RESEARCH ARTICLE

Comparative clinical evaluation of herbal formulation with multivitamin formulation for learning and memory enhancement

Jigna Shah [*1], **Ramesh Goyal** [2]

[1]Shri Sarvajanik Pharmacy College, near arvind baug, Mehsana, Gujarat, India.
[2]The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India.

Address for correspondence: Prof. Mrs. Jigna S Shah, Department of Pharmacology, Shri Sarvajanik Pharmacy College, near arvind baug, Mehsana, Gujarat, India. E-mail: jignashah74@yahoo.com

Epidemiological studies of Indian population reveal that dementia is largely a hidden problem in India. Ayurveda claims several plants are beneficial in cognitive disorders. Pharmacoepidemiological studies reveal that herbal and allopathic learning and memory enhancing medicines are becoming very popular among Indian population. The objective of study was to clinically evaluate polyherbal formulation (PHF) and compare with multivitamin (MV) preparations used as learning and memory enhancer. It was randomized, placebo controlled, double blind clinical study approved by Institutional Human Ethics Committee. Forty-seven healthy human volunteers from colleges of Mehsana (18-24 years) ready to sign informed consent form were included in study. All these subjects were given either one capsule of placebo or two capsules of PHF (500 mg) at night or MV (500 mg) 1 capsule two times a day for a period of three months. They were monitored for neuropsychological tests initially, after first and third month of active treatment with PHF / MV. Results showed that there was significant increase in IQ score and short term memory score in PHF treated group between 0 and 90 days treatment. In the other battery test significant alterations were observed in all three groups. Our data suggest both PHF and MV supplementation specifically improves learning and memory as compared to placebo in healthy young subjects. PHF appears to be more active than MV.

Keywords: *Learning and memory, Antioxidant property, Short-term memory, Micronutrients.*

INTRODUCTION

An enhanced life expectancy in developed countries has been accompanied by an increased number of people suffering from age-associated dementia. This syndrome not only causes a terrible reduction in the quality of life of the sufferer, it also places tremendous burden on both the career and the welfare systems. Epidemiological studies of Indian population reveal that dementia is largely a hidden problem in the country. Prevalence rates for dementia increase essentially with advancing age [1]. Persons above 60 years of age show 0.43% prevalence whereas persons aged above 65 years show 2.44% prevalence. The prevalence rate rises to 54.8% in individuals above 95 years of age [2]. There is a paucity of modern drugs/agents facilitating acquisition, retention, and retrieval of information and knowledge. Nootropic agents such as piracetam [3], nefiracetam, aniracetam [4] and choline esterase inhibitors like donepezil are being primarily used to improve memory, mood and behavior. However, the resulting adverse effects associated with these agents have limited their use [5, 6].

Ayurveda, the Indian system of medicine had developed certain dietary and therapeutic measures to delay ageing and rejuvenating whole functional dynamics of the body organs. This revitalization and rejuvenation is known as the '*Rasayana chikitsa*' (rejuvenation therapy) [7]. Ayurveda claims that several plants, the "Medhya" plants (intellect promoting) herbs such as, *Convolvulus microphyllus (C. pluricaulis), Centella asiatica, Bacopa monnieri, Acorus calamus, Zingiber officinale* and *Celastrus paniculatus* are beneficial in cognitive disorders [8].

Micronutrients play a central part in metabolism and in the maintenance of tissue function. An adequate intake therefore is necessary. Single micronutrient deficiency states are comparatively easily recognized and treated. Subclinical deficiency, often of multiple micronutrients, is more difficult to recognize and laboratory assessment is often

complicated by the acute phase response [9]. Furthermore, several studies have found that multivitamin/multimineral supplements can improve immunity in older people.[10-16] General nutritional supplements may also help improve response to stress [17]. More clinical trials are required with good clinical outcomes to optimize intake in prevention and treatment of disease [9].

Pharmacoepidemiological studies reveal that herbal and allopathic learning and memory enhancing medicines (LMEM) are becoming very popular among Indian population. The herbal drugs commonly used include Amalaki (*Emblica officinalis*), Brahmi (*Bacopa monniera*), Punarnava (*Boerhaavia diffusa*), Mandukaparni (*Hydrocotyle asiatica*), Ashwagandha (*Withania somnifera*), Galo (*Tinospora cordifolia*), Yashti-madhu (*Glycyrrhiza glabra*), Shankhapushpi (*Convolvulus pluricaulis* and *Evolvulus alsinoids*), Vacha (*Acorus calamus*), Shatavari (*Asparagus racemosus*) [18].

In view of various reports indicating that supplementation with various herbal and allopathic drugs may enhance learning and memory and the fact that there are hardly any published trial reports on the comparative evaluation of various formulations used in India. A preliminary clinical study was undertaken in healthy human subjects on PHF, the polyherbal formulation and MV, the allopathic formulation which were found to be comparatively effective in animal models for enhancing learning and memory.

MATERIALS AND METHODS

This investigation was a placebo controlled, double blind, and single center clinical study for control, PHF and MV. The Institutional Human Ethics Committee of Shri Sarvajanik Pharmacy College, Mehsana, approved the protocol and informed consent form. All the subjects were examined clinically for height, weight, blood pressure, heart rate, any other complications and absence of infections. Prior to participation, each volunteer signed an informed consent form and completed a medical health questionnaire. No specific dietary prescription was provided to avoid any diet fluctuation. The subjects were asked not to make any changes in their usual diet habits or physical exercise during the course of the study. The subjects were asked to come for check up at the regular interval of one month. The monitoring of blood pressure, pulse rate and assessment of subject for various neuropsychological tests were made before beginning the treatment and afterwards at the interval of first and third month of the active treatment.

The following was the exclusion criteria considered during selection of the subjects.

1) Those with serious cardiovascular, cerebrovascular, respiratory, liver or renal disease or any other disorder.
2) The subjects who have used any drugs, alcohol or indulged in smoking for at least 1 week prior to the study.
3) Subjects with a strong history of food or drug allergy of any kind.
4) Subjects using any weight reducing diets within 3 months prior to the start of the study.
5) Subjects ready not to use any other drugs (including aspirin) to be ingested during the course of the study unless there is serious illness.

Participants

Forty-seven healthy human volunteers from various colleges of Mehsana were enrolled for the clinical study during the period of August 2005 to October 2005. All the subjects were between the age group of 18-24 years.

Cognitive measures

Verbal test of intelligence [19], Bhatia battery of performance tests of intelligence (Picture construction test, Pass along test) [20] and Short-term memory working memory (3 unrelated meaningful words test and CCC trigram test) [21] were used to assess the memory parameters and IQ of these students. IQ was calculated in terms of scores by the formula given in the test. The memory scores also were calculated in terms of scores by the standard formula given in the all the tests.

Treatment

All these subjects were given either one capsule of placebo or two capsules of PHF (500 mg) at night with warm milk or MV (500 mg) 1 capsule two times a day for a period of three months. All the subjects were asked to report immediately if at all

they had any side effects like uneasiness, gastric pain, headache etc.

Drugs

The drugs used in the present study were obtained from following sources: PHF (Tonix Healthcare, Ahmedabad, India) and MV (Ami Healthcare, Ahmedabad, India). PHF is polyherbal formulation containing ashwagandha, jatamansi, sarpagandha, shankhpushpi, shatavari, amalki, kauch bij extract, khurasani ajmo, shilajit, mukta shukhti pishti and praval pishti. MV is vitamin B complex capsule containing vitamin B_1 (10 mg), vitamin B_2 (10 mg), vitamin B_6 (3 mg), vitamin B_{12} (15 μg), niacinamide (100 mg), calcium pantothenate (50 mg) and folic acid (1.5 mg), ascorbic acid (150 mg), biotin (100 μg).

Procedure

Each participant was required to attend a total of three study days. Testing for the cognitive measures took place in a suite of laboratories with participants visually isolated from each other. On arrival at their first session, participants were randomly allocated to a treatment regime using a Latin square design. The first day was identical to the following two, except that no treatment (active or placebo) was administered to allow familiarization with the test battery and procedure. Each study day comprised identical testing sessions. The first was a pre-dose testing session that established baseline performance for that day and was followed by the treatment from the next day. Each testing session included completion of tasks for cognitive measures.

Statistical analysis

The observations are reported as mean ± SEM. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison post test.

RESULTS

During the period of 3 months study after following inclusion and exclusion criteria 47 subjects participated (21 males and 26 females) in the study. 10 subjects were randomized as control (placebo treated), 15 subjects as PHF treated and 22 subjects as MV treated. The average age of the subjects was 18.78 ± 0.09 years. The average weight, blood pressure and pulse rate did not differ significantly among the subjects of different groups. Three participants dropped out of the study (for reasons unrelated to any aspect of the study) and data from these subjects were not analyzed. Volunteer compliance was noted by capsule count at the end of the study. The compliance was found to be 100%. No volunteer complained of any adverse effects during and after the period of drug intake. No neurological changes were observed at the given dose and for the given duration (Table 1).

Table 1. Various initial epidemiological parameters

Parameters	Control	PHF	MV
Age (years)	24.7±0.84	19.66±0.39	18.4±0.12
Sex: Male	30 %	53.33 %	45.45 %
Female	70 %	46.66 %	54.54 %
Weight: Male	55 kg	52.75 kg	56.50 kg
Female	44 kg	50.28 kg	45.58 kg
Systolic BP (mmHg)	118.8±3.38	120.8±2.17	118.7±1.34
Diastolic BP (mmHg)	72.66±1.51	79.73±1.36	76.63±0.91
Pulse rate/ min	78±0.59	78.53±0.83	77.18±0.71

Results showed that there was a significant ($p<0.001$) increase in IQ score in PHF treated group between 0 day and 90 days treatment. The IQ score was not significantly changed in control group or MV treated group (Table 2).

In picture construction test, there was no significant change in control group at 30 days and 90 days treatment interval. In PHF treated group the score increased significantly between 0 day (6.38±0.45) and 30 days (8.15±0.58, $p<0.05$) and also between 0 day 90 days (10.3 ±0.34, $p<0.01$). In MV treated group significant change was observed between 0 day (7.33 ±0.37) and 30 days (8.83±0.24, $p<0.01$) and also between 0 day and 90 days (10.16±0.16, $p<0.01$) (Figure 1).

In pass along test and 3-unrelated meaningful words short term memory test significant change was observed not only in MV or PHF treated group but also in control as compared to initial value after 30 days and 90 days of treatment (Figure 2).

In CCC trigram test, there was no significant change in control group. In PHF treated group

Table 2. Effect of PHF and MV on various learning and memory paradigms

Parameter	Group / Time (in Days)	Control	PHF	MV
Picture Construction Test	**0 Day**	7±0.64	6.38±0.45	7.3±0.37
	30 Days	8±0.55	8.15±0.58*	8.83±0.24**
	90 Days	8.9±0.45	10.3±0.34**	10.16±0.16**
Pass Along Test	**0 Day**	6.4±0.76	3.84±1.21	7±0.53
	30 Days	9.6±0.6**	9.77±0.41**	9±0.41
	90 Days	10.2±0.5**	10.6±0.18**	10.6±0.19**
CCC Trigram Test	**0 Day**	48.8±3.33	50±7.74	57.14±6.67
	30 Days	51.11±4.59	64±7.78	74.28±6.54***
	90 Days	60±4.47	80±5.44***	85.71±4.26***
3 Unrelated Words Test	**0 Day**	28±3.26	32.72±4.56	32±2.61
	30 Days	48±3.26***	40.00±6.25*	56±5.23***
	90 Days	56±2.66***	61.81±4.14***	62.67±3.83***
Verbal Test of Intelligence	**0 Day**	101.3±8.91	133.87±13.36	95±6.10
	90 Days	110.4±11.8	161.66±12.77***	98.22±6.2

Values are each a mean±SEM * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.00$ when compared to day 0 values (one way ANOVA followed by tukey's multiple comparison post test).

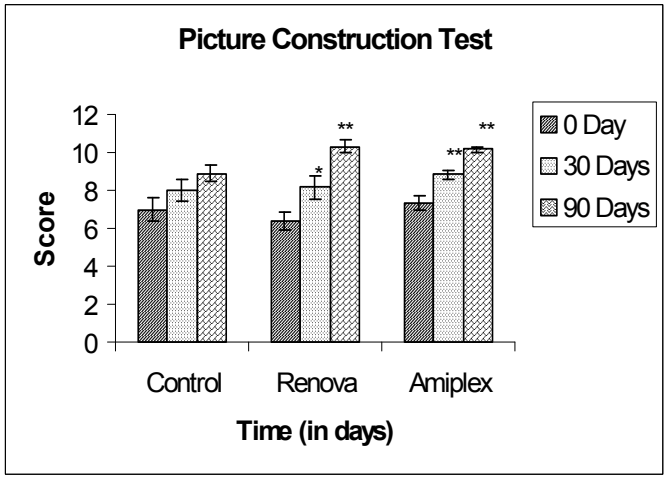

Figure 1. Effect of PHF and MV on picture construction parameter

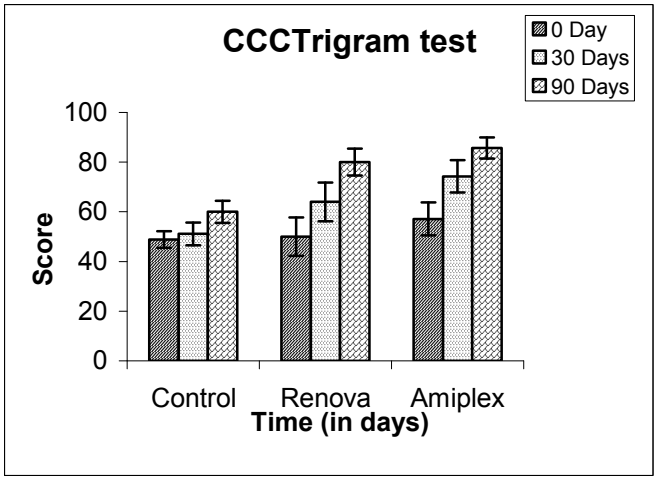

Figure 3. Effect of PHF and MV on CCC trigram parameter

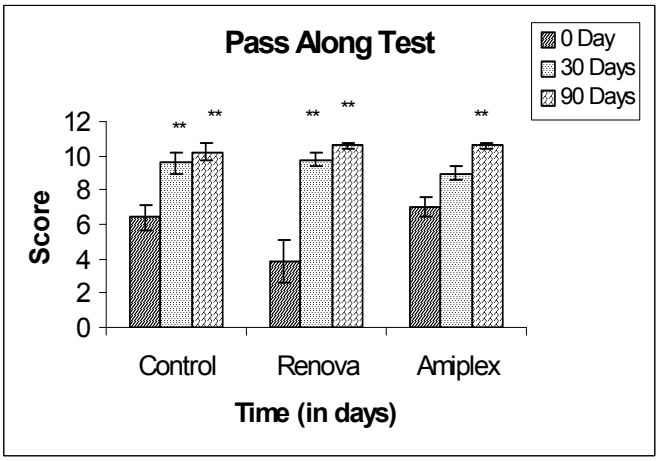

Figure 2. Effect of PHF and MV on pass along parameter

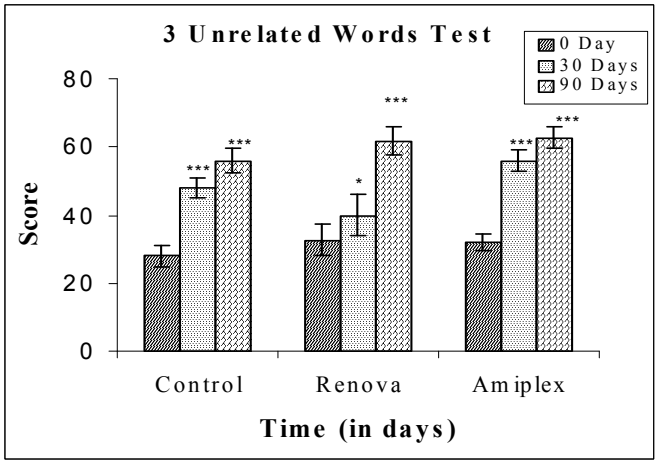

Figure 4. Effect of PHF and MV on 3 unrelated words parameter

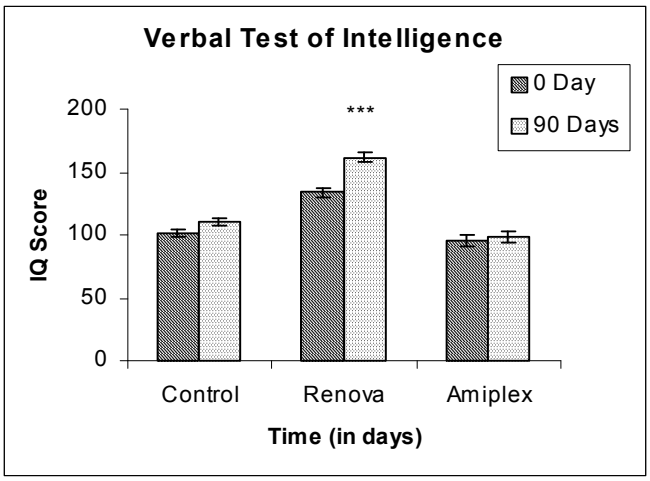

Figure 5. Effect of PHF and MV on verbal intelligence parameter

significant change in score was observed between 0 day (50±7.74) and 90 days (80±5.44, $p<0.001$) whereas there was no change in score between 0 day and 30 days treatment. In MV treated group significant changes were observed between 0 day and 30 days as well as 0 day and 90 days interval ($p<0.001$) (Figure 3, 4, 5).

DISCUSSION

The PHF (herbal formulation), MV (multivitamin formulation) and the placebo, all have shown a significant influence on intelligence and memory quotient. Various clinical models have been used to evaluate learning and memory. Out of five clinical models of learning and memory used in our study, an improvement was observed in both formulations in four models, out of which there was improvement in learning and memory in two models in control group also.

In pass along test significant change was observed not only in MV or PHF treated group but also in control. In 3-unrelated meaningful words test also there was a significant increase after 30 days and 90 days of treatment as compared to initial value in all the three groups. Pass along test as well as 3-unrelated meaningful words test contribute to the evaluation of immediate visual memory, since the subject arranges the designs immediately from memory. Visual spatial and constructional abilities are all considered together and all these are related to the right hemisphere functions. The right hemisphere is superior to the left in discriminating and remembering spatial patterns [22, 23]. Since the effects on learning and memory were observed in control group also in these two tests it can be concluded that these models can not be considered to be authentic to rule out the possibility of the placebo effect with the drugs used.

In CCC trigram short term memory task, the percentage recall improved significantly at the end of 90 days both in PHF treated group and MV treated group. Unlike pass along test and 3 unrelated meaningful words test control group did not show significant improvement. Hence, both PHF as well as MV appear to be effective as LMEM. The improvement in CCC trigram test may be due to improvement in visual imaging and visuospatial abilities [24]. It was found that on the first few trials the subjects recalled most of the letters. However, after some trials, the previous letters produced interference and the recall was poor in control subjects. It has been reported that after a mere 5 seconds delay; subjects forget approximately half of what they had seen earlier [25]. Baddeley interpreted short-term memory as working memory, which is a three-part system that temporarily holds and manipulates information as we perform cognitive tasks [26]. Brown showed that very small amount of material, well within the memory span were rapidly forgotten if rehearsal was prevented [27].

There was a significant improvement in the scores of picture construction test after the intake of MV or PHF for 30 days and 90 days. Brain's frontal areas are vulnerable to lifelong oxidative stress. These areas are related to cognition and memory functioning and are most likely to show possible benefits of antioxidants. Benton administered vitamin supplements or placebo (double blind) for a year to healthy college students ranging from 17 to 27 years, for 1 year and found no significant correlation between changes from baseline in blood serum levels of either vitamin C or vitamin E and changes in performance on any of the cognitive tests for both males and females at 3 months and 1 year mark [28]. As antioxidants work as a system, their effectiveness can depend on levels of other vitamins and minerals.

PHF and MV supplementation also might be enhancing learning and memory by virtue of their antioxidant property so that the metabotropic receptors are coupled to G protein allowing the G protein to initiate the intracellular cascade inside the

neural membrane that produces long- term changes in the neuron [29].

Memory does improve with age. As persons grow they use their experiences to establish, elaborate meaningful relations in the information to be remembered, as a consequence, to remember more accurately [30]. Our results have shown that there was a significant increase in IQ score in PHF treated group ($p<0.001$) between 0 day and 90 days treatment. The IQ score was not significantly changed in control or MV treated group. Thus, results of verbal test of intelligence clearly showed that while MV can not improve long term memory, PHF has a potential to produce a long term improvement in learning and memory. As mentioned above, antioxidant and vitality enhancing factors may be present in both the drugs by virtue of which both can produce improvement in short term memory. However, PHF appears to contain additional constituents possibly the herbal constituents responsible to produce long term effects.

Further, in addition to antioxidant and vitality, immunomodulation may also be involved in long term memory improvement. PHF contains *Asparagus racemosus* which is an indigenous agent with immunostimulant properties and has been reported to increase the activity of macrophages [31]. Clinical trials and animal research has shown that the use of *Ashwaganda* (another constituent present in PHF) produces a positive effect on anxiety, cognitive and neurological disorders including Parkinson's disease [32]. It also produces significant intellectual improvement in mentally retarded children [33]. The roots of *Nardostachys jatamansi* (yet another constituent in PHF) has been clinically employed for its anti-ischemic, antioxidant, anticonvulsant, and neuroprotective activities. *N. jatmansi* has been proved to be a useful memory restorative agent in the treatment of dementia seen in elderly persons. The underlying mechanism of action has been attributed to its antioxidant property or because of facilitation of cholinergic transmission in the brain [34]. Certain substances present in PHF can be synergistic or antagonistic to other substances. A mechanism that ordinarily would convert substances to a more toxic form could be arrested by another chemical that may be present in the same formulation [35]. Ayuredic treatises claim that drugs should not be used alone. Moreover it has been noted that raw form of the drug in ayurveda is more efficacious than its isolated and extracted active ingredients [36]. They also might be contributing to the production of important brain neurotransmitters such as acetylcholine, adrenal hormones, steroids and cortisone or by maintaining adequate levels of various micronutrients, which ultimately affect mental functions and enhance learning abilities [37].

CONCLUSION

Our data suggest that both PHF and MV supplementation improves learning and memory as compared to placebo in healthy young subjects. PHF appears to be more active than MV. Both the drugs might be enhancing learning and memory due to their antioxidant property or by contributing to the production of important brain neurotransmitters such as acetylcholine, adrenal hormones, steroids and cortisone or by maintaining adequate levels of various micronutrients, which ultimately affect mental functions and enhance learning abilities.

In conclusion, our studies authenticate the efficacy of the two products used to enhance learning and memory. However, in the light of differences observed in the two formulations studied extensively, more such placebo-controlled trials in this patient population are indicated as are trials in non-demented elderly and those with mild cognitive impairment are required to be carried out to authenticate and avoid misuse of learning and memory enhancing medicines (LMEM).

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