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Isorhamnetin (IRN) Attenuates Cognitive Dysfunction Induced by the Intracerebroventricular Injection of Amyloid beta 25-35 (Aβ 25-35) in Sprague Dawley rats.

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Abstract:
Amyloid beta (Aβ 25-35) is reported to induce oxidative stress in the brain and there are growing evidences of its possible play in Alzheimer's disease. The exact cause and pathogenesis of neurodegenerative diseases is not certain but oxidative stress, excitotoxicity and neuroinflammation play important role in its pathogenesis. Dietary intake of antioxidant rich food and fruits has been shown to benefit, delay, prevent and treat many aging related disorders, inflammatory diseases. Bioflavonoid, Isorhamnetin has strong antioxidant and anti-inflammatory properties. Therefore, the present study was conducted to check the neuroprotective effect of Isorhamnetin (IRN) against Aβ induced neurobehavioral changes. Intracerebroventricular injection of Aβ 25-35 caused learning and memory deficits as assessed by Radial arm maze (RAM), Morris water maze (MWM). It has also showed impairment in visual recognition memory and exploratory activity in Novel object recognition test and open field test respectively. Treatment with IRN (25mg/kg b.wt.) reduced all the behavioral abnormalities significantly than the IRN 50 mg/kg b.wt. treated rats. Our finding concluded that Isorhamnetin has potent neuroprotective effect against Amyloid beta induced neurotoxicity in rat.

Key words:
Morris water maze, Aβ 25-35, Cognitive deficit, neurobehavioral, Isorhamnetin.

INTRODUCTION

An experimental model that mimics the progression of Alzheimer's disease (AD) was developed using an intracerebroventricular (i.c.v.) injection of Aβ in rats. Oxidative stress is involved in the mechanism of Aβ-induced neurotoxicity and AD pathogenesis [1-4]. The i.c.v. injection of Aβ into mice provides a model of AD progression [5]. It causes learning and memory impairments in addition to biochemical changes and neuronal degeneration. There are many evidences that small aggregates of amyloid beta can cause synaptic dysfunction such as blocking of long term potentiation [6]. Minor fragments were also identified including the highly toxic Aβ 25–35 peptide [7, 8]. Aβ fragment 25–35 ($A\beta_{25-35}$) has been reported to be responsible for toxic and oxidative events leading to brain damage, such as oxidative stress-mediated changes in hippocampal long-term potentiation [9], protein nitration, induction of inducible nitric oxide synthase [10, 11] and protein oxidation in fibroblasts derived from AD patients [12]. Behavioral disturbances associated with AD are agitation, aggression, depressive mood, sleep disorder and anxiety. Much attention has been focused on the correlation between neurotrophic factors and depression. The upregulation of NGF, BDNF, GDNF, and other neurotrophic factors is considered for treatment of depression and neurodegenerative diseases [13]

Isorhamnetin, a flavanol aglycone present in a variety of plants, has potent antioxidant and anti-inflammatory activities. IRN has been shown to increase the neurite growth promoting activity of NGF and to increase the BDNF content in brain [14]. Memory loss and cognitive dysfunction are the main clinical symptoms of AD patients so any treatment for AD requires identification of the factors that can confer protection against learning and memory impairment. Hence we formed hypothesize that Isorhamnetin may have protective effects against amyloid beta induced neurodenegeration. To address this hypothesis, we performed the behavioral analysis to investigate the potential preventive effect of IRN against Aβ25-35 induced memory impairment in animal model of AD.

MATERIALS AND METHODS:

Chemicals:

$A\beta_{25-35}$ and Isorhamnetin were purchased from Sigma-Aldrich. Other chemicals were analytical grade.

Animals and Drug treatments:

Male albino rats weighing between 250–300g bred in Central Animal House, Dr. ALMPGIBMS, University of Madras, Taramani campus, Chennai 113, Tamil Nadu, India were used. The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle, and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) (IAEC NO. 01/05/2014) Dr. ALMPGIBMS, University of Madras, Taramani campus, Chennai 113, Tamil Nadu, India.

The rats were divided into five groups and six animals of each group. Group I: sham-operated control received 5µl of vehicle (PBS/DMSO) through intracerebroventricular injection. Group II: Rats were given Aβ 25-35(10µg/rat) through intracerebroventricular injection on 1st day. Group III: Rats were given Aβ 25-35 (10µg/rat) through intracerebroventricular injection on 1st day (after one

hour) followed by intraperitoneal administration of isorhamnetin (25mg /kg in PBS/DMSO) for 21days. Group IV: Rats were given Aβ 25-35 (10µg/rat) through intracerebroventricular injection on 1st day (after one hour) followed by intraperitoneal administration of isorhamnetin (50 mg /kg in PBS/DMSO) for 21 days. Group V: Rats were given isorhamnetin (25mg/kg in PBS/DMSO) intraperitoneally for 21 days. . Group VI: Rats were given isorhamnetin (50mg/kg in PBS/DMSO) intraperitoneally for 21 days.

Preparation of aggregated amyloid beta 25-35 and Intracerebroventricular injection:.

Aβ25–35 was "aged" by incubation at 37°C for 4 days as described previously [15]. Rats were anesthetized by intraperitoneal (i.p.) injections of ketamine and xylazine and placed in a stereotaxic holder (Instruments and Chemicals, Ambala, New Delhi). A midline sagittal incision was made in the scalp and hole was drilled in the skull over the intracerebro ventricle using the following coordinates: 0.8 mm posterior to Bregma, 1.5 mm lateral to the midline and 3.8 mm beneath the dura. All injections were made using a 10-µl Hamilton syringe equipped with a 26-gauge needle. The dura was perforated with the needle of the microsyringe. Animals were infused with 5µl of sterile distilled water (vehicle-treated), aggregated $A\beta_{25-35}$ (2µg/µl) into cerebral lateral ventricle at a rate of 1µl/min; the needle was left in place for an additional 5 min to permit sufficient diffusion and to avoid pressure induced damage. The scalp was then closed with a suture.

Postoperative Care

Recovery of anesthesia took approximately 4–5 h. The rats were kept in a well-ventilated room at 25 ± 3 ºC in individual cages until they gained full consciousness. Food and water were kept inside the cages for the first week, allowing animals' easy access, without physical trauma due to overhead injury. Animals were then treated normally; food, water, and the bedding of the cages were changed often.

Behavioral Assessment

Morris water maze

Spatial learning and memory abilities of rats were assessed in the Morris water maze, including place navigation trial and spatial probe trial on 14th and 21st day. Morris water maze was consisted of a black circular pool (diameter 160 cm, height 50 cm) filled with water (23 ± 1 °C) and the pool was divided into four quadrants (I, II, III, IV). The general testing process had been described in other reports [16]. Animals were placed into the pool, facing the wall of the pool, and were allowed to circumnavigate the pool in search of the escape platform (in the center of one of the four quadrants submerged 2 cm below the water surface) 4 trials (60 s per trial) per day after the treatment of Aβ25–35. Escape latency (s) was used to indicate the learning results. After the last trial, the platform was removed from the tank and each rat received a 60 s swim probe test. On day 21, a spatial probe trial was conducted by removing the platform and placing the rat next to and facing the pool wall and each rat was allowed to swim freely for 60 s. During the probe trial, the swimming time in the quadrant of platform were recorded to indicate the memory results.

Open field test

All rats were subjected to an open field test. Each rat was placed individually into the center of the open field apparatus. The open field apparatus was a square shaped box made of wood, 80 cm in length and 40cm in height. The floor is divided into 16 squares and each of them is 20x20 cm. Each animal was placed in the center of the open field and the activity of rat for 3 min was recorded by a video camera. Ambulation frequency, rearing frequency and grooming frequency were measured for 3min. The floor was cleaned with a wet sponge and a dry paper towel between rats.

Novel Object Recognition Test

The NOR test consisted of two sessions: a training session followed by a retention trial 24 h later. Rats were habituated to the testing arena for 2 consecutive days before the test. During the training session, two different objects (A and B) were placed in the testing arena. Each animal was allowed to explore the objects for 5min. The rat was considered to be exploring the object when the head of the animal was facing the object, or the animal was touching or sniffing the object. The total time spent exploring each object was recorded and expressed as percentage of total exploration time. In the retention session, one identical and one novel object (A and C) were used. The rat was allowed to explore the objects for 5min, and the time spent exploring each object was recorded. Exploration time was normalized as percentage of total exploration time. Preference for the novel object was considered as successful retention of memory for the familiar object.

Radial Arm Maze

Spatial learning and memory were assessed using a radial maze according to the paradigm described previously [17]. The apparatus consisted of a 50-cm-elevated (above the floor) eightarmed radial maze (RAM) made of black Plexiglas. The maze was placed in a sound-attenuated room. The 60-cm-long, 10-cm-wide, and 15-cm high arms extended radially from a central octagonal starting platform (35 cm in diameter), and there was a recessed food cup at the end of each arm. In some of the arms, the cup contained a single small food pellet as a reinforce. The rats were allowed to move freely. The RAM was surrounded by various extra-maze cues; their orientation relative to the maze was kept constant throughout the experiment. The maze was cleaned with 70% ethanol between trials. The rats were trained to visit each arm, eat the pellet, and not re-enter the arm that had been visited during the same test. Each entry into each arm with all four paws was scored during a period of 10 min. The number of correct choices or errors was used to assess the performance of the animal in each session. An error was defined as a re-entry into an already visited arm. Rats that made at least seven correct choices in each of three consecutive sessions were used in the subsequent behavioral experiments. Training was performed at 24-h intervals.

Statistical analysis

The data was analyzed by using analysis of variance (ANOVA) followed by Tukey's test using SPSS version 20. All the values are expressed as mean±S.D. In all tests, the criterion for statistical significance was $P < 0.05$.

RESULTS

Effect of IRN on $A\beta_{25-35}$ induced memory impairment in Morris water maze:

The effect of isorhamnetin on transfer latency in morris water maze in $A\beta_{25-35}$ induced rats was shown in figure 1. $A\beta_{25-35}$ induced group showed significant (P <0.01) decrease in the mean transfer latency in on 14th and 21st days in Morris water maze as compared to the control rats (Figure.1a). Isorhamnetin 25mg/kg b.wt. treated rats showed a significant (P <0.01) decrease in transfer latency on the 21st day as compared to the $A\beta_{25-35}$ injected group whereas the rats treated with and IRN 50mg/kg, b.wt. (P <0.05) showed less significant effect than the Isorhamnetin 25mg/ kg b.wt. treated rats. There was no significant changes observed in the isorhamnetin (25 mg/kg, b.wt and 50 mg/kg, b.wt) alone treated group as compared to control group. The time spent in target quadrant in probe test was shown in Figure1b. The $A\beta_{25-35}$ induced group (P <0.01) spent less time in target quadrant compared to control rats. Isorhamnetin 25mg/ kg b.wt. treated rats showed a significant (P <0.01) increase in time spent in target quadrant on the 21st day than the IRN 50mg/ kg b.wt (P <0.05). No significant difference between the IRN (25 and 50 mg/kg, b.wt) alone treated groups and control group.

Effect of IRN on $A\beta_{25-35}$ induced changes in Open field test:

The effect of isorhamnetin on open field test in $A\beta_{25-35}$ induced rats was shown in figure 2. $A\beta_{25-35}$ induced rats (P <0.01) showed reduction in the number of squares crossed (figure 2a), rearing (figure 2b), and head dippings (figure 2c), as compared to the control. Isorhamnetin treated group 25mg/ kg b.wt. had increased the rearing activity, head dipping and increase in the number of squares crossed significantly (P <0.01) as observed on 14th and 21st day as compared to the $A\beta_{25-35}$ injected group whereas treatment with isorhamnetin treated groups 50mg/ kg b.wt. showed less significant effect (P <0.05) as compared to IRN 25 mg/kg b.wt.. There was no significant change in the isorhamnetin (25mg/kg and 50 mg/kg, b.wt) alone treated groups as compared to control group.

Effect of IRN on $A\beta_{25-35}$ induced changes in exploratory behavior in novel object recognition test:

The effect of isorhamnetin on novel object recognition in $A\beta_{25-35}$ induced rats was shown in figure 3. There was a significant (P <0.01) decrease in exploratory behavior in $A\beta_{25-35}$ injected group as compared to the control whereas treatment with isorhamnetin 25mg/ kg b.wt. (P <0.01) showed increase in exploratory behaviors as compared to the $A\beta_{25-35}$ injected group on 21st day. The rats treated with IRN 50mg/kg b.wt. showed less significant effect (P <0.05) than the IRN 25mg/kg treated rats, There was no significant change in the isorhamnetin (25mg/kg and 50 mg/kg, b.wt) per se group as compared to the control group.

Effect of IRN on $A\beta_{25-35}$ induced impairment in spatial memory in Radial arm maze task:

The effect of isorhamnetin on spatial learning and memory in $A\beta_{25-35}$ induced rats was presented in figure 4. $A\beta_{25-35}$ injected group showed significant decrease (P <0.01) in the number of correct choices made in RAM task as compared to control group on 14th and 21st day, thereby showing significant impairment in spatial cognition of $A\beta_{25-35}$ induced rats. Significant improvement in spatial cognition (P <0.01) was noted in isorhamnetin (25mg/ kg b.wt.) treated groups than the IRN 50mg/kg b.wt. treated rats (P <0.05). No significant change was found in the isorhamnetin (25mg/kg and 50 mg/kg, b.wt) per se group when compared to the control group.

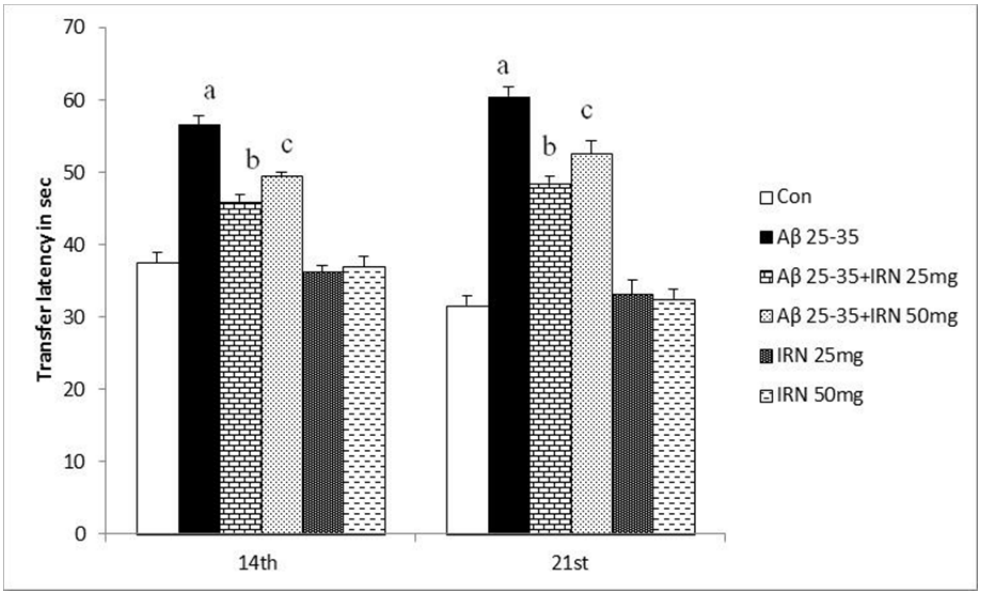

Figure.1a

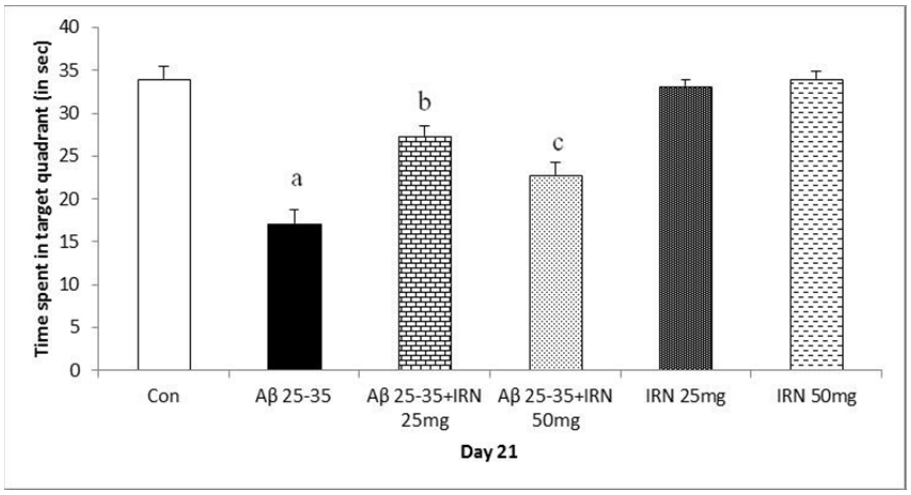

Figure.1b

Fig.1 Effect of isorhamnetin in morris water maze in $A\beta_{25-35}$ induced rats.

a$P < 0.01$ versus vehicle-treated group, b$P < 0.01$ and c b$P < 0.05$ versus $A\beta_{25-35}$ induced group, (one-way ANOVA followed by Tukey's test). Data presented are mean ± SD (n=6). The values are expressed as transfer latency in seconds (Figure 1a) and time spent in target quadrant in sec (Figure 1b)

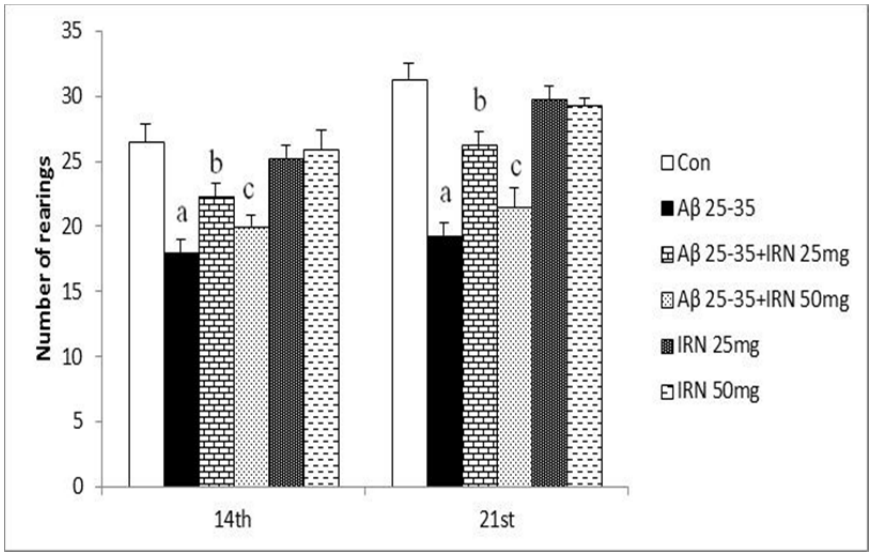

Figure.2a

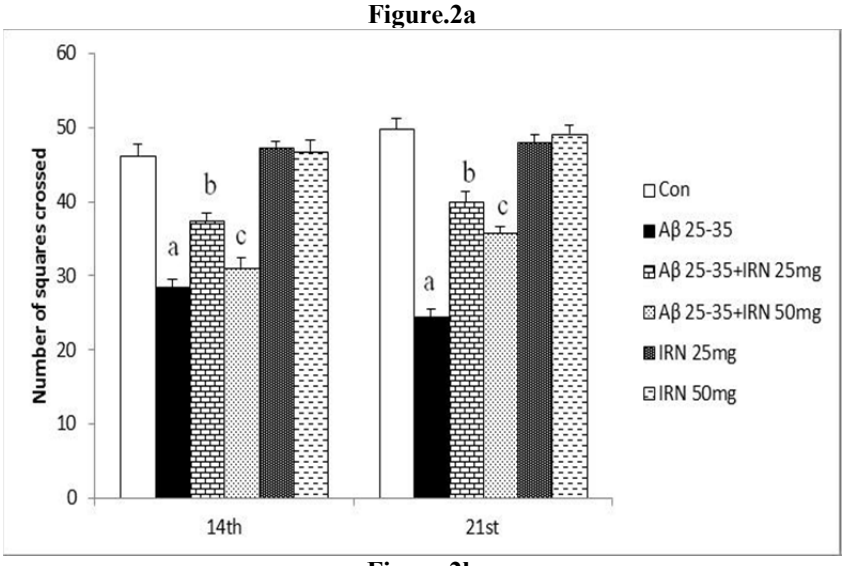

Figure.2b

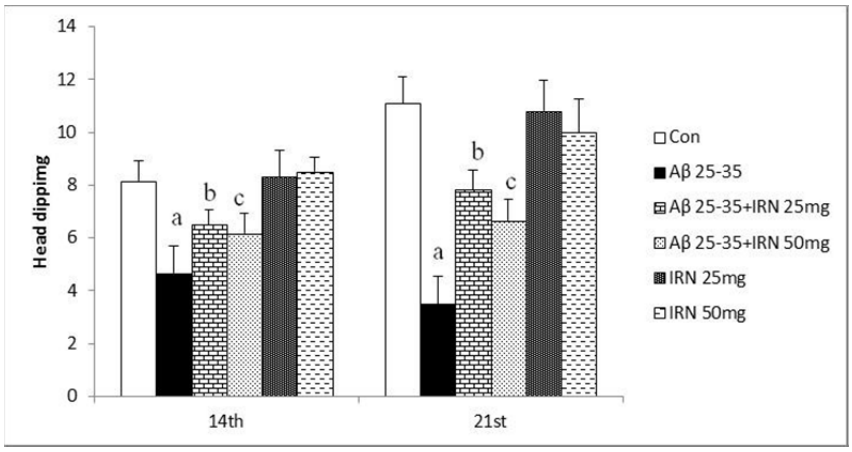

Figure.2c

Fig.2 Effect of isorhamnetin in open field task in $A\beta_{25-35}$ induced rats.

aP < 0.01 versus vehicle-treated group, bP < 0.01 and c bP < 0.05 versus $A\beta_{25-35}$ induced group, (one-way ANOVA followed by Tukey's test). Data presented are mean ± SD (n=6). The values are expressed as number of squares crossed (Figure 2a), rearings (Figure 2b) and head dipping (Figure 2c).

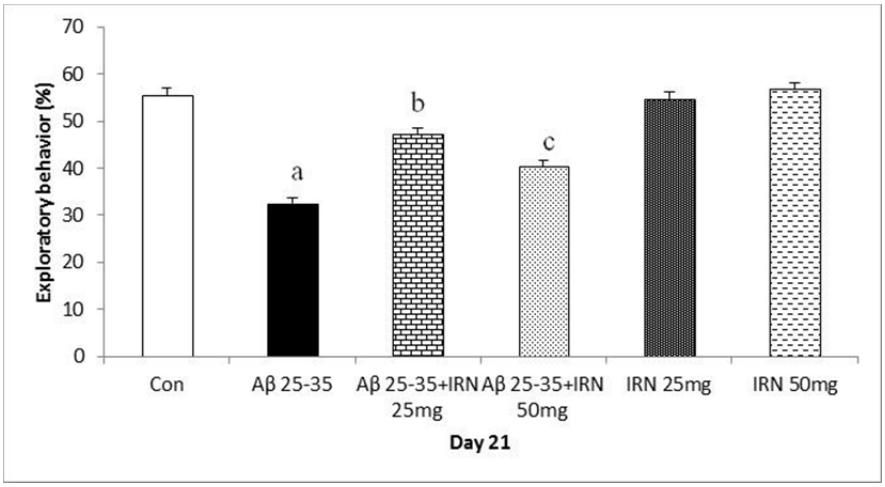

Fig.3 Effect of isorhamnetin on exploratory behavior in Novel object recognition test in $A\beta_{25-35}$ induced rats.

aP < 0.01 versus vehicle-treated group, bP < 0.01 and c bP < 0.05 versus $A\beta_{25-35}$ induced group, (one-way ANOVA followed by Tukey's test). Data presented are mean ± SD (n=6). The values are expressed as exploratory behavior in percentage (Figure 3).

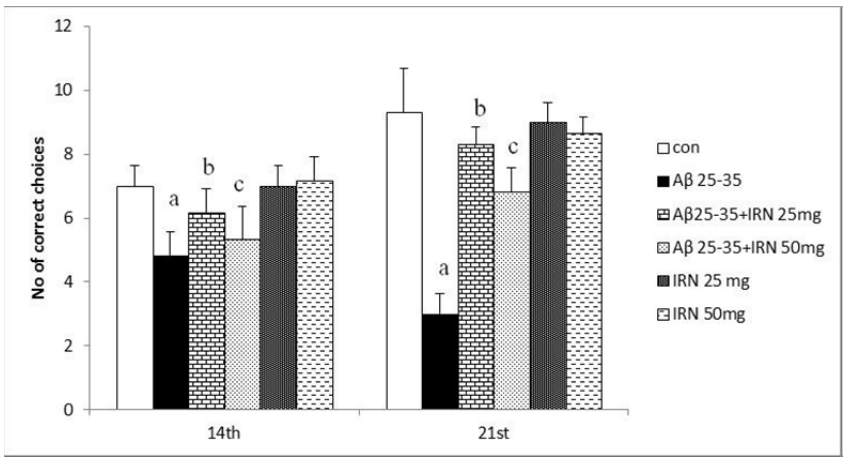

Fig.4 Effect of isorhamnetin on spatial cognition in radial arm maze task in $A\beta_{25-35}$ induced rats.

aP < 0.01 versus vehicle-treated group, bP < 0.01 and c bP < 0.05 versus $A\beta_{25-35}$ induced group, (one-way ANOVA followed by Tukey's test). Data presented are mean ± SD (n=6). The values are expressed as number of correct choices (Figure 4).

DISCUSSION:

The major findings of the present study were that the IRN treatment significantly reverted the abnormal neurobehavioral changes induced by the $A\beta_{25-35}$.Cholinergic system in the brain is responsible for memory and learning [18]. Morris water maze and radial arm maze are traditionally used to evaluate spatial learning and memory. Spatial memory is a form of short term memory utilising neuro-circuitry that provides temporary storage and manipulation of information necessary for complex cognitive tasks such as language comprehension, learning and reasoning [19]. Its impairment is analogous to memory disorder in Alzheimer's dementia [20]. In the cholinergic system, especially the basal forebrain projections to hippocampus, is known to be particularly affected in Alzheimer's disease [21]. The water maze test results showed impairment of spatial learning in rats induced with Aβ 25-35 and decrease in time spent in target quadrant in probe test. This effect was significantly ameliorated by IRN (25mg/kg b.wt). It has been reported that IRN (3′-methylquercetin) exhibited an anti-inflammatory activity similar to quercetin in RAW264.7 cells [22].Isorhmanetin, a flavanol aglycone is present in most of the neuroprotective plants.This is one of the constituents of *Ginkgo biloba* extract, EGD 761 which has been used for treatment of brain disorders (including dementia), neurosensory syndrome, peripheral blood flow disorders, and cerebral insufficiency [23]. *Ginkgobiloba* extract contains many polyphenols including the flavonols quercetin, kaempferol, and isorhamnetin and has been shown to have antidepressant-like effects that could be due to its properties of increasing BDNF which would increase neuronal survival and plasticity or due to its increase of pCREB through glutamate-invoked activation which would enhance synaptic strength and neuronal plasticity [24].

The amount and effectiveness of the neurotrophic factors in the brain were found to be decreased during the process of aging, and the decrease was prominant in the pathological condition of Parkinson's and Alzheimer's diseases [25, 26]. Much attention has been focused on the correlation between neurotrophic factors and depression. The upregulation of NGF, BDNF, GDNF, and other neurotrophic factors is considered for treatment of depression and neurodegenerative diseases [13].In the current study, Isorhamnetin has been shown to exert memory enhancing effects against $A\beta_{25-35}$ induced neurotoxicity. The open field test [27] provides simultaneous measures of locomotion, exploration and anxiety. The number of line crosses, head dipping and the frequency of rearing are usually used as measures of locomotor activity, but are also measures of exploration and anxiety in OFT. A high frequency of these behaviors indicates increased locomotion and exploration and/or a lower level of anxiety.

$A\beta_{25-35}$ induced rats showed significant decrease in exploratory behavior in OFT whereas the IRN treatment had significantly increased the activity in open field test. In the present study we also employed the Novel object recognition test to examine whether IRN normalized the visual recognition memory impairment induced by the i.c.v. administration of aggregated Aβ25-35 in rat for evaluating its potential value for the treatment of Alzheimer's disease. The exploration time for the novel object was longer than the familiar object in IRN treated animals whereas the $A\beta_{25-35}$ induced rats showed reduction in exploration time for the novel object. The results denote that $A\beta_{25-35}$ induced impairment in visual recognition memory was reverted significantly with the IRN treatment. The spatial cognition was reduced in $A\beta_{25-35}$ induced rats in RAM task whereas the IRN treatment was able to increase the spatial cognition and memory, thereby exhibiting their protective activity against $A\beta_{25-35}$ induced neurotoxicity.

CONCLUSION

The present study shows the protective effects of Isorhamnetin against $A\beta_{25-35}$ induced neurotoxicity in rat. Intracerebroventricular injection of $A\beta_{25-35}$ caused behavioral impairment. Isorhamnetin exerted significant neuroprotection by reducing the memory deficits.

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