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NOOTROPIC ACTIVITY OF ISORHAMNETIN IN AMYLOID BETA 25-35 INDUCED COGNITIVE DYSFUNCTION AND ITS RELATED mRNA EXPRESSIONS IN ALZHEIMER'S DISEASE

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ABSTRACT: Oxidative stress appears to be an early event involved in the pathogenesis of Alzheimer's disease. The present study was designed to investigate the neuroprotective effects of isorhamnetin (IRN) against amyloid beta 25-35 (A β 25-35)-induced memory impairment and oxidative damage in rats. Memory task was assessed using Y-arm maze and it revealed the impairment in spatial memory. The IRN treated rats showed improvement in memory task. A β 25-35 induced animals also exhibited increase in hydrogen peroxide (H₂O₂), Monoamine oxidase activity (MAO) and decrease in choline acetyltransferase (ChAT) activity. It also enhanced the expression of inducible nitric oxide synthase (iNOS) and proinflammatory cytokine, IL- β whereas all these abnormalities were reduced significantly in IRN treated rats showing the neuroprotective effect of IRN against A β 25-35 induced Alzheimer's disease (AD). Thus, IRN may be a potential therapeutic agent for Alzheimer's disease.

INTRODUCTION: The deposition of A β in the brain is assumed to initiate a pathological cascade that results in synaptic dysfunction, synaptic loss, neuronal death, and cognitive dysfunction¹. Amyloid beta (A β) is thought to be a major pathological cause of Alzheimer's disease. The A β (25–35) is a partial fragment of A β that similarly forms a β -sheet structure² and induces neuronal cell death²⁻³, neurite atrophy⁴⁻⁵, synaptic loss⁴⁻⁶ and memory impairment^{5, 6, 8}. There are many reports showing that mice injected with A β (25–35) suffer from memory impairment and neurite and synaptic atrophy^{5, 6, 8, 9}.

It has been confirmed that peroxynitrite-mediated damage contributes to A β -induced neuronal toxicity and cognitive deficits^{10, 11} and is widespread in the brain of AD patients¹². In AD patients, learning and memory are impaired by the concomitant loss of the cholinergic marker enzyme, choline acetyltransferase (ChAT), in the cerebral cortex¹³.

Flavonoids exert a multiplicity of neuroprotective actions within the brain, including a potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning and cognitive function. Isorhamnetin is an abundant Flavonolaglycone in herbal medicinal plants, such as sea buckthorn (*Hippophaerhamnoides* L.) and *Ginkgo biloba* L., which are frequently used in the prevention and treatment of cardiovascular diseases¹⁴⁻¹⁵. A number of studies suggested that isorhamnetin can

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protect endothelial cells from injury caused by oxidized low-density lipoprotein¹⁶, decrease blood pressure¹⁷, and alleviate the damages of ischemia-reperfusion (I/R) to ventricular myocytes¹⁸.

In the present study, to confirm the protective activity of isorhamnetin against A β -induced neurotoxicity, we investigated whether IRN prevents memory impairment in an A β 25–35-injected animal model of AD. Biochemical analysis of neurotransmitter enzyme was carried out to check the improvement in cognitive function. In addition, expressions of iNOS and IL- β mRNA in the hippocampus of A β 25–35-injected rats were analyzed using reverse transcriptase PCR.

MATERIALS AND METHODS:

Chemicals:

A β 25–35 and Isorhamnetin were purchased from Sigma-Aldrich. Acetyl coenzyme A, ethylene diamine tetra acetic acid (EDTA) and dithiothreitol (DTT) were purchased from Sisco Research Laboratories (SRL). Other chemicals were analytical grade.

Animals and Grouping:

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 six groups and six animals of each group. Group I: sham-operated control received 5 μ l of vehicle (PBS/DMSO) through intracerebroventricular injection. Group II: Rats were given A β 25-35 (10 μ g/rat) through intracerebroventricular injection on 1st day. Group III: Rats were given A β 25-35 (10 μ g/rat) through intracerebroventricular injection on 1st day (after one hour) followed by intraperitoneal administration of isorhamnetin (25 mg/kg in

PBS/DMSO) for 21 days. Group IV: Rats were given A β 25-35 (10 μ g/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:

A β 25–35 was “aged” by incubation at 37°C for 4 days as described previously⁶. 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 intracerebroventricle 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- μ l Hamilton syringe equipped with a 26-gauge needle. The dura was perforated with the needle of the microsyringe. Animals were infused with 5 μ l of sterile distilled water (vehicle-treated), aggregated A β 25–35 (2 μ g/ μ l) into cerebral lateral ventricle at a rate of 1 μ l/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 \pm 3°C 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.

Y-maze task:

Y-maze task is used to measure the spatial working through the spontaneous alternation of behaviour¹⁹. The maze is made of black painted wood. Each arm is 40 cm long, 13 cm high, 3 cm wide at the

bottom, 10 cm wide at the top, and converges at an equal angle. Each mouse is placed at the end of one arm and allowed to move freely through the maze during an 8-min session. Rats tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the rat know which arm they have already visited. The series of arm entries, including possible returns into the same arm, are recorded visually. Alternation is defined as the number of successive entries into the three arms, on overlapping triplet sets. The percentage of alternation is calculated as the ratio of actual alternations, defined as the total number of arm entries minus two, and multiplied by 100.

Dissection and Homogenization:

On day 22, animals were sacrificed for biochemical estimations. The animals were sacrificed and the brain was removed by decapitation. Hippocampus was separated from each isolated brain. A 10% (W/V) tissue homogenate were prepared in 0.1 M phosphate buffer (pH 7.4). The aliquots of supernatant were separated and used for biochemical estimations.

Biochemical Analysis:

Estimation of protein: The total protein content was measured according to the method of Lowry et al. (1951) ²⁰ using bovine serum albumin as standard.

Hydrogen Peroxide Assay:

The hydrogen peroxide (H₂O₂) generation was assayed by the method of Pick and Keisari ²¹. Horseradish peroxidase converts hydrogen peroxide in to water and oxygen. This causes oxidation of phenol redforms adduct with dextrose which has maximum absorbance at 610nm and can be recorded spectrophotometrically. Levels of H₂O₂ generation were expressed as nM of H₂O₂-generated/mg protein.

ChAT activity:

The choline acetyltransferase (ChAT) activity was determined spectrophotometrically according to Wolfgram(1972) ²². The reaction mixture contained sodium phosphate buffer (pH 7.0), acetyl coenzyme A, chloride choline, physostigmine, NaCl, EDTA, hydrochloric creatinine and DTT. The mixture was preincubated at 37 °C for 5 min then mixed with the hippocampus homogenates, incubated at 37 °C for 20 min and finally stopped the reaction in boiling water. Sodium arsenate was added to each tube for precipitation. The supernatant was mixed with 3 nM 4-PDS and incubated at 25 °C for 15 min. Absorbances were read at 324 nm.

Monoamine oxidase:

Monoamine oxidase (MAO) levels were measured by the method of Tabor *et al.* ²³ this method is based on measurement of benzaldehyde formed with benzyl-amine hydrochloride (0.1 M) used as substrate. The absorbance was read at 340 nm and expressed as nmolbenzaldehyde/min/mg protein.

Total RNA isolation and mRNA expression of iNos and IL-β by RT-PCR:

Total RNA was purified from freshly isolated brain tissue using 1 mL of the TRI reagent by the method of Chomczynski and Sacchi (1987) ²⁴. The RNA purity and concentration were determined using Nano drop (Thermo Scientific) at A260/A280 nm. The purity of RNA obtained was 1.8–2.0. One microliter of total RNA was reverse transcribed by RT-PCR kit (Thermo scientific) according to the manufacturer's instructions and further amplified by PCR. The details of the primer used, and size of the PCR amplified products are listed in **Table 1**. The PCR products were resolved by electrophoresis through 1.5% agarose gel and stained with ethidium bromide. The densities of PCR products in the agarose gel were scanned with a Gel Doc image scanner (Bio-Rad, USA) and quantified by Quantity One Software (Bio-Rad, USA).

TABLE 1: THE DETAILS OF PRIMERS USED

Target gene	Primer sequences	Annealing temperature	Product Size (bp)
IL-6	F: 5' TCAAGGCATAACAGGCTCATC 3' R:5' TCTGTCAGCCTCAAAGAACAGG 3'	56	150
iNOS	F: 5'TCCTCTTTGCTACTGAGACAAGG3' R:5' TACTGGTCCATGCAGACAACC3'	56	103

Statistical analysis:

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

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

$A\beta_{25-35}$ injected group showed significant decrease ($P < 0.01$) in the percentage alternation in Y-arm

task as compared to control group on 14th and 21st day (**Fig.1**), 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.

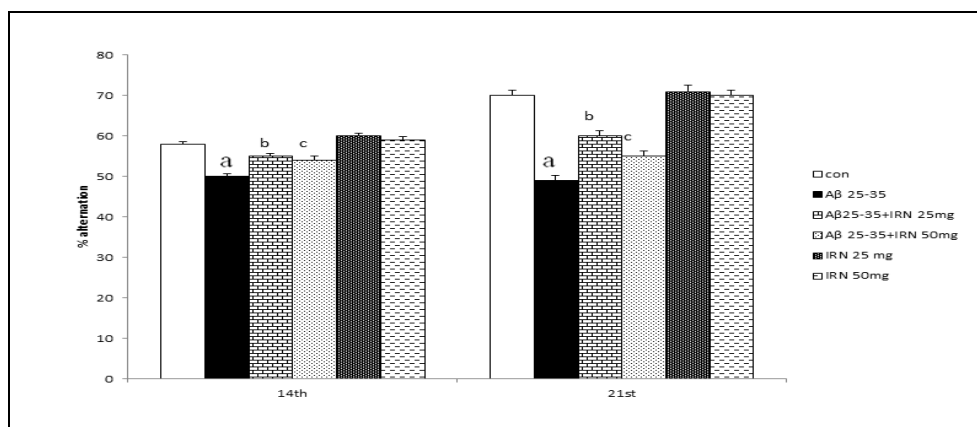


FIG. 1: EFFECT OF ISORHAMNETIN ON SPATIAL COGNITION IN Y- ARM MAZE TASK IN $A\beta_{25-35}$ INDUCED RATS a $P < 0.01$ versus vehicle-treated group, b $P < 0.01$ and c $P < 0.05$ versus $A\beta_{25-35}$ induced group, (one-way ANOVA followed by Tukey's test). Data presented are mean ± SD (n=6). The values are expressed as number of correct choices.

Effect of isorhamnetin on $A\beta_{25-35}$ induced changes in level of H_2O_2 in hippocampus of control and experimental rats:

There was significant ($P < 0.01$) increase in the H_2O_2 level in hippocampus of $A\beta_{25-35}$ injected group as compared to control group (**Fig.2**). Isorhamnetin (25mg/ kg b.wt.) was able to counteract and reduced H_2O_2 level significantly

($P < 0.01$) as compared to the $A\beta_{25-35}$ injected group whereas the rats treated with IRN 50mg/kg, b.wt. ($P < 0.05$) showed less effect than the Isorhamnetin 25mg/ kg b.wt. treated rats. The group treated with isorhamnetin (25 and 50 mg/kg, b.wt) alone did not show significant effect on level of H_2O_2 as compared to the control group.

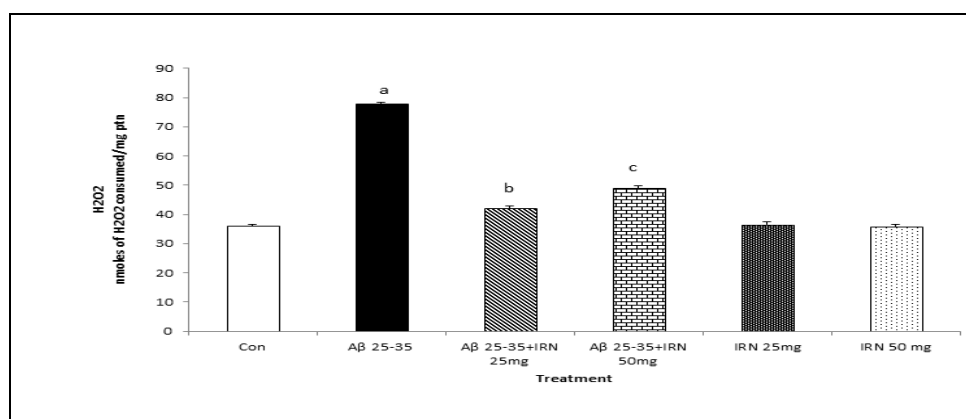


FIG. 2: EFFECT OF ISORHAMNETIN ON $A\beta_{25-35}$ INDUCED CHANGES IN LEVEL OF H_2O_2 IN HIPPOCAMPUS OF CONTROL AND EXPERIMENTAL RATS.

Data presented are mean± SD (n = 6).The units expressed as nmoles of H_2O_2 consumed/mim/mg protein. a $P < 0.01$ as compared to the vehicle-treated group; b $P < 0.01$ and c $P < 0.05$ as compared to $A\beta_{25-35}$ induced group (one-way ANOVA followed by Tukey's test).

Ameliorative effect of isorhamnetin on A β 25–35 induced changes in activity of ChAT in hippocampus of control and experimental rats:

ChAT activity was found lowered significantly in A β 25–35 induced group ($P<0.01$) as compared to control animals (Fig.3). Treatment with isorhamnetin (25mg/ kg b.wt.) significantly

($P<0.01$) increased the ChAT activity than the group treated with isorhamnetin (50mg/kg b.wt.) ($P<0.05$) as compared to the A β 25–35 induced group. There was no significant change found on the activity of ChAT in isorhamnetin (25 and 50 mg/kg, b.wt) alone treated group while compared to the control group.

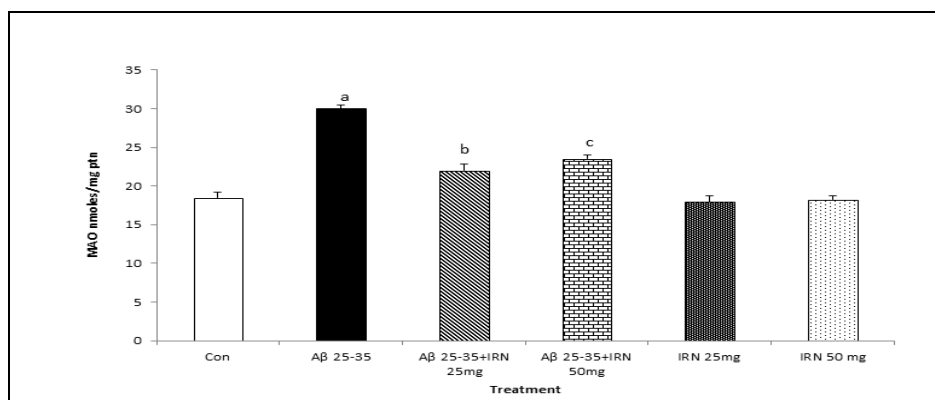


FIG. 3: EFFECT OF ISORHAMNETIN ON THE ACTIVITY OF CHAT IN HIPPOCAMPUS OF A β 25–35 INDUCED RATS

Data presented are mean \pm SD (n = 6). The units expressed as nmoles/g protein. a $P<0.01$ as compared to the vehicle-treated group; b $P<0.01$ and c $P<0.05$ as compared to A β 25–35 induced group (one-way ANOVA followed by Tukey's test).

Attenuating effect of isorhamnetin on A β 25–35 induced changes in the activity of MAO in hippocampus of control and experimental rats:

A β 25–35 injected group showed significant ($P<0.01$) increase in the activity of MAO in hippocampus compared to control group (Fig.4). Treatment with isorhamnetin (25mg/ kg b.wt.) significantly ($P<0.01$) attenuated the abnormal increase in MAO activity and reverted it to normal

when compared with A β 25–35 induced group whereas the group treated with isorhamnetin (50mg/kg b.wt.) showed less significant ($P<0.05$) effect compared to the isorhamnetin 25mg/ kg b.wt. treated rats. Treatment with isorhamnetin (25mg/kg b.wt. and 50mg/kg b.wt.) alone did not show significant change on activity of MAO when compared with the control group.

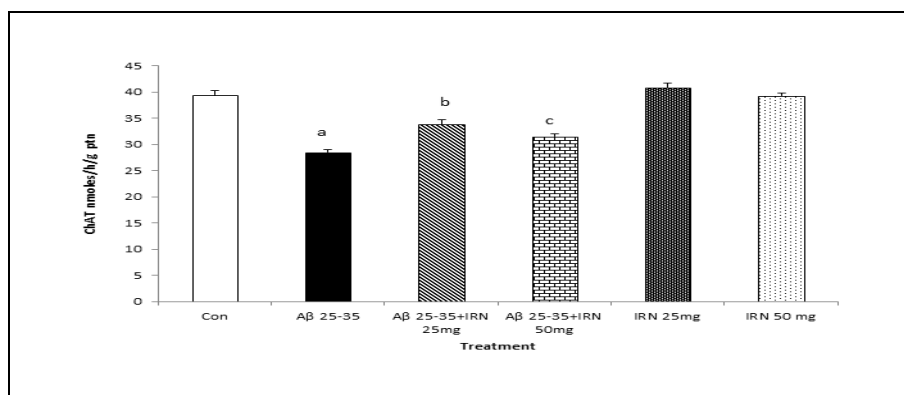


FIG. 4: EFFECT OF ISORHAMNETIN ON THE ALTERATIONS IN THE MAO LEVELS IN HIPPOCAMPUS OF CONTROL AND EXPERIMENTAL RATS.

a $P<0.01$ as compared to the vehicle-treated group; b $P<0.01$ and c $P<0.05$ as compared to A β 25–35 induced group, (one-way ANOVA followed by Tukey's test). Data presented are mean \pm SD (n=6). MAO-Monoamine Oxidase. MAO units expressed as nmoles/mg protein.

Ameliorative effect of isorhamnetin on A β 25–35 induced changes in mRNA expression of iNOS in hippocampus of control and experimental rats:

There was an abnormal ($p<0.01$) increase in

iNOS mRNA expression in hippocampus of A β 25–35 induced group as compared to control animals whereas isorhamnetin(25mg/kg b.wt.) treated group showed the significant decrease ($p<0.05$) in

abnormal expression of iNOS (**Fig.5**). The group treated with isorhamnetin (25mg/kg b.wt.) alone

showed normal expressions of iNOS when compared with the control group.

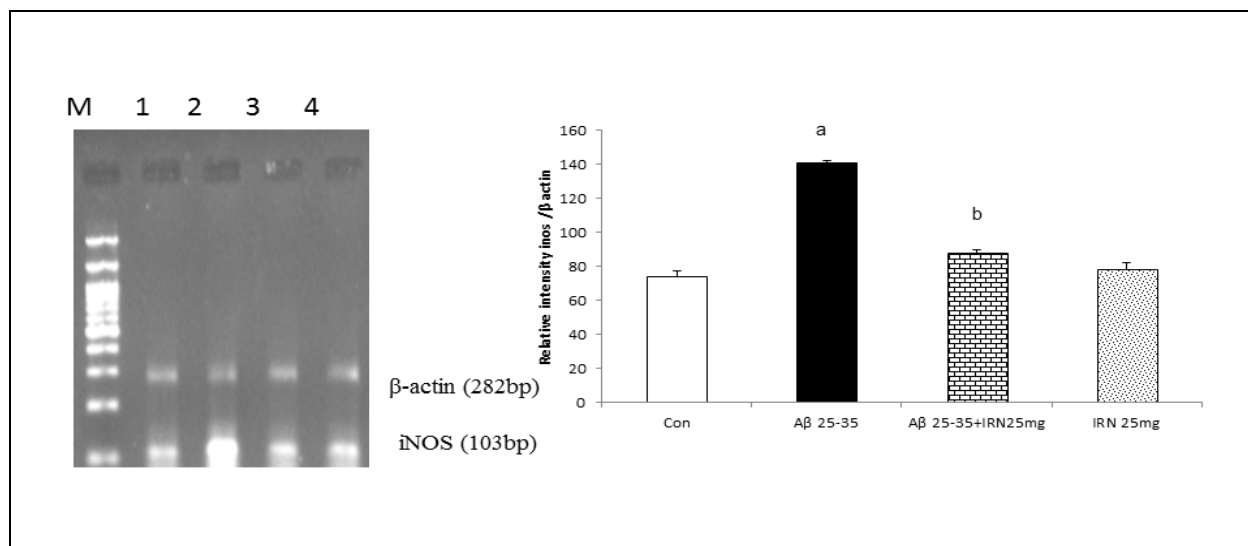


FIG.5: THE EFFECT OF ISORHAMNETIN ON A β ₂₅₋₃₅ INDUCED mRNA EXPRESSION OF iNOS IN CONTROL AND EXPERIMENTAL RATS

Lane M: DNA marker Lane.1. control group showing normal expression of iNOS. Lane.2. Increased expression of iNOS in A β ₂₅₋₃₅ induced group. Lane.3 A β ₂₅₋₃₅ + IRN 25mg/kg b.wt. treated group showing normal expression of iNOS (Lane .3). Lane.4. IRN alone treated group showing normal expression of iNOS. Data represents mean \pm SD of six rats in each group. Levels of mRNA were normalized to that of β actin. Statistical significance (P value): *ap*<0.01 significantly different from control group. *bp*<0.05 different from A β ₂₅₋₃₅ induced group.

Effect of isorhamnetin on A β ₂₅₋₃₅ induced changes in mRNA expression of IL- β in hippocampus of control and experimental rats:

A β ₂₅₋₃₅ induced group showed an abnormal increase (*p*<0.01) in IL- β expression in hippocampus as compared to control animals (**Fig.6**). There was the significant decrease (*p*<0.05) in abnormal expression of IL- β in isorhamnetin (25mg/kg b.wt.) treated group. The group treated with isorhamnetin (25mg/kg b.wt.) alone showed normal expressions of IL- β when compared with the control group.

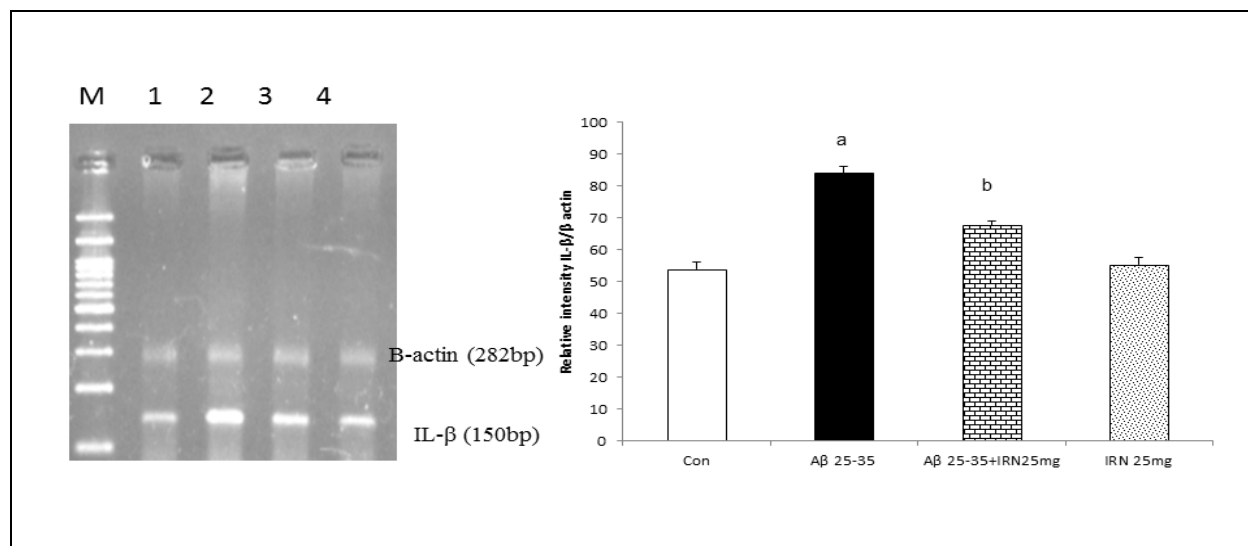


FIG.6: THE EFFECT OF ISORHAMNETIN ON A β ₂₅₋₃₅ INDUCED mRNA EXPRESSION OF IL- β IN CONTROL AND EXPERIMENTAL RATS

Lane M: DNA marker Lane.2. control group showing normal expression of IL- β . Lane.3. Increased expression of IL- β in A β 25–35 induced group. Lane.3 A β 25–35 + IRN 25mg/kg b.wt. treated group showing normal expression of IL- β (Lane.3). Lane.4. IRN alone treated group showing normal expression of IL- β . Data represents mean \pm SD of six rats in each group. Levels of mRNA were normalized to that of β *actin*. Statistical significance (P value): *ap*<0.01 significantly different from control group. *bp*<0.05 different from A β 25–35 induced group.

DISCUSSION: A β (25–35) is potential neurotoxic peptide for primary neuronal cortical cells which produces neurofibrillary tangles²⁵⁻²⁶ and the toxicity induced by A β (25–35) in rodents resembles to AD which is suitable for the evaluation of Alzheimer's type of dementia. Isorhamnetin, which is an O-methylated metabolite formed in the small intestine and liver, and increased in the human brain, has a higher BBB permeability than aglycone by its apparent lipophilicity²⁷. The O-methylated metabolites of derived flavonoids have the potential to improve human memory and neuro-cognitive performance via their ability to protect vulnerable neurons and long-term potentiation, which is considered as major mechanism of memory in the brain.

In this study the A β (25–35) induce impairment in short term memory was significantly alleviated by the isorhamnetin treatment. It has been reported that A β induces the production of hydrogen peroxide and lipid peroxide in hippocampal neurons of the rat brain²⁸. There was an increase in the level of hydrogen peroxide indicating increase in oxidative stress and decrease in antioxidant activity in hippocampus. Isorhamnetin has been shown to alleviate the hydrogen peroxide induced oxidative stress in previous studies. In this study also IRN reduced the level of hydrogen peroxide via its antioxidant activity.

In AD patients, learning and memory are impaired by the concomitant loss of the cholinergic marker enzyme, choline acetyltransferase (ChAT), in the cerebral cortex²⁹. In this study, the A β (25–35) induced decrease in ChAT activity was reverted to normal by the IRN treatment and which may

account for the improvement in the short term memory as assessed by y arm maze.

A β 25-35 has the potential to induced oxidative stress in the brain cholinergic hypo function, elevation of AChE and MAO³⁰⁻³². It is well accepted that oxidative stress (associated with MAO) contributes to the disturbance of neurotransmitters, including NE and the cholinergic system, which have a critical role in the cognitive impairment in AD³³⁻³⁵. Elevations in MAOA in Alzheimer neurons have been linked to increase in neurotoxic metabolites and neuronal loss. Compelling studies have shown that the involvement of MAO in AD and neurodegenerative diseases plays an important role in several key pathophysiological mechanisms³⁶⁻³⁷.

Elevated monoamine levels in the brain resulting from MAO induce changes in other neurotransmitter systems and lead to cognitive impairment. The isorhamnetin has been reported to inhibit MAO- A by 55% at 1.26 μ M³⁸. A β 25-35 induced animals exhibited the increase in MAO activity whereas IRN reduced the abnormal increase in activity of MAO. It might be the reason for improving the spatial memory in Y-arm maze.

In this study, we found that IRN significantly inhibited the increase of iNOS mRNA in the hippocampus induced by A β 25–35. It has been demonstrated in vitro that the stimulation of neuronal cell lines with TNF- α leads to increased expression of inducible nitricoxide synthase and subsequent apoptosis³⁹. We analyzed the expression of TNF- α in hippocampus of control and experimental rats. There was marked elevation in TNF- α expression which was reduced by IRN treatment (data not given). Hence, decrease in TNF- α would be the reason for reduction in the iNOS expression. In contrast to the NO synthesized by nNOS, which facilitates learning and memory formation under physiological conditions⁴⁰⁻⁴³, the NO synthesized by iNOS is deleterious⁴⁴. The iNOS produced a large amount of NO, which may cause neurotoxicity *in vitro*, has been previously reported^{45, 46}. It has been reported that isorhamnetin treatment down regulated the expression of iNOS, a key enzyme for catalyzing NO production⁴⁷⁻⁴⁸. IRN alleviated the oxidative

stress in hippocampus by reducing the peroxynitrite formation via inhibiting iNOS expression.

TNF- α and IL-1 β are involved in the inflammatory response, and they may also be toxic to neurons and glial cells⁴⁹. The increased level of pro-inflammatory cytokines in the central nervous system was shown to impair cognitive function⁵⁰ and play a fundamental role in the pathogenesis of AD⁵¹⁻⁵². In corroboration with previous report, there was an increase in the expression of IL- β in A β 25-35 induced rats⁵³. The current study reveals that A β 25-35 induced the expression of proinflammatory cytokine, IL- β which was significantly reduced by IRN treatment. Pro-inflammatory cytokine upregulation and excessive NO production play important roles in severe inflammatory diseases⁵⁴. Inhibition of pro-inflammatory cytokines and NO release in inflammatory cells could therefore beneficially attenuate excessive inflammatory response. In conclusion the IRN was able to revert the abnormal expression of iNOS and IL- β to normal, thereby improved the cognitive function and reduced the oxidative stress subsequently apoptosis.

CONCLUSION: In summary, this study highlights the potential neuroprotective mechanisms of IRN involved in improvement of cognitive function. Together, the results of the present study suggest that administration of isorhamnetin prevents A β 25-35 induced cognitive impairment and associated oxidative stress. Hence, neuroprotective activity of IRN may be mediated through free radical scavenging activity and inhibition of proinflammatory cytokine and iNOS.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interests.

REFERENCES:

- Walsh DM and Selkoe DJ. Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron*.2004; 44:181-193.
- Pike CJ, Walencewicz-Wasserman AJ, Kosmoski J, Cribbs DH, Glabe CG, Cotman CW. Structure-activity analyses of

- b-amyloid peptides: contribution of the b25-35 region to aggregation and neurotoxicity. *J Neurochem*.1995; 64: 253-265.
- Yankner BA, Duffy LK, Kirschner DA. Neurotrophic and neurotoxic effects of amyloid b protein: reversal by tachykinin neuropeptides. *Science*. 1990; 250: 279-282.
- Grace EA, Rabiner CA, Busciglio J. Characterization of neuronal dystrophy induced by fibrillar amyloid b: implications for Alzheimer's disease. *Neuroscience*. 2002; 114: 265-273.
- Tohda C, Matsumoto N, Zou K, Meselhy MR, Komatsu K. Ab(25-35)-induced memory impairment, axonal atrophy and synaptic loss are ameliorated by M1, a metabolite of protopanaxadiol-type saponins. *Neuro psychopharmacology*. 2004; 29: 860-868.
- Tohda C, Tamura T, Komatsu K. Repair of amyloid b(25-35)- induced memory impairment and synaptic loss by a Kampo formula, Zokumei-to. *Brain Res* 2003; 990: 141-147.
- Maurice T, Lockhart BP, Privat A. Amnesia induced in mice by centrally administered b-amyloid peptides involves cholinergic dysfunction. *Brain Res*. 1996; 706: 181-193.
- Kuboyama T, Tohda C, Komatsu K. Neuritic regeneration and synaptic reconstruction induced by withanolide A. *Br J Pharmacol*2005;144: 961-971.
- Kuboyama T, Tohda C, Komatsu K. Withanoside IV and its active metabolite, sominone, attenuate Ab(25-35)-induced neurodegeneration. *Eur J Neurosci*.2006; 23: 1417-1427.
- Tran MH, Yamada K, Nakajima A, Mizuno M, He J, Kamei H, and Nabeshima T Tyrosine nitration of a synaptic protein synaptophysin contributes to amyloid beta-peptide-induced cholinergic dysfunction. *Mol Psychiatry*.2003;8:407-412
- Alkam T, Nitta A, Mizoguchi H, Saito K, Seshima M, Itoh A, Yamada K, and Nabeshima T Restraining tumor necrosis factor- α by thalidomide prevents the amyloid β -induced impairment of recognition memory in mice. *Behav Brain Res* .2008; 189:100-106.
- Smith MA, Richey Harris PL, Sayre LM, Beckman JS, and Perry G Widespread peroxynitrite-mediated damage in Alzheimer's disease. *J Neurosci*.1997;17: 2653-2657
- Wilcock, G.K., Esiri, MN, Bowen, D.M. & Smith, C.C. Alzheimer's disease: Correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. *J. Neurol. Sci*. 1982; 57, 407-417.
- Zhao G, Duan J, Xie Y, Lin G, Luo H, et al. Effects of solid dispersion and self-emulsifying formulations on the solubility, dissolution, permeability and pharmacokinetics of isorhamnetin, quercetin and kaempferol in total flavones of *Hippophae rhamnoides* L. *Drug Dev Ind Pharm* 2012;3: 3.
- Bao M, Lou Y Isorhamnetin prevents endothelial cell injuries from oxidized LDL via activation of p38MAPK. *Eur J Pharmacol*.2009; 547: 22-30.
- Ibarra M, Moreno L, Vera R, Cogolludo A, Duarte J, et al. Effects of the flavonoid quercetin and its methylated metabolite isorhamnetin in isolated arteries from spontaneously hypertensive rats. *Planta Med*2003; 69: 995-1000.
- Zhang N, Pei F, Wei H, Zhang T, Yang C, et al. Isorhamnetin protects rat ventricular myocytes from ischemia and reperfusion injury. *Exp Toxicol Pathol*2011; 63: 33-38.

18. Sun B, Sun GB, Xiao J, Chen RC, Wang X, et al. Isorhamnetin inhibits H₂O₂-induced activation of the intrinsic apoptotic pathway in H9c2 cardiomyocytes through scavenging reactive oxygen species and ERK inactivation. *J Cell Biochem.*2012; 113: 473–485.
19. Tangui Maurice, Brain P Lockhart, Alain Privat, Amnesia induced in mice by centrally administered β -amyloid peptide involves cholinergic dysfunction, *Brain Research* 1996; 706: 181-193.
20. Lowry OH, Rosebrough NJ, Farr AL, Ranell RJ. Protein measurements with the Follin's phenol reagent. *J BiolChem* 1951; 193: 265–275.
21. Pick, E., Keisari, Y. Superoxide anion and H₂O₂ production by chemically elicited peritoneal macrophages-induction by multiple nonphagocytic stimuli. *Cell. Immunol.* 1981; 59, 301–318.
22. Wolfram, F. Spectrophotometric assay for choline acetyltransferase. *Anal. Biochem.* 1972; 46, 114–118.
23. Tabor H, Mehler AH, Stadtman ER. The enzymatic acetylation of amines. *J Biol Chem.* 1953;204:127–38
24. Chomczynski P and Sacchi N. "Single - step method of RNA isolation by acid guanidiniumthiocyanate phenol chloroform extraction". *Anal. Biochem.* 1987; 162: 156-159.
25. Glenner, G.G., Wong, C.W., 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and Biophysical Research Communications* 120, 885–890.
26. Masters, C.L., Gail, S., Nicola, A.W., Gerd, M., Brian, L., McDonald, Konrad, B. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proceedings of the Indian National Science Academy* 1985;82: 4245–4249.
27. Spencer, J.P.E. Food for thought: The role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. *Proceedings of the Nutrition Society.*2008; 67(2): 238–252.
28. Yatin SM, Varadarajan S, Butterfield DA, Vitamin E prevents Alzheimer's amyloid beta-peptide (1-42) induced neuronal protein oxidation and reactive oxygen species production. *Journal of Alzheimer's disease* 2000; 2: 23–131.
29. Noshita T, Murayama N, Nakamura S. Effect of nicotine on neuronal dysfunction induced by intracerebro ventricular infusion of amyloid- β peptide in rats. *Eur Rev Med Pharmacol Sci.* 2015; 19(2):334-43.
30. Casanova MF, Walker LC, Whitehouse PJ, Price DL, Abnormalities of the nucleus basalis in Down's syndrome. *Ann. Neurol* 1985; 18: 310– 313.
31. Alvarez A Opazo C, Alarcon R Garrido J, Inestrosa NC, Acetylcholinesterase promotes by forming a complex with growing fibrils. *J.Mol.Biol* 1997; 272: 348-/361.
32. Takehashi M, Tanaka S, Masliah E, Ueda K, Association of monoamine oxidase Agene polymorphism with Alzheimer's disease and Lewy body variant. *NeurosciLett* 2002; 327: 79–82.
33. Engelborghs S and De Deyn PP: The neurochemistry of Alzheimer's disease. *ActaNeurol Belg.*1997; 97:67–84.
34. Ishrat T, Parveen K, Khan MM, Khuwaja G, Khan MB, Yousuf S, Ahmad A, Shrivastav P and Islam F: Selenium prevents cognitive decline and oxidative damage in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. *Brain Res.* 2009; 1281:117–127.PubMed/NCBI
35. Schaeffer EL and Gattaz WF: Cholinergic and glutamatergic alterations beginning at the early stages of Alzheimer disease: participation of the phospholipase A2 enzyme. *Psychopharmacology (Berl).* 2008; 198:1–27. View Article : Google Scholar
36. Huang L, Lu C, Sun Y, Mao F, Luo Z, Su T, Jiang H, Shan W and Li X: Multitarget-directed benzylideneindanone derivatives: anti- β -amyloid (A β) aggregation, antioxidant, metal chelation, and monoamine oxidase B (MAO-B) inhibition properties against Alzheimer's disease. *J Med Chem.*2012; 55:8483–8492.PubMed/NCBI
37. Zheng H, Fridkin M and Youdim MB: From antioxidant chelators to site-activated multi-target chelators targeting hypoxia inducing factor, beta-amyloid, acetylcholinesterase and monoamine oxidase A/B. *Mini Rev Med Chem.*2012; 12:364–370. View Article : Google Scholar
38. Sloley BD, Urchuk LJ, Morley P, Durkin J, Shan JJ, Pang PK, Coutts RT Identification of kaempferol as a monoamine oxidase inhibitor and potential neuroprotectant in extracts of Ginkgo biloba leaves. *J Pharm Pharmacol.*2000;52:451–459
39. Heneka MT, Lo'schmann PA, Gleichmann M, Weller M, Schulz JB, Wu' llner U, and Klockgether T Induction of nitric oxide synthase and nitric oxide-mediated apoptosis in neuronal PC12 cells after stimulation with tumor necrosis factoralpha/ lipopolysaccharide. *J Neurochem.*1998; 71:88–94.
40. Böhme, G.A., Bon, C., Lemaire, M., Reibaud, M., Piot, O., Stutzmann, J.J.M., Doble, A.,and Blanchard, J.C. Altered synaptic plasticity and memory production in nitric oxidesynthase inhibitor-treated rats. *Proc. Natl. Acad. Sci. USA* 1993;90: 9191-9194
41. Yamada, K., Noda, Y., Nakayama, S., Komori, Y., Sugihara, H., Hasegawa, T., and Nabeshima, T. Role of nitric oxide in learning and memory and in monoamine metabolism in the rat brain. *Br. J. Pharmacol.* 1995; 115: 852-858
42. Yamada, K., Hiramatsu, M., Noda, Y., Mamiya, T., Murai, M., Kameyama, T., Komori, Y.,Nikai, T., Sugihara, H., and Nabeshima, T. Role of nitric oxide and cyclic GMP in thedizocilpine-induced impairment of spontaneous alteration behavior in mice. *Neuroscience.* 1996;74: 365-374
43. Kendrick, K.M., Guevara-Guzman, R., Zorrilla, J., Hinton, M.R., Broad, K.D., Mimmack,M., and Ohkura, S. Formation of olfactory memories mediated by nitric oxide. *Nature*1997;388: 670-674
44. Rahimian R¹, Fakhfour G, EjtemaeiMehr S, Ghia JE, Genazzani AA, Payandemehr B, Dehpour AR, Mousavizadeh K, Lim D. Tropicsetron attenuates amyloid-beta-induced inflammatory and apoptotic responses in rats. *Eur J Clin Invest.* 2013; 43(10):1039-51. doi: 10.1111/eci.12141. Epub 2013 Aug 13.
45. Dawson, T.M., Dawson, V.L., and Snyder, S.H. Molecular mechanisms of nitricoxide actions in the brain. *Ann. N. Y. Acad. Sci.* 1994;738: 76-85
46. Hewett, S.J., Csernansky, C.A., and Choi, D.W. Selective potentiation ofNMDA-induced neuronal injury following induction of astrocyticNOS. *Neuron* 1994;13:487-494
47. Vasquez-Vivar J, Whitsett J, Ionova I et al Cytokines and lipopolysaccharides induce inducible nitric oxide synthase but not enzyme activity in adult rat cardiomyocytes. *Free RadicBiol Med.* 2008;45:994–1001
48. Stadler K, Bonini MG, Dallas S et al Involvement of inducible nitric oxide synthase in hydroxyl radical-mediated lipid peroxidation in streptozotocin-induced diabetes. *Free RadicBiol Med.*2008; 45:866–874

49. Smith JA, Das A, Ray SK, et al. Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. *Brain Res Bull.* 2012; 87(1):10-20.
50. Viel JJ, McManus DQ, Smith SS, et al. Age- and concentration-dependent neuroprotection and toxicity by TNF in cortical neurons from beta-amyloid. *J Neurosci Res.* 2001; 64(5):454-465.
51. Rubio-Perez JM, Morillas-Ruiz JM. A review: inflammatory process in Alzheimer's disease, role of cytokines. *Scientific World Journal.* 2012;2012:756357
52. Shaftel SS, Griffin WS, O'Banion MK. The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. *J Neuroinflammation.* 2008; 5:7.
53. Zhang, L.L., Sui, H.J., Liang, B., Wang, H.M., Qu, W.H., Yu, S.X., Jin, Y. Atorvastatin prevents amyloid- β peptide oligomer-induced synaptotoxicity and memory dysfunction in rats through a p38 MAPK-dependent pathway. *Acta Pharmacol Sin* 2014; 35: 716-726.
54. Southan, G.J., Szabo, C. Selective pharmacological inhibition of distinct nitric oxide synthase isoforms. *Biochem. Pharmacol.* 1996; 51: 383-394.

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