CHAPTER 7

EFFICACY OF POLYHERBAL FORMULATION ON HUMAN SUBJECTS

7.1 INTRODUCTION

The existence of herbal medicines have been recorded with a world-wide long history used in ancient Chinese, Greek, Egyptian and Indian medicine for various therapeutic purposes. According to the world health organization 80% of the world's population still relies on the traditional medicines for their health care. The India subcontinent is one of the known mega biodiversity centers with huge flora and fauna, about 45,000 plant species are found In India and 15,000 plants have been recorded for its medicinal property. Of this number 7000-7500 plants are used by tribal communities for curing different diseases. Ayurveda is one of such ancient medical system which has employed the use of single and multiple herbs (polyherbal) for curing diseases.

The concept of polyherbal formulation was highlighted in the *Ayurvedic* literature *Sarangdhar Samhita*' in order to achieve greater therapeutic efficacy. At a certain point of time the active phyto-constituents of individual plants are insufficient to achieve the desirable therapeutic affects, hence the idea of combining the multiple herbs in a particular ratio arose which would give a better therapeutic effect with lower toxicity. This strategy of exploiting or combining of several medicinal herbs to achieve extra therapeutic effectiveness is known as polypharmacy or polyherbalism.

The present study was carried out to determine the efficacy of the polyherbal formulation on its neuroprotective and cognitive enhancement activity in aged and SDAT subjects through a randomized, double blind, conventional and placebo control trail.

7.2 METHODOLOGY

7.2.1 Study Design- A multi center, randomized, double blind, placebo, conventional controlled clinical trial to evaluate the efficacy and tolerability of a polyherbal formulation in the prevention and management of age related neurodegenerative disorders.

7.2.2 Investigational products:

Treatment 1, Test – Polyherbal formulation 500 mg capsules, containing samples of three plants *Bacopa monnieri, Dioscorea bulbifera and Hippophae rhamnoides*.

Treatment 2, Conventional– Donepezil 5 mg tablets.

Treatment 3, Control– Placebo 500 mg tablets.

7.2.3 Study groups

Group I

Subjects above the age of 60 years, who are normal without cognitive impairment and other severe disorders, were recruited to study the prevention of neurodegeneration.

Group II

Patients who have age related neurodegenerative disorder and who fulfill the Inclusion & Exclusion criteria was screened and recruited to study management of disease progression.

7.2.4 Inclusion criteria

Group I normal aged

- Age above 60 years, without cognitive impairment with (SMMSE score 30-26).

Group II SDAT cases

- Age above 60 years.
- Cognitive decline.
- Memory loss
- Attention deficits
- Anxiety and depression
- Sleep disorder

- Vertigo
- Willingness to provide written informed consent to participate in the study

Presence of minimum above 3 complaints was included in this study.

7.2.5 Exclusion criteria

Group I

- Subjects below the 60 years
- Subjects with any severe medical illness.

Group II

- Severe renal, hepatic, cardiac, gastrointestinal, neurological, hematological or respiratory disorders, in view of the investigator.
- Presence showing neuro-endocrine or metabolic disorder like high glycemic index, high obesity index, malignancy, tuberculosis, established cases of nephropathy etc. was not included for clinical trial.
- Patients receiving conventional treatment for Senile Dementia of Alzheimer's Type (SDAT) and Parkinson Disease (PD) was also not be included under present study.
- History of smoking (more than 10 cigarettes/day) or alcohol intake > 20 gm/day.
- History or evidence suggestive of complications of diabetes such as • Retinopathy • Nephropathy • Presence of ketone bodies in urine analysis • Ischemic heart disease with any cardiac event in last 6 months • Neuropathy
- Patients who are likely to undergo surgery during the study period.
- Patients who have participated in any investigational study in the last 12 weeks.
- Known hypersensitivity to the study drugs.
- Patients with severe infection, in view of the investigator
- History of intake of any Ayurvedic/herbal/homeopathic/dietary supplements in the last two months

- Pregnant or nursing mothers and women in childbearing age refusing to use contraceptives

7.2.6 Withdrawal Criteria

- Irregular treatment procedure
- Desire of the subject to discontinue the trial drug
- Development of chronic disease like Cancer and tuberculosis
- Development of severe adverse reactions
- During the course of therapy development of chronic hepatic or renal abnormalities.
- Development of psychiatric disorders

The subjects who meet the above criteria were withdrawn from the study and suitable alternate treatments were advised as per the currently followed medical practice.

Subjects should be encouraged to complete all study evaluations in case of withdrawal. However, subjects may withdraw consent to participate in this study at any time without penalty or loss of benefits to which the subject is otherwise entitled. Every reasonable effort should be made to determine the reason if a subject withdraws prematurely and this information should be recorded on the appropriate page(s) of the Case Report Form (CRF). In addition, for subjects who prematurely discontinue study drug, reasonable efforts should be made to obtain all protocol specified assessments to avoid losing outcome data needed to test the study objectives and evaluate safety. All efforts should be made to complete all study follow-up procedures.

7.2.7 Withdrawal from study protocol

Subjects who withdraw completely from the clinical study should be encouraged to complete all protocol-specified safety evaluations. The reason for withdrawal should be recorded on the appropriate page(s) of the CRF. Reasons for withdrawal from the study may include, but are not limited to the following:

- Significant subject noncompliance, defined as refusal or inability to adhere to the investigational product administration regimen
- Subject lost to follow-up

- At the request of the subject, investigator, or study sponsor
- Lack of efficacy as judged by the investigator
- Discovery that the subject entered the study in violation of the protocol or occurrence of a significant protocol violation during the study
- Development of intolerable adverse event due to study participation as determined by the investigator and/or subject
- Data not known before starting the trial become available and raise concern about the safety of the study drug so that continuation would pose potential risk to any particular subject

To the extent possible, any situation(s) that may warrant a subject to withdraw from the study, regardless of reason, must be discussed with the Medical Monitor prior to subject withdrawal.

In the event of study discontinuation due to the occurrence of a non-serious AE, the study site should notify the CRO as soon as possible. In the event of study discontinuation due to the occurrence of an SAE, the Medical Monitor must be notified within 24 hours. Subjects withdrawn secondary to an ongoing non-serious AE regardless of causality or SAE that is not possibly or probably related to investigational product must be followed clinically until the final study visit. Subject's withdrawn to an ongoing investigational product-related SAE must be followed clinically until resolution or stabilization.

7.2.8 Replacement of subjects

Subjects will not be replaced, if there are drop outs.

7.2.9 Prior and concomitant medication

All concomitant medications necessary for the health and well-being of a subject was permitted.

All prior and concomitant medications/topical medications taken during the trial were recorded on the appropriate CRF with indication, daily dose, frequency, and date(s) of drug administration.

7.2.10 Study procedure

7.2.10.1 Informed consent

Eligible subjects were asked to read, comprehend and sign an informed consent form. No study procedures were conducted until the informed consent form is signed.

7.2.10.2 Demographic data and medical history

Demographic data was collected for each subject during Screening and will include sex, date of birth, race & ethnicity. The subject's pertinent medical history will also be recorded. Any illness or injury that occurs before the administration of investigational product was recorded as medical history and evaluated to determine if the occurrence affects the subject's eligibility to participate in the trial.

7.2.10.3 Laboratory assessments

Laboratory assessments will be done as per protocol requirements. All lab investigations will be done in the laboratories approved by the medical monitor and the results will be evaluated using normal ranges provided by the laboratory.

7.2.10.4 Physical examination and vital parameters

A complete physical examination was performed during all visits.

The physical examinations will evaluate the major body systems in order to ensure subject safety and to identify any clinically significant disease or abnormality that, in the opinion of the investigator, would exclude or discontinue a subject from the study. The investigator or a design who is experienced and routinely conducts physical examinations will perform physical examinations.

Body weight, height and BMI, Abdominal girth, blood pressure and pulse rate were measured during all the visits.

7.2.10.5 Study drug administration

Subjects will self-administer study medication and the study personnel will educate them the method and frequency of drug administration.

The subjects should take two formulations every day.

7.2.10.6 Compliance to treatment

Subjects were given a diary card and asked to record the details of medication consumption in the card. They were advised to bring the empty containers and strips of medications during the follow up visits.

7.2.11 Randomization and blinding

The study is a multi center, randomized, double blind, placebo, conventional controlled clinical trial. Randomization will be generated using SAS, SPSS or any other appropriate software. Placebo investigational products were organized to ensure compliance to the design. Blinding was executed through authorized personnel who do not engage in study related activities.

7.2.**12 Assessment of safety and efficacy**

Efficacy of the treatment was assessed by periodic measurements of biochemical parameters and neuroelectrophysiological and psychological parameters.

7.2.12.1 Primary efficacy measures

- Reduction in initial general complaints regarding the quality of life.
- Improvement in sleep pattern.
- Reduction in high anxiety level.
- Improvement in attention span and memory.
- Improvement in quality of life particularly general feeling of well being.
- Showing no adverse reaction in comparison to conventional therapy.

7.2.12.2 Secondary efficacy measures

- Showing the improvement in the oxidative stress markers.

- Showing the reduction in biogenic amines in comparison to conventional drug therapy.
- Showing the reduction in inflammatory cytokines responsible for neurodegeneration.
- Showing the increase in adiponectin.

7.2.12.3 Assessment of safety

Safety was assessed through monitoring of adverse events (AEs), physical examinations and vital signs (blood pressure and pulse). All the adverse events will be recorded with the information about the onset, severity, duration and site of the adverse reaction.

Unscheduled protocol assessments (including but not limited to vitals, etc.) may be conducted or repeated as necessary in case any subject develops signs and symptoms suggestive of a clinically significant and/or abnormal event. All data collected was assessed for severity, changes from baseline and their relationship to the investigational product.

Any deaths, serious adverse events and premature terminations was tabulated and summarized.

7.2.13 Product information

The plants present in test formulation are *Bacopa monnieri*, *Hippophae rhamnoides* and *Dioscorea bulbifera*. The dose of each plant in the test formulation is *Bacopa monnieri* 225mg/capsule, *Hippophae rhamnoides* 125 mg/capsule, and *Dioscorea bulbifera* 125 mg/capsule.

7.2.14 Ethics

The study methodology was explained to the subjects and informed consent was signed from those interested in participation. The present study was approved by the institutional ethics committee of IMS, BHU and SRM University. The clearance was issued by the Ethics Committee, approval number- IMS BHU No. Dean/2009-10/1421 and 473/IEC/2013.

7.2.15 Expected Duration of Subject Participation

Each subject will participate in the study approximately from day -14 to 0 (pre-enrollment) and 1 to 365 days (including the allowable window period for the final follow up visit).

Subjects were randomized into two groups and further into two sub-groups:

Normal elderly- treated with formulation and placebo,

SDAT cases- treated with formulation and Donepezil.

Only the researchers were able to gain access to the supplements provided by industry, Varanasi bio science Pvt Ltd, UP, India. The physicians and subjects were blinded form the supplement details. Subjects were followed through telephone on weekly basis to avoid loss of sample and to keep an account on their capsule consumption. The capsule containers were not given as a whole, they were distributed once in every 15 days and the subjects were asked to remit the used container before they receive a new box. Subjects were asked to follow their regular diet and physical activities during the study. Subjects were also stopped from other medical supplements during the course of study.

7.2.16 Assessments

Basic information's of the subjects like age, weight, height, gender, marital status, occupation, education, physical activity, etc., were recorded. The dietary habit of the subjects was also noted throughout the study to check the interaction of diet on the study. The BMI was calculated by weight in (kg) divided by height squared (m) and blood pressure were also recorded.

The cognitive function was assessed by the scores obtained from MMSE, short term memory (STM), long term memory (LTM) and attention span. Short term and long term memory was done using digital memory span apparatus (Medicaid India) attention span using attention span apparatus (Medicaid India).

After overnight fasting, 5ml of venous blood sample was withdrawn from each subject with the help of medical lab technician. Venous blood was centrifuged, serum

separated and stored in -80 till use. Inflammatory markers such as IL6, TNF alpha and homocysteine were measured using ELISA kit, R& D system, India, Pvt

7.2.17 Statistical analysis

The data are reported as mean± SD. Statistical analyses were performed using Graphpad prism software version 6.0 and SPSS. Differences in the level of biomarkers from pre and post treatments were statistically compared within group using student's t- test. The results were considered statistical significant if the P value was less than 0.05 indicates statistical significance.

7.3 RESULTS

Subjects were selected after screening out of which they were randomized into the various groups. Basic demographic details of the subjects are given in Table 7.1. There was equal distribution of male female were in each group. Results of the various other physiological, biochemical and psychological parameters at baseline and after one year of the two treatment groups are given in Table 7.2 and 7.3. There was improvement in the levels of inflammatory and oxidative stress biomarkers in polyherbal treated group compared to placebo and Donepezil groups. The cognitive function was enhanced in formulation treated group along with improvement in the blood pressure and body mass index after one of treatment when compared to placebo and donepezil groups.

Table 7.1 Demographic detail of the subjects in each group

Variables	Group I		Group II	
	Formulation (N=53)	Placebo (N=54)	Formulation (N=51)	Donepizil (N=52)
Age (years)	65.3±3.3	64.5± 3.1	66.3±3.7	63.8± 3.5
BMI (kg/m$^{2)}$	24.8±3.9	23.6±4.2	24.3±3.7	24.7±3.6
Gender				
Male	27	29	27	28
Female	26	25	24	23

Results of age and BMI are given as mean values ± SD and for gender as whole number.

Table 7.2 Effect of polyherbal formulation on biomarkers associated with ageing and cognitive decline pre and post intervention in polyherbal formulation and placebo groups in Normal human subjects

Variables	Polyherbal formulation		Placebo	
	Baseline	After 1 Year	Baseline	After 1 Year
DBP (mm Hg)	126.7±12.9	120.3±10.1*	126.3±6.9	127.5±10.4
SBP (mm Hg)	79.1±6.9	74.5±6.2*	80.5±6.7	79.8±6.5
BMI (kg/m^2)	24.2±4.1	22.6±4.2*	24.6±3.9±	24.1±4.1
W/H ratio	0.92±4.4	0.86±3.8	0.90±0.5	0.9±0.6
TNF-α (pg/ml)	4.2±1.2	2.7±1.2**	4.1±1.3	4.2±1.3
IL- 6 (pg/ml)	4.9±1.0	3.0±1.1**	5.0±1.1	4.9±1.1
CRP (mg/l)	1.8±0.7	0.9±0.5**	1.9±0.8	1.8±0.8
HCY (µM/l)	15.5±3.9	10.8±3.6**	15.1±3.6	15.2±3.4
SOD (U/mgHb)	5.9±0.9	3.4±0.8**	5.8±0.9	5.9±0.9
TBARS (µM/l)	3.8± 1.1	1.6±1.0**	3.7±0.9	3.8±1.1
MMSE Score	21.8±1.1	25.27±1.0**	21.9±1.2	20.7±1.4
STM Score	5.9±0.8	7.2±0.8*	5.9±0.7	5.4±0.9
LTM Score	4.9±0.7	6.3±0.7*	4.8±0.6	4.6±0.7
Attention span core	11.8±1.1	16.1±0.7**	11.6±0.9	11.5±0.9

Note. All values are expressed as mean±SD. Student t test was used for comparison results.*P< 0.05 and**P< 0.01. Abbreviations- IL-6- interleukin 6, CRP- C- reactive protein, TNF-α tumor necrosis protein α, TGL- triglyceride, TC- total cholesterol, BMI- body mass index, HCY- homocysteine, H/W ratio- hip waist ratio, STM- short term memory, LTM- long term memory, MMSE- mini mental state examination, DBP- diastolic blood pressure, SBP- systolic blood pressure, SOD- Superoxide dismutase.

Table 7.3 Effect of polyherbal formulation on biomarkers associated with age related neurodegeneration and cognitive decline pre and post intervention in polyherbal formulation and Donepezil groups in SDAT subjects

Variables	Formulation		Donepezil	
	Baseline	After 1 year	Baseline	After 1 year
DBP (mm Hg)	134.2±13.5	128.3±9.1*	135.4±7.1	134.5±7.0
SBP (mm Hg)	82.1±7.2	77.5±6.2*	81.6±7.1	77.8±6.0
BMI (kg/m^2)	23.9±3.9	23.2±3.2*	24.2±3.5	24.1±3.1
W/H ratio	0.94±0.4	0.89±0.8	0.96±0.7	0.94±0.6
TNF-α (pg/ml)	5.2±1.6	3.6±1.1**	5.4±1.7	4.9±1.5
IL- 6 (pg/ml)	5.4±1.1	3.4±1.1**	5.4±1.2	4.9±1.1
CRP (mg/l)	2.8±0.9	1.9±0.6**	2.9±0.8	2.7±0.8
HCY (µM/l)	20.5±4.9	15.8±3.6**	21.5±4.8	20.2±4.4
SOD (U/mgHb)	6.8±1.0	4.3±1.0**	6.9±1.1	6.8±0.9
TBARS (µM/l)	4.8± 2.5	2.6±2.0**	5.0± 2.7	4.9±2.1
MMSE score	14.8±3.5	19.29±3.0**	13.9±3.2	20.7±1.4*
STM score	3.9±0.9	5.1±0.9*	3.7±0.7	5.0±0.9*
LTM score	3.2±0.8	4.9±0.7**	3.1±0.6	4.2±0.7*
Attention spans core	9.9±2.1	12.1±1.4*	9.8.6±1.9	11.5±1.5*

Note. All values are expressed as mean±SD. Students t test was used for comparison*P< 0.05 and**P< 0.01. Abbreviations- IL-6- interleukin 6, CRP- C- reactive protein, TNF-α tumor necrosis protein α, TGL- triglyceride, TC- total cholesterol, BMI- body mass index, HCY- homocysteine, H/W ratio- hip waist ratio, STM- short term memory, LTM- long term memory, MMSE- mini mental state examination, DBP- diastolic blood pressure, SBP- systolic blood pressure, SOD- Superoxide dismutase.

No renal, hematological, hepatic or any other adverse effects were observed after six months of test product treatment, results not provided.

7.4 DISCUSSION

The study provides the safety and efficacy profile of polyherbal formulation on cognitive enhancement and neuroprotective effect in the ageds. Polyherbal formulation improved the cognitive function and regulated the factors associated with ageing such as inflammation and oxidative stress markers.

The use of poly herbal formulation had been carried out in many researches. Researchers have followed this in order to combat the complications associated with particular disease as the use of single plant may not satisfy the need. In the current study also polyherbal formulation was used to act markers associated with ageing. Age is the major risk factor for cognitive dysfunction as there is a gradual decrease in cognition over age and it is also seen that ageing is positively correlated with inflammation and oxidative stress. Low grade inflammation is a characteristic associated with ageing and it is determined that elderly subjects were found with two to four times higher levels of inflammatory markers in their systemic circulation compared to young adults, even in absence of chronic disease.

In the present study we have found increased levels of inflammatory markers such as TNF alpha, hsCRP, IL-6 and homocysteine in the geriatric population and there was reduction in these markers on treatment with polyherbal formulation. Similar results were portrayed in several other studies where it was seen that treatment with *H. rhamnoides* reduces lymphocytes proliferation, C- reactive protein [375] and inhibit nitric oxide production in CVD cases.[376] *H. rhamnoides* was also found to reduce the level of inflammatory marker- C-reactive protein.[377]. The vitamins, flavonoids, lycopene, carotenoids, and phytosterols contents of the plant makes it an excellent antioxidant, further adding to its existing pharmacological properties as cardioprotective, anti- inflammatory and immunomodulatory agent [378-380].

Geriatric subjects are found with increased circulating blood pressure and the administration of polyherbal formulation has maintained the levels of blood pressure in treated subjects. Our result correlates with previous study which states that hypotensive property was observed in high sucrose diet induced rats treated with 150 mg/kg/day of *H.*

rhamnoides, the plant was found to have anti-hypertensive, anti-hyperinsulinemia, dyslipidemic effect [381].

Ageing is associated with accumulation of oxidative damage to cells and tissues, which leads to increase in morbidity and mortality [382]. Cognitive decline occur over age and it has been associated with inflammation and oxidative damage to lipids, proteins and nucleic acids [383-385] and higher level of free radicals causes behavioral changes in experimental animals [386, 387]. The results of the present study demonstrated that aged subjects were found with increased level of oxidative stress markers which may have been associated with cognitive decline in these subjects and the use of polyherbal formulation reduced the level of oxidative stress in PFG.

Numerous factors are found to improve memory impairment including cerebral blood flow, brain oxidative stress status and neurotransmitters level [388, 389]. *B. monnieri* sample may directly inhibit the formation of superoxide anion [390] and increases the level of antioxidants such as superoxide dismutase, catalase, glutathione peroxidase in the hippocampus [391]. *B. monnieri* has an ability to enhance memory function [392, 393]. *B. monnieri* is a nootropic drug known for cognitive enhancement [394, 395] and it decreases the loss of acquired information. *D. bulbifera* has phytoestrogen and antioxidant content which helps in protection of the brain from early ageing and ROS activity. Thus the use of polyherbal formulation helped in improving the cognitive function by regulating the inflammatory and oxidative stress markers in geriatrics.

The clinical relevance of the present study is that till date not many researchers have focused on controlling inflammation and oxidative stress associated with ageing though they have worked on cognitive improvement. Also the present combination of plants has not been used in other research. Our results were satisfactory that the polyherbal formulation enhanced the memory in treated subjects and simultaneously acted on inflammatory cytokines and oxidative stress markers.

On considering the result of the present study, the polyherbal formulation may be used as a drug for cognitive enhancement of the ageds and also for patients under the initial stage of senile dementia. But as this is a pilot study with small population data, there

is a need for more additional validation that may support the present pharmacological activities.

The strength of the study is proving the efficacy of polyherbal formulation on cognitive enhancement in elderly subjects and also in the present study we have found 98% compliance in capsule consumption by the subjects, proving its safety on consumption. The duration and the sample size stands on the limitation side of the study.

7.5 CONCLUSION

We can conclude from the above study that the daily consumption of polyherbal formulation for a period of six months enhances the cognitive function in elderly subjects along with the regulation of the inflammatory cytokines and oxidative stress markers. However a large multicentre clinical trial with sufficient data is required for validating the present study and for the approval of drug in the market. Also a longer duration of investigation is required for much assured results in order to further prove the safety and efficacy of polyherbal formulation.

SUMMARY

The current research work ultimately peruse the efficacy of novel polyherbal formulation which consists of *B. monnieri, H. rhamnoides* and *D. bulbifera* on the ageing and age related neurodegeneration biomarkers such as neurotransmitter system, psychological functions, oxidative stress and inflammation using three systems of investigation-*In vitro, In vivo* and clinical studies to identify the neuroprotective mechanism of action of polyherbal formulation in order to promote healthy brain ageing and to prevent the early progression of SDAT.

The polyherbal formulation was found to possess excellent antioxidant activity and neuroprotective effect in IMR32 human neural cell line against exogenous stress induced cytotoxicity. The pretreatment of IMR32 cells with formulation protected the cells against 24 hrs H_2O_2 induced cellular toxicity. This neuroprotective action of the formulation may be attributed towards its antioxidant and anti stress property.

The study illustrated the *In vivo* neuroprotective and cognition enhancing property of the polyherbal formulation. The formulation actively acted on the age related decline in the neurotransmitter levels i.e. acetylcholine, serotonin, dopamine, and nor-epinephrine system and cognitive dysfunction. The results of the findings revealed the neuroprotective action of the formulation on the cholinergic system. The formulation modulated the biosynthesis and uptake of the acetylcholine and AchE enzyme activity in treated rats. The formulation also showed a considerable effect on the monoaminergic neurotransmitter system on aged rats. There was considerable improvement in the cognitive function and decrease in Lipofuscin content in treated rats.

The formulation was also effective clinically in aged and SDAT subjects. The polyherbal formulation acted on the physiological, immunological, antioxidant and cognitive parameters. Therefore the present polyherbal formulation may be effective drug in early prevention of age related neurodegenerative disorders.