

RESEARCH ARTICLE

Received on: 01/04/2016

Published on: 22/04/2016

Corresponding Author

Sowjanya

Department of Pharmacology,
Srinivas College of Pharmacy,
Mangalore

Email: prabhu.sowju@gmail.com



QR Code for Mobile users

Conflict of Interest: None Declared !

Evaluation of Nootropic Potential of Apple Cider Vinegar in Validated Experimental Animal Models

Sowjanya^{1,*}, Dr. Satish.S¹, Dr. Karunakar Hegde¹, Dr. Shabaraya AR²

¹Department of Pharmacology, Srinivas College of Pharmacy, Mangalore.

²Department of Pharmaceutics, Srinivas College of Pharmacy, Mangalore.

ABSTRACT

Cognitive enhancing activity of ACV on scopolamine induced memory impairment in mice was investigated by using elevated plus maze and estimation of biochemical parameter in terms of AChE activity. Two doses (0.51ml/kg) and (1.02 ml/kg b.w.p.o) of ACV were subjected for the evaluation of nootropic activity against the amnesia induced by scopolamine (0.4mg/kg, i.p) in young mice. Pirecetam (400 mg/kg i.p) was served as standard in both the models. The animals were challenged for twice examination in 27 days study. The long term administration of both the lower (0.51ml/kg) and higher dose (1.02ml/kg) of ACV produced significant reduction of TL ($P < 0.01$) and ($P < 0.05$) in EPM model on both 19th and 27th day when compared with control and induced groups. ACV at higher dose significantly ($p < 0.01$) reduce the activity of AchE in the brain indicates the improvement in learning and retention of memory in young mice, concluded that apple cider vinegar was found to be effective against amnesia induced by scopolamine.

Keywords: Nootropic, Scopolamine, ACV, EPM, Young mice.

Introduction

Memory is special facility of brain which retains the events developed during the process of learning and both are mediated by nervous system. Probably learning and memory are most evolutionary advantageous developments for human. A nootropic is a cognitive enhancer or Memory are drugs, supplements, nutraceuticals and functional foods that are purported to improve mental functions such as cognition, memory, intelligence, motivation, attention and concentration are also used for mental retarded and learning deficit in children. Substances classified as nootropics ("smart drugs", "cognitive enhancers", "smart nutrients", "brain boosters", and "brain enhancers"). Causes of memory loss are normal aging, alzheimers disease, depression, head injury, seizures, chronic alcohol abuse etc. Typically, nootropics are alleged to work by increasing the levels of neurochemicals in the brain.¹

As per the estimates of the WHO aging population is increasing in developing countries and dementia is going to become epidemic among elderly in the coming decades. This demands early action to prevent the disease and treatment of the affected persons. It is estimated that the number of people living with dementia will almost double every 20 years to 42.3 million in 2020 and 81.1 million in

2040.² Many countries such as Malaysia, Indonesia, India and China have a rich tradition of folk medicine from centuries and provided effective remedies to various ailments using plants and plants derived compounds³.

One of these natural substances known for hundred years and nowadays living its renaissance is the apple cider vinegar (ACV) which has been helping people to healthier lives. Apple cider vinegar is a solution of acidic acid produced by fermentation of apples. The name vinegar comes from a French words "vin aigre", meaning "sour wine". Acetic acid is a volatile organic acid that identifies the product as vinegar consist of about 3 to 5% of acetic acid content and is responsible for the tart flavor and pungent, biting odor of vinegars.⁴ There is a long history of its use in medicine dating back to 400 B.C. when the father of medicine, Hippocrates used it to treat his patients. It has been particularly used during the American Civil War for disinfecting the wounds of soldiers.⁵ The aim of the research is to find out new nootropic drugs from fruits which are potent and nontoxic agents as they are low cost medicines which will be beneficial for the people.

MATERIALS AND METHODS:

Drugs and Chemicals: All the drugs and chemicals used in the study were obtained from authorized dealers. Sodium hydroxide, Acetylcholine iodide was purchased from lobachemie. Phenolphthalein, Dithionitrobenzoic acid (DTNB) was purchased from high media. Scopolamine (sigma Aldrich) and Piracetam (mangalore).

Collection and authentication of fruit material:

The fresh fruits of Red delicious apples used for the present studies were collected from Mangalore supermarket in September 2015. It was authenticated by Mr. Dinesh Nayak, Advisor (Green belt), Mangalore SEZ Limited. Red delicious apples used to produce natural apple cider with inclusion of maceration.

Preparation of Apple Cider Vinegar:⁶

The fresh fruits were washed and the cores and peels of apples are separated. Glass jar was selected and to this warm water and apple pieces, organic sugar added and stirred it well. Later covered the jar with a cotton cloth and put a rubber band around it and stored the glass jar in a cool and dry place. Allowed it stand for 2 to 3 weeks so that fermentation takes place. During this month long storage, every day the stirring of apple cider vinegar done. This is very important step it will help in fermentation process. On 27th day the peels are separated and filtered the liquid and tested for acidity of apple cider vinegar.

Experimental animals:

Healthy albino mice (20-25 g) of either sex were used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore. The project was approved from Institutional Animal Ethical Committee of Srinivas College of Pharmacy, Mangalore.

ESTIMATION OF TOTAL ACIDITY % OF VINEGAR.⁷

Total acidity was evaluated by acid – base titration with standardized solution of 0.1 N sodium hydroxide, using phenolphthalein as a indicator and the results were expressed as acetic acid content. The formula to calculate %TA as acetic is as below:

$$\%TA = \frac{\text{ml of NaOH} \times (\text{N of NaOH}) \times (60.05)}{\text{Sample Weight}} \times 10$$

Acetic acid is a volatile organic acid that identifies the product as vinegar consist of about 3 to 5% of acetic acid content and is responsible for the tart flavor and pungent, biting odor of vinegars.⁴ In present study

the apple cider vinegar showed the total titrable acidity was found to be 4.235%.

DOSE SELECTION OF ACV⁸

Two doses of apple cider vinegar were selected from the previous research carried out, was administered post orally at a constant volume of 0.51 ml/kg and 1.02 ml/kg for each animals.

EXPERIMENTAL DESIGN

MODEL 1: ELEVATED PLUS MAZE (EPM):^{9,10,11}

Treatment: Mice of either sex weighing between 20-25g were divided into 5 groups of six animals each.

Group I : Animals serve as control and was receive only vehicle.

Group II: Animals were administered Scopolamine on 19th and 27th day (0.4 mg/kg i.p.)

Group III : Animals were treated with standard drug Pirecetam (400mg/kg i.p.)

Group IV : Animals were treated with low dose (0.51ml/kg) of Apple cider vinegar for 27 days orally.

Group V : Animals were treated with high dose (1.02 ml/kg) of Apple cider vinegar for 27 days orally.

Procedure: From day 1-15 Animals was administered with drugs as mentioned above. On day 16, 17 and 18 animals were trained once daily on Elevated plus Maze (EPM) and drug administration was continued. On day 19, scopolamine (0.4 mg/kg i.p.) was administered to all animals (except group I) 30 min after the respective treatment, After 45 min assessment of final TL (transfer latency) was noted by using EPM. The respective treatment with drug was continued for 1week. (Days 20-26).

On day 27, scopolamine 0.4 mg/kg i.p. is injected to all the animals, 30 min prior to the last drug treatment. Assessment of learning and retention of memory is done after 45 min using EPM.

Evaluation: In this model the change in the latency to go from open arm to closed arm of the Elevated plus Maze is an indicator of learning and memory. On the first day each mice was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) is the time taken (in sec) by the animal to move from the open arm into any one of the enclosed arms with all its four legs. Retention of this learned-task (memory) was examined 24h after the 19th day and on 27th day of the treatment. Significant reduction in TL value of retention indicates improvement in memory.

ESTIMATION OF BRAIN AChE ACTIVITY: ^{9,12}

Procedure: On the 28th day same animals used in EPM model was euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE activity was measured using the Ellman method. Briefly, 0.4 mL of brain homogenate was added to a test tube containing 2.6 mL of phosphate buffer. 0.1 mL DTNB reagent was added to the above mixture and absorbance was noted at 412 nm. 0.02 mL of acetylcholine iodide solution was added and again absorbance was noted 15 min thereafter. The end point is the formation of the yellow color because of the reaction of thiocholine with dithiobisnitrobenzoate ions. Protein estimation will be done using Folin's method. AChE activity was calculated using the following formula:

$$R = \delta OD \times \text{volume of assay (3 ml)} / E \times \text{mg of protein,}$$

Where R is the rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed per minute per mg of protein. δ OD is the change in absorbance per minute and E is the extinction coefficient, which is 13 600 M⁻¹ cm⁻¹.

Statistical analysis : All data were expressed as Mean \pm SEM. The statistical significance between groups were compared using one way ANOVA, followed by Dunnett's (multiple comparison test).

RESULTS:**Estimation of total acidity % of apple cider vinegar:**

In 10 ml of apple cider vinegar the percentage of acidic acid was found to be 4.235%

1. ELEVATED PLUS MAZE:

The results of EPM in mice after administration of apple cider vinegar are given in (Table no.1). On day 16, 17 and 18, animals were trained once daily on Elevated plus Maze and drug administration was continued. TL of mice was noted on 19th day (learning) and after 24 hours i.e on 20th day (retention). ACV at lower dose (0.51ml/kg) significantly (P<0.05) decreased TL for 19th and 20th day. Higher dose of ACV (1.02ml/kg) produced more significant (P<0.01) decrease in TL for 19th and 20th day.

On 27th day (learning) and after 24 hours i.e on 28th day (retention) TL was noted, ACV at lower dose (0.51ml/kg) significantly (P<0.05) decreased TL. Higher dose of ACV (1.02ml/kg) produced more significant (P<0.01) decrease in TL. Thus both doses of apple cider vinegar produced significant reduction in TL. Hence it shows activity against scopolamine induced amnesia.

Group No	Treatment	On 19th day TL (sec) Mean \pm S.E.M [n=6]	After 24 hrs 20 th day	On 27th day TL (sec) Mean \pm S.E.M [n=6]	After 24 hrs 28 th day
I	Control	32.48 \pm 0.1627	31.38 \pm 0.326	30.17 \pm 0.2217	29.63 \pm 0.158
II	Scopolamine 0.4 mg/kg	51.74 \pm 0.2403	50.39 \pm 0.712	48.92 \pm 0.1342	47.48 \pm 0.753
III	Scopolamine 0.4mg/kg + Piracetam 400 mg/kg	36.57 \pm 0.253***	35.82 \pm 0.548***	34.34 \pm 0.212***	33.64 \pm 0.462***
IV	Scopolamine 0.4mg/kg+ ACV 0.51ml/kg	40.46 \pm 0.2119*	39.57 \pm 0.372*	38.85 \pm 0.320*	37.45 \pm 0.821*
V	Scopolamine 0.4 mg/kg + ACV 1.02ml/kg	38.32 \pm 0.365**	37.92 \pm 0.416**	36.12 \pm 0.425**	35.37 \pm 0.523**

Table no.1: Effect of ACV on transfer latency of young mice on Elevated Plus Maze (19th and 27th day)

Mean latencies (learning and memory scores) across 19th & 27th day in the EPM task; n=6; Values are expressed as Mean \pm SEM; * P<0.05, ** P<0.01 *** P<0.001 compared to scopolamine group. (ANOVA followed by Dunnett's multiple comparison test) ACV: Apple cider vinegar.

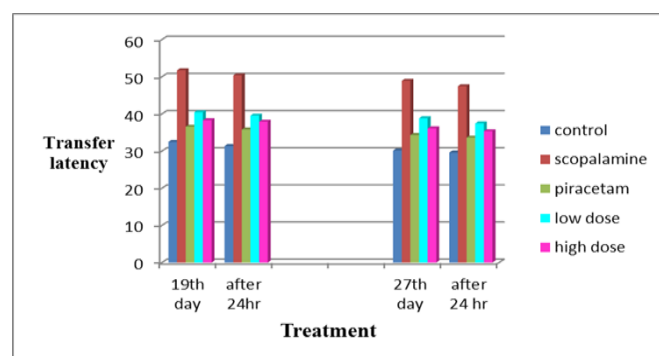


Figure no.1: Effect of apple cider vinegar on EPM task on 19th and 27th day.

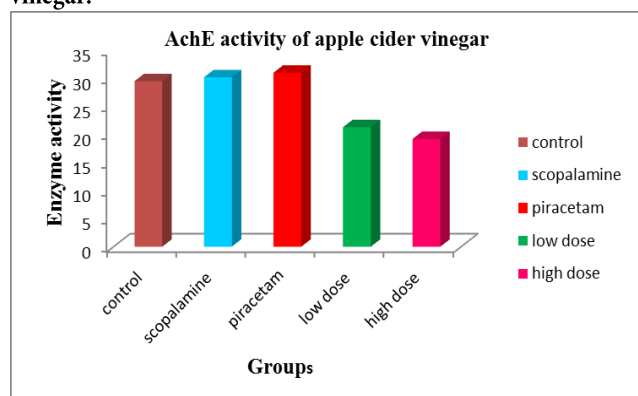
ESTIMATION OF BRAIN AchE ACTIVITY:

Changes in acetylcholine esterase (AChE) activity in isolated mice's whole brain homogenate after administration of apple cider vinegar are given in (TABLE 2), compared to control group (Group I) animals. Treatment with ACV (0.51ml/kg) significantly ($p < 0.05$) reduced the levels of AChE in the brain. Similarly (Group V) animals ACV (1.02 ml/kg) produced significantly ($p < 0.01$) reduced the level of AChE.

Table no.2: Effect of ACV on Acetylcholine esterase (AChE) activity.

Group No.	Treatment	AChE activity mean \pm S.E.M(μ mol)
I	Control	29.5 \pm 0.691
II	Scopolamine 0.4 mg/kg	30.19 \pm 0.710
III	Piracetam 400 mg/kg	31.01 \pm 0.551 ^{ns}
IV	ACV low dose 0.51ml/kg	21.20 \pm 0.610*
V	ACV high dose 1.02 ml/kg	19.20 \pm 0.630**

Figure no.2: Estimation of brain AChE activity of apple cider vinegar.

**Discussion:**

Acetic acid is a volatile organic acid that identifies the product as vinegar. Vinegar consists of about 3 to 5% of acetic acid. Apple cider vinegar pH value 3.15 and its total titrable acidity (% w/v as acetic acid) are 5. The acetic acid content is responsible for the tart flavor and pungent, biting odor of vinegars.⁴In the present study apple cider vinegar showed the total titrable acidity 4.235%. In general, vinegar samples with darker colour tended to present a higher content of total polyphenols and antioxidant capacity. The long period of maturation and aging processes yield a vinegar product with high acidity, sugar and density.

Scopolamine induced amnesic rodent model is one of the well-established animal model for memory dysfunction. Scopolamine is a nonselective muscarinic antagonist block cholinergic neurotransmission and produce impairment in

learning and memory processing. Both long term and short term memory deficit are produced by scopolamine. Scopolamine can provide a use full pharmacological tool to generate a partial model of the disorder. The cognitive enhancing activity of apple cider vinegar on scopolamine induced memory impairment in mice was investigated by using Elevated plus maze. On day 16, 17 and 18 animals were trained once daily and drug administration was continued.

In EPM model the significant decrease in TL of mice was noted Out of the two effective doses of ACV (0.51 and 1.02 ml/kg, i.p.) the long term administration of higher dose (1.02 ml/kg) produced ($P < 0.01$) better memory enhancing effect in mice as compared to the long term administration of lower dose ($P < 0.05$) in EPM model.

Central cholinergic system plays an important role in learning and memory. Decreased cholinergic firings in the brain, increased oxidative stress, hypercholesterolemia, and neuro inflammatory reactions are some of the reasons for decline in memory. Estimating the acetylcholine esterase activity provides valuable information on cholinergic function, which can correlate with cognitive function. In the present study mice brain homogenate was subjected for its AChE activity. ACV at higher dose (1.02 ml/kg) showed better result compared to lower dose which facilitates retention of learned task, therefore it may be considered as potential AChE inhibitor as anticholinesterase agent. By applying the knowledge on various researches carried out in the area of nootropic several mechanism in the form of hypothesis can be proposed to justify the activity of ACV in experimental models, various phytochemical studies showed presence of flavonoids (gallic acid, caffeic acid, ferulic acid) trace elements (iron, calcium, phosphorous, magnesium, chlorine etc) and vitamins (vitamin C, E,A,B1.B2,B6) provitamin beta carotene and polyphenol¹³. Recent studies highlighted the role of polyphenols in memory, which improves the cerebral circulation and brain metabolism which is a key factor in improvement of memory.¹⁴

Some researcher's also concluded that flavonoids (gallic acid, caffeic acid, ferulic acid) and vitamins (vitamin C, E,A,B1.B2,B6) from fruit and vegetable derived phytochemicals have a beneficial effect on

learning and memory.¹⁵ ACV produced significant memory enhancing effect in mice probably due to its antioxidant property by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improvement of neuronal function. Biochemical parameters data also suggest that ACV showed inhibition of brain acetylcholinesterase activity. It is worth for further investigations for isolation of more bioactive molecules for the treatment and using more experimental paradigms are required for further confirmation of nootropic potential of apple cider vinegar in the treatment of cognitive disorders.

Conclusion:

The ACV shown promising Nootropic activity and we can conclude that it may be due to synergistic effect of phytoconstituents like flavonoids, (gallic acid, caffeic acid, ferulic acid) vitamins (vitamin C,E,A,B1.B2,B6) and polyphenols present in apple cider vinegar. Therefore we can conclude that flavonoids, polyphenols and vitamins present in ACV may be responsible for the Nootropic activity.

Acknowledgement

The authors sincerely thank Dr. A R Shabaraya, Principal, Srinivas College of Pharmacy, Mangalore, for his support and encouragement and for providing all the necessary facilities.

References:

1. Anita Rani Shiksharathi, Stuti Mittal and JaspreetRamana. Systematic Review of Herbals as Potential Memory Enhancers. International Journal of Research in Pharmaceutical and Biomedical Sciences 2011; 2 (3): 918-924.
2. Shyamal K, Sandip Pal, Malay K Ghosal Dementia Indian scenario. Neurol India 2012; 60(6):618-624.
3. Ajaykumar T V, Anandarajagopal K, Jainaf R A M, Venkateshan N, Ananth R Antihyperlipidemics. Effect of apple cider vinegar on Lipid profiles. International Journal of Biological & Pharmaceutical Research 2012; 3(8):942-945.
4. Amin T. Hamed, Raed A. Matar. The Effect of Apple Cider Vinegar and Grape Vinegar on Lipid Profile in Albino White Rats. Jordan Journal of Pharmaceutical Sciences 2014; 7(3):163-170.
5. Zahra Beheshti1, YiongHuakChan,Hamid Sharif Nia. Influence of apple cider vinegar on blood lipids .Life Science Journal 2012; 9(4):2431-2440.
6. How To Make Apple Cider Vinegar At Home <http://onehundredoneways2life.hubpages.com/hub/HowToMakeAppleCiderVinegarAtHome>
7. PoojaSaha, Soumitra Banerjee. Optimization Of Process Parameters For Vinegar Production Using Banana Fermentation. International Journal of

Research in Engineering and Technology 2013; 2(9):501-513.

8. Mohamed eOA Mohamed SM, Mohamed KA. The effect of cider vinegar on some nutritional and physiological parameters in mice. J Egypt Public Health Assoc. 2001; 76 (1-2):17-36.
9. MeeraSumanth ,Mamatha K. Assessment of learning and memory enhancing Activity of *phoenix dactylifera* (date) in rats. World Journal of Pharmaceutical Research 2014;3(10):1215-1225.
10. Mani V, Milind P. Memory Enhancing activity of *Thespesiapopulnea* in rats. Journal of pharmacy and bioallied science 2007; 45: 267-73.
11. Dinesh D, Milind P, Kulkarni S.K. Memory Enhancing activity of *Glycyrrhizaglabra* in mice. Journal of Ethanopharmacology 2004; 91:361-65.
12. AshwlayanVrishDhwaj, Ranjit Singh. Reversal Effect Of Asparagus Racemosus Wild (Liliaceae) Root Extract On Memory Deficits Of Mice. International JournalOf Drug Development & Research 2011; 3 (2): 314-323.
13. Carol S. Johnston PhD, RD Cindy A. Gaas, BS. Vinegar Medicinal Uses and Antigliyemic Effect. Medscape Journal Medscape 2006; 8(2):61.
14. SharadhaSrikanth, Joel Chandrakanth ,G. Krishna Mohan et al Evaluation Of Green Tea For Its Nootropic Activity. Asian Journal of Pharmaceutical Research and Development 2013; 1(6): 121-12.
15. MeeraSumanth, K Mamatha. Learning and memory enhancing activity of *Ficuscarica* an experimental study in rats. Drug development and therapeutics 2014; 5(2):123-126.

Cite this article as:

Sowjanya, Dr. Satish.S, Dr. Karunakar Hegde. Dr. Shabaraya AR. Evaluation of Nootropic Potential of Apple Cider Vinegar in Validated Experimental Animal Models. Asian Journal of Pharmacology and Toxicology, 04(14), 2016, 16-20.