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Comparative study of the nootropic activity of the aqueous and methanolic extracts of onion and medharasayana

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Abstract

Nootropics are agents that enhance cognition and memory and are alleged to work by increasing the levels of neurochemicals in the brain, especially cholinergic transmission or by neutralizing free radicals. Onion (*Allium cepa* L.) is a known traditional medicinal plant that has been consumed for its putative nutritional and health benefits for centuries. Onion is a rich source of several phytonutrients such as thiosulphinate, volatile sulfur compounds and many polar components of phenolic origin. The present study was aimed to evaluate the cognitive and memory enhancing effect of aqueous as well as methanolic extract of onion bulb on mice. The effects were also compared with some commercially available memory enhancer supplements. The evaluation parameter, transfer latency, showed significant ($p<0.01$) change in methanolic extract when compared with the aqueous extract. Whole brain acetyl cholinesterase enzyme activity was studied which indicated that the methanolic extract of onion may be useful as a memory enhancer. The rich storehouse of antioxidants in the form of flavonoids and its ability to improve the cholinergic transmission may be attributed to the nootropic activity of onion extracts.

Key words : Nootropic, onion, antioxidants, piracetam, acetyl cholinesterase enzyme

1. Introduction

Learning and memory are two fundamental cognitive functions that confer us the ability to accumulate knowledge from our experiences (Liu *et al*., 2009). Memory is one of the most complex functions of the brain and eventually involves multiple neuronal pathways and neurotransmitters. Impairment of memory is defined as "loss of intellectual ability of sufficient severity to interfere either with occupational functioning, usual social activities or relationship of a person with the surroundings in absence of gross clouding of consciousness or motor involvement". A sudden surge in an array of mental illness due to the stressful life that one leads now-a-days has been seen in recent years. Cognitive decline is the central symptom associated with some of such mental illnesses and stressful living.

Nootropics are drugs, supplements, nutraceuticals, and functional foods that improve mental functions such as cognition, memory, intelligence, motivation, attention, and concentration (Mishu, 2012; Rutger, 2006). Synthetic nootropics such as Piracetam and Donepezil which have been widely used and recommended, are associated with adverse effects such as diarrhea, insomnia, nausea, bronchitis and muscular cramps. This prompts the need for safer remedies that may be used on a regular basis without addiction or toxicity. Plants and their products have been the reliable remedy for humankind since ancient times for various ailments. The ingredients in herbals are closer to the nature and usually can be absorbed and processed better in the human body.

Antioxidants work against oxidative stressors to prevent oxidative damage (Chidanand Chandrakant *et al*., 2012). Increased intake of dietary antioxidant is believed to maintain an adequate antioxidant status and therefore, the normal physiological function of living system (Rang *et al*., 2010; Pandey and Rizvi, 2009). Antioxidants have great importance in terms of preventing oxidative stress that may cause several degenerative diseases (Rajeshwari *et al.,* 2013). Antioxidants may be used to counteract the memory loss in which oxidative stress play a major role (Lokhart and Lestage, 2003).

Alliums vegetables have been employed for a long time in traditional medical practice to treat a variety of diseases. Onion (*Allium cepa* L.), one of the representative of Alliums vegetables, has been used for centuries for its pungency, flavoring value and medicinal properties. The bulb of onion is used medicinally and has been consumed as seasoning food for many centuries. Studies have proved that onion is rich in flavonols and organosulfur compounds, which have exhibited antioxidant activity (Chitrashenoy *et al.,* 2009).

The present study was aimed to investigate the nootropic potential of onion (*Allium cepa* L.), using standard animal models (Bhattacharya, 2005). The present study was also carried out to validate the chosen plant extracts with the standard Nootropic, Piracetam and marketed product, Medharasayana, to see if they were found to possess Nootropic activity.

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2. Materials and Methods

2.1 Materials

2.1.1 *Drugs and chemicals*

All the drugs and chemicals used in the study were obtained from authorized dealers. Nootropil (Piracetam 150 mg/kg) and Hyoscine (Scopolamine 0.4 mg/kg) were purchased from Yashoda Hospital, Secunderabad. Dithiobisnitrobenzoic acid (DTNB), Acetyl thiocholine iodide (ATCI), and Thiobarbituric acid were purchased from Chemicals and Chemicals Stores, Shahpur, Hyderabad. Onions were purchased from Reliance Fresh market, Alwal, Secunderabad. The collected onions were identified and authenticated by Botanical Survey of India (BSI), Hyderabad.

2.1.2 *Extract preparation*

The red onions were pealed, weighed and crushed. Then the crushed product was filtered, using sterile filter paper with 40 micrometers mesh size and the collected product served as fresh onion juice. Later few other fresh onions were made into juice by using mixer, the juice was filtered and mixed with methanol in the same ratio, taken in a separating funnel and allowed to stand for few hours. Then it was separated and dried. The products so obtained were then used for experimental work and also stored in a well closed container for further use.

2.1.3 *Acute toxicity studies*

The acute toxicity studies were performed in mice by giving the fresh onion juice and methanolic extracts at doses 1, 5, and 10 ml / kg body weight. The animals did not exhibit any toxic symptoms even at 10 ml/kg body weight and the dose was fixed at 1 ml and 0.5 ml/kg body weight based on the OECD (Organisation for Economic Co-operation and Development) guidelines 425.

2.1.4 *Animals*

Male Swiss albino mice (25-30 gms) were used throughout experiment. Animals had free access to feed and water ad libitium during quarantine period. Experimentation was carried out according to CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines and experimental work also approved by Institutional Animal Ethics Committee.

2.2 Methods

2.2.1 *Elevated plus maze*

Elevated plus maze (EPM) was used to evaluate learning and memory in mice. Procedure for testing learning and memory was followed as per the neuro psychopharmacological principle (Bhawani Singh Reddy *et al.,* 2012). The apparatus consists of two open arms (16 cm × 5 cm) and two enclosed arms (16 cm × 5 cm). The maze was elevated to a height of 25 cm from the floor. The center platform extended 5 cm × 5 cm from the arms. Transfer latency (TL) is the time taken by the mouse to enter into one of the enclosed arms (Dhansekaran and Palayan, 2010). Mouse was placed at the end of the open arm, facing away from the Centre platform. TL was recorded when the mouse entered with all its four legs into one of the enclosed arms. TL was recorded on the 1st and 8th day.

Before the 1st day, the mouse was exposed to plus maze by spending time in it for 10 seconds. Cut-off time was taken as 90 seconds for the model (Colucci, 2012).

2.2.2 *Morris water maze*

Morris water maze consists of a large circular tank with a depth of 30 cm, diameter 50 cm. In the center, a platform of 15 cm having dimensions 5 cm × 5 cm is mounted. The pool was filled with water admixed with milk in order to make it opaque (Reddy, 1997). Later animals were allowed for training before the experimental day. On the 1st day, animals were treated with different doses of standard and test samples. The animal was placed at the corner of the tank and allowed to swim until it identifies the hidden platform. The cut-off time was 90 seconds. The transfer latency is the time taken by the mouse to identify the platform. TL was recorded on 1st day and 8th day (Yalla Reddy *et al.,* 2010).

2.2.3 *Step down*

Step down passive avoidance test was used to examine long term memory. The apparatus consists of transparent acrylic cage (30 × 30 × 40 cm in height) with a grid floor; a platform (4 × 4 × 4 cm) is fixed in the centre of the grid floor. Electric shocks of 1Hz, 500 msec, 40V DC are delivered to the grid floor. The training was carried out before the experimental day (Sunil and Kshirsagar, 2011). On the experimental day, mouse was placed on platform in the centre of the platform, when the mouse steps down and places all its paws on the grid floor, shock was delivered. Later animal was placed again on the platform after 60-90 minutes and Step down latency (SDL), was recorded with an upper cut of time of 300 seconds (Sharma and Kulkarni, 1992).

2.2.4 *Estimation of acetyl cholinesterase enzyme activity of whole brain*

On the 8th day, animals were treated with scopolamine and after 90 mins., the animals were decapitated. The whole brain was taken out in normal saline, later suspended in phosphate buffer pH 8 (Dhingra, 2004). The brain was homogenized in tissue homogenizer and then 0.4 ml of the homogenate was mixed with 10 microliters of DTNB.

The absorbance was recorded in UV-Spectrometer. After few minutes, the sample was mixed the acetyl thio choline (ATC) and readings were taken and change in the absorbance per minute was noted (Farshichi *et al.,* 2010).

The rate of moles of substrate hydrolyzed per minute per gram of tissue was later calculated as per the following equation:

$$R = \frac{\Delta A}{1.36(10^4)} \times \frac{1}{(400/3120)C_0} = 5.74(10^{-4})\frac{\Delta A}{C_0}$$

where

ΔA = Change in absorbance per minute (mean change in absorbance)

C_0 = Original concentration of the tissue

R = Rate in moles substrate hydrolyzed per minute per gram of tissue

2.2.5 *Statistical analysis*

The transfer latency, step-down latency and AchE activity were analyzed by One-Way Analysis of Variance (ANOVA), followed by Dunnett's test for comparison of groups using Graphpad PRISM Version 5.01(GraphPad Software,Inc., USA)A probability level of $p<0.01$ was considered as significant.

3. Results

Table 1: EPM (Elevated plus maze) results for onion

S.No.	Treatment	Transfer Latency on 1st day Mean ± SEM	Transfer Lantency on 8th day Mean± SEM
1	Control (C)	23.3 ± 1.30	16.3 ± 1.20**
2	Neg.control(N.C)	23.3 ± 1.30	23.3 ± 0.91**
3	Standard (STD)	29.8 ± 2.00	14.6 ± 1.17**
4	Marketed product(M.P)	25.6 ± 0.88	16.0 ± 1.09**
5	Onion aqua(OTA)	36.1 ± 1.60	16.6 ± 1.17**
6	Onion methonal(OTM)	36.5 ± 0.42	16.6 ± 0.71**

The results are expressed as Mean ± SEM. n = 6 in each group. The analysis of results were done by comparing the sample groups with the positive control and also by comparing the positive control with control by one-way-analsyis of variance (ANOVA), followed by Dunnet's 't' test. In all tests, $p < 0.05$, *i.e.*, 95% confidence level was chosen to interpret the results and measure statistical significance. The analysis was performed by using GraphPad Prism 6. p value $p < 0.5$ is considered as significant $*p < 0.05$, $**p < 0.01$.

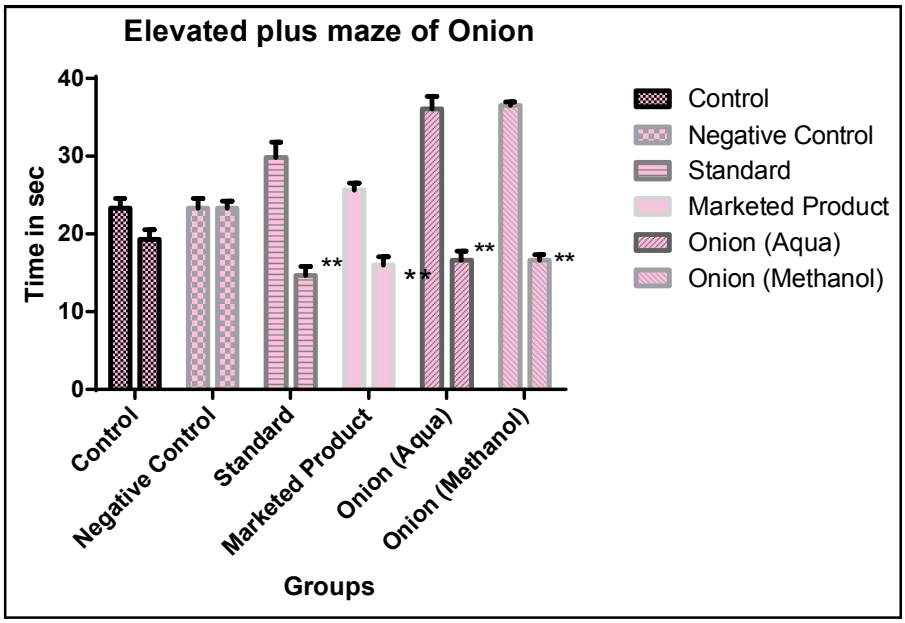

Figure 1: Graph showing EPM results for onion extract

Table 2: WM (Water Maze) results for onion

S.No.	Treatment	Transfer Latency on 1st day Mean ± SEM	Transfer Lantency on 8th day Mean ± SEM
1	Control (C)	10.50 ± 0.50	10.50 ± 0.47ns
2	Neg.control(N.C)	8.16 ± 0.47	9.16 ± 0.55
3	Standard (STD)	5.30 ± 0.55	8.33 ± 0.56**
4	Marketed product(M.P)	7.50 ± 0.56	7.50 ± 0.89ns
5	Onion aqua(OTA)	16.60 ± 0.91	6.00 ± 0.88*
6	Onion methonal(OTM)	14.30 ± 0.55	6.30 ± 0.50*

The results are expressed as Mean ± SEM. n = 6 in each group. The analysis of results were done by comparing the sample groups with the positive control and also by comparing the positive control with control by one-way-analsyis of variance (ANOVA), followed by Dunnet's 't' test. In all tests, $p < 0.05$, *i.e.*, 95% confidence level was chosen to interpret the results and measure statistical significance. The analysis was performed by using GraphPad Prism 6. p value $p < 0.5$ is considered as significant $*p < 0.05$, $**p < 0.01$.

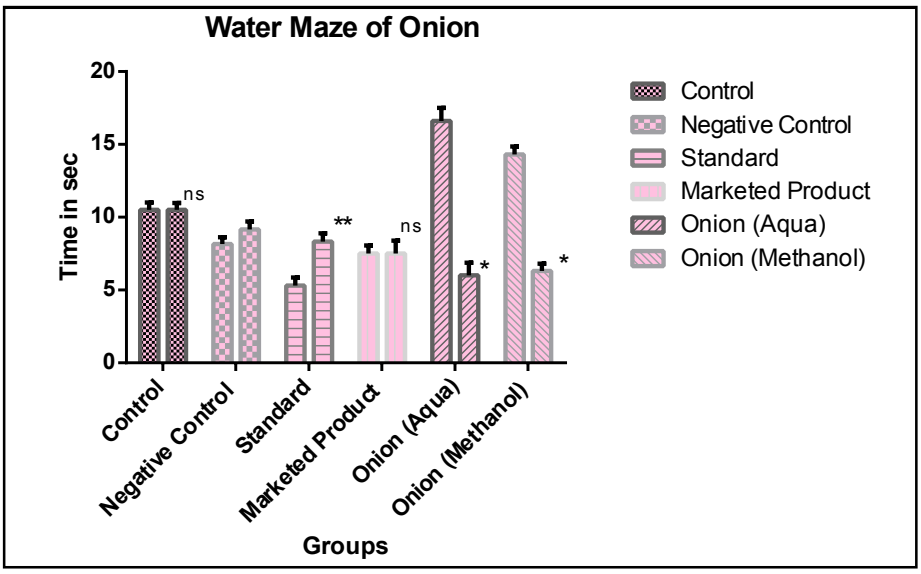

Figure 2: Graph showing WM results for onion extracts

Table 3: SD (Step Down) results for onion

S.No.	Treatment	Step down Latency on 1st day Mean ± SEM	Step down Kantency on 8th day Mean ± SEM
1	Control (C)	10.33 ± 0.421	10.83 ± 0.70ns
2	Neg.control (N.C)	11.83 ± 0.524	10.16 ± 1.40
3	Standard (STD)	9.167 ± 0.79	24.50 ± 0.76**
4	Marketed product (M.P)	10.83 ± 0.83	19.83 ± 0.60**
5	Onion aqua (OTA)	9.83 ± 0.60	14.83 ± 0.83**
6	Onion methonal(otm)	10.83 ± 0.30	15.16 ± 0.30**

The results are expressed as Mean ± SEM. n = 6 in each group. The analysis of results were done by comparing the sample groups with the positive control and also by comparing the positive control with control by one-way-analsyis of variance (ANOVA), followed by Dunnet's 't' test. In all tests, $p < 0.05$, *i.e.*, 95% confidence level was chosen to interpret the results and measure statistical significance. The analysis was performed by using GraphPad Prism 6. p value $p < 0.5$ is considered as significant $*p < 0.05$, $**p < 0.01$.

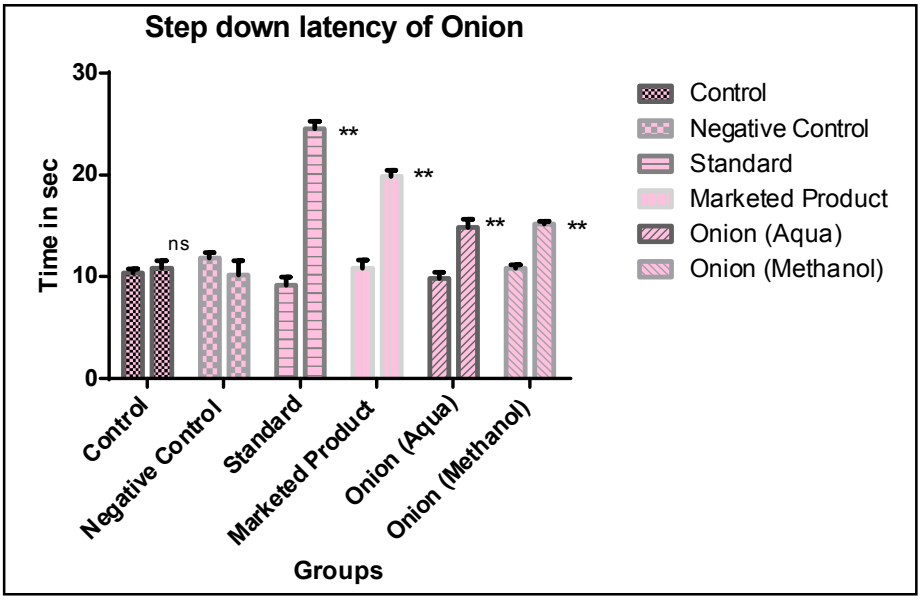

Figure 3: Graph showing SDL results for onion extracts

Table 4: Results for ache estimation

S.No	Treatment	Dose	Acetyl choline esterase enzyme activity. (Mean ± SEM)
1	Control (c)	10ml/kg	29.5 ± 0.691^{ns}
2	Neg.control (N.C)	0.4mg/kg	31.19 ± 0.710
3	Standard (STD)	150mg/kg	11.01 ± 0.551**
4	Marketed product (M.P)	10ml/kg	30.11 ± 0.712ns
5	Onion aqua (OTA)	1ml/kg	16.01 ± 0.632**
6	Onion methonal (OTM)	0.5ml/kg	15.12 ± 0.615**

The results are expressed as Mean ± SEM. n = 6 in each group. The analysis of results were done by comparing the sample groups with the positive control and also by comparing the positive control with control by one-way-analsyis of variance (ANOVA), followed by Dunnet's 't' test. In all tests, $p < 0.05$, *i.e.*, 95% confidence level was chosen to interpret the results and measure statistical significance. The analysis was performed by using GraphPad Prism 6. p value $p < 0.5$ is considered as significant $*p < 0.05$, $**p < 0.01$.

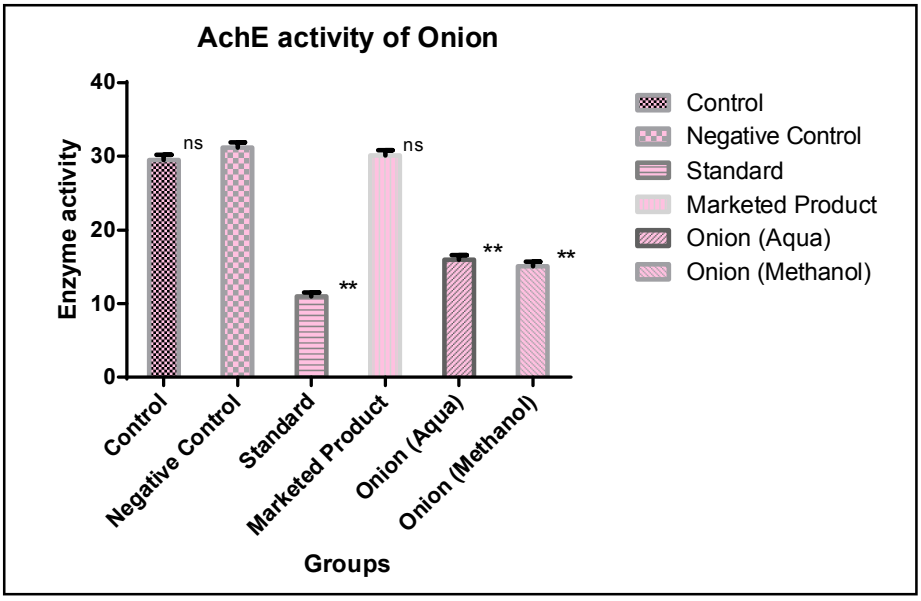

Figure 4: Graph showing ache estimation results for onion extracts

4. Discussion and Conclusion

The models showed the ability of the plant extracts in improving cognitive functions, impaired by scopolamine (centrally acting acetylcholine blocker), thereby emphasizing its utility in cognitive disorders (Goodman and Gilman, 2001). The present study revealed that the methanolic extract of onion showed better facilitatory effect on retention (memory) of acquired learning in mice when compared with the aqueous extract of onion. This observation has been supported by the administration of (1 ml/kg and 0.5 ml/kg) of aqueous and methanolic extracts of onion to Swiss albino mice. The findings showed shortened transfer latency by elevated plus maze and morris water maze and prolonged step down latency in the step down model for 0.5 ml/kg body wt of methanolic extract of onion.

This can be probably attributed to the more content of polyphenols in the methanolic extract and has to be further supported by an in depth study of the concentration of polyphenol content in methanolic and aqueous extracts of onion. Alterations in the levels of various neurochemicals have found to play a crucial role in the pathophysiology of memory deficits. However, the central cholinergic pathways play a prominent role in the learning and memory processes (Rai, 2001). Interference with cholinergic function produces disruption of memory/cognitive performance in both animals and man. Damage to the cholinergic system in the brain has been shown to be plausibly associated with memory deficits as well as with Alzheimer's disease. Plasticity of cholinergic synapses as well as other acetyl choline dependent operations in the brain is crucial to memory. In the central nervous system, acetyl choline has a variety of effects as a neuromodulator. It has an important role in the enhancement of sensory perception when we wake up and in sustaining attention which is a key requirement for memory consolidation and retrieval.

The degree of cholinergic neuron degeneration correlates positively with severity of memory impairment. Methanolic onion extract

(0.5 ml/kg, *p.o*) significantly lowered AChE activity ($p<0.01$), showing that it has a positive reinforcement in the cholinergic axis which is considered as one of the key markers of cognitive function. The marketed product medharasayana did not show marked improvement in memory compared to methanolic extract of onion when tested by the exteroceptive models described earlier. The cholinergic transmission was also not remarkably enhanced by medharasayana as the AChE levels were high when compared to the aqueous and methanolic extracts of onion.

Onion (*Allium cepa* L.) extract was found to improve learning abilities and memory capacities in mice. After 7 days of chronic treatment, the animals were dissected to estimate AChE level in whole brain, and that is considered as an additional parameter as a cholinergic marker of learning and memory. Data obtained from the study show significant memory enhancement by extract of onion (*Allium cepa* L.) at a dose of 0.5 mi/kg for methanolic onion extract. The marketed product, medharasayana failed to elicit a similar or better response in comparison to the onion extracts. This reinforces the need to validate all Ayurvedic products scientifically. Moreover, it does not disprove the use of medharasayana as a cognitive enhancer, but just reinforces the need to look for alternative better options available from the storehouse of nature.

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Conflict of interest

We declare that we have no conflict of interest.

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