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Original Article

Role of ((E)-(E)-4-(4-hydroxy-3-methoxyphenyl)-2-oxobut-3-en-1-yl 3-(4-hydroxy-3-methoxyphenyl) acrylate in preservation of spatial cognitive functions of rats with chronic epilepsy

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Abstract: The present study demonstrates the effect of ((E)-(E)-4-(4-hydroxy-3-methoxyphenyl)-2-oxobut-3-en-1-yl 3-(4-hydroxy-3-methoxyphenyl) acrylate (CA) on spatial cognitive functions of rats with lobal cerebrovascular hypoperfusion. The bilateral common carotid arteries occlusion (2VO) surgery was performed to prepare the cerebrovascular hypoperfusion rat model. The effect of CA on spatial cognitive function was analysed using Morris water maze (MWM) test prior to and after 2VO operation. Sixty rats were randomly assigned into two groups of 30 each; long-term memory (LTM) and short-term memory (STM) groups. Both the groups were further divided into 3 subgroups: control, untreated and CA treated groups. The animals received 50 μg/kg of CA for 10 weeks of 2VO operation following which all the subgroups were tested with MWM. Both the escape latency time and total distance travelled were significantly lower for control and CA treated groups compared to untreated group revealed by working memory test. The maze test performance for control and CA treated groups was found to be improved markedly. Similarly, the results from probe memory test performance revealed significant improvement for CA treated groups compared to untreated group. Therefore, CA exhibits significant effect on the spatial cognitive preservation in rats with chronic epilepsy.

Keywords: Chronic epilepsy, Morris water maze, cognitive, escape latency, working memory test

Introduction

Alzheimer's disease (AD), the most common cause of progressive cognitive dysfunction affects four million Americans and causes 100000 deaths every year [1, 2]. AD is characterised by extracellular deposition of *β*-amyloid as senile plaques infiltrated by reactive microglia and astrocytes [3, 4]. The approaches used currently for the treatment of AD include antioxidant therapy, acetylcholinesterase inhibitors, nicotinic and muscarinic agonists, estrogen, nerve growth factor, low molecular lipophilic compounds that can activate neurotrophic factor signalling pathway, non-steroidalanti-inflammatory drugs [3].

Reports demonstrate that no relation exists between amyloid plaque density and the level of memory and learning impairment [4, 5]. No effect of plaque removal was observed on the disease symptoms or the survival rate of the patients [6]. These findings suggested the involvement of other causes behind neurodegenerative changes in AD [7]. AD is associated withchronic reduction in cerebral blood flow leading to reduction in glucose and oxygen supply to cerebral neurons and finally neurodegeneration and cognitive decline [8]. Thus AD is considered to be a brain vascular disease [9]. Researchers have frequently used the two vessel occlusion (2VO) rat model of cerebral hypoperfusion for investigation of the neurodegenerative disorders. The 2VO rat model involves permanent bilateral ligation of common carotid arteries [8]. There is cerebral hypoperfusionfollowed byneurodegeneration of the pyramidal

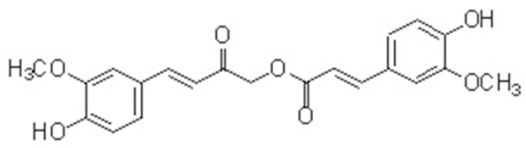

Figure 1. Structure of compound A.

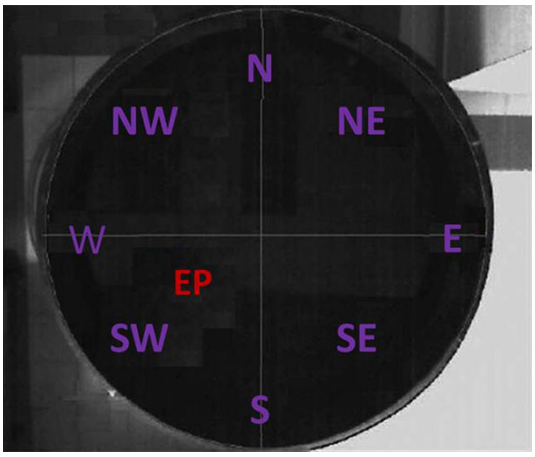

Figure 2. The four hypothetical random starting points of MWM pool for the test. The working memory test (WMT) was carried out for STM group after changing the position of EP every day to different zones from day 67-69. Four trials each of 120 seconds with 1 minute intervening period were performed every day. The final test trial was followed by cued version test beginning with the SE pole. During the test EP platform was raised 1 cm above the water surface and a yellow flag 10 cm in height was placed on it. Each panel of the flag was 1 cm × 5 cm in dimensions.

hippocampal neurons which control spatial learning and memory [10]. The spatial reference and working memory impairment is evaluated by employing Morris water maze (MWM) test [11, 12]. The spatial reference memory denotes the brain activity used to recall consolidated positions and places [13, 14]. One of the earliest symptoms of AD is the progressive weakening of spatial memories [15]. The 2VO operated rats show a significant poor MWM performance in learning as well as memory after 2VO surgery compared to healthy control rats [16].

The plants Ginkgo biloba L. Huperziaserrata Trevis have been extensively studied for the treatment of AD patients [17]. The compound A, ((E)-(E)-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-but-3-en-1-yl 3-(4-hydroxy-3-methoxyphenyl) acrylate, **Figure 1**) was isolated from the plant turmeric. The herb is indigenous to southern Asia and its extract protects PC12 cells from *β*-amyloid deposition [18]. In the present study, effect of compoundA (**Figure 1**) on spatial reference long-term memory (LTM), short-term (STM) and spatial working memory (WM) of cerebrally hypoperfused rats were examined.

Materials and methods

Animals and drug

Sixty male Sprague Dawley rats (21 week old) were purchased from Beijing Vital River Experimental Animal Technology Co., Ltd. SIBS Guide for the Care and Use of Laboratory Animals approved by the Animal Care and Use Committee of the Beijing Institutes for Biological Sciences was used to perform all the animal procedures. All efforts were made to minimize animal suffering and the number of animals used.

Treatment strategy

The 60 rats were divided randomly into two groups, a) long-term memory (LTM), and b) short-term memory (STM) and working memory (WM) groups. The animals of LTM group received MWM training before 2VO surgery and were retested 10 weeks after 2VO surgery. The STM and WM test group rats were naïve to MWM at the time of 2VO surgery and tested at the 10th week after operation. Both the groups were further divided into 3 subgroups: (a) Control group; The rats operated were neither double ligated nor CA treated, (2) Untreated group; rats were operated with bilateral double ligation but not CA treated and (3) CA treated group; rats were operated, double ligated and CA treated. The oral CA treatment was started 1 week before 2VO surgery and continued with the daily oral dose of 50 μg/kg for further 70 days (10 weeks) after 2VO surgery.

2VO procedure

The rats were administered ketamine and xylazine at the dose of 85 mg/kg and 8 mg/kg, respectively as anesthetic dose. Just above the sternal bone, a 2 cm skin incision was made to each rat for the identification of carotid sheath. Under sterilized conditions the carotid arteries were separated from the vagus nerve carefully. Immediately below the point of bifurcation into

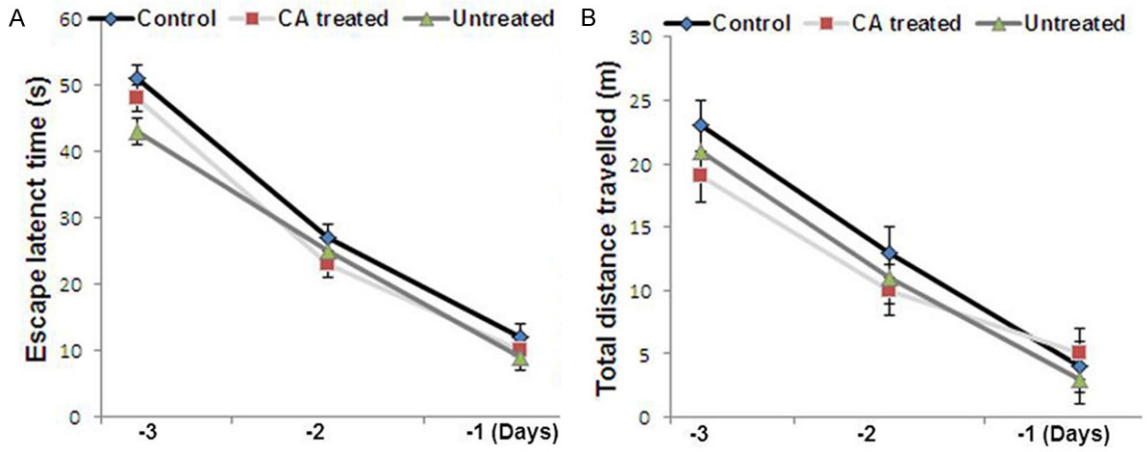

Figure 3. Differences in escape latency time and total distance travelled during the three successive days in MWM acquisition test before surgery.

internal and external carotid arteries, the carotid artery was doubly ligated with silk suture and the arteries were cut between the two ligatures.

Apparatus

To perform the memory tests we used a modified MWM apparatus with circular black fiber glass tank (diameter 2, height 60 cm). The apparatus was filled with water up to the height of 30 cm. It was provided with a black escape platform (EP) which served as the rescue island for the rats. To avoid odour, the water in the apparatus was changed regularly and the 26°C temperature was maintained. To facilitate animals build up their spatial memory the walls around the pool were pasted with colourful posters. For the purpose of recording and analysing swimming time, distance and speed of the animals, ANY-maze video tracking software (Stoelting Co., USA) was employed. The pool was divided hypothetically into 4 equal imaginary quadrants (SW, SE, NW and NE) and the tests were performed from 9 am and 5 pm (**Figure 2**). For each of the animal the quadrant was changed every day. For LTM and STM groups, the habituation training was performed on the day 5 and 4 before surgery and day 61 and 62 after surgery, respectively. During these days, a four trial per day MWM training each for a maximum of 120 seconds was given to each animal. On reaching EP, the animals were allowed to stay for 30 seconds. Prior to the actual acquisition test animals were adapted to the water tank and the surrounding extra-maze visual cues. On the day 1-3 before and 62-63 after operation for LTM and STM groups, respectively the acquisition tests were carried out. The EP was set in the SW zone of the pool during this phase and water level was raised 2 cm above the EP surface (**Figure 2**). For 4 consecutive days 4 trials each for 120 seconds followed by a 4 minute break was given to each animal. The distance travelled and time taken was calculated for each rat. LTM test was performed on the day 68 after operation. The retention (probe) memory test was performed for LTM and STM groups on day 69 and 66 after operation, respectively. To each animal a 60 second single swimming trial was given from the NE pole in absence of EP. The time spent and the number of annulus crossings in the target zone was calculated.

Statistical analysis

Two way analysis of variance (ANOVA) was employed to analyse the total distance travelled and swimming speeds. For retention probe memory test data one-way ANOVA was used. The results presented are the mean of mean ± SEM. The differences were considered statistically significant at $P < 0.05$.

Results

Mortality and blindness rates after the operation

Fourteen (14) animals from the untreated group of 30 died during the study (mortality rate = 46%) and 1 got blindness on the day 5 of surgery (blindness rate = 3.3%). The rats with

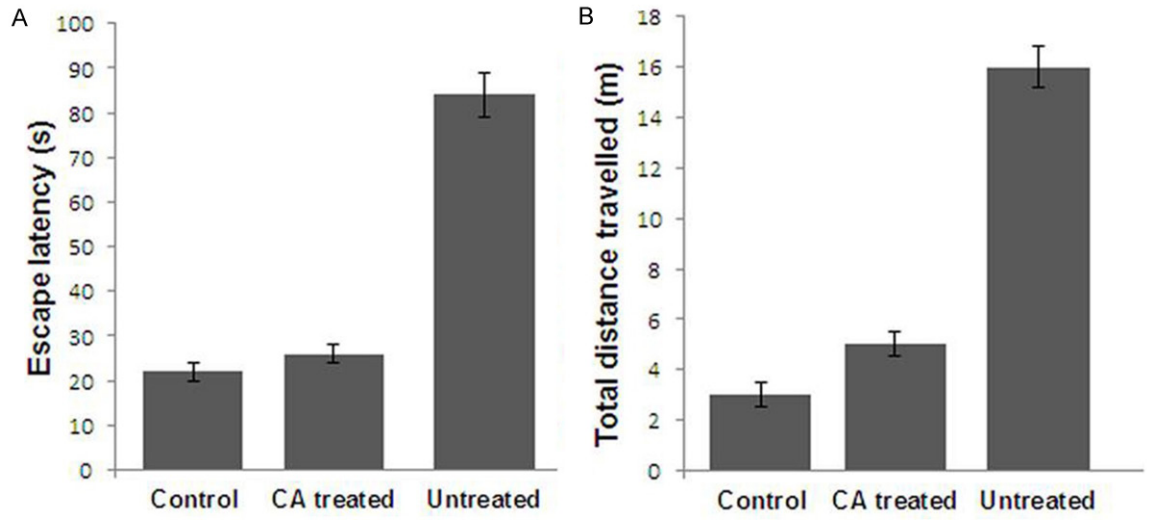

Figure 4. Differences in escape latency time and total distance travelled among control, NVB treated and untreated groups during LTM test on 68th after surgery.

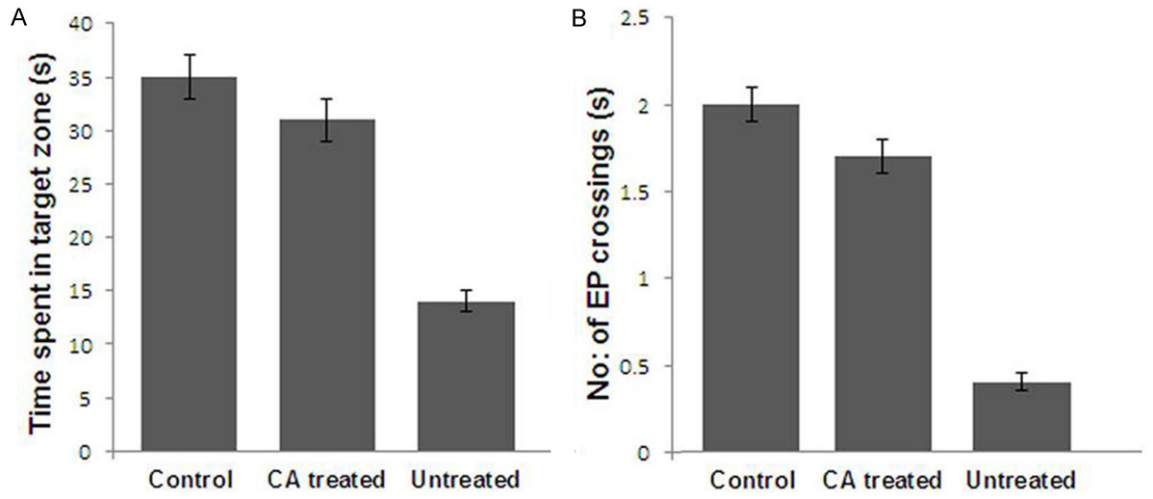

Figure 5. Differences among study groups in time spent in the target (SW) zone and number of annulus crossings retention probe LTM test.

blindness were excluded from the MWM study. Among 30 rats of the CA treatment group 1 died (mortality rate = 3.3%) and no one suffered from blindness. On the other hand, none of the animals from the control group either died or suffered from the blindness.

LTM test

On the days prior to the training, the rats showed similar results for escape latency time and the total distance travelled to reach the EP zone (**Figure 3**). The rats in the control and CA treatment groups showed significantly short mean escape latency time compared to those in the untreated group after the operation. The values of the mean escape latency time for control, CA treated and untreated groups were 19.43 ± 3.67, 21.78 ± 3.13 and 89.98 ± 5.65 sec, respectively (**Figure 4**). The swimming distances of rats in untreated group were also markedly different compared to the control and CA treated groups. For the control, CA treated and the untreated groups swimming distances covered were 3.2314 ± 1.02, 3.8967 ± 1.23 and 17.5464 ± 3.35 m, respectively (**Figure 4**). The rats in the control and CA treated groups

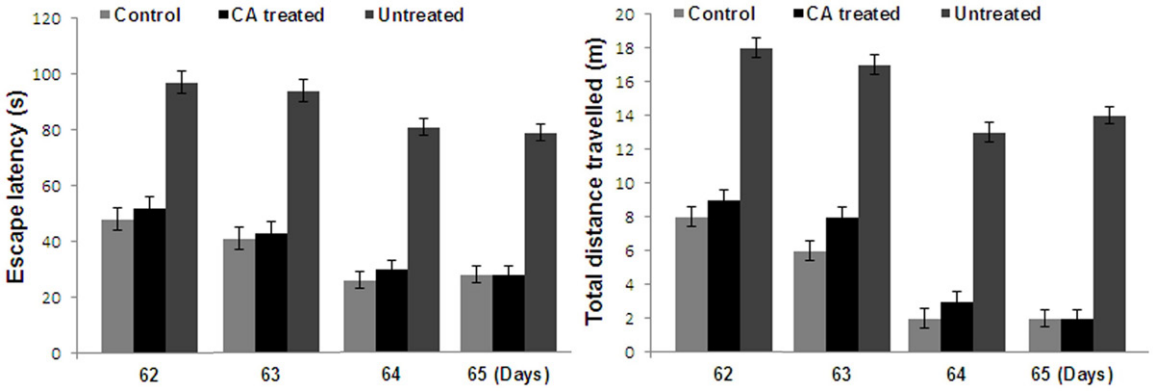

Figure 6. Differences between the study groups during the STM test in escape latency and total distance travelled.

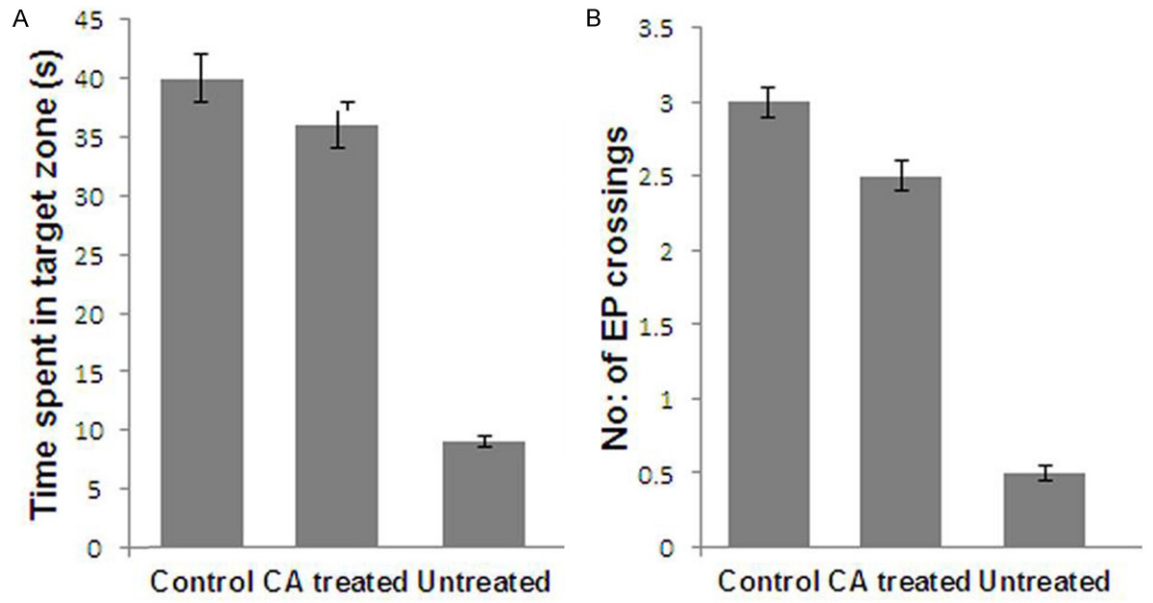

Figure 7. Differences among time spent in the target (SW) zone and number of annulus crossings.

spent significantly longer mean time in the target (SW) zone compared to the untreated group. The values for mean time spent in the target (SW) zone were 42. 42 ± 3.29, 35.57 ± 3.21 and 14.56 ± 2.65 s, respectively by the animals in control, CA treated and untreated groups (**Figure 5**). Results also revealed marked difference in the average number of annulus (EP zone) crossings. The number of annulus crossings was higher forthe control and CA treated groups than untreated group (**Figure 5**).

STM test

Results from the acquisition test of MWM carried out on the days 62-65 post operation revealed that the mean escape latency time and total distance travelled by control and CA treated groups were significant different compared to untreated group (**Figure 6**). For the control, CA treated and untreated groups the mean time spent in the target zone was 42.78 ± 3.75, 31.82 ± 3.45 and 13.09 ± 2.32 s, respectively (**Figure 7**). During the time period of 60 seconds the average number of annulus crossings in the EP zone were 4.43 ± 1.05, 2.97 ± 0.32 and 0.19 ± 0.06 for control, CA treated and untreated groups (**Figure 7**).

WMT results

Analysis of the results for mean escape latency on 3 successive days revealed significant difference among the three groups of rats (**Figure**

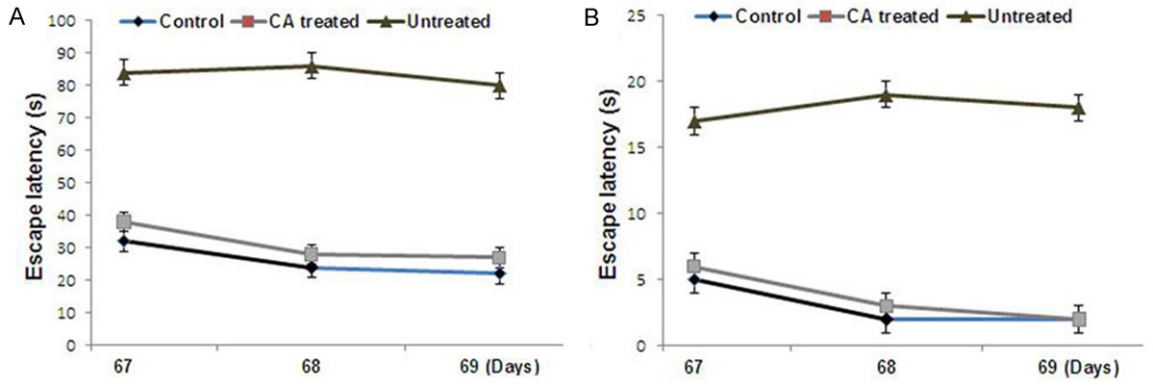

Figure 8. Differences in the escape latency time and total distance travelled with daily changing of EP location.

8). The control and CA treated groups showeda marked difference from that of untreated group for the mean distance travelled. The swimming distance travelled by the rats in control and CA treatment groups was different than untreated group (**Figure 8**).

Discussion

Alzheimer's disease, the most common cause of progressive cognitive dysfunction affects four million Americans and causes 100000 deaths every year [1, 2]. In the current study effect of CA on cognitive learning and memory performance was investigated in the rats. In order to prevent the animals from respiratory distress pre-anesthetic atropine doses were administered. It is well known that 2VO surgery is associated with the complication of optic tract acute ischemic injury induced blindness within the 7 days [19-21]. The results revealed a significantly higher mortality rate in untreated group compared to CA treated group. These findings indicated the acute ischemic injury to the vital brain centers. The lower mortality rate in CA treated group compared to untreated group suggests the possible neuroprotective effect of CA. CA prevents cerebral neurons from oxidative stress and neuroinflammation associated with acute ischemia. No significant differences were observed during 2 day habituation training followed by cued version MWM task test among all the tested animals. This indicated that sensory-motor function of the animals remained intact. All the three rat groups showed negligible difference for escape latency and swimming distance before operation because the animals were in cognitively healthy state. During LTM test, the performance of untreated group was very much deterioration compared to CA treated animals. The loss of remote spatial memory in untreated group was significantly observed in the retention memory test. Earlier reports demonstrate that cerebral hypoperfusion becomes chronic between 8th and 12th week following 2VO surgery. Then the blood flow gets normal but MWM performance continues to deteriorate resulting inneurodegeneration [22].

The 2VO induced LTM impairments were considerably improved on treatment with CA revealed by marked difference between CA treated and the untreated group. The improved LTM water maze performance by CA treated group was confirmed by the probe memory test. This test excludes spatial bias, adoption of non-spatial strategies and possibility that rats reached EP by chance [23]. In this study the results observed for the CA treated and the control group were comparable. This clearlyindicated the role of CA to preserve remote spatial reference memories. The average number of annulus crossings for CA treated rat group was also comparable to that of the control group. In CA treated group the improved results of MWM performance are believed to be due to adenylatecyclase (AC) enzyme. It is reported that AC 1 is o-enzyme plays a vital role for maintaining intact hippocampal impulse transmission for reference memory [23]. For working memory, AC 8 isoenzyme is believed to play a crucial role pre-synaptically [24]. Therefore, the neuroprotective effect of CA was more pronounced at the postsynaptic than the presynaptic neurons creating a significant improve-

ment in the reference memory and only an intermediate enhancement of spatial working memory.

Conclusion

The CA treatment to 2VO operated animals modulates neurotransmitters within the CNS thereby enhancing the cognitive function.

Disclosure of conflict of interest

None.

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