ejpmr, 2018,5(02), 412-415

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

LEARNING AND MEMORY ENHANCING ACTIVITY OF A CLASSICAL FORMULATION- ASHVAGANDHADHYA GHRITA, IN SWISS ALBINO MICE.

Dr. Vasavdutta Kothari*[1], Dr. B. J. Patgiri[2], Dr. Mukesh Nariya[3] and Prof. P. K. Prajapati[4]

[1]*Head, RS & BK Dept., IIAPS, GAU, Jamnagar.
[2]Head, RS & BK Dept, IPGT & RA, GAU, Jamnagar.
[3]Head, Pharmacology laboratory, IPGT & RA, GAU, Jamnagar.
[4]Director & Head, RS&BK Dept., AIIA, New Delhi.

***Corresponding Author: Dr. Vasavdutta Kothari**
Head, RS&BK Dept., IIAPS, GAU, Jamnagar.

Article Received on 11/12/2017 **Article Revised on 01/01/2018** **Article Accepted on 22/01/2018**

ABSTRACT

Ashvagandhadya ghrita is a classical formulation stated in Chakradutt, Vatavyadhi chikitsa. Its ingredients are Ashvagandha kvatha, Ashvagandha kalka, godugdha and goghrita. The individual ingredients have medhya, rasayan, jivaniya etc. properties. Medhya means that which is beneficial for the brain - i.e. beneficial for the intellect, memory and mental abilities. Thus this formulation may have learning and memory enhancing effect and so this was studied by animal experimentation in Swiss albino mice. However, regarding the preparation of this ghirta as per reference, it can be prepared in three days as it contains milk and it can also be prepared in twelve days as it contains roots. So this ghrita was prepared in three days (sample B) and in twelve days (sample C). One sample of this ghrita with Gir cow goghrita and fresh root decoction was also prepared (sample E) as Gir cow goghrita is considered to be superior and as per classical reference Ashvagandha should be used in the fresh state. The result showed that all the samples had learning and memory enhancing activity, the sample E having greatest effect followed by sample B, at a statistically significant level. Sample C did show this activity but it was not at a statistically significant level.

KEYWORDS: Nootropic, Ashvagandhadhya ghrita, learning and memory enhancing activity, Alzheimer's disease.

INTRODUCTION

Learning and memory enhancers or nootropics are the need of the hour in this era of competition and fast life. Studies have found that Ashvagandha improves memory, decreases oxidative brain stress and helps prevent nerve cell degeneration. It has a cognition promoting effect and benefitted children with memory deficit and was found to be useful in loss of memory in people of old age. It was also found useful in neurodegenerative diseases such as Parkinson's, Huntington's and Alzheimer's disease.[1]

As stated in the text Astanghridaya, Goghrita is beneficial for the intellect, memory, digestion (Jatharagni), strength, semen and eyes. It is useful for child and the aged.[2] As it aids in better digestive power it produces appropriate Dhatu (body tissues) and thereby helps to maintain the body and health.

Godugdha Milk i.e. here Godugdha (cow's milk) has the properties of being Rasayan, Medhya (beneficial for the intellect, memory and mental abilities) and is also Jivaniya (endows vitality in life and increases life span).[3]

Ashvagandhadhya Ghrita -this classical formulation is stated in Cakradutt, Vatavyadhi chapter [4] and constitutes of the above ingredients i.e. Ashvagandha kvatha, ashvagandha kalka , godugdha and goghirta. So looking to the properties of the individual ingredients it was inferred/hypothesised that the formulation as a whole would have nootropic effect and this was put forth for animal experimentation.

However as per classical reference, as this formulation contains milk it should be prepared in three days and as it contains roots it should be prepared in twelve days.[5] So the two samples were prepared –sample B –the ghrita prepared in three days and sample C- the ghrita prepared in twelve days. The sample E was also prepared with Gir cow ghrita and fresh root decoction in twelve days as gir cow ghrita is considered to be superior and as per classics Ashvagandha should be used in the fresh state.[6]

MATERIALS AND METHOD

Animals

Healthy Swiss albino mice of either sex were used for the experiment. Animals were obtained from Animal house attached to Pharmacology laboratory, IPGT&RA,

Gujarat Ayurved University, Jamnagar. The experimental protocol was approved by the Institutional Animal Ethics Committe (IAEC/20/2016/08) in accordance with the guideline formulated by CPCSEA, India.

The animals were kept under standard environmental conditions and fed standard diet (Amrut brand rat pellet feed supplied by Pranav Agro Ltd.). Animals were acclimatized for 15 days before experimentation.

Preparation of test formulation

The test drug i.e. Ashvagandhadhya ghrita was prepared in RS&BK dept., IPGT&RA, Gujarat Ayurved University, Jamnagar, as per reference of Cakradutt, Vatavyadhi.[4]

Ingredients

Goghrita-1 part

Ashvagandha Kalka (paste)- 1/4th part

Ashvagandha kvatha (decoction)- 4 parts [the decoction is prepared by adding eight times water to the yavakuta churna (coarse powder) of Ashvagandha, heating it till reduced to 1/4th part, then filtered and taken for use].

Godugdha -4 parts.

Procedure: The paste and decoction of Ashvagandha root are prepared and this along with Goghrita and Godugdha are put together in a vessel and heated till all moisture evaporates as indicated by the tests of this kalpana (dosage form) i.e., sneha kalpana. Then it is filtered and the ghrita obtained is allowed to cool and then stored in appropriate container.

In this way this ghrita was prepared in three days(sample B) and twelve days (sample C). A sample of this ghrita was also prepared with Gir cow goghrita and fresh root decoction (sample E).

Dose fixation

Dose of the drug was calculated by extrapolating the human therapeutic dose to animal on the basis of body surface area ratio (conversion factor 0.0026 for mice) by referring to the table of Paget and Barnes (1964).

The adult human dose was taken as 24 g/day

Mice dose =Therapeutic human dose × conversion factor for mice of 20 g.

=24g× 0.0026 =62.4mg for 20g mice=3.12gm/kg body weight of mice.

Anupan: Anupan of lukewarm water was given to the groups B, C and E.

Route of drug administration:

The ghrita was administered according to body weight of the animal via oral route with the help of oral feeding canula. The test drug and vehicle were administered between 8.30 to 9.30 am.

Evaluation for learning and memory enhancing (nootropic) effect

Transfer latency test in mice (Kulkarni ,1999)

Mice of either sex weighing between 25-35 gms were randomly divided into four groups each consisting of six as follows.

Control Group –Received distilled water (10ml/kg, po)

Group B-Ashvagandhadya ghrita prepared in three days (3.12g/kg,po)

Group C-Ashvagandhadya ghrita prepared in twelve days (3.12g/kg,po)

Group E-Ashvagandhadhya ghrita prepared with fresh root decoction and Gir cow ghrita (3.12g/kg,po)

Drugs were administered to respective groups B, C & E for one week and on 8^{th}, 9^{th} and 10^{th} day the actual experiment protocol was carried out. i.e. the drug was administered for total 10 days.

This test was carried out using an elevated plus maze for mice. In this test the effect of test drug on the transfer latency that is latency of first entry into the closed arm after placing the animal at the end of open arm of the elevated plus maze was noted down. Each mouse was placed at the end of the open arm of the elevated plus maze. This resulted in prolonged latency for the first entry in the close arm on the first day. The latency of entry will be shortened on the second exposure of the animals to the plus maze on the second day. This indicates establishment of memory about the first exposure to the instrument. Three different protocols were assessed.

a. Acquisition(learning)
b. Retention (memory)
c. Drug discrimination model (learning and memory)

On the first day, the animals were placed individually at the end of an open arm. If the animals do not enter an enclosed arm within 180 seconds, it was pushed on the back into one enclosed arm and the transfer latency was given as 180 seconds. The animal was allowed to move freely to explore the apparatus for some time. The transfer latency was again recorded 24 hour after first exposure (on 2^{nd} day). The transfer latency on the first day trial serve as acquisition (learning) and the retention/ consolidation (memory) was examined on 2^{nd} day, 24 hour after the first exposure.

Further on third day, one hour after test drugs, scopolamine (an amnesic drug) was administered (5mg/ kg, orally) to all groups. After one hour the animals were exposed to the elevated plus maze and the transfer latency was recorded. The difference between this transfer latency and the previous day's transfer latency may serve as an "index of cognition". Memory enhancing (nootropic) drug reverse this effect of the amnestic drug. This model is a representative test for testing spatial memory.

RESULT AND OBSERVATION

I Evaluation for learning and memory enhancing (nootropic) effect

Groups	Transfer latency (Sec.)							
	Initial on 1st day	After 24 hr on 2nd day	% change to initial	% change to control	1 hr after Scopolamine on 3rd day	% change to initial	% change to control	% change to 2nd day
Control	137.00±37.09	117.20±31.30	14.50↓	--	292.60±7.40*$$	113.57↑	--	149.65↑
Group B	106.33±25.45	42.50±10.46*#	60.03↓	63.73↓	130.33±41.00##	22.57↑	55.45↓	206.65↑
Group C	92.00±06.36	77.00±13.81	16.30↓	34.30↓	236.67±43.53*$	157.25↑	19.11↓	207.36↑
Group E	90.33±09.61	62.33±17.74	30.99↓	46.81↓	150.50±49.35#	66.61↑	48.56↓	141.45↑

Data: Mean ± SEM, ↑-Increase, ↓-Decrease

*P<0.05, **P<0.01 when compared to initial on 1st day; $P<0.05, $$P<0.01 when compared to initial on 2nd day (Paired't' test)

#P<0.05, ##P<0.01when compared with normal control group (Unpaired't' test)

The effect of test drugs on memory was evaluated by noting performance of the animals on elevated plus maze. Protocol of three consequent days represents the first day for acquisition, the second day for retention/consolidation (memory) and on the third day effect of drugs on scopolamine induced amnesia in the elevated plus maze was noted.

The transfer latency on the second day decreased in all groups which show the establishment of memory after the first exposure of the animals.

Significant increase in transfer latency in control group on third day shows that the amnestic effect of scopolamine had developed at a significant level in the control group.

Similarly group B shows significant establishment of memory on second day and it also shows a significant decrease in transfer latency compared to control on second day which indicates that the Ashvagandhadhya ghrita prepared in three days has a learning and memory enhancing effect at a significant level. Moreover, this group also shows a significant decrease in transfer latency on the third day one hour after giving scopolamine which indicates that the effect of amnesic drug is counteracted .i.e. Sample B of Ashvagandhadhya ghrita has a memory enhancing effect at a statistically significant level.

Groups C and E also show decrease in transfer latency on second day compared to control but the effect was non- significant.

Group C shows a significant increase in transfer latency on third day compared to second day and on third day compared to first day which shows the significant effect of scopolamine on the animals. However, though there is a decrease in transfer latency on third day compared to control the effect was non-significant.

Group E shows a significant decrease in transfer latency on third day compared to control which shows that the sample E of Ashvagandhadhya ghrita also has a significant effect in counteracting the amnestic effect of scopolamine i.e. sample E has a memory enhancing effect at a statistically significant level.

DISCUSSION

(All the groups i.e. B, C and E show memory enhancing effect, B and E are better being effective at a significant level and show almost similar efficacy but E has the highest significance.).

In the elevated plus maze paradigm scopolamine was used to induce amnesia in the mice. This cholinergic muscarinic antagonist is widely used to induce amnesia in the experimental animals. Studies show that acetylcholinesterase inhibitors, which enhance the availability of acetylcholine(ACh) in the synaptic cleft, were able to reverse the scopolamine-induced deficit, indicating a neurotransmitter role of ACh in learning and memory. Muscarinic type 1 receptor antagonists, such as pirenzepine and the nicotinic antagonist mecamylamine, also have a negative effect on learning and memory performance. Also, many brain lesion studies in which specific cholinergic deafferentation of different brain structure have yielded a decline in cognitive performance.[7]Thus there is a role of ACh in memory and learning and from the above it can be inferred that Ashvagandhadhya ghrita acts at the neurotransmitter ACh level.

Further, HPLC analysis of Ashvagandhadhya ghrita showed the presence of Withanone and WithanosideIV in all the samples of this ghrita. Both of these are associated with neuroprotective property and are useful in learning and memory and in ameliorating dementia.

A study indicated that besides cholinergic blockade, scopolamine-induced memory loss may be associated with oxidative stress and that Ashwagandha leaf extract or its purified component-withanone showed recovery from this and may serve as potential preventive and therapeutic agents for neurodegenerative disorders.[8] Thus withanone present in Ashvagandhadhya ghrita may act at this level.

It has been found that oral administration of withanoside IV significantly improved memory deficits in Abeta (beta amyloid)-injected mice and prevented loss of

axons, dendrites, and synapses. Sominone, an aglycone of withanoside IV, was identified as the main metabolite after oral administration of withanoside IV. Sominone induced axonal and dendritic regeneration and synaptic reconstruction significantly in cultured rat cortical neurons damaged by Abeta. Thus withanoside IV may ameliorate neuronal dysfunction in Alzheimer's disease (a chief cause of senile dementia).[9]

Acharya Caraka states Ghrita to be Smritivi vardhak and Buddhi vivardhak i.e. promotes memory and intellect.[10]

Ghrita contains unsaturated fatty acids like Docosahexaenoic acid- DHA- Omega-3 long-chain polyunsaturated fatty acid, which is a major component of retinal and brain tissues and remains important in prevention of various diseases.[11] Godugdha i.e. cow's milk is considered to increase the Oja and is jivaniya and rasayan.[12] A research conducted suggests that drinking milk could improve the functions of the brain.[13]

Ayurvedic perspective

Classically, as per Acharya Charaka, Vayu is 'Tantrayantradhar' and 'Niyantaprerentaca Mannasaha' - means Vata Dosha is responsible for appropriate functioning of the body and body organs. Moreover it initiates the mind for conducting its functions and also controls it so that it works appropriately. The sense organs also are capable of perceiving their respective subjects due to vayu. In short, Vata Dosha is responsible for initiation and smooth conduction of all the bodily functions and maintaining the body. Amongst the five types of Vayu, Udana and Prana vayu are related with mind, uthsaha(enthusiasm), functioning of all Indriya (sense organs)etc. Thus vitiation of Vata leads to disruption of these functions. Ashvagandhadya Ghrita in Cakradutta has been stated for the treatment of Vata Vyadhi and so it can be inferred that it will pacify the vitiated Vata Dosha. So this formulation can be helpful in rectifying the vitiated Prana and Udana Vayu and thereby carry out their functions appropriately.[14]

Thus in light of above Ashvagandhadhya ghrita acts not only as learning and memory enhancer (nootropic) but also may ameliorate diseases like Alzheimer's, Parkinson's etc. neurodegenerative diseases.

CONCLUSION

As hypothesised Ashvagandhadhya ghrita produced learning and memory i.e. nootropic effect in animal experimentation. (in Swiss albino mice.). The group E showed the greatest effect which could be due to the use of Gir cow goghrita and fresh root decoction in the formulation. The group B showed better result compared to group C which indicates that when milk is an ingredient then the paka kala i.e. the ghrita should be prepared in three days. This ghrita may also be useful in neurodegenerative diseases like Alzheimer's, Parkinson's etc.

REFERENCES

1. Bhattacharya SK, Goel RK, Kaur R, Ghosal S. Anti - stress activity of Sitoindosides VII and VIII;'New Acylsterylglucosides from *Withania somnifera'*. Phytother Res, 1987; 1: 32–37.
2. Kaviraj Atrideva Gupta.Vidhyotini commentary of Astangahriday, Sutrasthan 5, Varanasi; Chaukhambha prakashan; reprint, 2007; 44-45 .
3. Kaviraj Atrideva Gupta. Vidhyotini commentary of Astangahriday, Sutrasthan 5, Varanasi; Chaukhambha prakashan; reprint, 2007; 42-43
4. Priya Vrat Sharma. Cakradutt (English translation), 3rdedition,Varanasi;Chaukhambha publishers, 2002; 209.
5. Kaviraj Shri Ambika Dutt Shastri, Vidhyotini commentary of Bhaishajya Ratnavalli, 5th chapter, Varanasi, Chowkhambha sanskruts sansthan, 2002, verse, 1310-1312, p.133
6. Dr. Brahmanand Tripathy, Dipika commentary of Sharangdhar samhita, Purva khand 1st chapter,Varanasi, Chowkhambha surbharati prakashan, 2006; verse 45: 16.
7. S.K.Kulkarni, Handbook of experimental pharmacology, Chap.2, Third edition, Delhi, VallabhPrakashan, 1999, pg.50.
8. Protective role of Ashwagandha leaf extract and its component withanone on scopolamine-induced changes in the brain and brain-derived cells.Konar Aet el, https://www.ncbi.nlm.nih.gov/pubmed/22096544 accessed on 11/12/2017.
9. Bhattacharya SK, Goel RK, Kaur R, Ghosal S., Anti - stress activity of Sitoindosides VII and VIII;'NewAcylsterylglucosides from *Withania somnifera'*. Phytother Res, 1987; 1: pg 32–37.
10. Pt.Kashinath Shastri and Dr. Gorakhnath Chaturvedi, 'Vidhyotini' hindi commentary on Caraka samhita, Part-I,Sutrasthan 27, Varanasi;Chaukhambha Bharati Academy; Reprint 2005; verse 231-232 .
11. Kalpana S. Joshi, Docosahexaenoic acid content is significantly higher in *ghrita* prepared by traditional Ayurvedic method', https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4061595/J Ayurveda Integr Med. 2014, Apr-Jun; 5(2): 85–88, accessed on 5/11/2017.
12. Pt.Kashinath Shastri and Dr. Gorakhnath Chaturvedi, 'Vidhyotini' hindi commentary on Caraka samhita, Part-I,Sutrasthan 27, Varanasi;Chaukhambha Bharati Academy; Reprint 2005; verse 217-218 .
13. How Milk Can Benefit Your Brain & Boost Memory http://food.ndtv.com/health/how-milk-can-benefit-your-brain-boost-memory-749570, accessed on 5/11/2017.
14. Vasavdutta et al, Ashvagandhadhya ghrita –A classical formulation in a new perspective, IJAPR, May 2017; 5(5).