ORIGINAL RESEARCH

"EVALUATION OF NOOTROPIC ACTIVITY OF RUTIN AND BERBERINE BY SCOPOLAMINE INDUCED DEMENTIA IN *SPRAGUE DAWLEY* RATS"

S. Viswanth Reddy*, Kappera Swetha, Gajjela Avinash, Ramagiri Sagar

Deptt. Pharmacology, PNR College of Pharmacy, Shadnagar, Mahaboob Nagar (Dist) Telangana,

Submitted on: 11.06.2016; Revised on: 26.06.2016; Accepted on: 29.06.2016

ABSTRACT:

Alzheimer's disease is the most prevalent neurodegenerative disorder which leads to dementia that causes memory, thinking and behavior impairment. Treatment of this disease with drugs that enhance the cholinergic transmission in the brain such as N-methyl D-aspartate antagonists and Ache inhibitors are associated with unwanted side effects. So, alternative therapeutic strategies such as nutraceuticals with better safety profile were investigated for new safe nootropic agnets. In the present study nutraceutical products rutin and berberine were screened for nootropic activity. In Vivo nootropic activity was evaluated by scopolamine induced dementia using Morris water maze in Sprague Dawley rats by using donepezil as standard drug (2 mg/kg bd.wt) and scopolamine (0.5 mg/kg bd.wt) as inducing agent for dementia. Rutin and berberine at 500 mg/kg dose showed greater degree of memory improvement in both acquisition and probe trials. Our results indicate that these nutraceutical compounds posses memory enhancing activity which might be a potential treatment for impaired memory functions.

KEY WORDS: Dementia, nootropic, rutin, berberine, *Sprague Dawley*.

Corresponding Authour: S Viswanth Reddy
Mobile : +91-8125232429
E Mail : vishwanth555@gmail.com

Indian Research Journal of Pharmacy and Science; 9(2016) 602-609
Journal Home Page: https://www.irjps.in

1. INTRODUCTION:

Dementia is a broad category of brain disease that causes long term loss of the ability to think and reason clearly that is severe enough to affect a person's daily functioning. Dementia is the most common symptom observed in neurodegenerative disorders including Alzheimer's disease[1].Dementia becomes more common with age. While only 3 % of people between the ages of 65–74 have dementia, 47 % of people over the age of 85 have some form of dementia. As more people are living longer dementia is becoming more common. Symptoms of dementia include impulsivity, depression, agitation, balance problems, tremor, speech and language difficulty and memory distortions (believing that a memory has already happened when it has not, thinking an old memory is a new one, combining two memories, or confusing the people in a memory)[1,2,3].

Alzheimer's disease is the most common form of dementia. It is most common symptoms are short term memory loss and word finding difficulties. The main pathological features of AD comprise[1,2,3].

1. Amyloid plaque deposition in brain areas like hippocampus, amygdale.
2. Intraneuronal neurofibrillary tangles which comprise of the aggregates of highly phosphorylated form of normal neuronal protein (Tau).
3. Marked decrease in choline acetyltransferase and loss of cholinergic neurons in the brain.

The loss of cholinergic activity in the brain of Alzheimer's patients led o the use of cholinesterase inhibitors that can cross the BBB. These drugs block the degradation of Ach and increase the availability of Ach in the Synaptic Clefts. Tacrine, Donepezil,Rivastigmine are the reversible cholinesterase inhibitors used therapeutically for the management of dementia[1].

It is also postulated that excitotoxicity due to enhanced glutamate transmission via NMDA receptors, also contributes to path physiology of Alzheimer's dementia. Memantine an NMDA antagonist used for management of dementia it is better tolerated than AchE inhibitors. However, due to large side effect profile of above said drugs research is pointed towards the new safer and efficacy drugs which improve the memory functions like nootropics[1, 2].

Nootropics are the drugs which increase the memory functions and might be the better alternative for dementia patients. Nootropics are also referred to as smart drugs, memory enhancers and functional foods. Nootropics are thought to work by improving the brain's oxygen supply or by stimulating nerve growth. Examples of nootrpic agents are piracetam, aniracetam, Acetyl –L-carnitine, Brahmi, *Ginko biloba*[4, 5].

In the present study we investigated nootropic activity of the nutraceutical products rutin and berberine by scopolamine-induced dementia in rats using Morris water maze.

2. MATERIALS AND METHODS:

2.1 Drugs and Chemicals: Rutin, Berberine, Donepezil, Scopolamine, Sodium CMC, Saline.

Table 1: List of drugs and resources

S. no.	Drugs	Source
1	Scopolamine hydrobromide	Sigma-Aldrich, Mumbai, India
2	Rutin	Sigma-Aldrich, Mumbai, India
3	Donepezil hydrochloride	Sigma-Aldrich, Mumbai, India
4	Berberine	Sigma-Aldrich, Mumbai, India

2.2 Selection of animals:

Healthy male *Sprague Dawley* Rats used in this study. Animals were procured from M/s. Mahaveera enterprises, Hyderabad. All the animals are housed in poly propyelene cages with proper diet and animals are maintained according to CPCSEA guidelines.The approval has been documented in the committee for the purpose of control and supervision of experiments on animals (CPCSEA) specified 'Form B' plan (IAEC protocol approval number: IAEC/130309). The procedures used in this plan will be designed to conform to the accepted practices and to minimize/avoid risk of causing pain, distress or discomfort to the animals.

Table 2: Selection of animals

Species	**Rat**
Strain	*Sprague Dawley*
Sex	Male
Age at initiation of study	10-12 weeks
Body weight	200-250 g
Source	M/s. Mahaveera enterprises, Hyderabad
Number of animals per group	6
Number of groups	7
Number of animals	Acclimatization: 45; main study: 42
Identification of animals	By rat accession number

Environmental conditions:

Temperature : 22 ±3°C
Relative humidity : 40 – 70 %
Photoperiod : 12 hours light / 12 hours dark cycle

2.3 Randamization and Grouping of animals:

A group of 45 animals were examined and 42 healthy *Sprague Dawley* rats were selected for the study and randomly assigned to different groups based on the body weight.

Table 3: Study design:

Groups	**Scopolamine**		**Treatment**		**No. of animals**
	Dose (mg/kg bd. wt, *s.c)*	Dose conc. (mg/mL)	Dose (mg/kg bd. wt, *p.o*)	Dose conc. (mg/mL)	Male
G-I Vehicle control	-	-	-	-	6
G-II Induction control	0.5	0.2	-	-	6
G-III Rutin (250 mg/kg bd. wt)	0.5	0.2	250	25	6
G-IV Rutin (500 mg/kg bd. wt)	0.5	0.2	500	50	6

G-V Berberine (250 mg/kg bd. wt)	0.5	0.2	250	25	6
G-VI Berberine (500 mg/kg bd. wt)	0.5	0.2	500	50	6
G-VII Donepezil (2 mg/kg bd. wt)	0.5	0.2	2	0.2	6

2.4 Experimental Procedure:

Morris water maze apparatus: Water maze consists of a 72 inch diameter and 30 inch height circular water maze filled with water. An escape platform of 4 inch diameter and 12.76 inch height will be placed 1.0-1.5 cm below the water surface level in the center of one of the four imaginary quadrants, which remain constant for all rats and the movements of the animals will be tracked using an SMART video tracking system (Pan Lab) prominent visual cues surrounding the maze will be used as spatial cues around the arena[6, 7].

Acquisition trial:

The experiment consists of 4 days of acquisition trials and one probe trial on day 5 *i.e.* the day after the completion of acquisition trial. Rat was lowered gently, feet first into the water and allowed to swim for 60 seconds to find the hidden platform. If the platform is found during this time the trial was stopped and rat was allowed to stay on platform for 30 seconds before being removed from the maze. If the platform is not found during 60 seconds trial, the rat was guided to the platform and allowed to stay on platform for 30 seconds before being removed from the maze. Each rat was received 4 trials in a day. The maze has 8 starting points. On the first day animals were starting from 1st, 3rd, 5th and 7th starting point and on the second day the animals were starting from 2nd, 4th, 6th and 8th starting point and again on the third day the animals were starting from 1st, 3rd, 5th and 7th starting point, fourth day the animals were starting from 2nd, 4th, 6th and 8th starting point[6, 7].

Probe trial:

Retention of the task will be assessed on day 5 in which each animal received single 120 seconds probe trial with platform removed from the pool.

2.5 Drug Administration:

Rutin, berberine and donepezil hydrochloride monohydrate were administered *p.o.* approximately 40 minutes before the trial to respective groups. Scopolamine was administered through *s.c* route to all groups.

2.6 Measurement of Parameters:

Target latency (latency to reach escape platform in ms) was measured in acquisition trials for 4 days. Target latency (time to reach escape platform location in ms) was measured in probe trial on day 5.

2.7 Statistical analysis:

The data was expressed as mean±SEM Data was subjected to statistical analysis using ANOVA followed by dunnet test to draw a comparison between treatment group and control group.

3. RESULTS:

Scopolamine (0.5 mg/kg), an anticholinergic drug showed prolonged target latency in both acquisition and probe trials[8]. The test drug rutin at 250 mg/kg dose was showed no nootropic activity in both the acquisition and probe trials and no significant difference was observed when compared with scopolamine treated group. Rutin at 500 mg/kg dose showed, significant ($p<0.05$) nootropic activity in acquisition trail last day (Day 4) but in probe trail no significant difference was observed when compared with induction group.

Similarly, Berberine showed no significant nootropic activity at 250 mg/kg dose in both the acquisition and probe trials, however at higher dose (500 mg/kg bd. wt) berberine showed significantly increased nootropic activity in acquisition trial (on day 4 $p<0.01$) as well as in probe trial (day 5 $p<0.05$).

Berberine treated group showed the most potent memory enhancing activity in both the trials compared to rutin and scopolamine

treated groups. Berberine showed nootropic activity comparable to the standard drug donepezil, suggesting its potential as a nootropic agent in dementia.

Table 4: Summary of Target Latency during Acquisition Trial

Groups	Day 1	Day 2	Day 3	Day 4
G-I Normal control	56.25±2.26	47.29±8.85	33.58±5.77	24.25±5.85
G-II Induction control	59.95±0.85	58.87 ± 0.77	53.7±3.63	56.04 ±2.92
G-III Rutin (250 mg/kg bd. wt)	59.3±0.59 ns	54.87±2.44 ns	48.33±5.45 ns	46.16 ±6.77 ns
G-IV Rutin (500 mg/kg bd. wt)	58.4±1.63 ns	51.87 ± 2.89 ns	41.87± 5.63	37.95 ±5.38*
G-V Berberine (250 mg/kg bd. wt)	58.91±1.04 ns	56.04 ± 2.33 ns	43.41 ± 5.83 ns	40.00 ±6.12 ns
G-VI Berberine (500 mg/kg bd. wt)	57.29 ± 1.61 ns	51.54 ±3.38 ns	38.5±6.90 ns	26.79±6.49 **
G-VII Donepezil (2 mg/kg bd. wt)	56.75 ±1.28 ns	49.25±3.48 ns	37.79±7.11 ns	25±6.56***

Each value is the mean ± SEM for 6 rats, * P < 0.05; ** P < 0.01; ***P < 0.001 compared with Induction control. Data were analyzed by using Two-way ANOVA followed by Dunnett's test.

Table 5: Summary of Target Latency Time Spent in Target Quadrant during Probe Trial

Groups	Target latency (seconds)
G-I Normal control	33.33±5.55
G-II Induction control(Scopolamine 0.5 mg/kg bd. Wt)	56.33±2.40
G-III LNPR001 (250 mg/kg bd. wt)	52.6±3.40 ns
G-IV LNPR001 (500 mg/kg bd. wt)	40.83±4.94 ns
G-V LNPR002 (250 mg/kg bd. wt)	49.66±3.02 ns
G-VI LNPR002 (500 mg/kg bd. wt)	33.5±4.68*
G-VII Donepezil (2 mg/kg bd. wt)	29.16±6.35**

Each value is the mean ± SEM for 6 rats, * P < 0.05; ** P < 0.01; compared with Induction control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

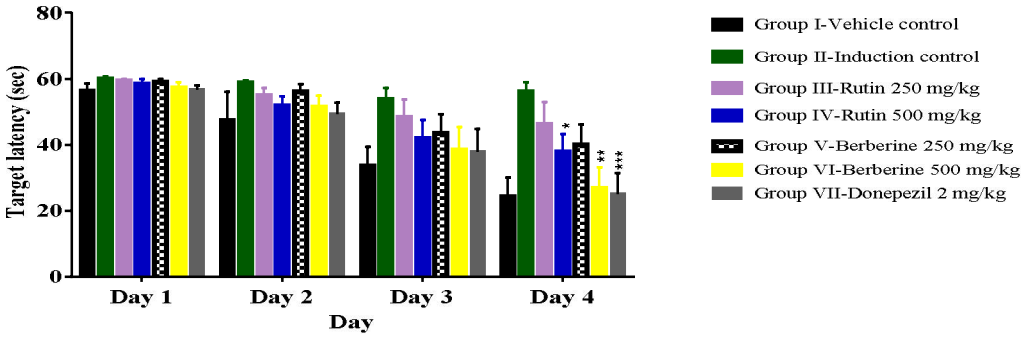

Figure 1: Target Latency in Acquisition trial

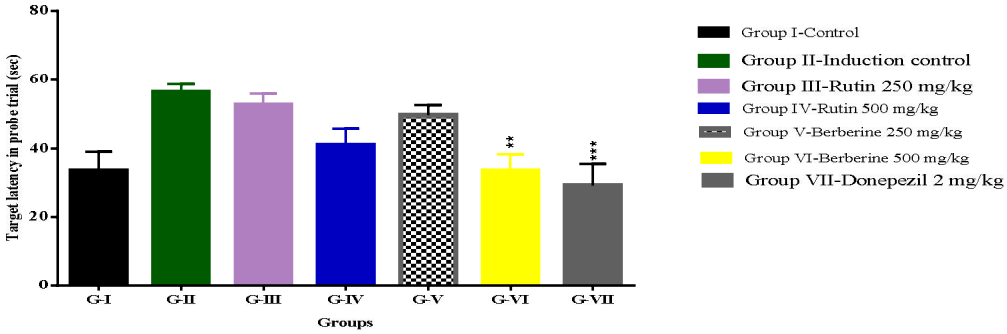

Figure 2: Target Latency in Probe trial

4. DISCUSSION:

There is an increasing amount of data showing that being decreased cholinergic transmission in the brain significantly associated with Alzheimer's disease. The compounds which inhibit the AchE in hippocampus region will helpful for treatment of impaired neurotransmission, previous studies reported the memory enhancing activity of rutin and berberine[9, 10].

Rutin is an bioflavonoidal compound before its oral absorption it will undergo deglycosylation. The absorbed aglycone is then conjugated by glucuronidation, both the aglycone and conjugate can pass the blood brain barrier. Rutin manifest its mechanism by decreasing the production of nitric oxide and proinflammatory cytokines leads to reducing the neuroinflammation, thereby decreasing the progression of Alzheimer's disease[11, 12].

Berberine is an isoquinoline alkaloid present in *Coptis chinensis*. Previous studies reported that an amount of 350 ng/g (~ 1 μM) berberine occurs in the hippocampus showing that berberine can cross the blood-brain barrier. Berberine attenuates the tau hyperphosphyryaltion and cytotoxicity of neuronal cells, suggested that berberine can be a potential therapeutic target for AD[12, 13].

In the present study rutin and berberine were evaluated for their effect on scopolamine induced impairment of memory in *Sprague Dwaley* rats. In this study scopolamine (0.5 mg/kg *s.c*) was used as an induction agent for dementia. None of the reported articles were used this dose and route administration for impairment of memory in rats. Similarly none of the literature reported the comparative study of rutin and bereberine, but in the present work we evaluated the comparative nootropic study of rutin and berberine.

Scopolamine treated animals were showed more time to reach the escape platform when compared to other group animals and both

the test drugs were showed significant nootropic activity at higher doses. In the present work berberine showed better nootropic activity compared to rutin.

5. CONCLUSION:

- Test compounds Rutin and berberine were screened for *in vivo* nootropic activity by scopolamine induced dementia using Morris water maze.
- Rutin and berberine at doses of 250 mg/kg and 500 mg/kg improved the scopolamine induced impairment of memory in both acquisition and probe trials when compared to induction control. Both of these test compounds possess significant memory enhancing activity at higher doses.
- Test compounds at a dose of 500 mg/kg showed greater memory improvement in acquisition and probe trials. Berberine at higher dose showed better nootropic potential than rutin.
- According to the reported literature the possible mechanism of these compounds might be due to increase in Ach levels and reduces the neuroinflammation in brain.
- Both the nutraceuticals rutin and berberine possess nootropic activity, suggesting its potential as a future nootropic agent for treatment of dementia.

6. REFERENCES:

1. HL Sharma, KK Sharma. Principles of Pharmacology. 2nd edition.2011.pp.541-542.
2. J G Hardman and L E Limbird. Goodman and Gilman's the pharmacological basics of therapeutics.12th ed. Mcgraw hill professional; 2010. pp. 560-562.
3. Greely, Henry, Sahakiran, Harris, John, Kessler Ronald, Gazzaniga, Michael, Campbell, Philip, Farah, J Martha. "Towards responsible use of cognitive-enhancing drugs by the healthy". *Nature (Nature Publishing Group)*. 2008; 456: 702-705.
4. D S Reddy. Assessment of nootropic and amnesic activity of centrally acting agents. *Indian Journal of pharmacology and Toxicology*. 1997; 29: 208-221.
5. B Annapurna, A Ramakrishna, V Murali, K Krishna kumar, V Prasad. Studies on nootropic activity of ramipril and losartan on scopolamine induced amnesia in rats. *Indian Journal of pharmaceutical sciences*. 2004; 66: 31-35.
6. D Dinesh, M Parle, S K Kulkarni. Memory enhancing activity of *Glycyrrihza glabra* in mice. *Journal of Ethno pharmacology* 2004; 91: 361-365.
7. Vikas Kumar, P N Singh, Murganandham, Bhattacharya .Effect of Indian *Hypericum perforatum Linn* on animal models of cognitive dysfunction. *Journal of Ethno pharmacology* 2000; 72: 119-128.
8. Nejat Gacara, Oguz Mutlu, Tijen Utkan , Ipek Komsuoglu, Celikyurt, Semil Selcen Gocmez, Guner Ulak. Beneficial effects of resveratrol on scopolamine but not mecamylamine induced memory impairment in the passive avoidance and morris water maze tests in rats. *Journal of Pharmacology, Biochemistry and Behavior*. 2011; 99: 316-323.
9. Chen Zhong. Reversal of Scopolamine-induced spatial memory deficits in rats by TAK-147. *Acta Pharmacologica Sinica*. 2002; 23: 355-360.
10. Ki Yong Lee, Sang Hyun Sung, Seung Hyun Kim, Young Pyo Jang, Tae Hwan Oh and Young Choong Kim. Cognitive-enhancing activity of loganin isolated from *Cornus officinalis* in scopolamine-induced dementia. *Archives of Pharmacal Research*. 2009; 32: 677-683.
11. Anna K. Jäger* and Lasse Saaby. Flavonoids and the CNS. *Molecules*. 2011; *16:1471*-1485.

12. Konathala Rajesh*, K.Phani Kumar, S.Kotaiah, Prof. A. Annapurna. Studies on nootropic activity of rutin and naringinon scopolamine induced amnesia in rats using morris water maze task. World journal of Pharmacy and Pharmaceutical sciences.2014; 3(7):1770-1776.

13. Xiaofeng Liu1.", Jie Zhou1.", Morad Dirhem Naji Abid2, Huanhuan Yan1, Hao Huang1, Limin Wan1,Zuohua Feng1, Juan Chen1*. Berberine Attenuates Axonal Transport Impairment and Axonopathy Induced by Calyculin A in N2a Cells. *PLOS ONE* .2014 ; 9 (4) : e93974.

CONFLICT OF INTEREST REPORTED: NIL; SOURCE OF FUNDING: NONE REPORTED