

# EFFECT OF ALPHA LIPOIC ACID, MELATONIN AND TRANS RESVERATROL ON INTRACEREBROVENTRICULAR STREPTOZOTOCIN INDUCED SPATIAL MEMORY DEFICIT IN RATS

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**Abstract :** In the present study, the effect of antioxidants-alpha lipoic acid, melatonin and trans resveratrol were studied against intracerebroventricular streptozotocin induced spatial memory deficit. Male Wistar rats were injected with intracerebroventricular streptozotocin bilaterally. The rats were treated chronically with alpha lipoic acid (200 mg/kg, po), melatonin (20 mg/kg, ip) and trans resveratrol (20 mg/kg, ip) for 18 days starting from day 1 of streptozotocin injection in separate groups. The spatial memory was evaluated using the Morris water maze task. The intracerebroventricular streptozotocin rats treated with antioxidants showed significantly less spatial memory deficit both in the acquisition and probe trials as compared to the vehicle treated rats. The study demonstrated the effectiveness of alpha lipoic acid, melatonin and trans resveratrol in preventing spatial memory deficit induced by intracerebroventricular streptozotocin and its potential in age related neurodegenerative disorders where oxidative stress is involved such as Alzheimer's disease.

**Key words :** antioxidants spatial memory ICV streptozotocin rats

## INTRODUCTION

Alzheimer's disease is the most common cause of dementia in the elderly population. The most widely used treatment for Alzheimer's disease at present are acetylcholinesterase inhibitors, which aim to prolong cognitive function through increased synaptic activity, without providing

neuroprotection. This treatment is only symptomatic and provides modest outcomes for patients (1).

The recent elucidation of the oxidative stress pathways involved in Alzheimer's disease however, has opened vistas for better treatment and prevention by targeting the cause of the disease rather than the

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symptoms. Reports indicate that administration of antioxidants may be useful in prevention and treatment of Alzheimer's disease (2). In this respect scavenging of free radicals by non-enzymatic/exogenous antioxidants seems to be the most practical approach.

This is due to the fact that many different nonenzymatic/exogenous antioxidants are known and many (e.g. vitamins E and C, melatonin, flavanoids and carotenoids) have no major side effect (3) and there is currently clinically no therapeutic approach known to increase levels of enzymatic/endogenous antioxidants in humans (4).

The intracerebroventricular streptozotocin (ICV STZ) model of cognitive impairment has been likened to sporadic dementia of Alzheimer's disease in rats. It is characterized by cognitive impairment, impaired glucose metabolism (5–7), oxidative stress (8) and a decrease in cholinergic markers in the brain.

Alzheimer's disease patients show a deficit in spatial memory (9–11). The Morris water maze is one of the choice apparatus to investigate the spatial memory deficits in rodent models of AD (12). As in the Morris water maze task, the cognitive performance is highly dependent on hippocampal integrity (13).

We have shown the protective effect of antioxidants-alpha lipoic acid (ALA), melatonin and trans resveratrol against ICV STZ induced cognitive impairment and oxidative stress in rats (14–16).

ALA is a free radical scavenger and antioxidant, which crosses the blood brain

barrier with ease (17). Melatonin is a neurohormone with potent antioxidant property (18), while trans resveratrol is a substance derived from red wine (19) and has shown potent antioxidant activity in several in vitro studies. However, the effect of these antioxidants on spatial memory deficit is unknown. Therefore, the present study was undertaken to evaluate the effect of ALA, melatonin and trans resveratrol on ICV STZ induced spatial memory deficit in rats.

## MATERIAL AND METHODS

### Animals

Adult male Wistar rats weighing 320–350 g were used. The animals were obtained from the central animal facility of All India Institute of Medical Sciences, New Delhi and stock bred in the departmental animal house. The rats were group housed in polyacrylic cages (38 × 23 × 10 cm) with not more than 4 animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. They were allowed free access to standard dry rat diet and tap water ad libitum. All procedures described were reviewed and approved by the Institutional Committee for Ethical Use of Animals.

### Experimental protocol and drugs

The animals were divided into seven different groups of 7 rats each. In the first group (sham), the rats were injected ICV on day 1 and 3 with artificial CSF instead of STZ. The second, third and fourth group were the vehicle treated, rats were injected with ICV STZ (day 1 and 3) and then treated

with the vehicle; 1% gum acasia, propylene glycol and 50% ethanol respectively for 21 days. The sham and vehicle control groups were run parallel to the drug treated groups.

In the next three groups, the rats were treated with ALA (200 mg/kg, po), melatonin (20 mg/kg, ip) and trans resveratrol (20 mg/kg, ip) after ICV injection (day 1 and 3) of STZ. ALA (Intas Ltd, Ahemdabad, India) was suspended in 1% gum acacia and administered at a dose of 200 mg/kg. It was administered every day using an intragastric cannula starting from day of 1st STZ ICV injection to day 18. On the day of the STZ ICV injections (day 1 and day 3), ALA was administered before the ICV injection. Melatonin (Courtesy, Dabur India Limited) was prepared in propylene glycol. It was administered chronically every day at a dose of 20 mg/kg ip starting from day of 1st STZ ICV injection to day 18. On the day of the ICV injections (day 1 and day 3), melatonin was injected just before the ICV injection. Trans resveratrol (Courtesy, Pharmascience, Canada) was freshly prepared in 50% alcohol. It was administered chronically at a dose of 20 mg/kg, ip from day 1 (day of 1st STZ ICV injection) to day 18.

The doses of ALA, melatonin and trans resveratrol chosen in this study are on the basis of our earlier studies in which these doses have been effective in protecting against ICV STZ induced cognitive impairment (14–16).

#### **Intracerebroventricular injection of streptozotocin**

STZ was injected intracerebroventricularly according to the protocol described earlier (8, 16). Briefly, rats were anesthetized with

chloral hydrate at a dose of 400 mg/kg, intraperitoneally. The head was positioned in a stereotactic frame and a midline sagittal incision was made in the scalp. Burr holes were drilled in the skull on both the sides over the lateral ventricles using the following coordinates: 0.8 mm posterior to bregma; 1.5 mm lateral to sagittal suture; 3.6 mm beneath the surface of brain. STZ (Sigma, St Louis, USA) (3 mg/kg body weight) was injected ICV bilaterally in the STZ group; vehicle treated and ALA treated group. The injection (3 mg/kg) was repeated on day 3. In the sham group, artificial CSF: 147 mM NaCl; 2.9 mM KCl; 1.6 mM MgCl<sub>2</sub>; 1.7 mM CaCl<sub>2</sub> and 2.2 mM dextrose was injected (20 µl on each site) on the same days as in STZ group.

#### **Assessment of spatial memory**

During behavioral testing only one animal was tested at a time.

#### **Morris water maze**

The Morris water maze task was carried on day 14–day 18 after the ICV STZ injection. The water maze used was adapted from Morris (13) and consisted of a white circular tank, 165 cm in diameter and 100 cm in depth. It was filled to a depth of 37 cm with water (20–22°C). Four arbitrarily points equally spaced around the perimeter of the tank were designated to serve as starting positions (N, S, W, E) and the tank was divided virtually into four equal quadrants. The escape platform located in the center of one of these platforms (quadrant II) was white and had a diameter of 10 cm. The water was made opaque using milk powder. A video camera was mounted on the ceiling

in the center of the circular pool. The room also contained several visible cues including a door, windows, air-conditioner and the experimenter. Following each day of testing the pool was drained and thoroughly cleaned. The heads of the rats were painted black using a hair dye (Godrej, India).

The testing procedure used was a modification of the procedure described earlier by van der Staay and Blokland, 1996 (20). Briefly, the animals received four trials during four daily acquisition sessions. A trial was started by placing a rat into the pool, facing the wall of the tank. Each of the four starting positions (N, S, W, E) was used once in a series of trials. The platform was always in the same quadrant (North West). The trial was terminated automatically as soon as the rat reached the platform or when 60 s had elapsed. The rat was allowed to stay on the platform for 5 s. Then it was taken out of the platform and a new trial was started. Rats that did not find the platform within 60 s were put on the platform gently by the experimenter and were allowed to stay there for 5 s. After the completion of the fourth trial, the rat was gently dried with a towel, kept warm for one hour and returned to its home cage.

On the fifth day, a spatial probe trial (60s) was given to detect the spatial memory of the rat. On this day the same protocol as described earlier was followed, however, the platform had been removed from the pool and the time spent and the distance swam in the quadrant previously containing the platform was recorded and also the time spent in the other quadrants. The path of each rat was analyzed by using the Videomex tracking software (Columbus, Ohio, USA). The parameters determined

were 1) latency to find the platform; 2) traveled distance and 3) the time spent in each quadrant.

The results are expressed as mean  $\pm$  SEM. Statistical analysis for the Morris water maze test, two-way analysis of variance was conducted. \* $P < 0.05$  represents level of significance.

## RESULTS

### Effect of ALA on spatial memory

There was a significant decrease in the escape latencies during acquisition task of the Morris water maze in the sham [F (3, 57) = 76.3] ( $P < 0.05$ ), vehicle treated ICV STZ group [F (3, 54) = 3.43] ( $P < 0.05$ ) and the ALA treated ICV STZ group [F (3, 57) = 21.82] ( $P < 0.05$ ). However, there was a significant difference between the escape latencies on day 2, 3 and 4 in the sham Vs vehicle treated ICV STZ ( $P < 0.05$ ) and the ALA treated ICV STZ group [F (2, 59) = 22.98; F (2, 56) = 18.9] ( $P < 0.05$ ) respectively. The mean escape latencies of the sham group and the ALA treated ICV group were significantly shorter than that of the vehicle treated ICV STZ group (Fig. 1). This signifies a poorer/ impaired acquisition of the task by the vehicle rats treated with ICV STZ and an amelioration of this impairment by ALA. The mean escape latencies on day 1, 2, 3 and 4 of the ICV STZ rat being  $51 \pm 11$ ,  $50 \pm 4$ ,  $43 \pm 1.3$  and  $39 \pm 1.8$  s respectively while the escape latencies on day 1-4 of the ALA treated ICV STZ group being  $52.6 \pm 2.6$ ,  $33.5 \pm 5$ ,  $15.8 \pm 3.8$  and  $14.4 \pm 3.5$  s respectively. The distance traveled by the rats paralleled the time spent therefore was not considered separately in the study.

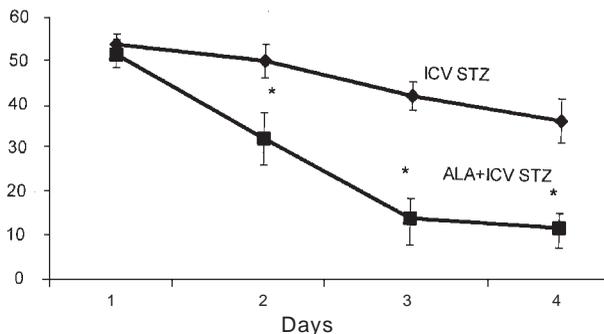


Fig. 1: Effect of alpha lipoic acid (200 mg/kg, po) on ICV STZ cognitive impairment (Morris water maze) (acquisition phase). ALA represents alpha lipoic acid in figure. Values are expressed as Mean  $\pm$  S.E.M. \* $P < 0.05$  vs vehicle treated ICV STZ.

In the probe trial, the sham rats and the ALA treated ICV STZ group spent significant time and distance in the quadrant which previously contained the platform (quadrant 2) while the vehicle treated ICV STZ rats showed no such preference either in terms of time spent or in terms of the distance swum. The time spent in quadrant 1, 2, 3 and 4 by the sham, vehicle treated ICV STZ and ALA treated ICV rats was: quad 1 ( $10.8 \pm 1.2$ ;  $17 \pm 11$  and  $8.36 \pm 2$  s); quad 2: ( $21.3 \pm 1.8$ ,  $15.3 \pm 2.7$  and  $20.1 \pm 1.8$  s); quad 3 ( $13.5 \pm 1.2$ ;  $13 \pm 2$ ,  $10.7 \pm 1.1$  s); quad 4 ( $14.1 \pm 1.5$ ,  $11 \pm 1$ ,  $11.1 \pm 1.3$  s) respectively (Fig. 2). The sham and the ALA treated rats spent significant time in quadrant 2 ( $P < 0.05$ ) as compared to the other quadrants. The distance swum by the vehicle treated ICV STZ rats in quadrant 1, 2, 3 and 4 was ( $300 \pm 40$ ,  $411 \pm 31$ ,  $490 \pm 60$  and  $513 \pm 36$  cm respectively) while the distance swum by the ALA treated group was ( $313 \pm 63.4$ ,  $629 \pm 39.8$ ,  $530 \pm 11.6$  and  $391 \pm 33.6$  cm respectively).

#### Effect of melatonin on spatial memory

There was a significant decrease in the escape latencies during acquisition task of

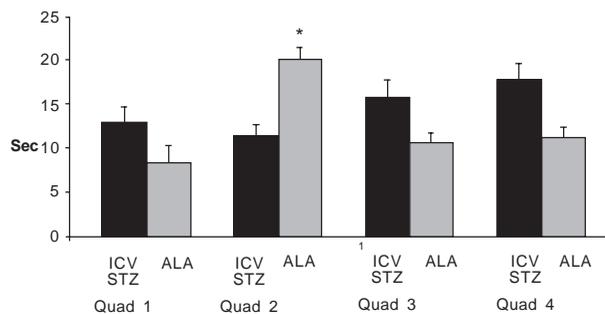


Fig. 2: Effect of alpha lipoic acid (200 mg/kg, po) on ICV STZ rats on time spent in different quadrants. ALA represents alpha lipoic acid in figure. Values are expressed as Mean  $\pm$  S.E.M. \* $P < 0.05$  vs ICV STZ+alpha lipoic acid group.

the Morris water maze in the sham [ $F(3, 51) = 76.3$ ] ( $P < 0.05$ ), vehicle treated ICV STZ group [ $F(3, 54) = 3.43$ ] ( $P < 0.05$ ) and the melatonin treated ICV STZ group [ $F(3, 57) = 43.7$ ] ( $P < 0.05$ ). However, there was a significant difference between the escape latencies on day 3 and 4 in the sham Vs vehicle treated ICV STZ ( $P < 0.05$ ) and the melatonin treated ICV STZ group [day 3  $F(2, 56) = 26.58$ ;  $F(2, 56) = 24.21$ ] ( $P < 0.05$ ) respectively. The mean escape latencies of the sham group and the melatonin treated ICV STZ group were significantly shorter than that of the vehicle treated ICV STZ group. This signifies a poorer/impaired acquisition of the task by the vehicle rats treated with ICV STZ and an amelioration of this impairment by melatonin. The mean escape latencies on day 1, 2, 3 and 4 of the ICV STZ rat being  $54.6 \pm 2.4$ ,  $51.9 \pm 3.5$ ,  $44 \pm 3.7$  and  $39 \pm 3.4$  s respectively while the escape latencies on day 1–4 of the melatonin treated ICV STZ group being  $56.4 \pm 2.8$ ,  $46.9 \pm 4.4$ ,  $11.35 \pm 2.9$  and  $11.8 \pm 2.6$  s respectively (Fig. 3). The distance traveled by the rats paralleled the time spent therefore was not considered separately in the study.

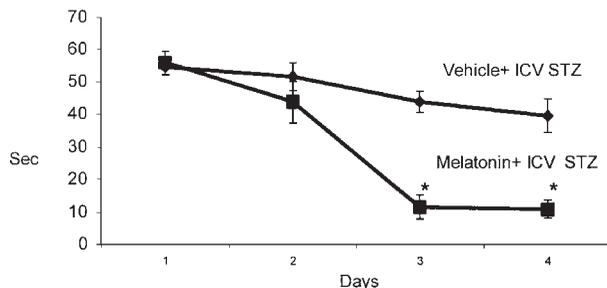


Fig. 3 : Effect of melatonin (20 mg/kg, ip) on ICV STZ cognitive impairment on escape latencies in the Morris water maze test (acquisition phase). Mel represents melatonin in figure. Values are expressed as Mean  $\pm$  S.E.M. \* $P < 0.05$  vs vehicle treated ICV STZ.

In the probe trial, the sham rats and the melatonin treated ICV STZ group spent significant time and distance in the quadrant which previously contained the platform (quadrant 2) while the vehicle treated ICV STZ rats showed no such preference either in terms of time spent or in terms of the distance swum. The time spent in quadrant 1, 2, 3 and 4 by the sham, vehicle treated ICV STZ and melatonin treated ICV STZ rats was : quad 1 ( $10.8 \pm 1.24$ ,  $13 \pm 1.8$  and  $6.3 \pm 0.5$  s) quad 2 ( $21.3 \pm 1.2$ ,  $11.3 \pm 1.4$  and  $23.5 \pm 0.26$  s); quad 3: ( $13.5 \pm 1.2$ ,  $15.8 \pm 2$ ,  $16 \pm 2.3$  s) and quad 4 ( $14.1 \pm 1.5$ ,  $17.6 \pm 1$  and  $11.1 \pm 1.4$  s) respectively (Fig. 4). The sham and the melatonin rats spent significant time in quadrant 2 ( $P < 0.05$ ) as

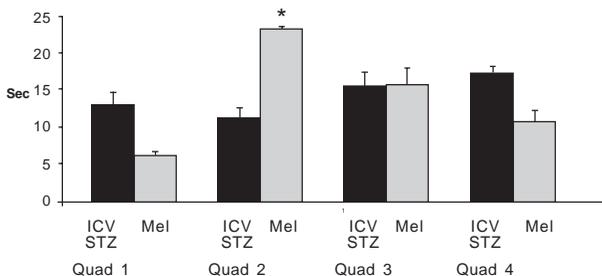


Fig. 4 : Effect of melatonin (20 mg/kg, ip) on ICV STZ in different quadrants in the Morris water maze task. Mel represents melatonin in figure. Values are expressed as Mean  $\pm$  S.E.M. \* $P < 0.05$  vs ICV STZ+ melatonin group.

compared to the other quadrants. The distance swum by the vehicle treated ICV STZ rats in quadrant 1, 2, 3 and 4 was ( $351 \pm 39.6$ ,  $383 \pm 76.6$ ,  $450 \pm 55.9$  and  $501 \pm 49.3$  cm respectively) while that swum by the melatonin treated group was ( $284 \pm 83$ ,  $660.7 \pm 147$ ,  $700 \pm 61.7$  and  $356 \pm 85$  cm respectively).

#### Effect of trans resveratrol on spatial memory

There was a significant decrease in the escape latencies during acquisition task of the Morris water maze in the sham [ $F(3, 57) = 76.3$ ] ( $P < 0.05$ ), vehicle treated ICV STZ group [ $F(3, 54) = 3.43$ ] ( $P < 0.05$ ) and the trans resveratrol treated ICV STZ group [ $F(3, 57) = 29.97$ ] ( $P < 0.05$ ). However, there was a significant difference between the escape latencies on day 2, 3 and 4 in the sham Vs vehicle treated ICV STZ ( $P < 0.05$ ) and the trans resveratrol treated ICV STZ group [ $F(2, 56) = 28.71$ ;  $F(2, 56) = 24.2$ ] ( $P < 0.05$ ) respectively. The mean escape latencies of the sham group and the trans resveratrol treated ICV group were significantly shorter than that of the vehicle treated ICV STZ group. This signifies a poorer/impaired acquisition of the task by the vehicle rats treated with ICV STZ and an amelioration of this impairment by trans resveratrol. The mean escape latencies on day 1, 2, 3 and 4 of the vehicle treated ICV STZ rat being  $57 \pm 1.3$ ,  $53 \pm 3$ ,  $43 \pm 1.1$  and  $40 \pm 1.8$  s respectively while the escape latencies on day 1–4 of the trans resveratrol treated ICV STZ group being  $51.1 \pm 4.3$ ,  $36.5 \pm 4.7$ ,  $13.2 \pm 2.4$  and  $17.3 \pm 2.7$  s respectively (Fig. 5). The distance traveled by the rats paralleled the time spent therefore was not considered separately in the study.

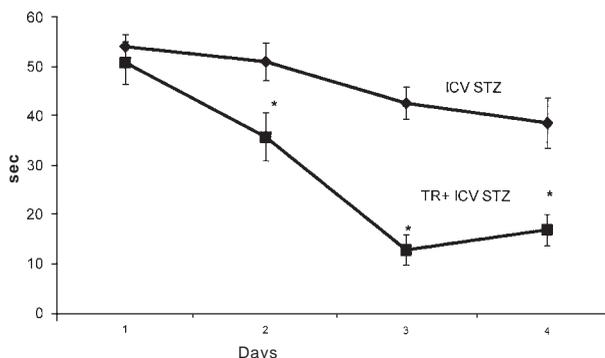


Fig. 5: Effect of trans resveratrol (20 mg/kg, ip) on ICV STZ cognitive Impairment on the escape latency in Morris water maze task (Acquisition phase). TR represents trans resveratrol in figure Values are expressed, as Mean  $\pm$  S.E.M. \*P<0.05 vs vehicle treated ICV STZ.

In the probe trial, the sham rats and the trans resveratrol treated ICV STZ group spent significant time and distance in the quadrant which previously contained the platform (quadrant 2) while the vehicle treated ICV STZ rats showed no such preference either in terms of time spent or in terms of the distance swum. The time spent in quadrant 1, 2, 3 and 4 by the sham, vehicle treated ICV STZ and trans resveratrol treated ICV rats was: quad 1 ( $10.8 \pm 1.2$ ;  $11 \pm 1.1$  and  $15.3 \pm 2.3$  s); quad 2: ( $21.3 \pm 1.8$ ,  $15 \pm 1.3$  and  $20.1 \pm 0.4$  s); quad 3 ( $13.5 \pm 1.2$ ;  $17 \pm 1.6$ ,  $11.5 \pm 0.73$  s); quad 4

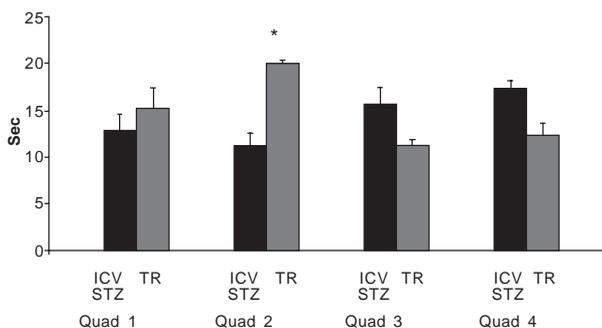


Fig. 6: Effect of trans resveratrol (20 mg/kg, ip) on ICV STZ rats on time 'Spent in different quadrants in the Morris water maze. TR represents trans resveratrol in figure. Values are expressed as Mean  $\pm$  S.E.M. \*P<0.05 ICV STZ+ trans resveratrol group.

( $14.1 \pm 1.5$ ,  $12 \pm 1.3$ ,  $12.7 \pm 1.30$  s) respectively (Fig. 6). The sham and the trans resveratrol rats spent significant time in quadrant 2 ( $P<0.05$ ) as compared to the other quadrants. The distance swum by the vehicle treated rats in quadrant 1, 2, 3 and 4 was ( $321 \pm 40$ ,  $313 \pm 70$ ,  $493 \pm 39$  and  $368 \pm 50$  cm respectively), while the distance swum by the trans resveratrol rats was ( $467 \pm 14.3$ ,  $609 \pm 53$ ,  $383 \pm 40$  and  $450.4 \pm 69$  cm respectively).

## DISCUSSION

During the past few years, there has been an increase in the understanding in the pathophysiology of Alzheimer's disease, however, effective therapeutics remain elusive. The results obtained in clinical trials with antioxidants are promising and there is a need to identify effective and safe drugs (21). In the present study, alpha lipoic acid, melatonin and trans resveratrol significantly protected against ICV STZ induced spatial memory deficit. The water maze task has been most extensively used to investigate specific aspects of spatial memory (12, 22, 23). The task is based on the premise that animals have evolved an optimal strategy to explore their environment and escape from the water with a minimum amount of effort i.e., swimming the shortest distance possible. The time it takes a rat to find a hidden platform in a water pool after previous exposure to the set up, using only available external cues, is determined as a measure of spatial memory. In the Morris water maze task, the vehicle treated ICV STZ rats showed a significant impairment in spatial memory as evidenced by significantly longer escape latencies as compared to the sham in the acquisition phase and no preference for the quadrant, which previously contained the platform in the probe trial.

Alpha lipoic acid (200 mg/kg, po), melatonin (20 mg/kg, ip) and trans resveratrol (20 mg/kg, ip) had significantly shorter escape latencies as compared to the ICV STZ group signifying an acquisition of memory and in the probe trial, the antioxidant treated groups showed significant preference for the quadrant

which previously contained the platform.

The present study indicates the protective effect of antioxidants against ICV STZ induced spatial memory deficit and further confirms the beneficial effect of antioxidants in neurodegenerative disorders such as Alzheimer's diseases.

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