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EFFECT OF *BRAHMI GHRITA* ON NEURO-INFLAMMATION IN MCI DUE TO ALZHEIMER'S DISEASE

Ayurveda

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ABSTRACT

The transitional stage between the cognitive changes of normal ageing and dementia is known as "Mild cognitive impairment" (MCI). MCI due to Alzheimer's disease is also an intermediated stage in the process of Alzheimer's disease.

Although vigorous research is going all over the world, the exact diagnosis as well as the most hopeful therapy i.e. disease alteration therapy is being compromised because of lack of biomarkers and other pathologic events. Significant evidence suggests that inflammation has a contributory role in Alzheimer's Disease pathogenesis[1,2] and understanding and control of interactions between the immune system and nervous system might be key to prevention or delay of disease [3,4]. In Alzheimer's Disease, neuro-inflammation plays almost equal role to the pathogenesis as senile plaques and neuro fibrillary tangles (NFTs). TNF-α[5], is one of the main inflammatory cytokines and Interleukin 10 (IL-10) is an anti-inflammatory cytokine involved in initiating and propagating an inflammatory response. This study shows the positive response of a *Brahmi Ghrita* (polyherbal *Ayurvedic* formulation) on these inflammatory markers.

KEYWORDS

Mild cognitive impairment, *Ayurveda, Brahmi Ghrita, Polyherbal*

INTRODUCTION

Alzheimer's Disease is a progressive terminal form of dementia. More recent research has pivoted focus to inflammation-mediated responses as a major contributor to the development of cerebrovascular diseases, and evidence suggests that inflammation promotes pathological processes that lead to AD.[11–13]

Pathological accumulation of Aβ plays a key factor in initiating neuroinflammatory responses in Alzheimer's Disease. The activated microglia are responsible for fibrillar Aβ deposition [4,6], and it is quiet early event in the pathogenesis of AD which leads to the accumulation of the Aβ and has been reported to precede extensive tau-related neurofibrillary pathology [7,8,9]. This finding of proteins at preclinical stage or at the stage of mild cognitive impairment may be able to predict clinical AD diagnosis with high accuracy [10,12].

Among the cytokines involved in neuro-inflammation, tumor necrosis factor α (TNF- α) is the most studied and plays an essential role in the cytokine cascade during an inflammatory response. Although the levels of TNF-α in the periphery and central nervous system (CNS) of healthy adults are maintained at very low levels, the levels of this cytokine are significantly elevated in blood[14] and CNS[12] of patients with AD, and many clinical and animal studies have demonstrated a link between excess TNF-α levels in the brain and AD[13]. Elevation in TNF-α levels in the cerebrospinal fluid (CSF)[18] and serum[19,20] of patients were found to be directly proportional to Alzheimer's Disease progression.[20] Also, reduction in production of IL-10 has been claimed as a characteristic of AD patients[15]. IL-10 is synthesized in the central nervous system and is known to restrict clinical symptoms of many neurological diseases, including AD. In fact, expression of IL-10 is elevated during the course of most major neurological diseases and promotes the survival of neurons and all glial cells in the brain by limiting inflammation[16,17]. On this basis, we have studied TNF-α and IL 10 as potential therapeutic criteria for evaluating the effect of therapy on the level of these cytokines for AD.

Our aim was to investigate the inflammatory response in serum samples of Mild Cognitive Impairment due to AD and to examine whether the prescribed *Ayurvedic* Drug *Brahmi Ghrita* (As. Hr. Ut. 6/23- 23) is associated with positive therapeutic response of inflammatory markers. We conducted the trial on 57 diagnosed patients of MCI due to AD. The cases were compared with patients undergoing conventional treatment of MCI (Donepezil 10mg/day-control) and the data was evaluated with respect to inflammatory markers and their rate of change in three months of trial period.

Diagnosis of MCI due to AD was performed according to standard clinical procedures and followed the revised guidelines of Alzheimer's Association (previously known as Alzheimer's Association and ADRDA, revised in 2011).[51]

MATERIALS AND METHODS

It was an open label, single centered, comparative, prospective, pragmatic trial. The trial protocol and related documents were reviewed and approved by the Institutional Ethical Committee Registration no ECR/526/Inst/UP/2014, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., India. The study was conducted in accordance with Indian Council of Medical Research (ICMR) ethical guidelines for biomedical research on human participants.

Objectives

To evaluate efficacy of *Ayurvedic* formulation *Brahmi Ghrita* in the subjects suffering from Mild cognitive impairment due to Alzheimer's Disease by assessing change in inflammatory markers TNF a and IL10 in all the samples.

Trial interventions – *Brahmi Ghrita* at a dose of 12 gm was given twice a day empty stomach (*Pranodana Kala*) with luke warm water as *Anupana* for 90 days. The trial drug was acquired from the Ayurvedic Pharmacopoeia of India complied GMP (Good manufacturing practice) certified company.

Inclusion criteria

Subjects of either gender, age between 40 years and above, fulfilling the criteria of MCI due to AD (progressive since minimum six months), without any other probable and possible cause of dementia and willing to participate in the study for 90 days were included in the study.

Exclusion criteria

Patients with any kind of kidney disorders, early onset of a focal seizure, previous or ongoing depression, early prominent gait disturbance with only mild memory loss, resting tremor with stooped posture, when dementia occurs after a well-established diagnosis of PD, the early appearance of Parkinsonian features in association with fluctuating alertness, visual hallucinations, chronic alcoholism, vitamin B12 deficiency, chronic drug intoxication and dementia with any other explainable cause. Further, patients with gross disability in performing daily normal routine, past history of atrial fibrillation, acute coronary syndrome, myocardial infarction, stroke or severe arrhythmia in the last 6 months, severe renal or hepatic disorders, pregnant and lactating woman were also excluded from the study.

Withdrawal criteria

The subjects were free to withdraw themselves from the trial at any time without the authorization of researcher or any reason.

Study procedures

Informed written consent was taken from the patient on the first visit.

General, systemic and biochemical examinations were done. Total 57 subjects who fulfilled the inclusion and exclusion criteria were enrolled for the trial. The patients who were on any kind of anti inflammatory treatment or anticholinergic treatment were kept on a 28 days washout period before enrollment. The screening of enrolled subjects were done by ADAS –cog and MMSE. The enrolled subjects were randomly divided into two groups. Group I – were treated with Donepezil 10 mg/day for next 90days. Group II – was treated with *Brahmi Ghrita* 12 gm empty stomach BD (*Pranodan Kala*) with luke warm water for 90 days.

Follow-up assessment

Subjects were visited for follow-up visits on day 30 (Visit 1), day 60 (Visit 2), day 90 (Visit 3). On each follow-up visit, patient's general and systemic physical examinations were done. Assessment of the cognitive and other symptoms of MCI were done on ADAS –cog and MMSE on Day 0 and day 90. Blood serum samples were collected on Day 0 and Day 90 for recording the change in level of inflammatory markers.

Statistical analysis

The analysis of the data using statistical software SPSS 15.0 data describing quantitative measures are expressed as median or mean ± SD or SE or the mean with range. Comparison of variables representing categorical data was performed using paired t test and one-way ANOVA. All p (probability) values are reported based on two-sided significance test and all the statistical tests are interpreted as significance at 5% level ($p < 0.05$)

Sample Preparation and Data Acquisition

Sample collection- All participants were required to fast for two hours before blood sample collection; only water or fluids containing no milk or sugar were allowed during the fasting period. Serum samples were collected in plain bulbs and centrifuged at 3000 rpm for 10 minutes at 4°C before being aliquoted and then frozen at −20°C.

Human TNF-a ELISA kit and IL-10 ELISA kit was purchased from E lab science and the samples were prepared according to manufacturer's manual.

RESULTS

The results were assessed on the basis of the presence of pro-inflammatory and anti-inflammatory cytokines TNF-a and IL-10 respectively in blood serum of patients suffering from MCI due to AD. The samples of Day 1 and Day 90 were compared in the trial.

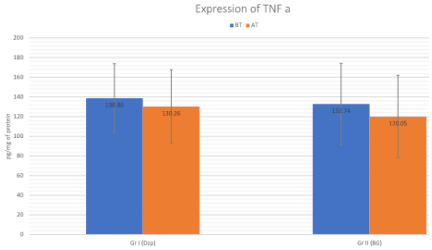

Fig 1 Effect of Donepezil and Brahmi Ghrita on the level of TNF a. the graph shows initial and final recording at the interval of 90 days. The levels of TNF a was found to be decreased in both the groups which are statistically significant in both groups

The intergroup comparison of mean TNF a after treatment was not statistically significant.

IL-10

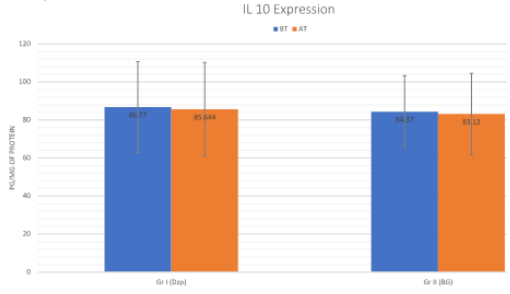

Fig 1 Effect of Donepezil and *Brahmi Ghrita* on the level of IL10. The graph shows initial and final recording at the interval of 90 days. The levels of IL 10 was found to be decreased in both the groups in which it was statistically significant in Donepezil group while it was not significant in *Brahmi Ghrita* group making *Brahmi Ghrita* better.

The intergroup comparison of mean IL 10 after treatment was not statistically significant.

DISCUSSION

- In AD, several mechanisms which aggravate the neuro-inflammatory response like- accumulation and sustained exposure to Amyloid ß, chemokines, cytokines, interleukins, nitric oxide, neuronal debris and other potentially cytotoxic molecules seems to be responsible for persistent functional impairment of microglial cells seen at plaques site.
- Physiologically, APP is broken down by sequential cleavage in soluble part and less soluble Amyloid ß fibrils. These fibrils when accumulate they form senile plaques which may be termed as "*Aam*" (undigested byproducts) as per *Ayurvedic* definition. There *Dhatwagni* (metabolic catalysts) in the form of scavenging receptors try to get rid of this "*Aam*" as a result of which there is production of proinflammatory cytokines and chemokines. *Manda* (feeble) *Dhatwagni* is responsible for downregulation of expression of Amyloid ß and phagocytosis receptors, increased cytokine concentration and neuronal debris resulting in inefficient clearance of Amyloid ß establishing chronic, non-resolving inflammation.
- Likewise, astroglia which are responsible for neuroprotection and recovery of injured neuronal cells when undergo atrophy or when exposed to this Amyloid ß (*Aam*) aggravate the neuro-inflammatory response which directly affects the synaptic conduction.

So particularly *Agni Vardhan* (increasing the power of digestion), *Aam Pachan* (proper digestion of neuronal debris and other toxic undigested materials) , *Aam Shodhan* (flushing out of toxic metabolic byproducts) and neuroprotection are the desirable treatment for Alzheimer's Disease

Brahmi Ghrita Is a perfect combination of drugs for this condition.
Constitutions of *Bramhi Ghrit* (As. Hr. Ut. 6/23- 23)

- *Brahmi Swaras- Bacopa monnieri* Fam. Sorophulariaceae
- *Goghrit*
- *Shunthi – Zingiber officinale Roscoe* Fam. Zingiberaceae
- *Maricha – Piper nigrum Linn.* Fam. Piperaceae
- *Pippali – Piper longum Linn.* Fam. Piperaceae
- *Shyama Trivrit- Operculina turpethum* Fam. Convolvulaceae
- *Danti- Boliospermum montenum Muell- Arg* Fam. Euphorbiaceae
- *Shankhapushpi- Convolvulus pluricaulis, Chois.* Fam. Convolvulaceae
- *Chhitvan Twak – Alstonia scholaris R. Br.* Fam. Apocynaceae
- *Vidang – Embelia ribes Burm.* Fam. Myrsinaceae

Here we can see that the drugs have a unique action on neuroinflammation as well as neuroprotection and improvement of cognition. e.g.

***Shunthi* (Zingiber officinale)-** 10 gingerol found in fresh ginger is responsible for anti neuroinflammatory property. It was found to inhibit the production of many pro inflammatory factors including TNF-a[22]. Moreover 6 gingerol was also found to possess anti neuroinflammatory property in animal models[23]. Ginger extracts were found to inhibit ß -amyloid peptide induced cytokine and chemokine expressions in cultured TMP-1 monocytes[24].

Ayurveda: Indicated for *Shotha* (inflammation) and responsible for absorption of metabolic by-products.

***Pippali* – Piper *longum Linn.* -** *Piper longum* Linn. Extract inhibits TNF-α-induced expression of cell adhesion molecules by inhibiting NF-κB activation and microsomal lipid peroxidation[25].

Ayurveda: responsible for degradation of metabolic byproducts, increasing digestive cellular metabolism and have rejuvenating property.

***Maricha* – Piper *nigrum* Linn.** - The effect of isolated piperine from *Piper nigrum* fruits on memory and behavior mediated via monoamine

neurotransmitters was investigated. Elevated plus maze and passive avoidance paradigms were used as exteroceptive behavioral models, whereas scopolamine induced amnesia was the interoceptive behavioral model. The effects on lithium induced head twitches, clonidine-induced hypothermia, and halperidol-induced catalepsy were also observed to study the effect on serotonin, noradrenaline and dopamine mediated behavior, respectively. Piperine isolated from *P. nigrum* exhibited prominent nootropic activity, reversed clonidine-induced hypothermia, decreased lithium induced head twitches and significantly delayed haloperidol induced catalepsy at a dose of 10 mg/kg. The alkaloid modified 5-HT and NA mediated behavior. Hence, piperine from the fruits of *P. nigrum* can be employed as a potential nootropic agent[26].
Ayurveda: Increases digestive fire, hot, *Kapha Vata* pacifier

***Shankhpushi* (Convolvulus *pleurecaulis*)-** Scopolamine is known to produce amnesia due to blockade of the cholinergic neurotransmission. Oral administration of CP extract (150 mg/kg) in scopolamine treated rats was found to reduce the increased protein and mRNA levels of tau and APP levels followed by reduction in A levels compared with scopolamine treated group. The potential of extract to prevent scopolamine neurotoxicity was reflected at the microscopic level as well, indicative of its neuroprotective effects[27].
Ayurveda: Rejuvenator, improves cognition, enhances memory, suitable for psychological disorders

***Brahmi* (Bacopa *monnieri*) -** The effect of Bacopa M. on memory and cognition has been extensively studied. Various extracts of Bacopa were prepared and examined in the N9 microglial cell line in order to determine if they inhibited the release of the proinflammatory cytokines TNF-α and IL-6. Bacopa inhibits the release of inflammatory cytokines from microglial cells and inhibits enzymes associated with inflammation in the brain. Thus, Bacopa can limit inflammation in the CNS, and offers a promising source of novel therapeutics for the treatment of many CNS disorders[28].
Ayurveda: Improves cognition, Rejuvenating, anti-inflammatory, enhances memory,

***Saptaparna* (Alstonia *scholaris*) -** It also exhibits neuroprotective effect – Neuroprotective activity of Ethanolic Extract of Alstonia scholaris Linn. Leaves against global model of Ischemia in Rats.[29] An experiment done by Kumar et al in 2003[30] revels that Alstonia scholaris has strong In vivo and In vitro inhibitory effect on acetylcholine esterase of Lymnaea acuminate.

In *Ayurveda* although there is no direct reference of Alstonia scholaris acting as nootropic agent but it has been mentioned in treatment of *Unmaad* and *Apasmara* where there is predominance of *Kapha* and *Rakta Dosha* and purification of micro channels are required.

***Vidang* (Embelia *ribes*)** – In an experiment on ischemia followed by reperfusion in ischemic group rats, the treatment by *E.ribes* significantly reduced the grip strength activity and non-enzymatic (reduced glutathione, GSH) and enzymatic [glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST)] antioxidant levels in hippocampus and frontal cortex compared to sham-operated rats[31].

Ayurvedic properties consists of *Laghu, Ruksha, Teekshna, Kashaya, Ushna* and *Krimighna* property. It is also mentioned to possess rejuvenating property in children. It is constituent of variety of nootropic formulations in *Ayurvedic* texts eg. *Brahma Rasayan, Guda pippali, Manibhadra Yoga, Bhallatak Rasayan, Brahmi Ghrita* etc.

Also the drugs- ***Shyama Trivrit- Operculina turpethum*** Fam. Convolvulaceae **and *Danti- Boliospermum montenum Muell- Arg*** Fam. Euphorbiaceae , have the effects as *Virechan* i.e . They help in expelling the *Aam* and thus might help in getting rid of NFTs and Plaques.

CONCLUSION:

Collectively *Deepan* (ignition power), *Pachan* (digestive power), *Kapha Vata Shaman* (pacifier of *Kapha* and *Vata)*, *Medhya* (improving cognition), *Rasayana* (Rejuvinator) and *Shotha Hara* (Anti-inflammatory), *Srotoshodhak* (clearance of small channels), and *Rakta Prasadan* (blood purification) are the major collective work of *Brahmi Ghrita* which is perfect for clearing up the *Aam* (toxic metabolic byproducts) in brain and stopping neurodegeneration and providing neuro protection.

The study shows that there was significant decrease in TNF a level by Brahmi Ghrita and the decrease in IL 10 levels were comparatively less in Brahmi Ghrita group without any untowards effects making it a promising option for further elaborated research.

Conflicts of interest : None

REFERENCES

1. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, et al. ((2000) Inflammation and Alzheimer's disease. Neurobiol Aging 21: 383–421.
2. Mrak RE, Griffin WS (2005) Potential inflammatory biomarkers in Alzheimer's disease. J Alzheimers Dis 8: 369–375.
3. Bonifati DM, Kishore U (2007) Role of complement in neurodegeneration and neuroinflammation. Mol Immunol 44: 999–1010. doi: 10.1016/j.molimm.2006.03.007
4. Eikelenboom P, Veerhuis R (1996) The role of complement and activated microglia in the pathogenesis of Alzheimer's disease. Neurobiol Aging 17: 673–680. doi: 10.1016/0197-4580(96)00108-x
5. Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D (1989) Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol 24: 173–182. doi: 10.1016/0165-5728(89)90115-x
6. Rozemuller JM, Eikelenboom P, Stam FC, Beyreuther K, Masters CL (1989) A4 protein in Alzheimer's disease: primary and secondary cellular events in extracellular amyloid deposition. J Neuropathol Exp Neurol 48: 674–691. doi: 10.1097/00005072-198911000-00009
7. Arends YM, Duyckaerts C, Rozemuller JM, Eikelenboom P, Hauw JJ (2000) Microglia, amyloid and dementia in alzheimer disease. A correlative study. Neurobiol Aging 21: 39–47. doi: 10.1016/s0197-4580(00)00094-4
8. Eikelenboom P, Veerhuis R, Scheper W, Rozemuller AJ, van Gool WA, et al. (2006) The significance of neuroinflammation in understanding Alzheimer's disease. J Neural Transm 113: 1685–1695. doi: 10.1007/s00702-006-0575-6
9. Vehmas AK, Kawas CH, Stewart WF, Troncoso JC (2003) Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. Neurobiol Aging 24: 321–331. doi: 10.1016/s0197-4580(02)00090-8
10. Griffin WS (2006) Inflammation and neurodegenerative diseases. Am J Clin Nutr 83: 470S–474S.
11. Akiyama H, Barger S, Barnum S, et al. Inflammation and Alzheimer's disease. Neurobiol Aging. 2000;21:383–421.
12. Tarkowski E, Andreasen N, Tarkowski A, Blennow K. Intrathecal inflammation precedes development of Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2003;74:1200–1205.
13. McCaulley ME, Grush KA. Alzheimer's disease: exploring the role of inflam- mation and implications for treatment. Int J Alzheimers Dis. 2015;2015:515248.
14. Fillit H, Ding WH, Buee L, et al. Elevated circulating tumor necrosis factor lev- els in Alzheimer's disease. Neurosci Lett. 1991;129:318–320.
15. Cheng X, Shen Y, Li R. Targeting TNF: a therapeutic strategy for Alzheimer's disease. Drug Discov Today. 2014;19:1822–1827.
16. Strle K, Zhou JH, Shen WH et al. Interleukin-10 in brain. Crit Immunol 2001; 21:427-449. Rev
17. Szczepanik AM, Funes S, Petko W, Ringheim GE. IL-4, IL-10 and IL-13 modulate a beta (1-42) induced cytokine and chemokine production in primary murine microglia and a human monocyte cell line. J. Neuroimmunol 2001; 11:349-36
18. Tarkowski E, Liljeroth AM, Minthon L, Tarkowski A, Wallin A, Blennow K. Cerebral pattern of pro- and anti-inflammatory cytokines in dementias. Brain Res Bull. 2003;61:255–260.
19. Alvarez A, Cacabelos R, Sanpedro C, Garcia-Fantini M, Aleixandre M. Serum TNF-alpha levels are increased and correlate negatively with free IGF-I in Alzheimer disease. Neurobiol Aging. 2007;28:533–536.
20. Paganelli R, Di Iorio A, Patricelli L, et al. Proinflammatory cytokines in sera of elderly patients with dementia: levels in vascular injury are higher than those of mild-moderate Alzheimer's disease patients. Exp Gerontol. 2002;37:257–263.
21. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's Disease: Report of NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's Disease. Neurology1984; 34: 939–944
22. SC Ho, KS Chang, CC Lin - Food chemistry, 2013 – Elsevier
23. HG Kim, JG Choi, H Oh, , PKJ Lee , Sanh Keun Ha, Sun Yeou Kim, Yongkon Park, Myung sook ho, - Biochemical and Biophysical Research Communication, 2014 – Elsevier
24. Reinhard Grzanna, Ph.D., Phong Phan, M.S., Anna Polotsky, M.D., Lars Lindmark, Ph.D., Carmelita G. Frondoza, Ph.D., The Journal of Alternative and Complementary Medicine, 2005
25. Naresh Singh, Sarvesh Kumar, Prabhjot Singh, Hanumantharao G. Raj, Ashok K. Prasad, Virinder, S. Parmar, Balaram Ghosh – Phytomedicine, 2007- Elsevier
26. Anti-inflammatory activity of piperine, AM Mujumdar, JN Dhuley, VK Deshmukh, PH Raman, SR Naik - Japanese Journal of medical science and biology, 1990 - jstage.jst.go.jp
27. Supplementation of Convolvulus pluricaulis attenuates scopolamine induced increased tau and Amyloid precursor protein (AβPP) expression in rat brain SW Bihaqi, AP Singh, M Tiwari - Indian journal of pharmacology, 2012 - ncbi.nlm.nih.gov
28. Michelle D.NemetchekAndrea A.StierleDonald B.StierleDiana I.Lurie - Journal of Ethnopharmacology- 2017
29. Vishal B. Airao, P.R. Buch, Tejas Sharma, Devendra Vaishnav- Researchgate 2015
30. Sunil Kumar Singh, Ajay Singh Molluscicidal and Anti-choline esterase activity of Alstonia scholaris plant against fresh water snail Lymnaea acuminate.
31. Evaluation of antioxidant and neuroprotective effect of ethanolic extract of Embelia ribes Burm in focal cerebral ischemia/reperfusion-induced oxidative stress in rats- M. Nazam Ansari, Uma Bhandari, F. Islam, C.D. Tripathi- Fundamental and clinical pharmacology, 2008