased total antioxidant capacity, whereas PCO reversed these parameters. Our results demonstrated that PCO has neuroprotective effects and can protect learning and memory impairments via its hypolipidemic and antioxidant effects.

Introduction

Alzheimer's disease (AD) as the sixth leading cause of death, is a multifactorial and progressive disorder (Reitz, 2012). It affects certain areas of the brain (Wenk, 2003). One of the neuropathological features of this disease may be the accumulation of neuronal plaques called beta-amyloid (Aβ) plaques and neurofibril coils resulting from the accumulation of microtubule-dependent protein hyperphosphorylation, such as intracellular Tau protein (Huang and Jiang, 2009). In AD, memory and learning can be impaired (Barone et al., 2014). AD affects the neurons, and consequently thinking, memory, and behavior and it has a significant effect on work and social life (Singhal et al., 2012; Klimova et al., 2015). However, the exact AD etiology and pathogenesis are still unknown (Tanzi et al., 1996; McNeilly et al., 2012). Oxidative stress has been shown associated with cognitive disorders, such as AD (Halagappa et al., 2007).

The important effect of oxidative stress as the main cause on AD pathogenesis has been reported (Andersen, 2004; Butterfield et al., 2006). In this regard, oxidative stress causes several neurological diseases, like Parkinson’s disease, AD, and amyotrophic lateral sclerosis (Rojas and Gomes, 2013). The critical function of oxidative stress in the brain of AD cases has been also been indicated (Markesbery, 1997; Swerdlow, 2012; Bonda et al., 2014). Thus, antioxidants can attenuate Aβ-associated neurotoxicity and cell death, resulting in the amelioration of AD-related defects in cognition and memory (Lin et al., 2006; Aliev et al., 2008).

An unhealthy lifestyle leads to an increase in the incidence of obesity and hypertension, which are components of metabolic syndrome, which can be linked to AD (Halagappa et al., 2007). Metabolic defects lead to functional modifications associated with cerebral aging and AD pathogenesis (Chen et al., 2016). Obesity and a diet high in fat are associated with cognitive impairment (Pistell et al., 2010; Kanoski and Davidson, 2011; Moy and McNay, 2013). It has been shown AD is highly observed in states, in which the consumption of diets high in and calorie is high (Martin et al., 2014). Diet-induced obesity (DIO) is linked to cognitive defect and pathological alterations similar to changes observed in AD (Heyward et al., 2012; Yang et al., 2013; Boitard et al., 2014; Osborne et al., 2016). Although eating a high-fat diet (HFD) affects AD-associated pathology in different animal models and conditions (Grant, 1999; Grillo et al., 2011; Soares et al., 2013; Hsu and Kanoski, 2014), the mechanisms linking risk factors to AD pathogenesis are not still clear (Thériault et al., 2016). There is an association between obesity, insulin resistance, diabetes, and dementia (De Felice, 2013; Arnold et al., 2014; De Felice and Ferreira, 2014; Dineley et al., 2014; Ettcheto et al., 2016). Several factors, such as oxidative stress cause HFD-related damage to the brain, particularly with aging (Freeman et al., 2014; Tarantini et al., 2018). Overweight leads to neuronal damage (Kim et al., 2015), long-term memory loss (Komaki et al., 2015), and impaired synaptic plasticity (Karimi et al., 2013; Karimi et al., 2015). It has been reported that high blood cholesterol is linked to AD and is one of the important risk factors (Barone et al., 2014). Data from these epidemiological studies suggest that elevated serum/plasma cholesterol levels in middle age are associated with varying degrees of progression to AD (Kivipelto and Solomon, 2006; Solomon et al., 2009).

Policosanol (PCO) is an effective dietary and nutritional supplement for lowering total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and serum triglyceride (TG) in human and animal models (Lee et al., 2016). Octacosanol (66%), triacontanol (12%), and hexacosanol (7%) are the main constituents of the PCO mixture. The remaining 15% includes other alcohols, which are minor constituents (Arruzazabala et al., 2000). Hypolipidemic effects, a natural antioxidant, and inhibitors of platelet aggregation, endothelial lesions, and foam cell generation are among the effects of PCO (Nam et al., 2019). Octacosanol, as a main constituent of the PCO, is very effective in lowering LDLs and increasing high-density lipoproteins (HDLs), and also increases athletic performance (Taylor et al., 2003; Ma et al., 2018).

According to the various properties of PCO, including antioxidant, anti-inflammatory, cholesterol-lowering properties, and the fact that the effects of PCO on the treatment of AD have not yet been addressed, its effects on AD were assessed in the present experiment. We investigated the possible therapeutic effects of PCO as a therapeutic or protective compound in Aβ-related learning and memory impairment in an animal model of AD.

Material And Methods

Animals and experimental design

Healthy adult male Wistar rats (230–250 g) were prepared from Hamadan University of Medical Sciences. Environmental conditions in the animal included the temperature of 22 ± 2°C and the optical cycle was 12 hours of light and 12 hours of darkness (from 7 am to 7 pm). The rats were given adequate water and food during the experiment and all tests were performed throughout the day. The research protocol was confirmed by the Animal Ethics Committee Guidelines for the Use of Experimental Animals (IR.BASU.REC.1398.029), following the “NIH Guide for the Care and Use of Laboratory Animals”.

Adaptation to the environment was done one week the experiments and then, animals were randomly divided into seven experimental groups: (n=6-7):

1. Control group: This group had access to food and water indefinitely and did not undergo AD induction.
2. Sham group: The rats received phosphate-buffered saline (intracerebroventricular (ICV); 5 µL).
3. AD group: The rats received Aβ1-40 (5 µL; ICV).
4. PCO group: 50 mg/kg of PCO was given once daily by oral gavage for 8 weeks.
5. Acacia gum group (vehicle): 50 mg/kg of acacia gum was given once daily by oral gavage for 8 weeks.
6. AD + acacia gum group: The rats received Aβ1-40 (5 µL; ICV) and 50 mg/kg of acacia gum was given once daily by oral gavage for 8 weeks.
7. AD + PCO group: The rats received Aβ1-40 (5 µL, ICV) and 50 mg/kg of PCO was given once daily by oral gavage for 8 weeks.

Clinical dose of Policosanol: or Administration and dosage

Administration of PCO was done at 50 mg/kg body weight (Guerra et al., 2015). PCO is a water-insoluble substance (Luz et al.), and it was prepared by oral suspension with the use of acacia gum, which is a solvent of PCO (Guerra et al., 2015; Elseweidy et al., 2016). The rats were then gavaged by this suspension for 8 weeks (Elseweidy et al., 2016).

Aβ injections and surgery

Aβ1-40 (100 µg; Tocris Bioscience, Bristol, UK) was dissolved in 100 µL of PBS (vehicle solution), followed by incubation (37◦C / 7 days) before usage. As a result of this process, neurotoxic amyloid fibrils were obtained (Lorenzo and Yankner, 1994; Yaghmaei et al., 2013). The rats to generate an AD model were anesthetized using ketamine and xylazine (100 and 10 mg/kg, respectively) and transferred to the stereotaxic apparatus (Stoelting Co., Wood Dale, IL, USA). Using an electrically shielded heating pad, the rats’ body temperature was kept at 37.0 ± 0.2◦C during Aβ injection. Their skulls were uncovered and over the ventricular regions, the holes were drilled based on the coordinates of the appendix: 2 mm lateral to the midline, 1.2 mm posterior to bregma, and 4 mm ventral to the surface of the cortex (Paxinos and Watson, 2005). The rats were injected (1 µL/min) slowly using a 5 µL microsyringe (Hamilton Laboratory Products, USA). The injections lasted for 5 min and then, syringes were left untouched for 5

min after the injection and removed painlessly. The sham group received a vehicle solution. The recovery for rats lasted for 7 days (Asadbegi et al., 2017; Ahmadi et al., 2021a; Ahmadi et al., 2021b) (**Fig.1**).

Behavioral study

Locomotor activity in the open field (OF)test

Locomotor activity was assessed using the OF test. The apparatus is made of white acrylic with a surface area of 50 × 50 cm, and the walls are 38 cm in height. The field is lit by low ambient room lights (Bisagno et al., 2004). An overhead video camera recorded the time spent by rats in the central and peripheral zones and the data were analyzed through a video track software. The square-shaped central zone locates 25 cm from each wall. Animals were located in the middle of the central zone and could explore for 10 min (Drews et al., 2005). The total distance moved (locomotor activity) and average velocity in the apparatus were measured (Lalonde et al., 2003).

Novel object recognition test (NOR)

This test measures the visuospatial memory of rodents (Hansen et al., 2010; Ganji et al., 2017). Adaptation to the apparatus (60 × 60 × 45 cm) was done 24 h prior to test by placing animals in the device for 20 min. After 24 h, we placed two identical objects in the apparatus and the animals were placed independently in the middle part and close to the walls, and their heads were fixed to be opposite to the objects. In this phase, animals were given 10 min to explore the objects, and then they were transferred to their cages. After 24 h, one of the familiar objects was replaced with a new one, and the animals were placed in the device with a new and familiar objects for 10 min (Cohen and Stackman Jr, 2015). A video camera recorded this phase. The discrimination index was considered as the time taken to explore the new object to the total time spent with both objects. The experiment timeline represents the time taken exploring both objects. Objects were presented randomly between the groups and rats. Cleaning of the objects and the box was done during intervals using 70 % ethanol to get rid of olfactory cues (Hansen et al., 2010).

Morris water maze test

Spatial memory was tested using Morris water maze (MWM), which is a black circular pool with a diameter of 180 cm and a depth of 60 cm filled with water (22±1°C) (42 cm of depth). The pool has four quadrants and starting sites with an equal distance from each other called north, east, south, and west. There is an invisible platform (diameter: 10 cm) that is 1 cm below the water in the northern quadrant center. Training sessions were performed from 10:00 AM to 13:00 PM for four days, in which two blocks with four trials were considered. In the training phase, each rat could swim to find the invisible platform for 90s. Training was done from all starting sites. The rats could stay on the platform for 30 s between the two trials. A 5-min resting time was considered between two blocks. The parameters, such as time spent to reach the platform (escape latency) and traveled distance were recorded using a video camera (Nikon, Melville, NY, USA) installed above the pool and attached to a tracking software. The probe trial

was conducted on day 5, on which the platform was removed and animals could swim for 60s. Then, we recorded the time spent in the target quadrant (Zarrinkalam et al., 2016; Zarrinkalam et al., 2018).

Passive avoidance learning (PAL) test

Passive avoidance apparatus

A step-through device measured passive avoidance learning (PAL) and memory (Zarrinkalam et al., 2016), which has two light (transparent plastic) and dark (dark opaque plastic) compartments (both 20 cm × 20 cm × 30 cm) . Both chambers have a floor covered by stainless-steel rods (3 mm in diameter) spaced 1 cm apart. A shock generator (Borj Sanat, Tehran, Iran) electrifies the floor of the dark compartment. A rectangular opening guillotine door (6cm×8cm) separates two compartments (Komaki et al., 2015; Shiri et al., 2017).

Passive avoidance training

The habituation phase was done by giving the groups two primary trials. After placing the animals in the light compartment facing away from the door, the guillotine door was raised after 30 s. Rats prefer dark environments. Following the entrance of the rats to the dark chamber, the door was closed and 30 s later, they were transferred to their cages. This trial was repeated after 30 min, and 30 min later, the first acquisition trial was done. The latency to enter the dark chamber (step-through latency, STLa) was recorded after placing all four paws in the dark chamber. After entrance to the dark chamber, the guillotine door was closed and the animal received an electrical shock (50 Hz, 1.5 s, 0.4 mA intensity). After 30 s, the animal was transferred to its home cage and the process was repeated after 2 min. The training was finished when the animal remained in the light chamber for 120 s. The number of light-dark transitions was also noted (Zarrinkalam et al., 2016; Shiri et al., 2017).

Retention test

The retention test was conducted 24 h following the acquisition trial. Animals were located in the light compartment and after 30 s, the door was raised. The STLr and time spent in the dark compartment (TDC) were noted for 300 s. When the rats did not enter the dark chamber during 300 s, the retention test was finished and the animal received a ceiling score of 300 s (Barzegar et al., 2015; Zarrinkalam et al., 2016).

Biochemical analysis

After all behavioral tests, 5 ml of portal vein blood specimens were collected into heparinized tubes. The specimens were then centrifuged (3500 rpm / 10 min / 4°C) and serums were frozen at −80°C and transferred for biochemical assessments. Finally, total antioxidant capacity (TAC) and total oxidant status (TOS) were determined.

Histology

After all experiments, rats were deeply anesthetized using urethane and perfused via the heart using formol–saline (Komaki and Esteky, 2005; Komaki et al., 2007). Regarding Congo red staining, hippocampal coronal sections (5 μm) were prepared. Then, the slides were assessed using an optic microscope and Image J software. Congo red staining was applied to indicate Aβ plaque generation in the brain tissue (Mirzaei et al., 2018).

Data analysis

Data analysis was done by one-way and two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to compare groups. Data are presented as mean ± SEM. Statistical significance was set at $P < 0.05$.

Results

Effect of PCO and Aβ on locomotor activity in the open field test

Comparing the mean velocity and the distance moved showed no significant difference between the different groups (distance moved: $F(6, 55) = 2.018$, $P=0.0786$ and mean velocity $F(6, 55) = 2.015$, $P=0.0791$). Also, the motor activity did not change significantly after Aβ injection and PCO did not affect the motor activity (**Fig. 2**).

Effect of PCO and Aβ on NOR test

DI as an index of the NOR test is considered as the time spent to explore the new object divided by the total time to explore both familiar and new objects on the second day of the test ($F(6, 40) = 8.204$, $P<0.0001$). The time spent around the new object in the AD group decreased significantly in comparison with the control and sham groups ($P<0.001$). A significant decrease was detected in DI of the AD group in the comparison with PCO and acacia gum groups ($P < 0.001$). A significant increase was found in DI in the AD+PCO group in comparison with the AD group ($P < 0.01$) (**Fig. 3**).

Effect of PCO and Aβ on MWM test

The escape latency and the distance moved to reach the invisible platform on the first to fourth days of training were the criterion for learning in animals. In this period, the AD group showed a significant increase in the time spent to find the invisible platform compared with the control and sham groups. During this four-day learning period, significant differences were observed between the escape latency in the AD rats and the control and sham groups (($P < 0.05$), ($P < 0.0001$), ($P < 0.001$, $P < 0.01$), and ($P < 0.001$ and $P < 0.0001$, respectively)). Also, a significant difference was detected between the AD and PCO group on the second to fourth days ($P < 0.01$, $P < 0.01$, and $P < 0.0001$, respectively), in the acacia gum group on the second, third, and fourth days ($P < 0.05$, $P < 0.01$, and $P < 0.0001$, respectively), and in the AD+PCO group on the second and fourth days ($P < 0.05$) (**Fig. 4A**). The distance traveled to find the invisible platform in the AD group during the training days on the third and fourth days was significantly different from the other groups tested (**Fig. 4B**). The average

time spent in the target quadrant on the test day (the fifth day) was measured in the probe trial. The AD group had the least time spent in the target quadrant than all groups, but this parameter was significantly different between the AD group and other groups (Control, P < 0.01; PCO, P < 0.001; and AD+PCOP < 0.01) (**Fig. 4C**).

Effect of PCO and Aβ on PAL test

Comparing the latency to enter the dark compartment in the compromise stage (STLa) indicated is no significant difference between the groups, which indicates that the rats did not differ from each other in terms of entering the dark chamber before the shock (F (6, 38) = 1.825, P=0.1202) (**Fig. 5A**). The groups showed no significant difference regarding the number of shocks received until the learning criteria were met (NTa) (F (6, 59) = 2.682, P=0.0228) (**Fig. 5B**).

Regarding STLr, a significant difference was found between the AD group and groups, such as the control and sham groups (P < 0.01 and P < 0.001, respectively), acacia gum and PCO groups (P < 0.01 and P < 0.001, respectively), and the AD+PCO group (p < 0.05) (F (6, 42)= 6.146, P=0.0001) (**Fig. 6A**). There was a significant difference between the AD group and groups, such as the control and sham groups (P < 0.001), the acacia gum and PCO groups (P < 0.001), the AD + acacia gum groups (p < 0.01), and the AD+PCO group (p < 0.001) in terms of TDC (F (6, 42) = 6.146, P=0.0001) (F (6, 43) = 14.63, P<0.0001) (**Fig. 6B**).

Effect of PCO and Aβ on TAC and TOS

TOS is considered an oxidative indicator. As illustrated in **Fig. 7A**, PCO and AD+PCO groups were found with a markedly lower TOS level in comparison with the AD group (F (6, 35) = 9.379, P<0/0001). In general, the AD group had a significantly higher concentration of TOS compared with other groups. TAC is considered an antioxidant indicator. The AD group was found with a significantly lower TAC mean level in the plasma than the PCO group (F (6, 31) = 6.064, P=0.0003) (**Fig. 7B**).

Effects of PCO on brain Aβ plaques

To approve generation of Aβ plaques animals' brains, Congo Red staining was done. **Fig. 8** indicates the Aβ plaques (red spots) in the hippocampal coronal sections. These plaques were found in the brain sections related to the Aβ group. After staining, the PCO-treated Aβ rats were found with a significant decrease in Aβ plaque deposits in than the Aβ rats. No significant plaque was detected in the control and sham groups.

Discussion

In the current research, we studied the effects of an ICV injection of Aβ (1–40) on learning and memory, under the influence of PCO as a cholesterol-lowering, anti-inflammatory, and antioxidant supplement in

adult male rats. Our findings showed that the use of PCO is not effective in motor activity in the open field test in all groups. Consumption of PCO in AD male rats improved cognitive memory evidenced by the NOR test, spatial memory confirmed by the MWM test, and PAL and memory evidenced by the shuttle box test. Aβ plaques increased in the AD group, while PCO decreased the plaques. The ICV injection of Aβ increases TOS, which indicates an increase in and induction of oxidative stress, whereas the use of PCO increases TAC, which indicates an increase in antioxidant properties.

Prior to the behavioral tests, the rat's motor activity was assessed in an open field test, and our findings showed that PCO had no effect on motor activity. According to our previous findings, cinnamaldehyde with antioxidant properties had no effect on the animal's motor activity (Etaee et al., 2019). Also, the chronic use of Cyanidin-3-glucoside in diabetic rats showed that it does not significantly alter the motor activity of animals (Nasri et al., 2012).

According to the results of the MWM test, the Aβ injection had a significant effect on spatial memory. Numerous studies have shown that Aβ injection impairs memory and learning (Asadbegi et al., 2017; Ahmadi et al., 2021b). In line with our findings, different studies have shown that a single dose of Aβ 1–42 (ICV) (Choi et al., 2001), as well as an intrahippocampal (IHP) injection of Aβ (25–35), lead to a decrease in antioxidant activity and learning in the MWM test (Sohanaki et al., 2016). In our experiment, PCO improved learning and memory in the NOR, MWM, and PAL tests. Several studies have confirmed the role of antioxidant factors in improving memory and an improvement in memory has been reported after consuming antioxidants. The long-term use of thymol (as an antioxidant) by AD rats improved learning and memory in the MWM and PAL tests. Thymol reduces Aβ plaques, lipid peroxidation, and nerve damage (Asadbegi et al., 2017). *Glycyrrhiza glabra* extract improved spatial learning and memory. Accordingly, the memory-enhancing effects of *G. glabra* are possibly mediated by its antioxidant and anti-inflammatory effects. The *G. glabra* root extract exposes sensitive brain cells to less oxidative stress, which reduces brain damage and improves neural function (Chakravarthi and Avadhani, 2013). Vitamin E through its antioxidant effect improves learning and memory in rats in the PAL test following the learning and memory impairment after exposure to the lead (Khodamoradi et al., 2015).

Oxidative stress can be caused due to an imbalance between reactive oxygen species (ROS) generation and intracellular antioxidants (Allan Butterfield, 2002). The two most important sources of ROS in the cell are mitochondria and NADPH oxidase (NOX). In mitochondria, ROS are produced during the respiratory chain, whereas NOX is produced in the membrane of neutrophils and phagocytes (Scherz-Shouval et al., 2007; Azad et al., 2009; Chen et al., 2009). Mitochondria are vulnerable to oxidative stress (Halliwell, 2012). Free radicals and ROS and the accumulation of these free radicals in the body result in oxidative damage to lipids, proteins, and DNA that can lead to diabetes, cancer, and other neurological diseases (Chakravarthi and Avadhani, 2013). On the other hand, PCO can inhibit some deteriorating physiological activities through many mechanisms, including the modification of ROS (Wong et al., 2016).

Our results showed that memory impairment induced by Aβ was associated with a reduction in antioxidant capacity and an increase in oxidative stress. Evidence suggests that Aβ may directly impair

mitochondrial function, and also energy deficiency and neuronal death can be seen in AD patients (Du and Yan, 2010). PCO by increasing TAC levels and its antioxidant properties to some extent prevents the effects of Aβ. The brain is susceptible to oxidative stress because of its low level of antioxidants and cell membrane lipids; thus, injecting Aβ (which leads to the induction of AD) reduces the antioxidant power and learning in the MWM (Butterfield et al., 2007). Resveratrol, a polyphenolic phytoalexin, exerts many beneficial and neuroprotective effects. Resveratrol protects neurons by affecting the oxidative stress caused by Aβ plaques and also by reducing the formation of nitric oxide and lipid peroxides (Huang et al., 2011; Carrizzo et al., 2013; D Rege et al., 2015). In addition, intracellular Aβ accumulation causes severe damages to mitochondria via tau accumulation as well as dysfunction of metabolic enzymes. Tau phosphorylation is involved in the development of AD and causes microtubule instability and disturbance in axon transport (Gandbhir and Sundaram, 2020).

Biochemical, genetic, and epidemiologic evidence suggests that predisposition to AD may arise from altered cholesterol metabolism (van der Kant et al., 2019). In addition to AD, abnormal cholesterol metabolism in the brain also causes many neurological diseases, like Parkinson’s disease, Huntington's disease, and lateral amyotrophic lateral sclerosis (Jin et al., 2019). Diabetes, dyslipidemia or stroke increase the risk of AD (Hunsberger et al., 2019). We used PCO as a natural antioxidant with hypocholesterolemic, anti-aging, and hypoglycemic properties (Elseweidy et al., 2016; Nam et al., 2019). PCO lowers total cholesterol by inhibiting HMG-CoA reductase activity and inducing phosphorylation of AMP kinase, which can result in greater inhibition of HMG-CoA reductase activity and inactivation of acetyl CoA carboxylase, leading to the suppression of fatty acid biosynthesis (Carling et al., 1987; Singh et al., 2006; Viola et al., 2008; Elseweidy et al., 2016). Curcumin improved the cognitive memory in mice with AD (induced by an ICV injection of STZ) in the NOR test. This positive effect was attributed to the neurogenesis effects of curcumin by increasing the activation of the AMP-kinase pathway (Bassani et al., 2017). In this regard, PCO reduces total cholesterol by inhibiting the activity of HMG-CoA reductase and induces the phosphorylation of AMP-kinase similar to curcumin.

The PCO hypoglycemia effect may be due to the AMP-kinase activation that is similar to the mechanism, by which metformin (Met) acts. It activates the glucose absorption into the skeletal muscle, inhibits hepatic gluconeogenesis, and ultimately reduces circulating fat (Zhou et al., 2001; Shaw et al., 2005). In this regard, it has been shown that the pretreatment by Met via its neuroprotective effect can prevent the impaired synaptic plasticity caused by Aβ (Asadbegi et al., 2016). The biguanide Met, as a first-line antidiabetic treatment for type 2 diabetes, can act as an insulin sensitizer and reduce blood glucose through an increase in glucose uptake into muscles and a reduction in liver gluconeogenesis by activating AMP-activated protein kinase (AMPK) (Campbell et al., 2017). Met is also a possible treatment for dementia by reducing pTau (Farr et al., 2019). It also significantly reduces inflammatory markers, like TNF-α and CRP. The hypoglycemic effect of PCO is mainly related to AMP-kinase activation, which is similar to the mechanism used by Met (Elseweidy et al., 2016).

Conclusion

In summary, our findings obtained from the NOR, MWM, and PAL tests showed that Aβ impairs learning and memory, while PCO eliminates learning and memory impairment following the Aβ injection and even improves memory. The results of biochemical studies also showed that Aβ increases the TOS levels and decreases the TAC levels and vice versa, the consumption of PCO reduces the TOS levels and increases the TAC levels. These results indicate the antioxidant properties of PCO. Aβ plaques increased in the AD group, while PCO decreased these plaques. The effects of PCO on learning and memory can be due to its antioxidant, hypolipidemic, and anti-inflammatory properties.

Declarations

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Author contributions

Samaneh Safari: Study concept and design, Data acquisition, Data analysis and interpretation, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content, Statistical analysis

Naser Mirazi: Supervision, Conceptualization, Writing - Review & Editing, Data Curation

Nesa Ahmadi: Original draft preparation, Resources

Masoumeh Asadbegi: Data analysis and interpretation, Formal analysis, Software, Validation

Alireza Nourian: Methodology, Validation

Alireza Komaki: Study concept and design, Critical revision of the manuscript for important intellectual content, Study supervision

Availability of data and material

All relevant data and material are within the manuscript and its Supporting Information files.

Consent for Publication

All authors read and approved the final manuscript. All authors of this article are completely satisfied with its publication.

Compliance with Ethical Standards

Ethical Approval

The experiments were carried out according to Guidelines of the National Institutes of Health on the principles of laboratory animal care (NIH Publication 80-23, 1996). The Local Ethical Committee approved all planned experimental procedures.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

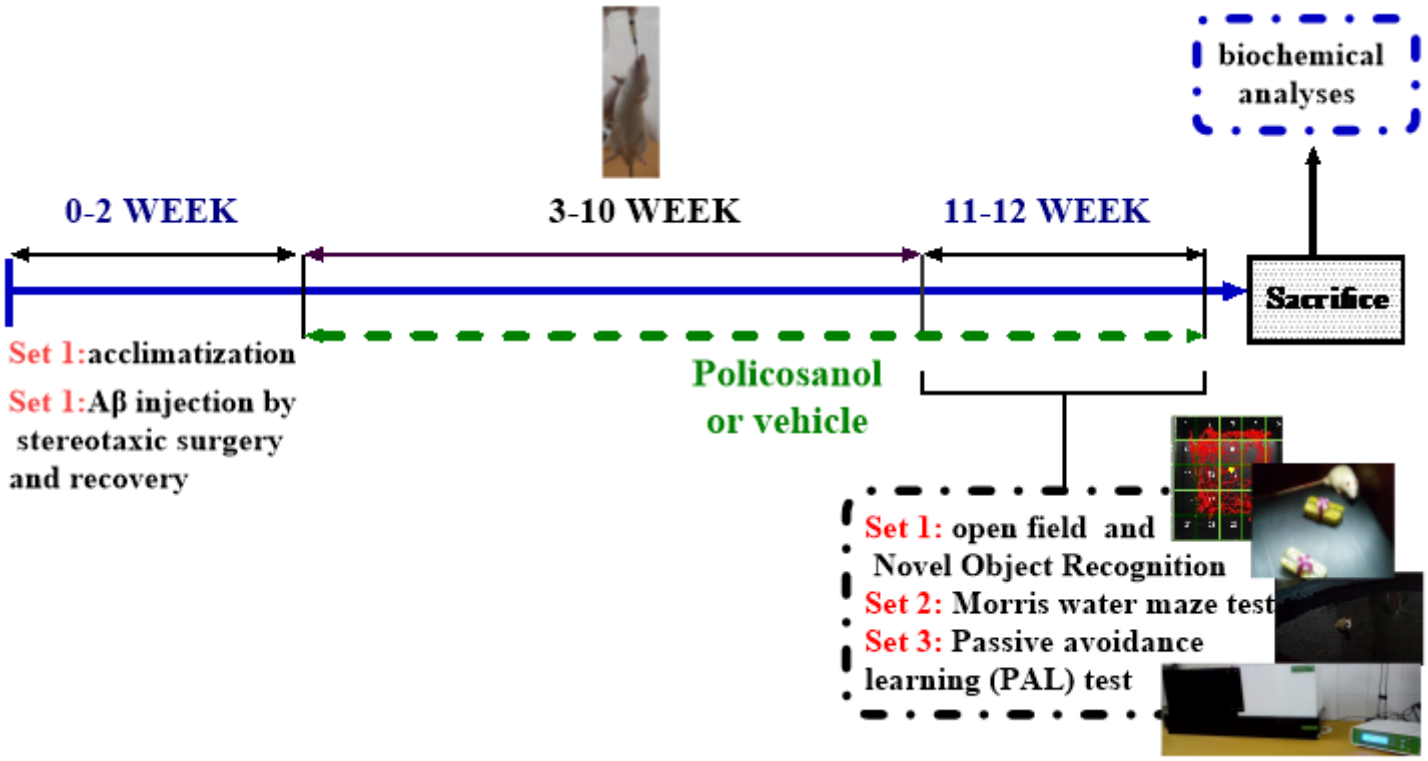

Figure 1

The experimental timeline. Following 2 weeks of acclimatization, to generate an AD model, the rats in the experimental groups were anesthetized with xylazine (10 mg/kg) and ketamine (100 mg/kg) and placed in a stereotaxic device. The Aβ solution (5 µL; 1 µL/1 min) was injected intracerebroventricularly (ICV). Following recovery, policosanol (PCO) was received by animals by gavage daily for 8 weeks. Then, the open field and novel object recognition (NOR) tests were performed. To measure spatial (acquisition and retention) and aversive (acquisition and retention) learning and memory following the training programs, the MWM and shuttle box tests were used, respectively. After the experiments, the biochemical parameters and the concentrations of the biomarkers of oxidative stress were calculated by serum assessment.

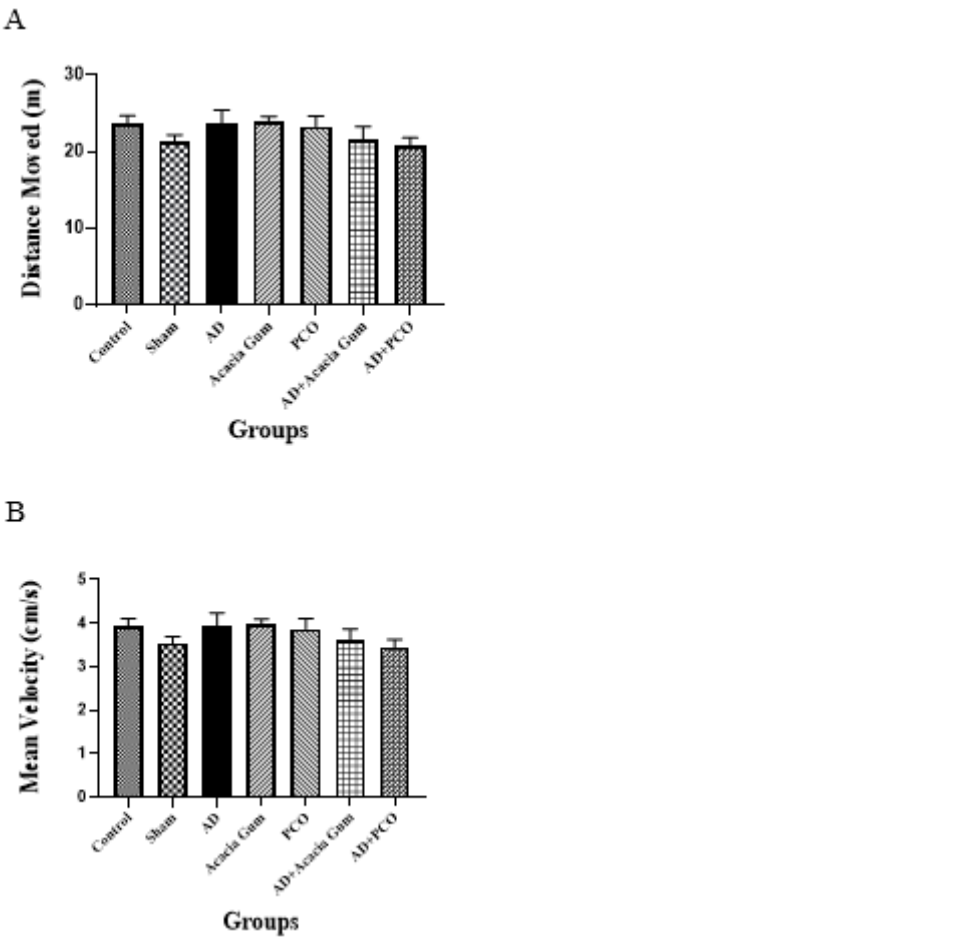

Figure 2

Comparing the mean velocity and the distance moved between groups. Data are represented as mean ± SEM. No significant difference was detected between groups (n=6-7).

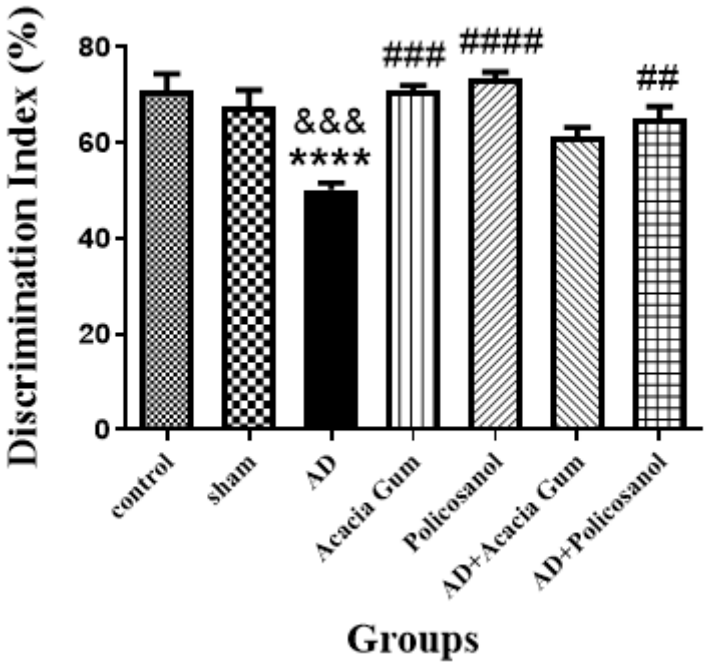

Figure 3

The effect of policosanol (PCO) on discrimination index on the second day. Data are represented as mean ± SEM. **** p<0.0001 versus the control group; &&& p<0.001 versus the sham group. ## p < 0.01; and ### p < 0.001 and #### p < 0.0001 versus the AD group (n=6-7).

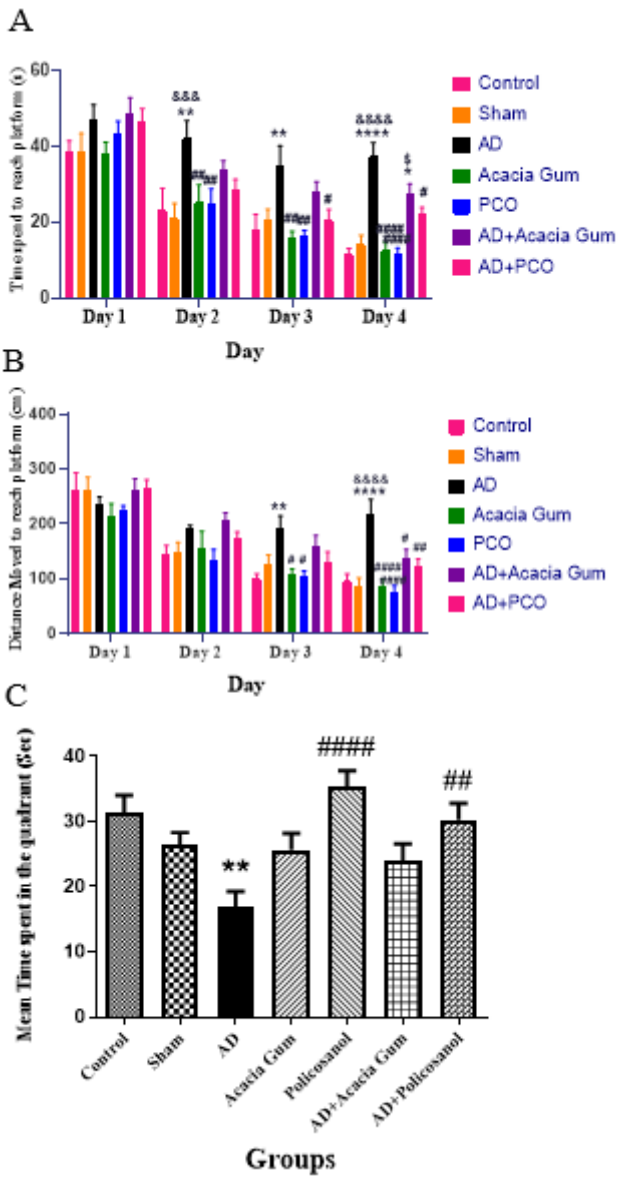

Figure 4

The time spent to reach the hidden platform (latency) (A). The entire distance moved to reach the hidden platform (total distance) (B). The mean time spent in the target quadrant on the test day (C). Data are presented as mean ± SEM. ** p<0.01 and **** p<0.0001 versus the control group; &&& p<0.001 and &&&& p<0.001 versus the sham group; # p < 0.05, ## p < 0.01, and #### p < 0.0001 versus the AD group (n=6-7).

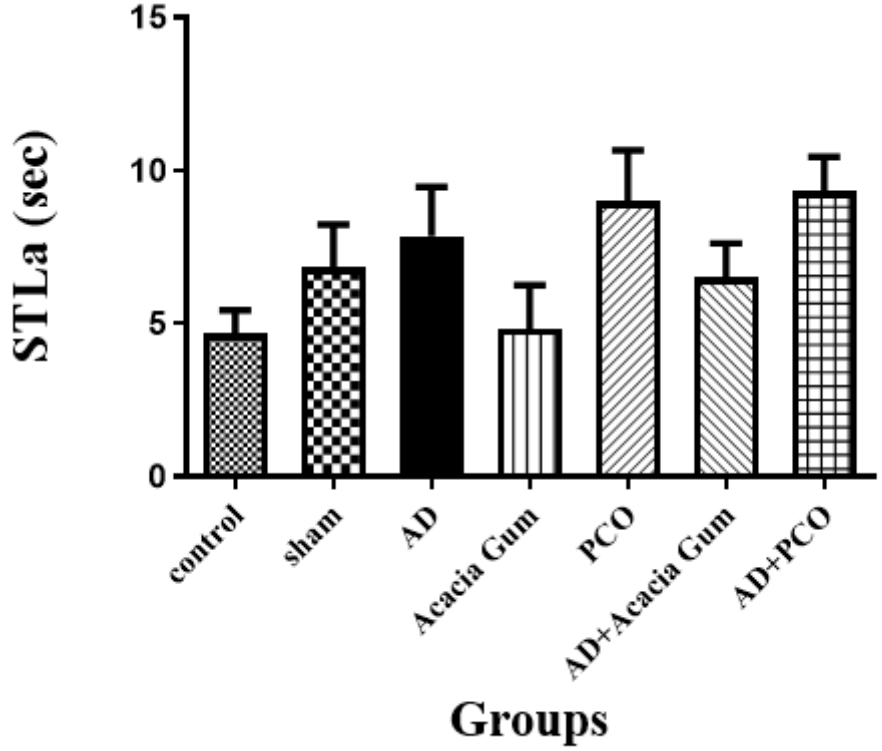

Figure 5

The effect of policosanol (PCO) administration after the ICV injection of amyloid-beta (Aβ). Comparing the latency to enter the dark compartment of the shuttle box in the compromise stage (STLa). Data are presented as mean ± SEM. No significant difference was detected between the groups (n=6-7).

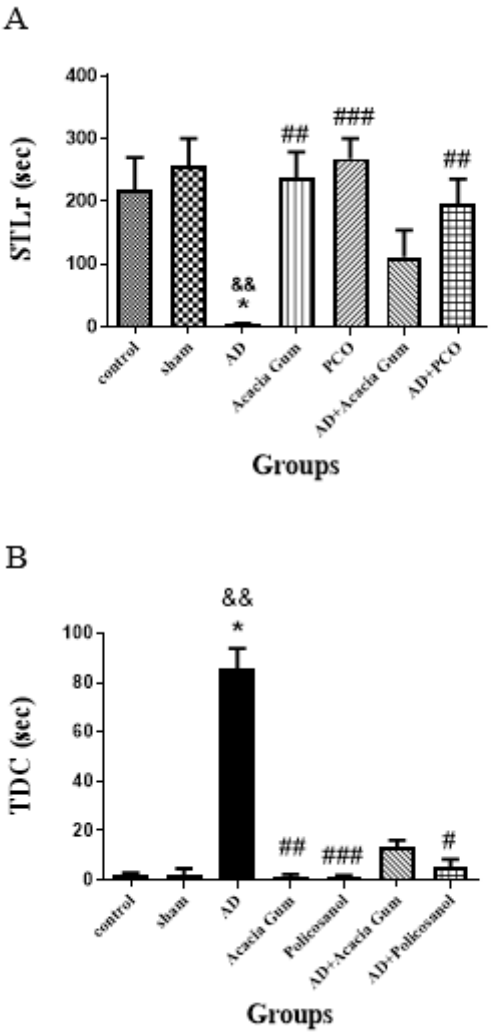

Figure 6

The effect of policosanol (PCO) administration after the ICV injection of amyloid-beta (Aβ). Comparing latency to enter the dark compartment on the test day (STLr) (A) and the time spent in the dark compartment on the test day (TDC) (B). Data are presented as mean ± SEM. * $p < 0.05$ versus the control group; && $p<0.01$ versus the sham group; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ versus the AD group (n=6-7).

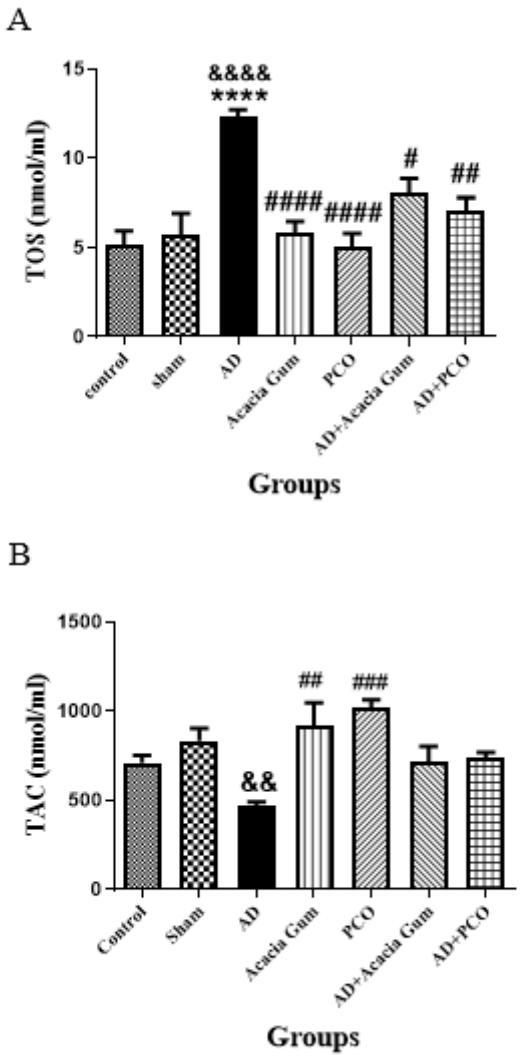

Figure 7

The effect of policosanol (PCO) on total oxidant status (TOS) and total antioxidant capacity (TAC) levels in the normal and AD rats. Data are presented as mean ± SEM. **** p<0.0001 versus the control group; && p<0.01 and &&&& p<0.0001 versus the sham group; # p < 0.05, ## p < 0.01, ### p < 0.001, and #### p < 0.0001 versus the AD group (n=6-7).

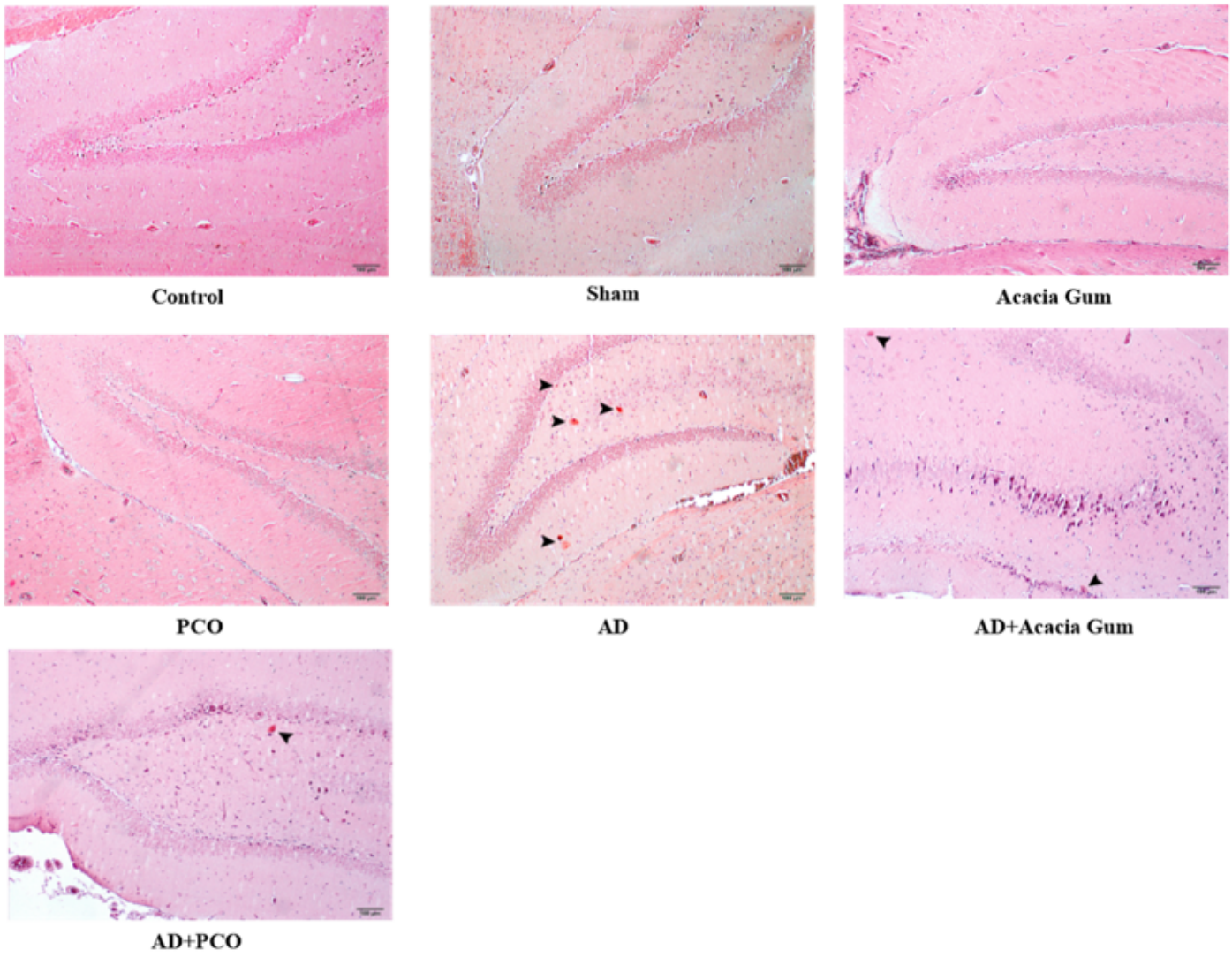

Figure 8

Congo Red staining of the hippocampal sections. Staining was performed to confirm the existence of Aβ plaque in the brain tissues (straight arrows) of the studied groups. The control (normal) group (A), sham group receiving an intracerebroventricular (ICV) injection of vehicle (PBS) (B), acacia gum (AG) group receiving acacia gum orally (C), policosanol (PCO) group receiving PCO orally (D), AD group receiving an ICV injection of Aβ (E), AD+AG group receiving an ICV injection of Aβ + acacia gum orally AG (F), and AD+PCO group receiving an ICV injection of Aβ + PCO orally (G). Images were observed with ×10 objective; scale bar: 200 μm.