ASSESSMENT OF NOOTROPIC ACTIVITY OF VACHADI GHrita, A MEDICATED GHEE FORMULATION USING ANIMAL MODELS

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ABSTRACT

Background: Vachadi ghrita is medicated ghee ayurvedic formulation, recommended to improve cognition. Synergism of herbal drugs of preparation of VG and extraction of lipid soluble extractives of these drugs in Goghrita [Cow Ghee] may show cumulative positive effect on cognition. Thus the cognitive-enhancing activities of medicated ghee formulation VG was evaluated in Morris water maze test and in diazepam induced amnesia mice model using Elevated plus maze.

Methodology: Piracetam, most widely used nootropic agent as positive control and Goghrita as negative control was employed in both tests. VG was given in X/2, X and 2X dose levels.

Results: Vachadi Ghrita at X and 2X dose showed significant reduction in decrease in transfer latency in EPM test. In Morris water maze test, escape transfer latency of Piracetam, VG X and 2X groups showed significant improvement in memory on eleventh and twenty sixth day. However VG X/2 group did not show significant results on improvement of memory compared to Goghrita group in EPM and MWM tests. Goghrita also showed positive result on retention of memory in comparison to plain control group in both models. Antioxidant properties of Vacha, Apamarga, Haritaki and Guduchi were proved in earlier researches. Synergism of these ingredients in VG might be responsible to produce neuro-protective and memory enhancement activities. Study findings suggest that Vachadi Ghrita at therapeutic dose, exhibited anti-amnesic and memory enhancement activity in EPM and MWM tests. Hence it can be employed as nootropic drug or adjuvant to conventional treatment to minimize adverse effects of these drugs.
KEYWORDS: Vachadi Ghrita, Medicated ghee, Goghrita, Nootropic activity, EPM, MWM models.

INTRODUCTION

Human learning acquires new or modifying existing knowledge, behaviors, skills and values.\textsuperscript{[1]} Memory is the process where the acquired knowledge is coded, stored and again retrieved.\textsuperscript{[2]} These processes of human being remain healthy constantly as all physical and psyche attributes exerted active functions. Owing to changed lifestyle, peer pressures, higher ambitions and dietary etiological factors; vitiation of physical entities, affliction in mind and intellect is seen. This phenomenon leads to develop increase in oxidative stress, inflammatory conditions in brain resulted into declination of memory or memory loss.

Well-planned, representative epidemiological surveys published report estimate that 24·3 million people have dementia today, with 4·6 million new cases of dementia every year. Most of the people with dementia live in developing countries and it is estimated that 60% in 2001 rising to 71% by 2040\textsuperscript{[1]} Mild cognitive impairment [MCI] is seen without dementia and crude prevalence of MCI ranged from 0.8% to 4.3% in India.\textsuperscript{[2]} Hence diagnosis and treatment of these conditions is a big challenge in front of medical fraternity. Modern system of medicine provides medicaments to treat cognitive deficits still limitations are seen in these treatment modalities, thus it is worthwhile to search for different medicines which helps to maintain memory loss of patients with neuropsychiatric disorders or improve memory functions.

Alternative medicine provides broad-spectrum therapeutic effects of herbal based medicines which would be useful in management of cognitive deficits. Universality of herbal remedies of Indian traditional medicine makes an avenue. Medicated ghee, one of the potent poly herbal dosage forms has been prescribed to treat different CNS disorders. These lipid base formulations might have potential to cross blood brain barrier and show beneficial effects on brain tissue.

Vachadi ghrita [VG] is one of the medicated ghee formulations claimed in Ayurved to improve cognition [intellect and memory].\textsuperscript{[3]}

Eight herbal drugs of VG have been reported for their antipsychotic, anti-stress, antidepressant, memory enhancer and nootropic activities.\textsuperscript{[4,5,6,7,8,9]} It is assumed that synergism
of these herbal drugs in preparation of VG and extraction of lipid soluble extractives of these
drugs in Goghrita[Cow Ghee] may show cumulative positive effect on cognition.

However, till date no any research work was carried out to assess Nootropic activity of VG.
Therefore attempt was made to find out pharmacological action of Vachadi Ghrita on animal
cognition using experimental models.

2. MATERIAL AND METHODS

2.1 Plant material and preparation of Vachadi Ghrita

Vachadi Ghrita was prepared as per standard ayurvedic guideline It contains Vacha (Acorus
calamus), Guduchi (Tinospora cordifolia), Haritaki (Terminalia chebula) Shankhapushpi
(Convolvulus pluricaulis). Vidang (Embelia ribes), Shunthi (Zingiber officinale), Shati
(Hedychium spicatum). Apamarg (Achyranthes aspera) and Goghrita s[Cow ghee].

Standard ayurvedic protocol for manufacture medicated ghee was followed.[10] Initially paste
of ingredients was made and added to liquid Goghrita. Then plain water was mixed with
continuous stirring. This whole mixture was heated on low flame till total evaporation of
water takes place and to achieve ayurvedic testing parameters.[11] It was then filtered with
cotton cloth and stored in well dried glass container. VG was then tested with organoleptic
and physico-chemical tests. It is interpreted in Table 1.

2.2 Animals

Experiments were carried out using in either sex animals. Wistar rats and Swiss albino mice
were procured from animal house, B.V.D.U. Medical College, Pune. Wistar rats weighing
around 180 gm to 200 gm and Swiss albino mice weighing around 18gm to 20gm were used.

Housing of animals was maintained with provision of 12 hours, day and night cycle. Standard
Rodent feed from Pranav Argo industry, and Aqua water ad libitum was given.

2.3 Drug doses

The recommended therapeutic dose [40 mg/70 kg] of medicated ghee[12] given in Ayurved was
extrapolated. The study was carried out in three dose levels as VGX/2, X and 2X (2.5g, 5g,
10g/ /kg) for mice and 1.75, 3.5, 7.0 gm /kg for wistar rats. Vehicle control [Goghrta] was
given in 5gm/kg for mice and 3.5 gm/kg for rat. Standard nootropic drug, Piracetam was
administered in 8mg/20gm for mice and 54mg/200gm for rats. Diazepam [CalmPose,
Ranbaxy, India] was used for induction of amnesia in animals.
Drug administration schedule: All drugs were administered to animals as per standard protocol. Thus 8 days treatment was given in diazepam induced animal model. In MWM test all animals were treated for 26 days.

2.4 Behavioral study

2.4.1: Diazepam induced amnesia in young mice[13]

Elevated plus-maze served as an exteroceptive behavioral model used to evaluate learning and memory in mice. 36 animals were grouped into six groups viz plain control, positive control, standard drug [Piracetam] and three groups of VG. All animals of each group were treated with the respective drugs orally for 8 days. On the eighth day after 45 minutes of administration of last dose of drugs, diazepam was administered (i.p.). Transfer latency [TL] was recorded after 45 min of administration of diazepam and was again noted after 24 hrs i.e. on 9th day.

2.4.2: Morris water maze [MWM] test[14]

This model is an established model used to assess learning & retention of memory activity. Circular water tank, 6 feet diameter and 3 feet in depth was used which is filled with opaque water at 26°C to 28°C temperature. Platform is circular in shape with 20 cm in diameter and 1.5 feet in height was placed in water tank at one of the quadrant. The method described by Papazova et al. was modified as follows. 36 wistar rats were grouped into six groups. All animals were treated with respective drugs orally for 7 days. Four consecutive learning trials were given to all animals on 8, 9 and 10th day in water maze with platform. Observations were made through escape transfer latency to assess acquisition of learning on 11th day. Continuous treatment with respective drugs was done for further two weeks. ETL was noted of each animal on 26th day. The obtained values of ETL on 11th and 26th day were analyzed.

Statistical Analysis: All the results are expressed as Mean ± S.E.M. Data was analyzed by one way analysis of variance (ANOVA) followed by Dunnett post hoc test in Graph Pad Prism version 5. P< 0.05 was considered as significant.

RESULTS

Table 1: Organoleptic and physico-chemical value of Vachadi Ghrita

<table>
<thead>
<tr>
<th>Organoleptic parameter</th>
<th>VG values</th>
<th>Parameter</th>
<th>VG values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Touch</td>
<td>Unctuousness</td>
<td>pH</td>
<td>5</td>
</tr>
<tr>
<td>Colour</td>
<td>Dark greenish</td>
<td>Specific Gravity</td>
<td>0.9198</td>
</tr>
<tr>
<td>Taste</td>
<td>Bitter++</td>
<td>Wt/ml</td>
<td>0.81 wt/ml</td>
</tr>
<tr>
<td>Odor</td>
<td>Aromatic</td>
<td>Free Fatty acid</td>
<td>Moisture</td>
</tr>
<tr>
<td>---------------</td>
<td>----------</td>
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<td>----------</td>
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<tr>
<td></td>
<td></td>
<td>0.81%</td>
<td>0.18</td>
</tr>
</tbody>
</table>

**Figure 1: Escape Transfer latency on 11th day in MWM test**

Values are expressed as Mean ± SEM. Data was analyzed by one way ANOVA followed by Dunnett post hoc test.

***-p<0.0001, **-p<0.001, *-p<0.01 when compared to Goghrita control group

**Figure 2: Escape Transfer latency on 26th day in MWM test**

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by Dunnett post-hoc test.

***-p<0.0001, **-p<0.001, *-p<0.01 when compared to Goghrita control group
Table 2: Transfer latency on 9th day in Elevated plus maze model

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Mean± SEM</th>
<th>95% CI of diff</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Goghrita</td>
<td>37.17±0.7923</td>
<td>35.13-39.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Distilled water</td>
<td>47.17±2.007</td>
<td>42.01-52.33</td>
<td>0.009</td>
<td>**</td>
</tr>
<tr>
<td>3. Piracetam</td>
<td>12.5±0.6708</td>
<td>10.78-14.22</td>
<td>&lt;0.0001</td>
<td>***</td>
</tr>
<tr>
<td>4. VG X/2</td>
<td>31.5±1.118</td>
<td>28.63-34.37</td>
<td>0.002</td>
<td>**</td>
</tr>
<tr>
<td>5. VG X</td>
<td>12.17±0.4773</td>
<td>10.94-13.39</td>
<td>&lt;0.0001</td>
<td>***</td>
</tr>
<tr>
<td>6. VG 2X</td>
<td>32.33±1.174</td>
<td>29.32-35.35</td>
<td>0.0066</td>
<td>**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by Dunnett post-hoc test.

***-p<0.0001, **-p<0.001 when compared to Goghrita control group

DISCUSSION

Ayurvedic classical formulation ‘Vachadi Ghrita’ contains eight herbal drugs along with Goghrita, is advocated to increase intellect and memory. Thus to create an evidence in present study assessment of effect of VG on animal memory was investigated in EPM and MWM models.

Recent studies on several nootropics and amnestic agents made EPM model a widely accepted paradigm to study learning and memory processes in rodents. In EPM, acquisition (learning) can be considered as transfer latency on first day trials and the retention of memory is examined 24hrs later. In the study pre-treatment with VG for 8 days successfully protected the animals from memory deficits produced by diazepam. VG at X dose demonstrated positive effect on retention of memory similar to Piracetam drug. These findings suggest the possible neuro-protective role of VG.

In Morris water maze model on eleventh day VG X (3.6gm/kg dose) and 2X [7.2 gm/kg] treated groups showed improvement in memory compared to Goghrita and plain control groups. VG 2X dose had better-quality results than VG X dose. However on twenty sixth day, VG X and 2X doses showed relatively equivalent effect on improvement of memory compared to both plain and Goghrita control groups. VG X/2 (1.8 gm/kg) group didn’t show any significant effect on animal memory. This indicates that lower dose of VG is not sufficient for producing desired effect in this animal model. Goghrita group showed decrease in ETL compared to plain control group which shows that Goghrita has better memory enhancement action.
Vehicle used in preparation of VG is Goghrita which makes the preparation highly lipid soluble, then easily crosses blood brain barrier. Thus helps to carry active components to specific target site [CNS]. Ayurveda has considered ghee to be the healthiest source of edible fat possesses beneficial properties and facilitate the positive effect of herbal drugs added to it in preparation of medicated ghee. It is well documented that Goghrita promotes longevity and protects normal functioning of body entities as well intellect and memory. In present study this effect of Goghrita is verified compared to plain control group in EPM and MWM models. Thus we can say that Goghrita increases the memory enhancing effects of Vachadi ghrita.

Ayurvedic science treatment focuses on use of different medicated ghee formulations in CNS disorders depending on involvement of Vata, Pitta, Kapha entities of individual. A combination of various herbal drugs with Goghrita produces different therapeutic effects as each has actions on different entities. Brahmyadi, Amalakyadi, Panchagavya, Kushmand and Vachadi Ghrita formulations are recommended in various CNS conditions.

Ayurvedic pharmacology explains actions of each ingredient of VG according to their properties. These drugs possesses Ushna (hot), Teekshna (penetrating) properties and Vata-Kaphashamak activities. As per ayurvedic theory it is said that the cumulative effect of these ingredients is seen in final product i.e. Vachadi Ghrita. Hence probably VG is useful in the treatment of memory impairment occurred due to Kapha-Vata dominance and can be used to improve intellect and memory.

In earlier research works, active constituents of Vacha [A- asarone, B-asarone] showed antioxidant activity towards brain tissue. Phenolics, flavonoids, tannins present in Vacha, Apamarga, Haritaki and Guduchi drugs have been proven for their antioxidant activities. Synergism of these ingredients of VG might be showing antioxidant and neuro-protective activities and thus producing memory enhancement activity. In our study also anti amnesic and improvement of memory actions of VG are determined in diazepam induced amnesia (Table 2) and in MWM animal models [Fig 1 and 2].

This study has created evidence that therapeutic dose [40 gm] of Vachadi ghrita given in ayurvedic literature is quite sufficient dose which has produced desired effect on animal cognition. As observed in study low dose of VG might be very less so couldn’t produce significant increase in memory compared to VGX and 2X doses. Therefore as a result,
Vachadi Ghrita can be used as nootropic agent to treat as well as prevent impairment of memory. It can also be used as adjuvant with conventional antipsychotic and antiepileptic drugs to minimize memory impairment effects of these drugs.

**Limitation:** Study should be conducted using other models for learning and memory. We have not assessed other neurological and systemic adverse effect.

**CONCLUSION**
Anti-amnesic and memory enhancement activity of Vachadi Ghrita at therapeutic dose is exhibited in Diazepam induced amnesia and Morriz Water Maze animal models. VG has potential to improve memory as shown in both models, thus it may serve as protective and therapeutic medicine in cognitive disorders like Alzheimers and Dementia.

**ACKNOWLEDGMENT**
The authors are sincerely grateful to Dr. Vijaya Pandit, Head of department of Pharmacology, Bharati Vidyapeeth Medical College for providing essential facilities to conduct this study and also the Lab-technicians for support of technical assistance.

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