Naunyn-Schmiedeberg's Archives of Pharmacology
https://doi.org/10.1007/s00210-024-03442-8

RESEARCH

# Carvacrol improves cognitive dysfunction by decreasing amyloid-β accumulation and regulating neuroinflammation in ovariectomized renovascular hypertensive rats

**Duygu Bayraktar[1] · Büşra Ertaş[2] · Yasemin Aydın[1] · Göksel Şener[3]**

Received: 15 August 2024 / Accepted: 8 September 2024


**Abstract**
Hypertension contributes to both the development and progression of brain damage and cognitive dysfunction in the post-menopausal period in women. Carvacrol (CAR), which can easily cross the blood-brain barrier, exhibits neuroprotective properties due to its antioxidant, anti-inflammatory, and anti-apoptotic effects. In the present study, we have examined the effect of CAR treatment on learning-memory impairment in a post-menopausal hypertensive rat model that was induced by ovariectomy following two-kidney, one-clip renovascular hypertension surgery. From the third week after the establishment of renovascular hypertension in ovariectomized rats, CAR (40 mg/kg) was administered once daily for consecutive 7 weeks by gastric gavage. Systolic blood pressure was estimated by the tail-cuff method once a week. At the end of the study, cognitive functions were evaluated with behavioral tests and also neurochemical changes were measured in serum, cortex, and hippocampus by ELISA test. Blood pressure was decreased with CAR treatment in hypertensive rats. Serum estrogen levels decreased in ovariectomized rats and did not change with CAR treatment. CAR demonstrated beneficial effects on learning and memory tests as determined by increased recognition index, the number of platforms crossed, and time spent in the target quadrant. Due to CAR treatment, there was a marked reduction in the hippocampal and cortex amyloid-β, osteopontin, interleukin-6 and tumor necrosis factor-alpha levels, and acetylcholinesterase activity, while an increment in neprilysin and interleukin-10 levels was found. In conclusion, since CAR suppressed amyloid-β deposition and neuroinflammation in ovariectomized-hypertensive rats, it is thought that it may be protective against memory disorders in postmenopausal hypertensive women.

**Keywords** Carvacrol · Hypertension · Postmenopause · Amyloid-β · Neuroinflammation · Cognitive impairment

## Introduction

Increasing evidence has shown that hypertension, a major vascular risk factor, causes cerebral vascular disease and brain tissue injury and leads to impaired brain function (Lin and Lee 2018). Studies have documented impairments in memory, executive function, and visual-spatial skills in patients with hypertension. High blood pressure promotes brain damage by a complex mechanism including neuroinflammation, blood-brain barrier disruption, microglial activation, and vascular amyloid-β deposition (Kivipelto et al. 2002). There is twice the rate of cognitive impairment in hypertensive women than in normotensives (Kuller et al. 2010). White matter lesions in the brains of hypertensive women over the age of 65 have been detected and have been associated with high blood pressure. Furthermore, since these lesions in hypertensive aged woman's brains were in the occipital, parietal, or temporal lobes that are responsible for memory and executive functions, it confirms the hypotheses suggesting deterioration in cognitive functions due to high blood pressure (Devore et al. 2019). Since estrogen plays an important regulatory role in brain functions with its neuroprotective effects, cognitive decline, anxiety, and hypertension observed in the postmenopausal period have

✉ Yasemin Aydın
yaseminydn@yahoo.com; yaydin@ogu.edu.tr

[1] Department of Physiology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

[2] Department of Pharmacology, Faculty of Pharmacy, Marmara University, Istanbul, Turkey

[3] Department of Pharmacology, Faculty of Pharmacy, Fenerbahçe University, Istanbul, Turkey

Published online: 16 September 2024

been relatively associated with estrogen deficiency (Yanes and Reckelhoff 2011). Chou et al. suggest that hypertension is likely to impair the cognitive functioning of postmenopausal women in the Taiwanese population (Chou et al. 2021).

Recently, preclinical drug development studies targeting different pathophysiological processes have been carried out for the treatment of cognitive deficits. Although there are controversial results about its safety and benefits, the effects of hormone replacement therapy against cognitive impairment in postmenopausal women are being investigated (Delgobo et al. 2019). The most important limitation of this treatment approach is that in clinical studies, estrogen therapy in postmenopausal women increases the risk of venous thromboembolism and breast cancer, despite a decrease in coronary heart disease and mortality (Wang et al. 2018). In this context, neurotrophic phytochemicals with antioxidant and anti-inflammatory activity are emerging as a new strategy to alleviate cognitive retardation related to the postmenopausal hypertensive state. Increasing evidence to date points to the presence of the renin-angiotensin system (RAS) in the brain, which is closely related to the pathological features of AD (Claflin and Grobe 2015). It is well-known that RAS modulates cognitive functions as well as regulates blood pressure. RAS activation causes BACE-1 expression, which both disrupts the blood-brain barrier and causes amyloid-β deposition in neurons (Hernández-Espinosa et al. 2019). The relationship between the inhibition of the angiotensin-converting enzyme (ACE, RAS component) and the consequent inhibition of angiotensin II formation and the modulation of memory functions reveals the involvement of this system in AD pathology (Mogi et al. 2012). Clinical studies have demonstrated enhanced levels of ACE in the cerebrospinal fluid (CSF) of patients with cognitive impairment (Miners et al. 2009). Furthermore, experimental studies have clearly demonstrated that perindopril (PER) improved learning-memory performance in rats (Alzahrani et al. 2020). A study by Ali et al. presented results supporting the cognitive performance improvement effect of PER treatment, which is a centrally acting ACE1 inhibitor, on amyloid-$\beta_{1-42}$, neuroinflammatory markers, and oxidative and nitrosative stress levels (Ali et al. 2016).

Carvacrol (4-isopropyl-2-methyl phenol; CAR) is a phenolic monoterpene phytochemical approved by the Federal Drug Administration (FDA) (Naeem et al. 2021). CAR easily crosses the blood-brain barrier with the aid of its low molecular weight and lipophilic structure (Sisti et al. 2021). Numerous experimental studies attribute its broad biological activity to its antioxidant, anti-inflammatory, anti-hypertensive, and neuroprotective properties (Baser 2008; Jamhiri et al. 2019). In our previous study, we revealed that carvacrol exerts neuroprotective effects by modulating Transient Reseptor Potential (TRP) channel expression in neurons and astrocytes in an animal model of Parkinson's disease (Akan et al. 2023).

Azizi et al. demonstrated that CAR has a neuroprotective effect against cytotoxicity induced by amyloid-$\beta_{25-35}$ in PC cells (Azizi et al. 2020). Furthermore, pieces of evidence suggest that CAR reduces chronic cerebral hypoperfusion-induced neuronal necrosis and decline in learning (Shahrokhi Raeini et al. 2020), and protects against LPS-induced neuroinflammation (Naeem et al. 2021). In our previous study (Aydin et al. 2007), results were observed that CAR treatment had a significant hypotensive effect in normotensive rats. In addition, reductions in systolic and diastolic blood pressure were observed in L-NAME-induced hypertensive rats in the same study. In a report presented by Demirci et al. (2022), it was emphasized that CAR has a significant angiotensin-converting enzyme inhibitory effect both *in vitro* and *in silico*. Numerous studies reported that CAR treatment prevented learning-memory impairment in rat models of diabetes, epilepsy, and AD (Azizi et al. 2012; Khalil et al. 2017). In light of the above knowledge, the present study aimed to investigate whether CAR administration could ameliorate behavioral deficits and neurodegeneration caused by postmenopausal hypertensive conditions in the animal model.

## Material and methods

### Chemicals

Standardized carvacrol (purity>98%, Catalogue No. 282197-10G), perindopril erbumine (Cat No. 107133-36-8), and dimethyl sulfoxide (DMSO) were from Sigma-Aldrich (St. Louis, MO, USA). Estrogen, sodium-potassium adenosine triphosphatase ($Na^+$, $K^+$-ATPase), interleukin (IL)-10, neprilysin (NEP), amyloid-β (Aβ), acetylcholinesterase (AChE), osteopontin (OPN), and choline acetyltransferase (ChAT) enzyme-linked immunosorbent assay (ELISA) commercial kits were obtained from YL Biotech Co. (Shanghai, China).

### Animals

The experimental protocol was approved by the Marmara University Experimental Animals Ethical Committee (no. 59.2017.mar) and all procedures were performed according to the scope of the experiment and were studiously implemented in accordance with the institutional principle. Sixty adult female Sprague-Dawley rats (10–12 weeks, 200 ± 250 body weight) were obtained from the Marmara University Experimental Animal Application and Research Center (DEHAMER). Rats were housed in rooms with controlled

temperature (22 ± 2°C), humidity (40–60%), and light (12-h light/dark cycle), which are specified as appropriate laboratory conditions. Throughout the experiment, a pellet diet and water were provided *ad libitum*.

## Experimental design

### Induction of ovariectomy

Rats were ovariectomized (OVX) under general anesthesia by intraperitoneal ketamine/xylazine mixture (100/0.75 mg/kg) injection. Firstly, a peritoneal cavity has been reached by making a single central skin incision (0.5 cm) in the abdominal area and separating the connective tissue. Both ovaries were ligatured with a 3/0 silk thread together with the blood vessels and ducts, and then they were cut out from the upper part of the ligature. The same incision was made for the sham group without ligation of the ovaries. After the uterus was reinserted, the incision was closed with a suture (Kadıoğlu Yaman et al. 2020). Local antibiotic ointment was applied over the sutured skin.

### Induction of renovascular hypertension (two-kidney, one-clip model)

Goldblatt's two-kidney, one-clip (2K1C) method was used to induce hypertension in rats that were left to recover for 3 days after ovariectomy surgery. Under anesthesia, a silver clip (internal diameter 0.25 mm) was placed around the left renal artery, and the right kidney was left intact. In the sham-operated control group, rats had similar surgical procedures but without clipping the renal artery. At week 3, rats with systolic blood pressure above 150 mmHg were considered hypertensive (Cevikelli-Yakut et al. 2020). Finally, rats were kept individually in polypropylene cages for a period of 2 days for complete healing of the wounds, and then they were re-grouped.

### Animal treatment

The animals were randomly divided into the following experimental groups ($n$=10):

- Sham-operated control group
- Ovariectomized group (OVX)
- Two-kidney, one-clip group (RVH)
- Ovariectomized and two-kidney, one-clip group (RVH/OVX)
- CAR-treated group (RVH/OVX + CAR)
- PER-treated group (RVH/OVX + PER)

All treatments were started 3 weeks after surgical operations and continued 7 weeks until decapitation. The animals received CAR (40 mg/kg) and PER (2 mg/kg) by gavage once a day, for 7 consecutive weeks. Sham-operated, OVX, RVH, and RVH/OVX groups received a 5% solution of DMSO (i.g.) for 7 weeks. All rats were weighed and their tail-cuff SBP measurements were measured once a week for 10 weeks (Fig. 1).

### Blood pressure measurement

Systolic blood pressure (SBP) was evaluated through the non-invasive tail-cuff method. Blood pressure was measured with a pneumatic pulse sensor cuff wrapped around their tails (Biopac MP35 Systems, Inc.; COMMAT Ltd., Ankara, Turkey). Measurements were performed while the animals were awake, in a plastic restraint cage. Before measurement, rats were placed in a chamber at 35 °C for 10 min to easily identify arterial pulsation in the tail and to stabilize their blood pressure. For measurement, they were positioned in individual plastic restrainers. Basal values were determined by taking measurements of rat blood pressure values before surgical operations. The mean of three consecutive measurements was determined as the reading values.

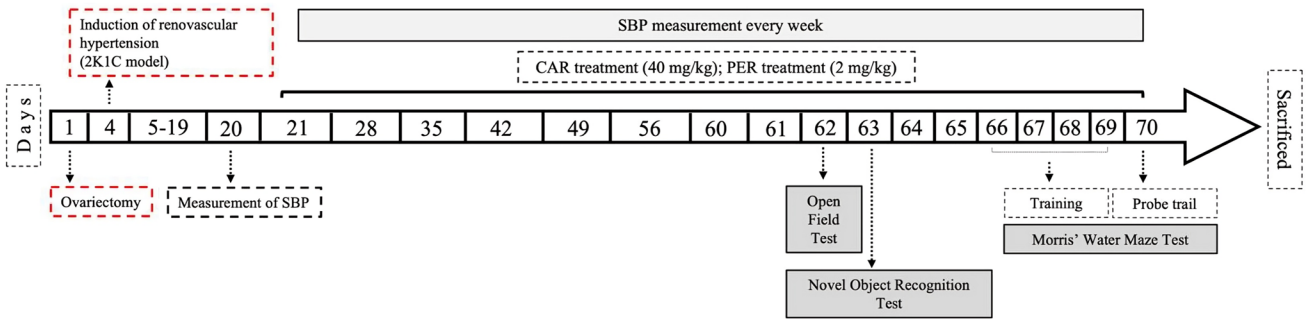


**Fig. 1** Schematic representation of the experimental design. CAR, carvacrol; SBP, systolic blood pressure; 2K1C, two-kidney, one-clip

### Behavioral testing procedures

**Open field test** Anxiety level and locomotor activity ability were evaluated in a square open field arena. The time spent in the central and peripheral regions, the number of passes to the central region, and the number of rearing and ornamentation were recorded.

**Novel object recognition test** The new object recognition (NOR) test is widely used to assess non-spatial memory function and various aspects of learning in rodent models (Ertas et al. 2021). At postoperative week 10, the NOR test was performed in a semidark environment in a restricted chamber (31 cm × 24 cm × 45.5 cm) with no external cues to assist learning and memory. The task procedure consisted of a 2-day process, 1-day habituation, and the other-day test phase. In the "habituation phase," rats spent 60 min in the arena exploring the restricted chamber without any objects. The test phase consisted of two 3-min periods separated by 60 min. On the test day, each animal was placed in the center of the arena and allowed to explore two identical objects (F1 + F2) by moving freely for 3 min. The objects were located in corners of the arena and could not be moved by rats. After this "familiarization phase," rats were allowed to stay in their housing cages for 1 h. In the "test phase," the rat was allowed to explore another 3 min after one of the objects was replaced by a different (F1 + N), and the rat is placed back into the chamber. After each trial, the apparatus and objects were cleaned with 70% ethanol solution to avoid the presence of olfactory effects. Both periods were recorded by a computer-aided camera. Discrimination index (DI) and preferential index (PI) were calculated for each rat and were expressed by the ratio:

$$DI = \frac{t(N - F)}{t(N + F)} \quad (1)$$

$$PI = \frac{tN}{tN + tF} \quad (2)$$

**Morris water maze task** Morris water maze (MWM) is used frequently to assess hippocampus-dependent spatial learning and memory in rodents. The experimental setting consisted of a black circular water tank (diameter, 150 cm; height, 40 cm) filled with black-colored water (24 ℃), and a movable escape platform (diameter, 10 cm; height, 20 cm) was placed. Firstly, the training trial stage was conducted on days 1–4 and all rats were trained to find the hidden escape platform, using cues placed on the walls around the pool, and the latency period was recorded. The rat was allowed to swim for four trials lasting for a maximum of 75 s with an inter-trial interval of 60 s. Regardless of whether the rat found the platform or not, it was allowed to stay on the platform for 20 s. During the probe trial, the platform has been removed. The rats were allowed to swim freely for 60 s. In this stage, the time spent in the target quadrant was recorded. The system automatically recorded the frequency of the rats in the target quadrant crossing station area during the test to examine the spatial memory of rats (Topal et al. 2022).

### Sampling

The blood pressure measurements of all rats were taken again after the completion of the behavioral tests. Rats were sacrificed and obtained cortical and hippocampal tissue samples. Blood samples were collected, and serum was collected by centrifuging at 3000*g* for 10 min.

Prefrontal cortex and hippocampus samples were weighed and placed in tubes. Phosphate buffered saline (PBS) was added to each tube to prepare 10% homogenization. Cellular proteins were released using two different homogenizers (IKA Ultra Turrax T25 and Bandelin Sonopuls). Tissues were centrifuged at 12,000*g* for 15 min to remove non-protein substances; the supernatants were collected and stored at −80 ℃ until use for measurement of biochemical parameters. As well, the heart and aorta were rapidly dissected for histopathological analysis.

### Enzyme-linked immunosorbent assay

In our study, noncompetitive ELISA (sandwich ELISA) method was used manually using rat-specific ELISA commercial kits for the analysis of serum and tissue samples. In the sandwich ELISA method, antibodies are immobilized in the solid phase of the wells of the microtiter well. The sample containing the antigen is then added and the antigen-antibody complex is expected to form. Unbound proteins are removed by washing steps. A second enzyme-labeled antibody binds to the antigen bound to the capture antibody at a different epitope. Substrate of the enzyme is added to the medium. The substrate reacts with the enzyme bound to the detection antibody, causing a color change. The resulting color change indicates the presence of antigen. This color change is measured with a spectrophotometer.

Serum and hippocampal estrogen, hippocampal and cortical acetylcholinesterase (AChE), choline acetyltransferase (ChAT), amyloid-β, neprilysin, $Na^+/K^+$-ATPase, and osteopontin, as well as serum and hippocampal interleukin (IL)-10, tumor necrosis factor (TNF-α), and IL-1β levels, were quantified according to the manufacturer's instructions and guidelines of the ELISA kits (Shanghai YL Biotech Co., China) specific for rats. Reading extinction was performed using ELISA Microplate Reader with a wavelength of 450 nm. Each sample was tested three times and the average was calculated.

### Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). The Shapiro-Wilk test was used to test whether our data fit the normal distribution. All data are presented as mean ± standard error (SEM). The scoring of the behavioral tests was evaluated using the Mann-Whitney *U* test. Biochemical data groups were compared with analysis of variance (ANOVA) after Tukey multiple comparison tests, and $p$ values <0.05 were considered statistically significant.

## Results

### Systolic blood pressure

Systolic blood pressure was measured weekly after surgery until the end of the study. There was no difference in baseline systolic blood pressure among all groups. As shown in Fig. 2, the average systolic blood pressures of the RVH, OVX, and RVH/OVX groups were dramatically higher than the sham-operated control group during the 3rd week ($p$<0.001). During the experiment, there were no significant differences in systolic blood pressure between the RVH and RVH/OVX groups. Blood pressure was significantly decreased in the CAR and PER-treated groups from the 4th week. At postoperative week 10, there was no significant difference in systolic blood pressure between CAR-treated and PER-treated RVH/OVX throughout the treatment.

### Serum estrogen levels

There was a significant decrease in serum estrogen level in the OVX group compared to the sham operation group. These levels also prove the accuracy of the ovariectomy procedure. Similarly, compared with the sham-operated group, the RVH/OVX group showed a significant decline in the level of serum estrogen by 28.91%. In addition, estrogen levels in the CAR- and PER-treated groups (6.857±1.063 pg/mL and 7.1 ± 0.56, respectively) had not exhibited a considerable difference compared with OVX and RVH/OVX groups (Fig. 3).

### Neurobehavioral observations

#### Open field test

The effects of surgical operations on the level of spontaneous locomotion and anxiety were evaluated in the open field test. The number of passes did not differ in all groups,

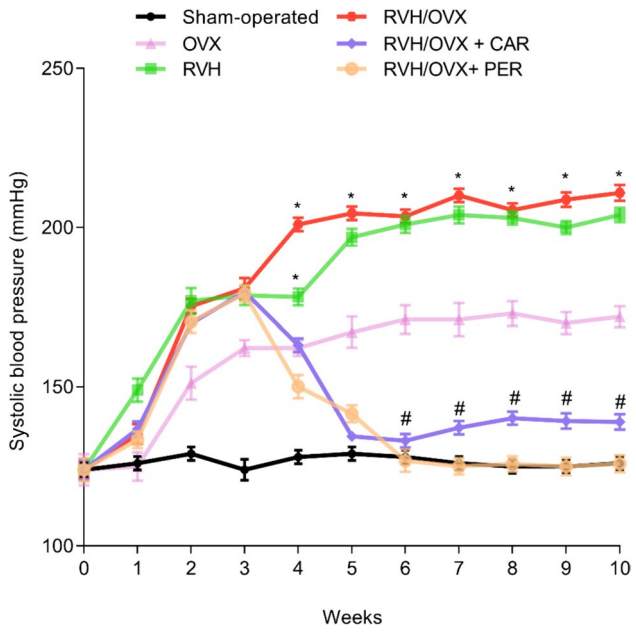


**Fig. 2** Systolic blood pressure in the experimental period. Data were shown as mean ± SEM ($n$ = 10 rats/group). * RVH/OVX vs. sham-operated rats, $p$ <0.001; # RVH/OVX + CAR vs. RVH/OVX $p$ <0.001. RVH, renovascular hypertension; OVX, ovariectomy; CAR, carvacrol; PER, perindopril (ANOVA followed by Tukey's post hoc test)

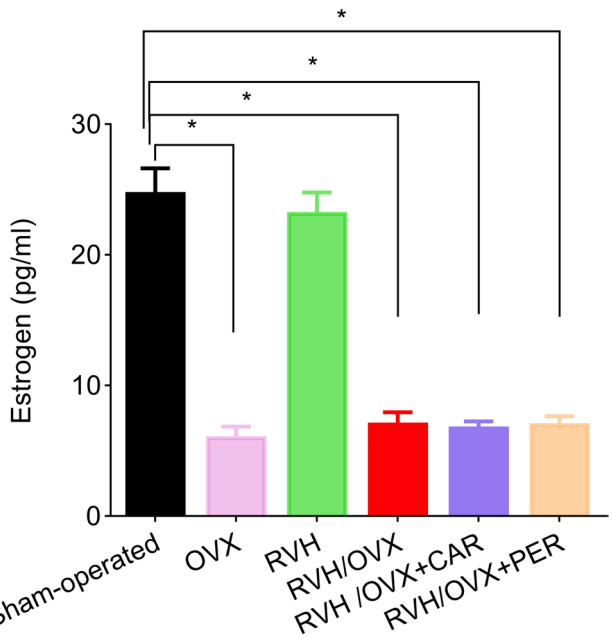


**Fig. 3** Effect of CAR treatment on serum estrogen levels. Data were shown as mean ± SEM ($n$ = 10 rats/group). *$p$ <0.05. RVH, renovascular hypertension; OVX, ovariectomy; CAR, carvacrol; PER, perindopril (ANOVA followed by Tukey's post hoc test)

indicating that with RVH and OVX interventions there was no impairment of locomotor function in the model rats. There was no difference between groups in the time spent in the middle zone of the open field arena as a criterion for assessing anxiety-like behaviors such as rearing and grooming (Fig. 4).

## Novel object recognition test

NOR test was performed to evaluate all groups' learning and memory performances. RVH/OVX showed impaired memory by reducing their exploratory capacities during the novel object test. The time for exploring the novel object and the recognition index was increased in the RVH/OVX + CAR group, compared with the RVH/OVX group (Fig. 5a). According to the test results, differences between the groups were seen in the discrimination index and preferential index values (Fig. 5b, c). It was observed that all groups were significantly higher than the RVH/OVX group in the DI ($p<0.01$) and PI ($p<0.001$). CAR administration reversed these high values to the sham-operated levels.

## Morris water maze task

Hippocampal-dependent spatial learning and memory were assessed with the two-stage MWM test. In learning trials, compared with the sham-operated group, the RVH/OVX group had longer latencies from day 2 to day 4, indicating that the RVH/OVX group had spatial cognitive impairments. In both treated groups, with CAR and PER, escape latency gradually decreased on training days ($p<0.01$) (Fig. 6a). These results signal that CAR-treated rats could learn to locate the submerged platform. The decrease in the path length showing that the animals travel to reach the hidden platform is related to memory (Fig. 6b). A significant difference was observed on the second day in terms of the RVH/OVX group and treatment groups' path length ($p<0.01$). The RVH/OVX group demonstrated a significant rise in the path length compared to the sham-operated group

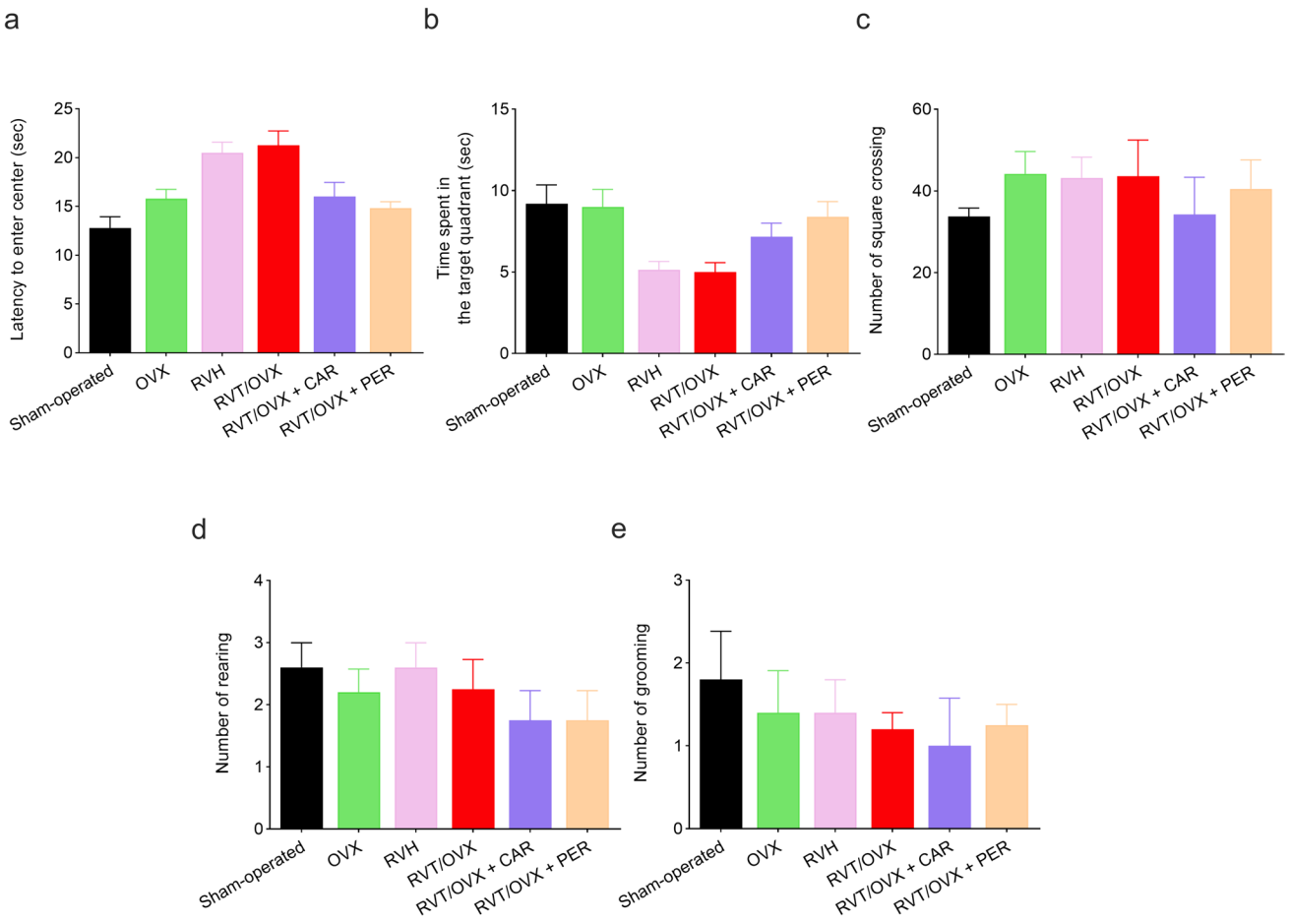


**Fig. 4** Effect of CAR treatment on **a** latency to enter center (s), **b** time spent in the target quadrant (s), **c** number of square crossing, **d** number of rearing, and **e** number of grooming in the open field test. Data were shown as mean ± SEM ($n=10$ rats/group). RVH, renovascular hypertension; OVX, ovariectomy; CAR, carvacrol; PER, perindopril (ANOVA followed by Tukey's post hoc test)

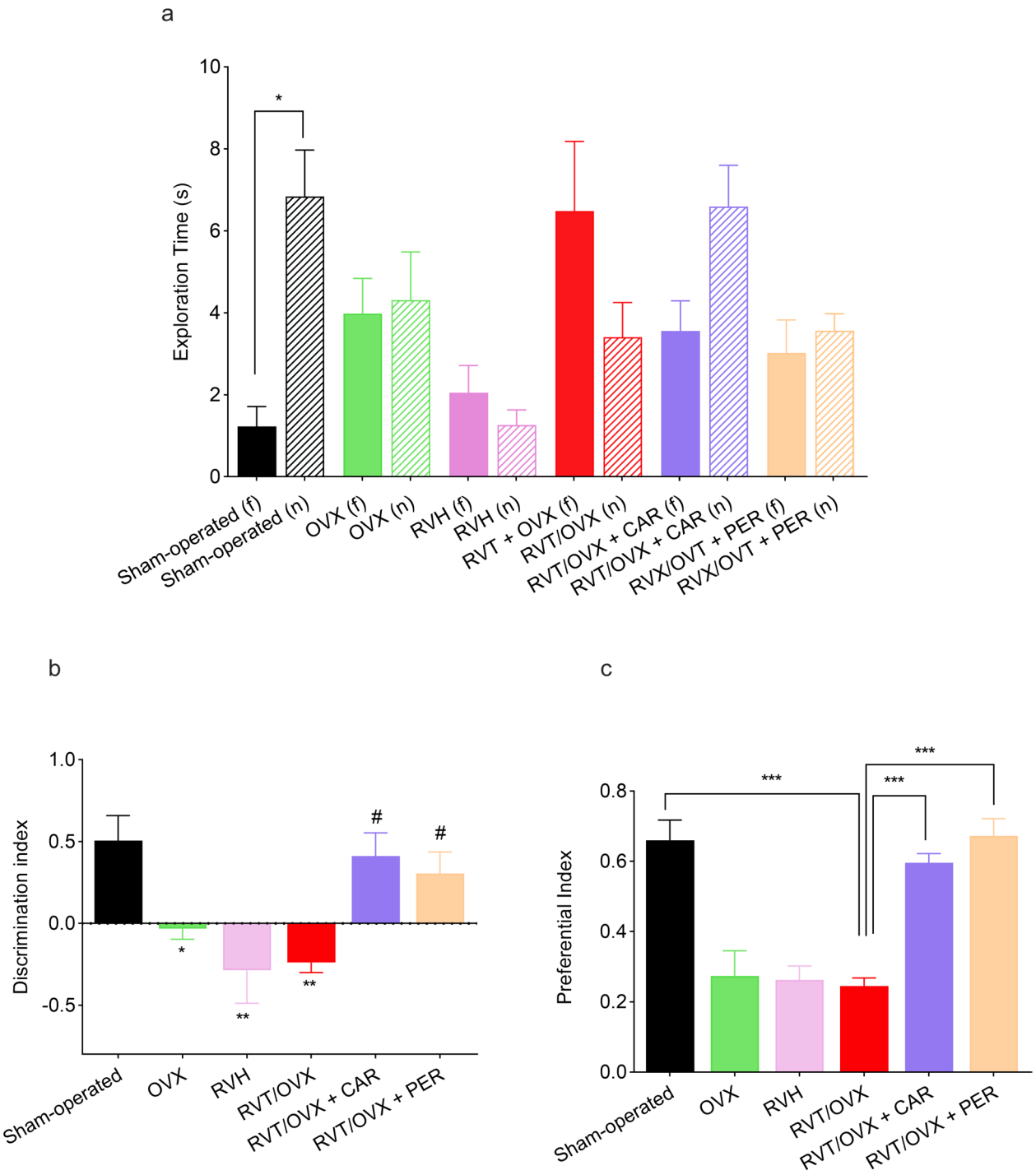


**Fig. 5** Effect of CAR treatment on **a** exploration time, **b** discrimination index, and **c** preferential index. Data were shown as mean ± SEM ($n = 10$ rats/group). *$p$ <0.05, **$p$ <0.01, and ***$p$ <0.001. # RVH/OVX + CAR vs. RVH/OVX $p$ <0.05. (f), familiar object; (n), novel object; RVH, renovascular hypertension; OVX, ovariectomy; CAR, carvacrol; PER, perindopril (Mann-Whitney $U$ test and ANOVA followed by Tukey's post hoc test)

throughout the trial period. The CAR treatment group demonstrated a significant decrease in the path length compared to the RVH/OVX group ($p$<0.01). These results showed that CAR treatment improved memory in a supportive manner compared to other parameters. Moreover, analyses of the swimming speed have shown that there were no remarkable differences throughout the training days between all groups (Fig. 6c).

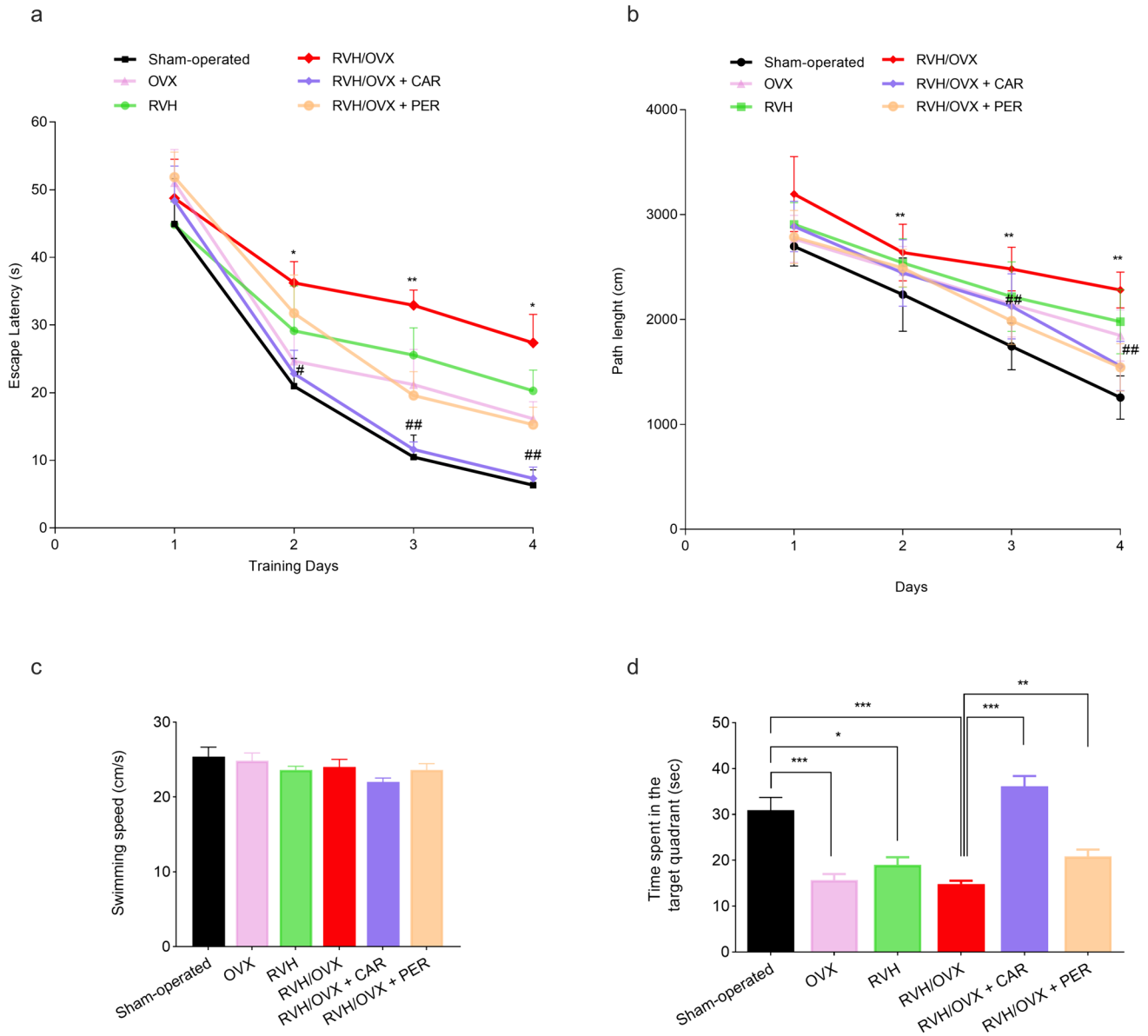


**Fig. 6** Performances in Morris water maze task training period and probe test of groups. **a** Escape latency. **b** Path length. **c** Swimming speed. **d** Time spent in target quadrant *$p$ <0.05, **$p$ <0.01, ***$p$ <0.001 vs. sham-operated group; # $p$ <0.05, ## $p$ <0.01 vs. RVH/OVX group. Data were shown as mean ± SEM ($n$ = 10 rats/group). RVH, renovascular hypertension; OVX, ovariectomy; CAR, carvacrol; PER, perindopril (Mann-Whitney $U$ test and ANOVA followed by Tukey's post hoc test)

The time spent in the target quadrant allows the evaluation of the memory performance for the probe trial (Fig. 6d). Compared to the sham-operated group, the number of crossing platform was significantly decreased in the RVH/OVX group ($p$<0.001). On the other hand, a significant rise in crossing the former platform site was observed in CAR-receiving animals when compared to the RVH/OVX group. The significant increase in the percentage of time spent in the correct quadrant confirmed the cognitive benefits of CAR in the probe test session ($p$<0.001). Our data suggested that spatial memory retrieval of CAR administration was better improved in the RVH/OVX group.

## Amyloid-$\beta_{1\text{-}42}$ levels

As shown in Fig. 7a, compared with the sham-operated group, hippocampal $A\beta_{1\text{-}42}$ levels significantly increased in the OVX, RVH, and RVH/OVX groups ($p$ < 0.05). Similar to the PER-treated group, the level of hippocampal $A\beta_{1\text{-}42}$ in the CAR-treated group was found to be significantly lower than that of the OVX, RVH, and RVH/OVX groups

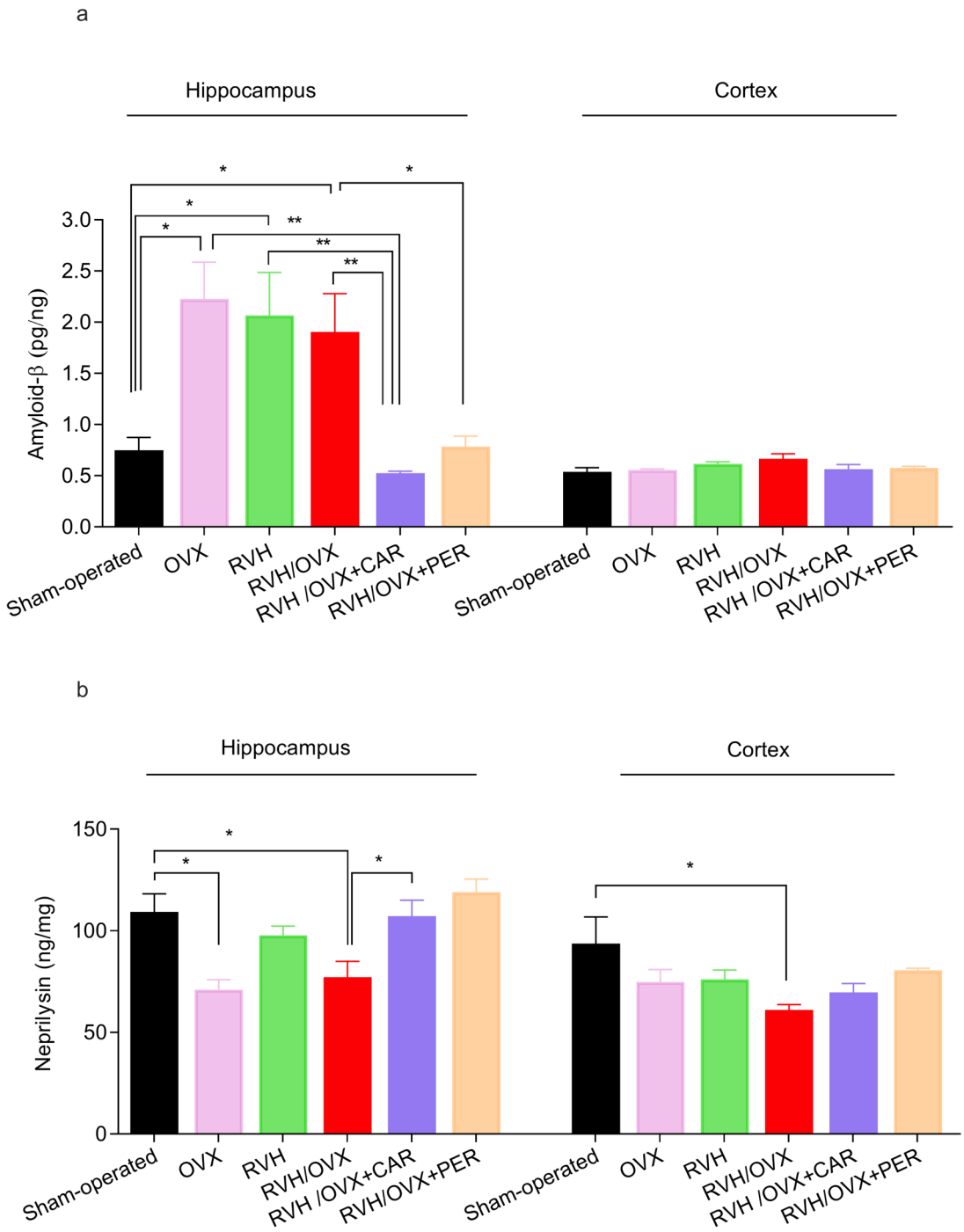


**Fig. 7** Effects of CAR treatment on **a** amyloid-$\beta_{1\text{-}42}$ levels and **b** neprilysin on the hippocampus and cortex. Data were shown as mean ± SEM ($n = 10$ rats/group). *$p$ <0.05, **$p$ <0.01. RVH, renovascular hypertension; OVX, ovariectomy; CAR, carvacrol; PER, perindopril (ANOVA followed by Tukey's post hoc test)

($p < 0.01$). Meanwhile, the cortex $A\beta_{1\text{-}42}$ concentrations of the RVH/OVX group did not differ compared to those in the sham-operated group, and similarly, there was no difference between the groups after the treatment.

## Neprilysin levels

Compared to the sham surgery group, the RVH/OVX group was found to have a significant decrease in neprilysin levels

in the hippocampus (Fig. 7b). CAR treatment produced a significant increment in the neprilysin level of the hippocampus when compared with RVH/OVX group. On the other hand, the RVH/OVX group exhibited a significant elevation of neprilysin on the cortex when compared with the sham-operated group ($p<0.05$). However, CAR and PER treatments had no remarkable difference when compared with the RVH/OVX group.

## Acetylcholinesterase and choline acetyltransferase levels

Estrogen deficiency and hypertension significantly increased AChE activity in the hippocampus and cortex compared to the sham-operated group. The CAR administration group showed a significant decrease ($p<0.001$) in AchE levels in the hippocampus and cortex in ovariectomized hypertensive rats (Fig. 8a). Similarly, compared to the RVH/OVX group, the administration of PER significantly decreased AchE activity ($p<0.001$ in the hippocampus and $p<0.01$ cortex). As shown in Fig. 8b, the ChAT levels in both hippocampus and cortex of the RVH/OVX group had a decrease compared to those in the sham-operated group ($p<0.05$ in the hippocampus and $p<0.05$ in the cortex). Hippocampus and cortex ChAT levels were significantly increased in the CAR-treated group compared with those in the RVH/OVX group ($p<0.01$ in the hippocampus and $p<0.01$ in the cortex).

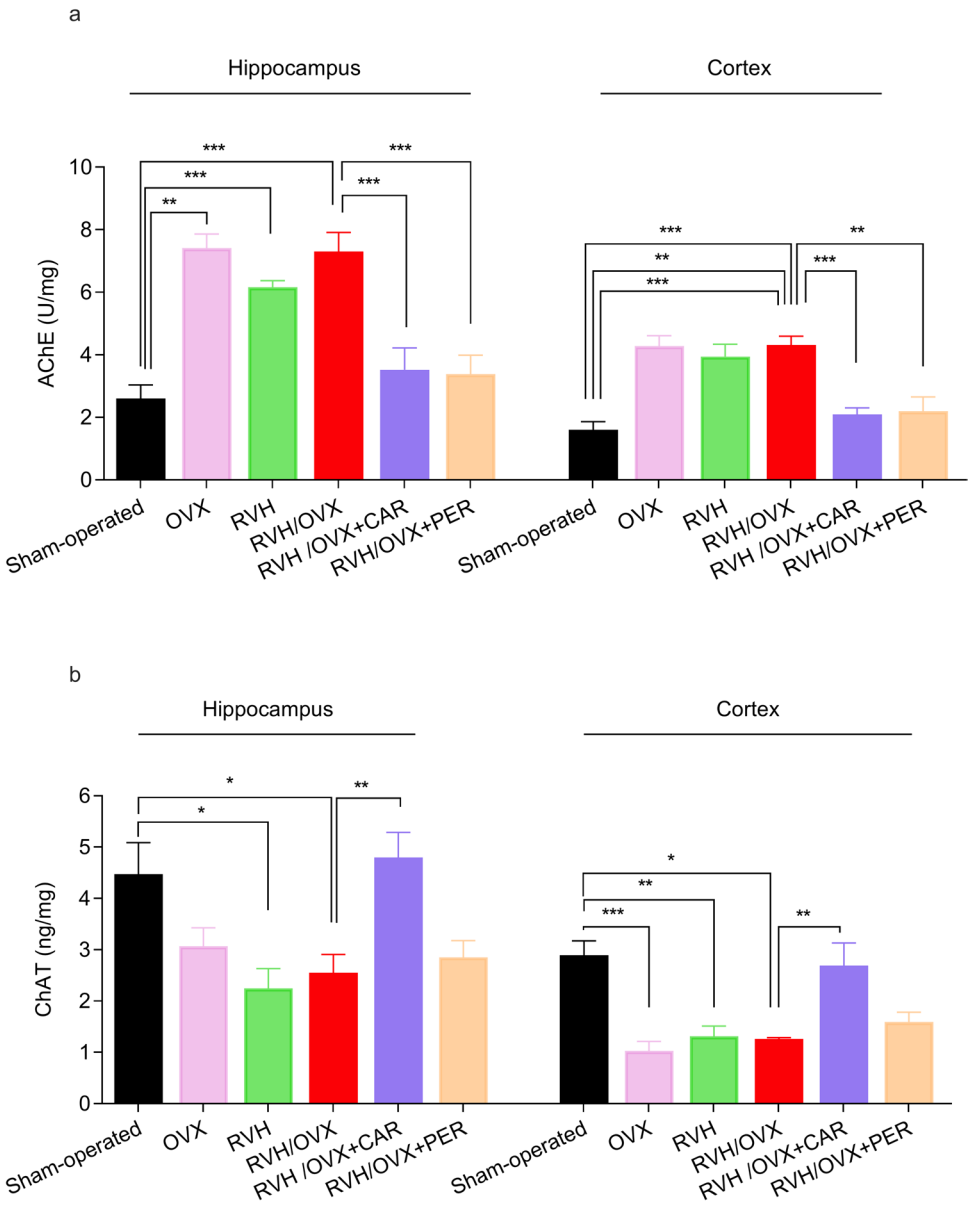


**Fig. 8** Effect of CAR treatment on **a** AChE activity and **b** ChAT levels on the hippocampus and cortex. Data were shown as mean ± SEM ($n=10$ rats/group) *$p<0.05$, **$p<0.01$, ***$p<0.001$. RVH, renovascular hypertension; OVX, ovariectomy; CAR, carvacrol; PER, perindopril; AChE, acetylcholinesterase; ChAT, choline acetyltransferase (ANOVA followed by Tukey's post hoc test)

### $Na^+/K^+$ ATPase level

Our findings demonstrated that hippocampal $Na^+/K^+$ ATPase levels in the RVH/OVX rats and RVH rats were significantly decreased ($p$ <0.05) in comparison with the sham-operated group. Administration of CAR increased $Na^+/K^+$ ATPase level in the hippocampus ($p$ <0.05). After PER treatment for 7 weeks, the $Na^+/K^+$ ATPase concentration was found to be higher than the concentration in the RVH/OVX group ($p$ <0.05). As shown in Fig. 9a, the $Na^+/$

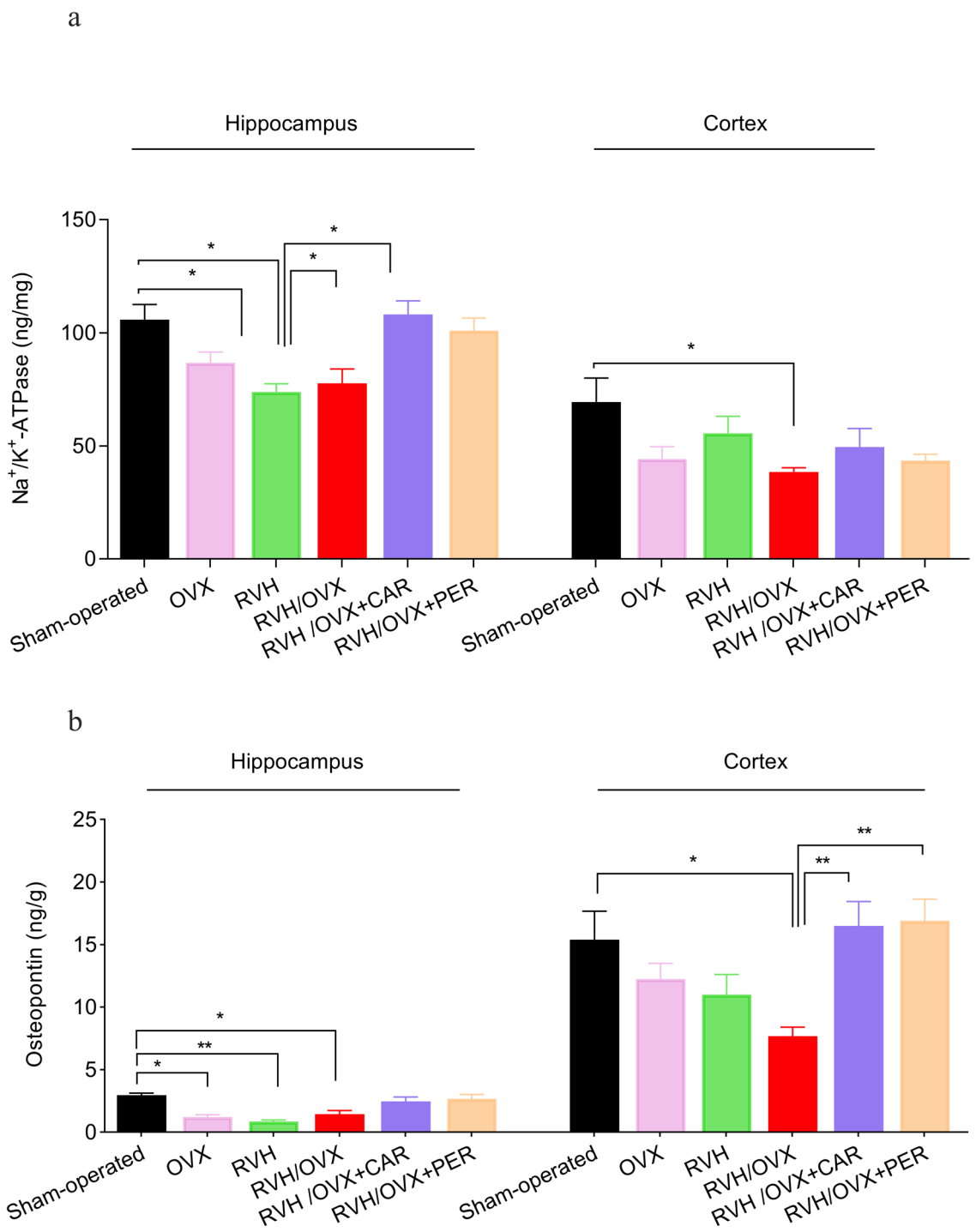


**Fig. 9** Effects of CAR treatment on **a** $Na^+/K^+$ ATPase levels and **b** osteopontin levels on hippocampus and cortex. Data were shown as mean ± SEM ($n$ = 10 rats/group). *$p$ <0.05, **$p$ <0.01. RVH, renovascular hypertension; OVX, ovariectomy; CAR, carvacrol; PER, perindopril (ANOVA followed by Tukey's post hoc test)

$K^+$ ATPase level of RVH/OVX is lower than in the sham-operated group in the cortex. In the RVH/OVX group, cortex $Na^+/K^+$ ATPase was significantly lowered as compared with that in the sham-operated group ($p$ <0.05), but no significant changes were observed in the CAR-treated group compared with the RVH/OVX group.

### Osteopontin level

Hippocampal osteopontin level in the RVH/OVX group was found to be significantly decreased as compared with that in the sham-operated group ($p$ <0.05). Similarly, the hippocampal osteopontin levels of both OVX and RVH groups were also significantly lower ($p < 0.05$ and $p < 0.01$, respectively) than in the sham-operated group. Furthermore, compared with the RVH/OVX group, the CAR-treated group had no significant difference in the level of osteopontin in the hippocampus (Fig. 9b).

### Inflammatory cytokine levels of brain tissues

In point of inflammatory markers, the RVH/OVX group demonstrated significant increments in hippocampus TNF-α and IL-1β reaching about 3-fold and 2.2-fold, respectively, compared with the sham-operated group, while a notable decrement in IL-10 reveals reaching about 2-fold compared with the sham-operated group (Fig. 10a). The hippocampus level of TNF-α was significantly decreased in the CAR-treated group compared with the RVH/OVX group ($p$<0.001); similarly, it was significantly decreased in the PER-treated group ($p$< 0.001) compared with the RVH/OVX group. The RVH/OVX +CAR group showed a significant increment in the level of hippocampus IL-10 to reach 1.3-fold, coupled with a significant decline in TNF-α and IL-1β levels by 40.7% and 57.3%, respectively, when compared with RVH/OVX animals (Fig. 10a). PER treatment produced a significant rise in IL-10 level by 48.6%, while it showed a significant decline in TNF-α and IL-1β levels to 2.03-fold and 2.25-fold, respectively, when compared with the RVH/OVX group. CAR treatment produced significant reductions in the serum levels of TNF-α and IL-1β by 75% and 78.89%, respectively, when compared with RVH/OVX. Moreover, on the level of IL-10 in serum, the RVH/OVX +CAR group showed a significant increase ($p$< 0.001) when compared with RVH/OVX (Fig. 10b).

## Discussion

The current study aimed to examine whether CAR improves cognitive decline that can occur in postmenopausal hypertensive status. The results demonstrated that CAR administration both improved learning-memory functions and reduced blood pressure in postmenopausal hypertensive rats. According to the results of the novel object recognition test in our study, the performance of the subjects in the RVH, OVT, and RVH+OVT groups was found to be significantly lower than in the control group. On the other hand, the performances of rats in perindopril and carvacrol treatment groups were significantly higher than those in the RVH+OVT group. Our results suggest that renovascular and postmenopausal hypertension models cause damage in learning and short-term memory in relation to the cortex and hippocampus, but carvacrol and perindopril treatments prevent hypertension-induced memory damage. To our knowledge, CAR treatment has not previously been shown to provide beneficial effects on cognitive function and neuropathology in postmenopausal hypertensive rats; and thus, the data from this study represent a new finding.

High blood pressure leads to the development of brain damage and various types of dementia by impairing the permeability of the blood-brain barrier and increasing amyloid-β accumulation and neuroinflammation (Cevikelli-Yakut et al. 2020; Loera-Valencia et al. 2021). In addition, postmenopausal women with hypertension have been reported to be more prone to deficits in functions such as working memory, memory recall, executive function, and psychomotor speed (El-Tahawy et al. 2019). The Goldblatt 2K1C model produces results similar to human hypertension by continuously increasing renin-angiotensin system activity and thus blood pressure. Moreover, more importantly, it is well known that increased angiotensin causes memory impairment (Willeman et al. 2019). Consistent with previous reports, blood pressure continued to increase throughout the experiment in 2K1C-induced rats, namely RVH and RVH/OVX groups. Furthermore, there is a noticeable reduction in blood pressure in rats treated with CAR for 7 weeks compared to the RVH/OVX group. In this study, the reduction of blood pressure by CAR treatment in rats with RVH/OVX is in line with the hypotensive effect of CAR in normotensive rats in our previous study (Aydin et al. 2007). Impaired cognitive functions in postmenopausal hypertension are thought to be due to both neuropathological effects mediated by high blood pressure-induced vascular damage and the disappearance of estrogen's neuroprotective effects (El-Tahawy et al. 2019). Also, estrogen suppression contributes to the neurodegenerative effects caused by increased renin levels by supporting RAS activity (Schunkert et al. 1997). The presence of the renin-angiotensin system in the brain and its relationship with the cholinergic system, and thus its contribution to the pathogenesis of AD, have been demonstrated (Sepehri et al. 2022).

Various studies have shown that estrogen deficiency causing RAS activation leads to cognitive impairment with amyloid deposition through this system (Abo-Youssef et al. 2020; Labandeira-Garcia et al. 2017). These findings show

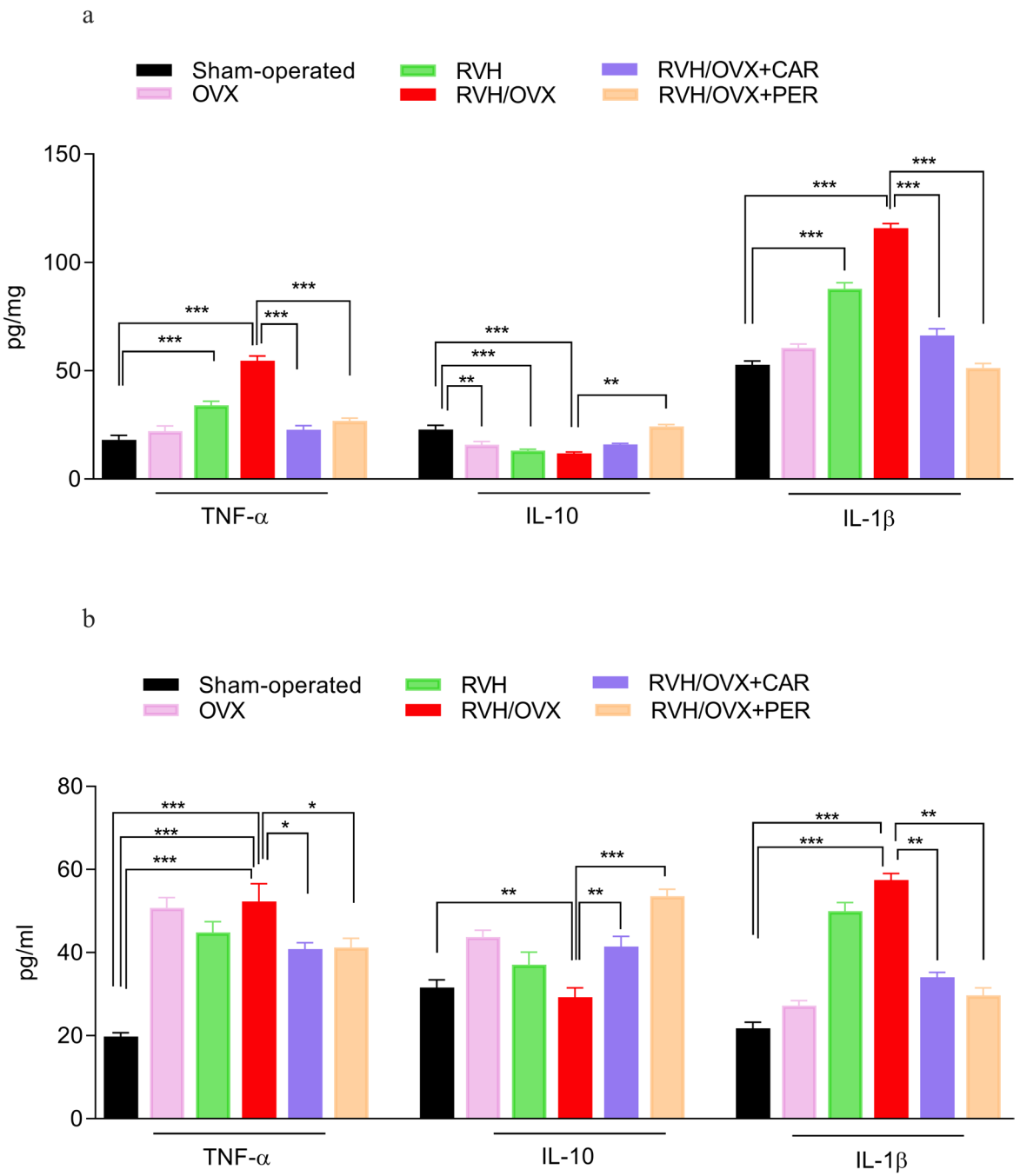


**Fig. 10** Effects of CAR treatment on inflammatory cytokine levels: **a** hippocampus and **b** the serum. Data were shown as mean ± SEM ($n$=10 rats/group). Carvacrol dose of 40 mg/kg was administered (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). RVH, renovascular hypertension; OVX, ovariectomy; CAR, carvacrol; PER, perindopril (ANOVA followed by Tukey's post hoc test)

that the neuroprotective effects of estrogen are mediated by its suppressive effect on RAS; on the other hand, they explain the cause of postmenopausal memory disorders. Indeed, Labandeira-Garcia et al., examining cognitive functions in rats with OVX, showed an increase in renin and thus angiotensin and a decrease in memory and emphasized the relationship between estrogen deficiency and increased renin in postmenopausal cognitive decline (Labandeira-Garcia et al. 2016).

Our results clearly showed that the serum estrogen level was decreased due to ovariectomy in the OVX and RVH/OVX groups. On the other hand, 7 weeks of CAR treatment did not have a noticeable effect on the decreased estrogen level. After CAR treatment, compared with the

RVH/OVX, the number of crossed platform and the time spent in the target quadrant increased in parallel with the increasing length of treatment in MWM. Additionally, the current data demonstrated that CAR significantly improved short-term and spatial memory and learning functions in postmenopausal hypertensive rats. Therefore, the functional improvement in cognitive performance in the RVH/OVX group is likely to be related to the estrogen-independent blood pressure-lowering efficacy of CAR. Moreover, we found that rats in the PER-treated groups had significantly longer escape latencies on the MWM and lower numbers of platform crossings compared to rats in the RVH/OVX group; however, these improvements were slightly less than in the CAR group. These findings pointed out that CAR, as a neurotrophic phytochemical (Sisti et al. 2021), can be used to protect against cognitive deficiency seen in postmenopausal hypertension. Lins et al. (2018) suggest that CAR shows a protective effect in a rat model of Parkinson's disease, preventing motor and neurochemical impairments induced by reserpine. In addition to our previous study, we demonstrated that carvacrol exerts neuroprotective effects by modulating Transient Receptor Potential (TRP) channel expression in neurons and astrocytes in an animal model of Parkinson's disease (Akan et al. 2023). The abovementioned results suggested that this effect of CAR treatment, which provides an estrogen-independent cognitive improvement, may be mediated by RAS or other mechanisms.

High blood pressure causes structural and functional changes in cerebral blood vessels, results in a disruption in the BBB, and deteriorates the balance between the peripheral and central nervous systems (Carnevale et al. 2012). The transport of amyloid-β proteins from the bloodstream to the intracerebral vessels is organized by the activation of the receptor for advanced glycated end products, the major bidirectional transporter of amyloid-β across the BBB (Carnevale et al. 2016). Accumulating evidence suggests a correlation between increased RAGE expression and amyloid-β deposition in the brains of transgenic models and patients with AD (Loera-Valencia et al. 2021). More importantly, hypertension induces amyloid-β accumulation by causing upregulation of RAGE (Katsouri et al. 2016). In this study, neprilysin, which provides amyloid-β degradation, was found to be lower while amyloid-β levels were significantly higher in the hippocampus of the RVH/OVX group than in the sham-operated group. Furthermore, CAR administration decreased the amyloid-β level, in parallel with an increase in neprilysin level in the RVH/OVX group. Diverse studies suggested that estrogen deficiency causes RAS activation, promoting the pathogenesis of AD through amyloid deposition and cognitive impairment (El-Tahawy et al. 2019; O'Donnell et al. 2014). These confirmed that CAR improved the cognitive function of postmenopausal hypertension-induced AD and may contribute to reducing amyloid-β through estrogen independence.

Searching the literature revealed the existence of many mechanisms that mediate the neuroprotective effect of acetylcholine by inhibiting ROS formation and inflammation, in addition to improving cognitive functions (Nestor et al. 2018). Alpha7 nicotinic acetylcholine receptor signalling plays a role in the inflammatory response mechanism in the cortex, and decreased receptor response leads to neuroinflammation and oxidant and eventually synaptic losses (Martinelli et al. 2021). Gao et al. (2018) showed that telmisartan, an angiotensin receptor blocker, improves cognitive retardation in spontaneously hypertensive rats with vascular dementia and suggested that reduction in blood pressure and improvement in cognitive tests were associated with improvement in cholinergic transmission in the hippocampus. In our study, the AChE level was significantly increased and the ChAT level was significantly decreased in the hippocampus and cortex tissues of the RVH/OVX group compared to the sham group. CAR treatment decreased the level of AChE while increasing the level of ChAT in the hippocampus and cortex tissues of rats with RVH/OVX.

In recent years, consistent studies provide evidence that hypertension inhibits $Na^+/K^+$-ATPase activity in the brain and triggers oxidative stress and inflammation in the paraventricular nucleus (Su et al. 2022). $Na^+/K^+$-ATPase, which is known to regulate cardiovascular function, neurotransmitter release, sympathetic activity, oxidative responses, and cytokine balance, has the effect of increasing blood pressure with RAS activation (Logan and George 1982). Treatment with CAR in RVH/OVX rats brought the $Na^+/K^+$-ATPase level to values close to the sham-operated and perindopril groups. Therefore, we presumed that CAR increases $Na^+/K^+$-ATPase reduced by hypertension, and may play a role in inhibiting both ROS and cytokine release. Osteopontin is well known to promote the migration, survival, and proliferation of neural stem cells and their differentiation into neurons (Rogall et al. 2018). In cases of inflammation and oxidant damage, osteopontin plays a neuroprotective role with its reparative effect on neurons (Doyle et al. 2008). In the RVH/OVX group, osteopontin levels were decreased in both tissues. With CAR treatment, the osteopontin level did not show much variability in the hippocampus but slightly increased in the cortex tissue.

Studies illuminating the mechanisms of cognitive damage caused by high blood pressure highlight a correlation between the increase in hypertension-induced inflammatory cytokines in the brain and Aβ aggregation and synaptic loss (Carnevale et al. 2012). Similarly, high blood pressure causes increased macrophage activation and inflammatory response, which underlies the decline in learning and memory functions due to neurodegeneration (Avolio et al. 2018). A study in postmenopausal rats

with hypertension reported that more cerebellar changes in RVH/OVX rats compared to rats with only hypertension or estrogen deficiency were due to the downregulation of IL-10 expression, accompanied by increased pro-inflammatory cytokine increase caused by both conditions (El-Tahawy et al. 2019). The present study showed that there were increased TNF-α and IL-1β and decreased IL-10 in both serum and hippocampal tissue in the RVH/OVX group, suggesting an increased expression of pro-inflammatory cytokines. It has been reported that CAR, which is a neurotrophic phytochemical, has a strong antioxidant, anti-inflammatory, and anti-apoptosis effect (Zamanian et al. 2021). Our findings indicated suppressive effects of pro-inflammatory cytokines such as TNF-α and IL-1β in serum and hippocampus. In addition, CAR revealed the ability to increase the level of the anti-inflammatory cytokine IL-10 in serum and hippocampus. These results can be interpreted as the effects of CAR on preventing neuroinflammation, such as increasing antioxidant capacity and inhibiting apoptotic pathways.

In conclusion, to our knowledge, this is the first study to show that CAR improves the cognitive function and neurochemical changes of hypertensive postmenopausal rats in an independent estrogen manner. Based on these findings, CAR exerts neurotrophic activities on hypertensive OVX rats that are attributed to the demolishing of amyloid peptide, anti-apoptotic activities, anti-inflammatory activities, and neurotrophic activities, as well as a decrease in blood pressure level. Our data will pave the way for studies aiming to obtain a better understanding of the mechanisms underlying the neuroprotective effects of CAR on cognitive decline. Moreover, these findings lay the groundwork for future research focused on treating hypertension-induced cognitive impairment during the postmenopausal period.

Our study is important in terms of demonstrating the strong neuroprotective effects of calvacrol in the central nervous system. However, there is a need for more research to explain the mechanisms by which calvacrol exerts these effects. In particular, the number of studies investigating the neuroprotective effects of calvacrol via calcium homeostasis and TRP channels should be increased; molecular mechanisms of action should be elucidated.

**Author contributions** D.B.: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Roles/Writing - original draft. B.E.: Methodology; Software; Supervision; Validation; Y.A.: Visualization; Roles/Writing - original draft; G.S.: Writing - review & editing. The authors declare that all data were generated in-house and that no paper mill was used.

**Funding** This research work was supported by the Eskisehir Osmangazi University Scientific Research Projects Commission University (ESOGÜ BAP-2017-1774) and by the Pharmacology Department/Faculty of Pharmacy, University of Marmara, Istanbul.

**Data availability** Processed data is provided within the manuscript, raw data can be obtained by us on reasonable grounds.

## Declarations

**Ethical approval** All animal procedures followed the laws for animal protection and were approved by the local animal care committee (Marmara University Animal Experiments Local Ethics Committee) as well as governmental authorities (Ethics committee approval protocol no. 59.2017.mar).

**Competing interests** The authors declare no competing interests.

## References

Abo-Youssef AM, Khallaf WA, Khattab MM, Messiha BAS (2020) The anti-Alzheimer effect of telmisartan in a hyperglycemic ovariectomized rat model; role of central angiotensin and estrogen receptors. Food Chem Toxicol 142:111441. https://doi.org/10.1016/j.fct.2020.111441

Akan T, Aydin Y, Korkmaz OT, Ulupinar E, Saydam F (2023) The effects of carvacrol on transient receptor potential (TRP) channels in an animal model of Parkinson's disease. Neurotox Res 41(6):660–669. https://doi.org/10.1007/s12640-023-00660-5

Ali MR, Abo-Youssef AM, Messiha BA, Khattab MM (2016) Tempol and perindopril protect against lipopolysaccharide-induced cognition impairment and amyloidogenesis by modulating brain-derived neurotropic factor, neuroinflammation and oxido-nitrosative stress. Naunyn Schmiedebergs Arch Pharmacol 389(6):637–656. https://doi.org/10.1007/s00210-016-1234-6

Alzahrani YM, Alim ASMA, Kamel FO, Ramadan WS, Alzahrani YA (2020) Possible combined effect of perindopril and Azilsartan in an experimental model of dementia in rats. Saudi Pharm J 28(5):574–581. https://doi.org/10.1016/j.jsps.2020.03.009

Avolio E, Pasqua T, Di Vito A, Fazzari G, Cardillo G, Alò R, Cerra MC, Barni T, Angelone T, Canonaco M (2018) Role of brain neuroinflammatory factors on hypertension in the spontaneously hypertensive Rat. Neuroscience 375:158–168. https://doi.org/10.1016/j.neuroscience.2018.01.067

Aydin Y, Kutlay O, Ari S, Duman S, Uzuner K, Aydin S (2007) Hypotensive effects of carvacrol on the blood pressure of normotensive rats. Planta Med 73(13):1365–1371. https://doi.org/10.1055/s-2007-990236

Azizi Z, Ebrahimi S, Saadatfar E, Kamalinejad M, Majlessi N (2012) Cognitive-enhancing activity of thymol and carvacrol in two rat models of dementia. Behav Pharmacol 23(3):241–249. https://doi.org/10.1097/FBP.0b013e3283534301

Azizi Z, Salimi M, Amanzadeh A, Majelssi N, Naghdi N (2020) Carvacrol and thymol attenuate cytotoxicity induced by amyloid β25-35 via activating protein kinase C and inhibiting oxidative stress in PC12 Cells. Iran Biomed J 24(4):243–250. https://doi.org/10.29252/ibj.24.4.243

Baser KH (2008) Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Curr Pharm Des 14(29):3106–3119. https://doi.org/10.2174/138161208786404227

Carnevale D, Mascio G, D'Andrea I, Fardella V, Bell RD, Branchi I, Pallante F, Zlokovic B, Yan SS, Lembo G (2012) Hypertension induces brain β-amyloid accumulation, cognitive impairment, and memory deterioration through activation of receptor for advanced glycation end products in brain vasculature. Hypertension 60(1):188–197. https://doi.org/10.1161/hypertensionaha.112.195511

Carnevale D, Perrotta M, Lembo G, Trimarco B (2016) Pathophysiological links among hypertension and Alzheimer's disease. High Blood Press Cardiovasc Prev 23(1):3–7. https://doi.org/10.1007/s40292-015-0108-1

Cevikelli-Yakut ZA, Ertas B, Sen A, Koyuncuoglu T, Yegen BC, Sener G (2020) Myrtus communis improves cognitive impairment in renovascular hypertensive rats. J Physiol Pharmacol 71(5):665–677. https://doi.org/10.26402/jpp.2020.5.07

Chou CC, Chien LY, Lin MF, Wang CJ (2021) Cognitive function and associated factors among postmenopausal women with hypertension and natural menopause in Taiwan. Geriatr Nurs 42(1):110–116. https://doi.org/10.1016/j.gerinurse.2020.12.007

Claflin KE, Grobe JL (2015) Control of energy balance by the brain renin-angiotensin system. Curr Hypertens Rep 17(5):38. https://doi.org/10.1007/s11906-015-0549-x

Delgobo M, Agnes JP, Gonçalves RM, Dos Santos VW, Parisotto EB, Zamoner A, Zanotto-Filho A (2019) N-Acetylcysteine and alpha-lipoic acid improve antioxidant defenses and decrease oxidative stress, inflammation and serum lipid levels in ovariectomized rats via estrogen-independent mechanisms. J Nutr Biochem 67:190–200. https://doi.org/10.1016/j.jnutbio.2019.02.012

Demirci F, Teralı K, Karadağ AE, Biltekin SN, Ak Sakallı E, Demirci B, Koşar M, Başer KHC (2022) In vitro and in silico evaluation of ACE2 and LOX inhibitory activity of Origanum essential oils and carvacrol. Planta Med. https://doi.org/10.1055/a-1828-2479

Devore E, Hall KE, Schernhammer ES, Grodstein F (2019) CE: original research: midlife hypertension and hypercholesterolemia in relation to cognitive function later in life in Black women. Am J Nurs 119(2):22–30. https://doi.org/10.1097/01.NAJ.0000553179.89954.bc

Doyle KP, Yang T, Lessov NS, Ciesielski TM, Stevens SL, Simon RP, King JS, Stenzel-Poore MP (2008) Nasal administration of osteopontin peptide mimetics confers neuroprotection in stroke. J Cereb Blood Flow Metab 28(6):1235–1248. https://doi.org/10.1038/jcbfm.2008.17

El-Tahawy NFG, Abdel Hafez SMN, Ramzy MM, Zenhom NM, Abdel-Hamid HA (2019) Effect of experimentally induced hypertension on cerebellum of postmenopausal rat. J Cell Physiol 234(8):12941–12955. https://doi.org/10.1002/jcp.27961

Ertas BTF, Gulhan R, Yanardap R, Sacan O, Sener G (2021) Beta vulgaris L. var. cicla improves memory deficits in intracerebroventricular streptozotocin injected rats: Role on neuroinflammation. J Res Pharm 25(5):589–599

Gao Y, Li W, Liu Y, Wang Y, Zhang J, Li M, Bu M (2018) Effect of telmisartan on preventing learning and memory deficits via peroxisome proliferator-activated receptor-γ in vascular dementia spontaneously hypertensive rats. J Stroke Cerebrovasc Dis 27(2):277–285. https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.01.025

Hernández-Espinosa DR, Massieu L, Montiel T, Morán J (2019) Role of NADPH oxidase-2 in the progression of the inflammatory response secondary to striatum excitotoxic damage. J Neuroinflammation 16(1):91. https://doi.org/10.1186/s12974-019-1478-4

Jamhiri M, Safi Dahaj F, Astani A, Hejazian SH, Hafizibarjin Z, Ghobadi M, Moradi A, Khoradmehr A, Safari F (2019) Carvacrol ameliorates pathological cardiac hypertrophy in both in-vivo and in-vitro models. Iran J Pharm Res 18(3):1380–1394. https://doi.org/10.22037/ijpr.2019.1100766

KadıoğluYaman B, Çevik Ö, Yalman K, Ertaş B, Şen A, Şener G (2020) Myrtus communis subsp. communis improved cognitive functions in ovariectomized diabetic rats. Gene 744:144616. https://doi.org/10.1016/j.gene.2020.144616

Katsouri L, Lim YM, Blondrath K, Eleftheriadou I, Lombardero L, Birch AM, Mirzaei N, Irvine EE, Mazarakis ND, Sastre M (2016) PPARγ-coactivator-1α gene transfer reduces neuronal loss and amyloid-β generation by reducing β-secretase in an Alzheimer's disease model. Proc Natl Acad Sci U S A 113(43):12292–12297. https://doi.org/10.1073/pnas.1606171113

Khalil A, Kovac S, Morris G, Walker MC (2017) Carvacrol after status epilepticus (SE) prevents recurrent SE, early seizures, cell death, and cognitive decline. Epilepsia 58(2):263–273. https://doi.org/10.1111/epi.13645

Kivipelto M, Helkala EL, Laakso MP, Hänninen T, Hallikainen M, Alhainen K, Iivonen S, Mannermaa A, Tuomilehto J, Nissinen A, Soininen H (2002) Apolipoprotein E epsilon4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. Ann Intern Med 137(3):149–155. https://doi.org/10.7326/0003-4819-137-3-200208060-00006

Kuller LH, Margolis KL, Gaussoin SA, Bryan NR, Kerwin D, Limacher M, Wassertheil-Smoller S, Williamson J, Robinson JG (2010) Relationship of hypertension, blood pressure, and blood pressure control with white matter abnormalities in the Women's Health Initiative Memory Study (WHIMS)-MRI trial. J Clin Hypertens (Greenwich) 12(3):203–212. https://doi.org/10.1111/j.1751-7176.2009.00234.x

Labandeira-Garcia JL, Rodriguez-Perez AI, Valenzuela R, Costa-Besada MA, Guerra MJ (2016) Menopause and Parkinson's disease. Interaction between estrogens and brain renin-angiotensin system in dopaminergic degeneration. Front Neuroendocrinol 43:44–59. https://doi.org/10.1016/j.yfrne.2016.09.003

Labandeira-Garcia JL, Rodríguez-Perez AI, Garrido-Gil P, Rodriguez-Pallares J, Lanciego JL, Guerra MJ (2017) Brain renin-angiotensin system and microglial polarization: implications for aging and neurodegeneration. Front Aging Neurosci 9:129. https://doi.org/10.3389/fnagi.2017.00129

Lin YY, Lee SD (2018) Cardiovascular benefits of exercise training in postmenopausal hypertension. Int J Mol Sci 19(9):2523. https://doi.org/10.3390/ijms19092523

Lins L, Souza MF, Bispo JMM, Gois AM, Melo TCS, Andrade RAS, Quintans-Junior LJ, Ribeiro AM, Silva RH, Santos JR, Marchioro M (2018) Carvacrol prevents impairments in motor and neurochemical parameters in a model of progressive parkinsonism induced by reserpine. Brain Res Bull 139:9–15. https://doi.org/10.1016/j.brainresbull.2018.01.017

Loera-Valencia R, Eroli F, Garcia-Ptacek S, Maioli S (2021) Brain renin-angiotensin system as novel and potential therapeutic target for Alzheimer's disease. Int J Mol Sci 22(18):10139. https://doi.org/10.3390/ijms221810139

Logan JG, George MJ (1982) Hypertension and (Na-K) ATPase activity in brain. Biochem Pharmacol 31(6):1156–1158. https://doi.org/10.1016/0006-2952(82)90358-6

Martinelli I, Tomassoni D, Roy P, Amenta F, Tayebati SK (2021) Altered brain cholinergic and synaptic markers in obese Zucker rats. Cells 10(10):2528. https://doi.org/10.3390/cells10102528

Miners S, Ashby E, Baig S, Harrison R, Tayler H, Speedy E, Prince JA, Love S, Kehoe PG (2009) Angiotensin-converting enzyme levels and activity in Alzheimer's disease: differences in brain and CSF ACE and association with ACE1 genotypes. Am J Transl Res 1(2):163–177

Mogi M, Iwanami J, Horiuchi M (2012) Roles of brain angiotensin II in cognitive function and dementia. Int J Hypertens 2012:169649. https://doi.org/10.1155/2012/169649

Naeem K, Tariq Al Kury L, Nasar F, Alattar A, Alshaman R, Shah FA, Khan AU, Li S (2021) Natural dietary supplement, carvacrol, alleviates LPS-induced oxidative stress, neurodegeneration, and depressive-like behaviors via the Nrf2/HO-1 pathway. J Inflamm Res 14:1313–1329. https://doi.org/10.2147/JIR.S294413

Nestor J, Arinuma Y, Huerta TS, Kowal C, Nasiri E, Kello N, Fujieda Y, Bialas A, Hammond T, Sriram U, Stevens B, Huerta PT, Volpe BT, Diamond B (2018) Lupus antibodies induce behavioral changes mediated by microglia and blocked by ACE inhibitors. J Exp Med 215(10):2554–2566. https://doi.org/10.1084/jem.20180776

O'Donnell E, Floras JS, Harvey PJ (2014) Estrogen status and the renin angiotensin aldosterone system. Am J Physiol Regul Integr Comp Physiol 307(5):R498-500. https://doi.org/10.1152/ajpregu.00182.2014

Rogall R, Rabenstein M, Vay S, Bach A, Pikhovych A, Baermann J, Hoehn M, Couillard-Despres S, Fink GR, Schroeter M, Rueger MA (2018) Bioluminescence imaging visualizes osteopontin-induced neurogenesis and neuroblast migration in the mouse brain after stroke. Stem Cell Res Ther 9(1):182. https://doi.org/10.1186/s13287-018-0927-9

Schunkert H, Danser AH, Hense HW, Derkx FH, Kürzinger S, Riegger GA (1997) Effects of estrogen replacement therapy on the renin-angiotensin system in postmenopausal women. Circulation 95(1):39–45. https://doi.org/10.1161/01.cir.95.1.39

Sepehri H, Ganji F, Nazari Z, Vahid M (2022) Effects of Goldblatt hypertension on rats' hippocampal cholinergic system. Transl Neurosci 13(1):72–79. https://doi.org/10.1515/tnsci-2022-0215

Shahrokhi Raeini A, Hafizibarjin Z, Rezvani ME, Safari F, Afkhami Aghda F, Zare Mehrjerdi F (2020) Carvacrol suppresses learning and memory dysfunction and hippocampal damages caused by chronic cerebral hypoperfusion. Naunyn Schmiedebergs Arch Pharmacol 393(4):581–589. https://doi.org/10.1007/s00210-019-01754-8

Sisti FM, Dos Santos NAG, Do Amaral L, Dos Santos AC (2021) The neurotrophic-like effect of carvacrol: perspective for axonal and synaptic regeneration. Neurotox Res 39(3):886–896. https://doi.org/10.1007/s12640-021-00341-1

Su Q, Yu XJ, Wang XM, Peng B, Bai J, Li HB, Li Y, Xia WJ, Fu LY, Liu KL, Liu JJ, Kang YM (2022) Na(+)/K(+)-ATPase alpha 2 isoform elicits Rac1-dependent oxidative stress and TLR4-induced inflammation in the hypothalamic paraventricular nucleus in high salt-induced hypertension. Antioxidants (Basel) 11(2):288. https://doi.org/10.3390/antiox11020288

Topal F, Ertas B, Guler E, Gurbuz F, Ozcan GS, Aydemir O, Bocekci VG, Duruksu G, Sahin Cam C, Yazir Y, Gunduz O, Cam ME (2022) A novel multi-target strategy for Alzheimer's disease treatment via sublingual route: donepezil/memantine/curcumin-loaded nanofibers. Biomater Adv 138:212870. https://doi.org/10.1016/j.bioadv.2022.212870

Wang Y, Lewin N, Qaoud Y, Rajaee AN, Scheer AS (2018) The oncologic impact of hormone replacement therapy in premenopausal breast cancer survivors: a systematic review. Breast 40:123–130. https://doi.org/10.1016/j.breast.2018.05.002

Willeman MN, Chawla MK, Zempare MA, Biwer LA, Hoang LT, Uprety AR, Fitzhugh MC, De Both M, Coleman PD, Trouard TP, Alexander GE, Mitchell KD, Barnes CA, Hale TM, Huentelman M (2019) Gradual hypertension induction in middle-aged Cyp1a1-Ren2 transgenic rats produces significant impairments in spatial learning. Physiol Rep 7(6):e14010. https://doi.org/10.14814/phy2.14010

Yanes LL, Reckelhoff JF (2011) Postmenopausal hypertension. Am J Hypertens 24(7):740–749. https://doi.org/10.1038/ajh.2011.71

Zamanian MY, Kujawska M, Nikbakhtzadeh M, Hassanshahi A, Ramezanpour S, Kamiab Z, Bazmandegan G (2021) Carvacrol as a potential neuroprotective agent for neurological diseases: a systematic review article. CNS Neurol Disord Drug Targets 20(10):942–953. https://doi.org/10.2174/1871527320666210506185042