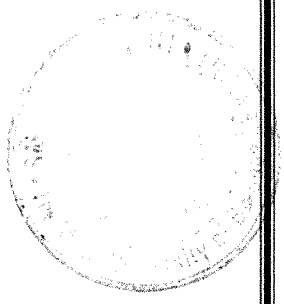


MATERIALS AND METHODS

MATERIALS AND METHODS

The present study was carried out with the broad objective to assess the diet, nutritional, physical and mental health profile of adult and older women suffering from depression and carry out interventional studies on folic acid and brahmi supplementation. Related objectives of the study included i. Assessment of socio-demographic profile, diet, nutritional, mental and physical health status and psychosocial profile of adult and older depressed women. 2. Evaluation of post intervention effects of folic acid and brahmi supplementation on nutritional, mental and physical health profile of adult and older depressed women above the age of 60 years. For this purpose the adult and older women were screened for depression from free living population of Vadodara city. A total of 180 women with moderate depression were identified for present study.

The details of study design, sample selection, parameters studied and the tools and techniques used for data collection are presented in three phases under following sub headings:

PHASE I	**COLLECTION OF BASELINE DATA ON SOCIO-DEMOGRAPHIC PROFILE, DIET PROFILE, NUTRITIONAL STATUS, MORBIDITY PROFILE AND PSYCHOSOCIAL PROFILE IN ADULT AND OLDER DEPRESSED WOMEN.**
PHASE II	**FOLIC ACID SUPPLEMENTATION FOR A PERIOD OF EIGHT WEEKS AND EVALUATION OF THE POST INTERVENTION EFFECT OF SUPPLEMENTATION ON OLDER WOMEN WITH DEPRESSION.**
PHASE III	**BRAHMI SUPPLEMENTATION FOR A PERIOD OF TWELVE WEEKS AND EVALUATION OF THE POST INTERVENTION EFFECT OF SUPPLEMENTATION ON OLDER WOMEN WITH DEPRESSION**

Details of each phase have been discussed and presented under following sub headings:

A. Selection of the Sample

B. Study Design

C. Experimental Plan

PHASE I

COLLECTION OF BASELINE DATA ON SOCIO-DEMOGRAPHIC PROFILE, DIET PROFILE, NUTRITIONAL STATUS, MORBIDITY PROFIE AND PSYCHOSOCIAL PROFILE IN ADULT AND OLDER DEPRESSED WOMEN.

For carrying out the study, women above 40 years of age from the free-living population were screened. After screening; a sample of moderately depressed women was selected to participate in the in-depth study. Further, these women were categorized into two groups as per the age. The details are mentioned below:

A. Selection of the sample:

A multi stage sampling technique was used to determine the sample for the in-depth interviews. Based on the database of the city obtained from Vadodara Urban Development Association (VUDA), wards wise city was equally distributed into 25 areas. Further high, middle and low-income localities were covered from each area. This was done to ensure equal representation of women from all parts of the city as well as socio-economic groups. Home visits were done to identify women above 40 years of age for each area. These women were screened for depression after obtaining their consent.

B. Study Design:

To find out the presence of depression in the free-living population amongst women above 40 years a standardized scale Beck's Depression Inventory(BDI) scale was used (refer Appendix-I).

A 13-itemed BDI developed for the purpose of the study was pilot tested on 37 women above the age of 40 years from the free-living population of Vadodara. After the pilot test it was found that certain symptoms of depression could not be identified with a 13-item abridged version of BDI. To overcome this limitation, the second edition of the 21-item BDI was used for screening for actual data collection.

A predesigned performa was used for collecting background information as well as the Beck's Depression Inventory for screening. The respondents were than classified into three categories of mild, moderate or severe depression.

After screening, respondents for the in-depth interviews were selected based on the following criteria.

Selection Criteria for In-depth Interview	
INCLUSION CRITERIA	**EXCLUSION CRITERIA**
1. Age: > 40 Yrs	**1. Age:** < 40 Yrs
2. Category of Depression ***Score on BDI:*** 20-28	**2. Category of Depression** ***Score on BDI:***<20 &>28
3. Income Group: - Lower - Middle - Upper	**3. Medical History** - Cancer - Cardiac Disease - Dementia - Alzheimer's Disease - Parkinson's Disease - Post Operative
	4. Mental Illness - Psychosis - Mental Retardation

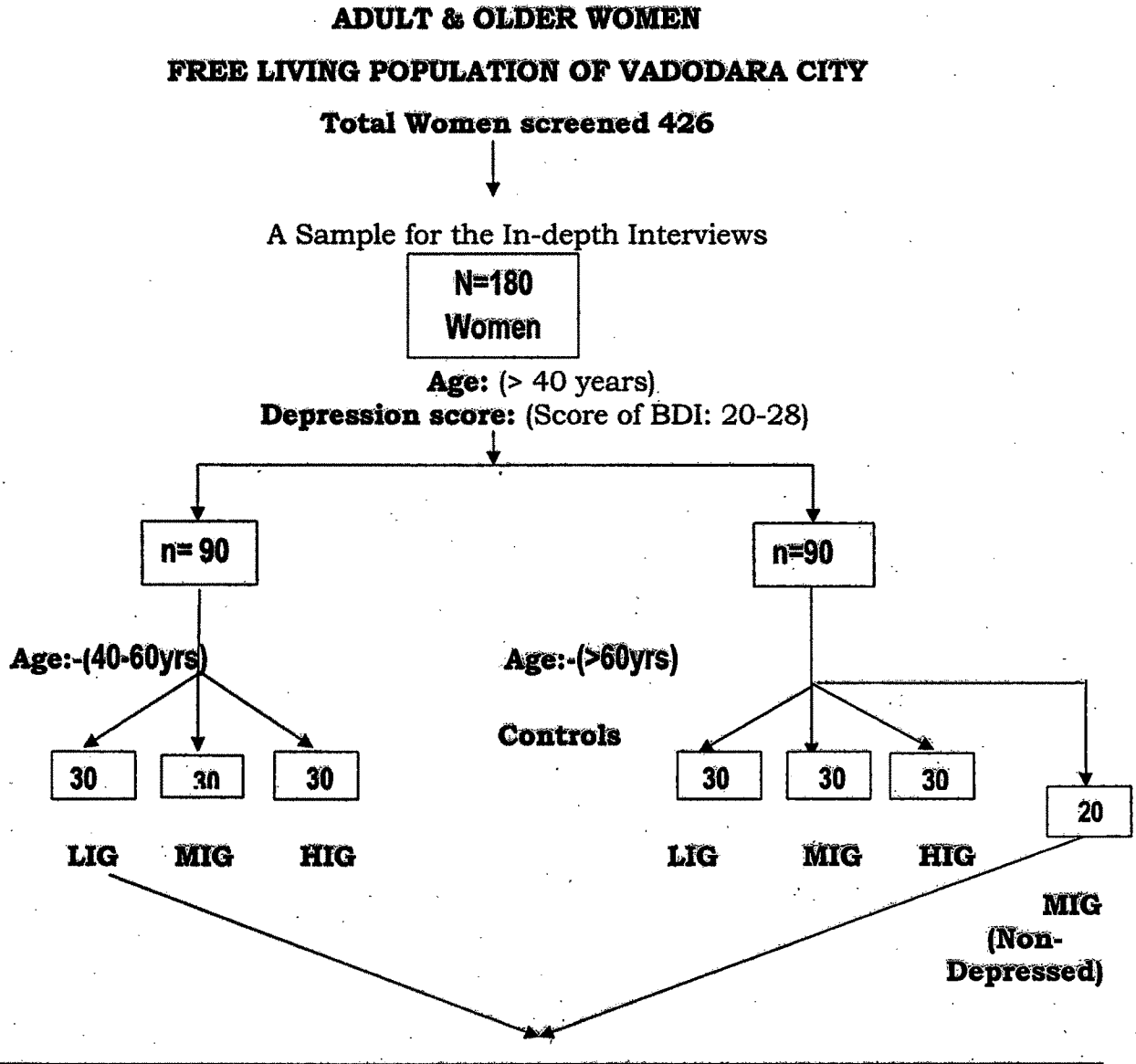
Those women who were selected according to above mentioned criteria were included for the in-depth study based on their consent and willingness to participate.

Using the snowball sampling technique, more subjects were identified from each locality of the city.The screening process was continued till the required sample of 180 women with moderate depression was identified. Total of 426 women were screened. On the basis of their scores on the BDI they were identified as having 'minimal', 'mild', 'moderate' and 'severe' depression. For the purpose of analysis, women were categorized into two age groups of adult (40-60 years) and older (above 60 years). The subjects from 40-60 years were referred to as adult or younger women and subjects above 60 years as older or elderly women in the

entire study. In addition to these 180 women, 20 women over 60 years of age who were non-depressed from the middle income group were selected as controls. These women had a score of 0-2 points on the BDI scale during the screening. The findings from the interviews with these 20 women had been used primarily for the purpose of comparison between health and nutritional status of depressed and non-depressed women.

C. Experimental Plan:-

Experimental plan followed for this phase has been described here in the form of flow sheet diagram as mentioned below:



Socio demography	Nutritional Status	Diet Survey	Clinical parameter	Morbidity Profile	Psychosocial Profile
Questionnaire	Anthropometry (height, weight, MUAC and BMI)	• General dietary aspect • 24-hr dietary recall • Food frequency	• Blood pressure check up	• Major and Minor health complaints checklist • Menopausal aspects	Questionnaire on Lifestyle factors, Power structure, life events stress, self esteem, loneliness and isolation

Figure no 3.1:- Flow sheet diagram of Phase I design of the study

PHASE II

FOLIC ACID SUPPLEMENTATION FOR A PERIOD OF EIGHT WEEKS AND EVALUATION OF THE POST INTERVENTION EFFECT OF SUPPLEMENTATION ON OLDER WOMEN WITH DEPRESSION.

In this phase the impact of intervention given to the moderately depressed elderly women has been presented.The details are mentioned below:

A. Selection of the sample:

Hundred and eighty women with moderate depression who were selected for in-depth interviews under phase I, 90 women out of these were above the age of 60 years. Amongst these 90 elderly women who were moderately depressed, 30 women irrespective of income were selected for the intervention.

Selection criteria for Folic acidintervention
1. Serum estimation for folic acid levels < 3 mg
2. Deficient in Calculation of folic acid intake based on the 24-hr recall method.
3. Consent and willingness to take supplementation for the respective period.

B. Study Design:

Folic acid tablets were distributed on daily basis to the selected sample of older women. It was confirmed each day, that the earlier days supplement had been consumed with the main meal and the compliance were recorded. The subjects were requested to inform in advance if they were likely to be away from the home for a day or two any time during the study period so that extra supplements could be given to them. Subjects who failed to inform before leaving were followed up till they had completed 60 days of supplementation. Folic acid tablets in appropriate doses were given to the 30 older depressed women for a period of eight weeks. The impact of intervention of folic acid supplementation was evaluated by comparing the pre and post intervention data on depression status, nutritional status, dietary intake, and prevalence of minor health complaints.

C. Experimental Plan:-

Experimental plan followed for this phase has been described here in the form of flow sheet diagram as mentioned below:

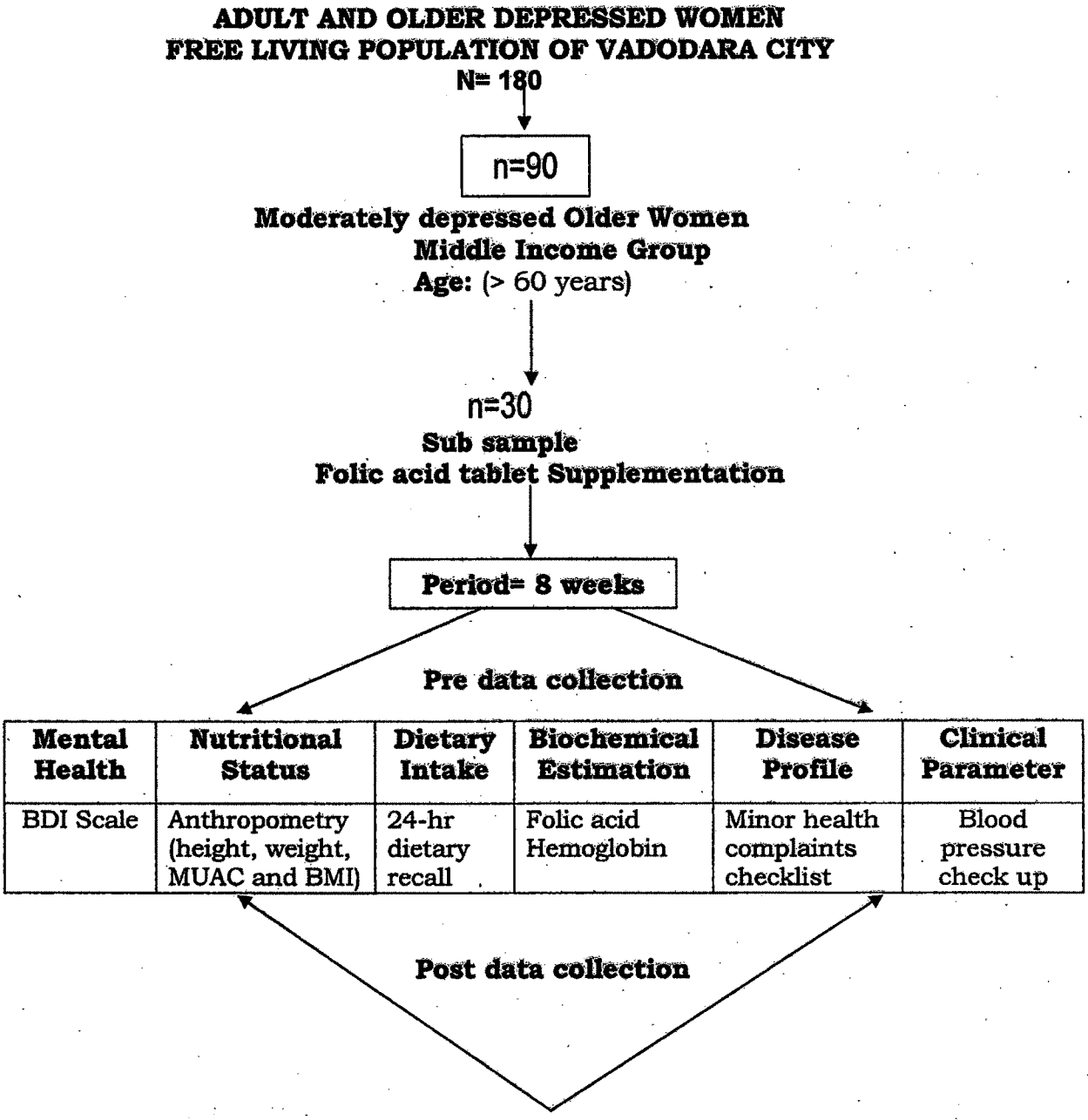


Figure no 3.2:- Flow sheet diagram of Phase II design of the study

PHASE III:

BRAHMI SUPPLEMENTATION FOR A PERIOD OF TWELVE WEEKS AND EVALUATION OF THE POST INTERVENTION EFFECT OF SUPPLEMENTATION ON OLDER WOMEN WITH DEPRESSION.

Ayurveda is the most ancient medical system widely accepted by the people. The role of herbal intervention for prevention of mental health problems in the elderly still remains unclear, the present study made an attempt to test the efficacy of Brahmi in depression.The details are discussed as under:

A. Selection of the sample:

As per categorization there were 90 older women above the age of 60 years. Out of these 90 elderly women who were moderately depressed, 30 women from middle income group were selected for the intervention. Further thirty selected subjects were categorized into experimental group receiving Brahmi supplements (n=15) and control group did not receive any supplements during the intervention period (n=15).

Selection criteria for Brahmi intervention
1. Abnormal cognitive impairment score > 10
2. Moderate mini mental state examination score
3. Low self esteem
4. Moderate depression score
5. Consent and willingness to take supplementation for the respective period.

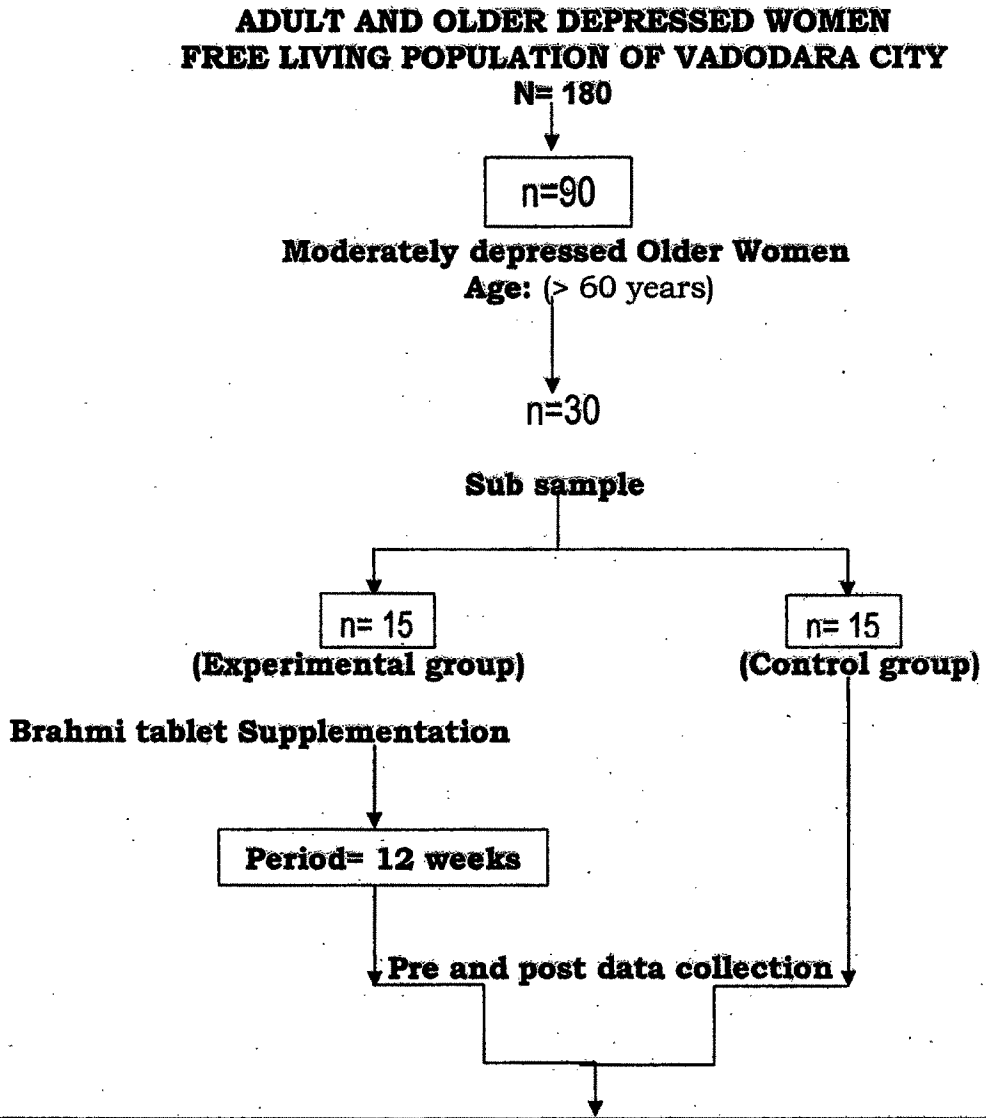
B. Study Design:

Amongst total 30 selected middle income depressed elderly subjects, a sub sample selected as experimental group (n=15) were given the intervention of Brahmi supplementation for a period of twelve weeks. At the same time control group were not given any intervention during that period. Brahmi capsules were given on weekly basis. Daily intake of supplements given was confirmed telephonically and compliance was confirmed during the visit. For each subject brahmi supplements were packed in a bottle. To cover one week of the treatment,

subjects were given a bottle containing capsules sufficient in number for the entire week.Subject would be provided with one bottle of brahmi supplements during the visits. Subjects were instructed to take one tablet three times a day orally with a glass of water. The subjects were requested to inform in advance if they were likely to be away from the home for a day or two any time during the study period so that extra supplements could be given to them. Subjects who failed to inform before leaving were followed up till they had completed 90 days of supplementation.The impact of intervention of Brahmi supplementation was evaluated by comparing the pre and post intervention data on mental health status, nutritional status, dietary intake and disease profile.

C. Experimental Plan:-

Experimental plan followed for this phase has been described here in the form of flow sheet diagram as mentioned below:



Mental Health	Nutritional Status	Dietary Intake	Biochemical Estimation	Disease Profile	Clinical Parameter
• BDI Scale • MMSE Scale • CIT Scale • Self Esteem scale	Anthropometry (height, weight, MUAC and BMI)	24-hr dietary recall	Hemoglobin	Minor health complaints checklist	Blood pressure check up

Figure no 3.3:- Flow sheet diagram of Phase III design of the study

The list of tool and techniques used for collection of data are discussed below.

TOOLS AND TECHNIQUES

The tools used for the present study are described as under:

a) Interview cum Questionnaire (pre-designed and pre-tested)

b) Beck's Depression Inventory scale

c) Gurmeet Singh's Presumptive Stressful Life Event Scale

d) Self Esteem Rating Scale

e) Mini Mental Status Examination scale

f) Cognitive Impairment Scale

g) Bathroom scale and measuring tape

h) Set of standard cups and spoon

i) Sphygmomanometer

Keeping in mind the objectives of the study, the tools for data collection were prepared after referring various standardized scales and tools related to study. Prior to the actual commencement of the study in-depth interviews, a detailed structured interview schedule and questionnaire was prepared to assess the overall quality of life of adult and older depressed women. The tool was pilot tested among 16 women. The tools were prepared in English and then translated into Gujarati for better comprehension by the respondents and ease in communication. The purpose of this test was to check the data gathering procedure and reliability of the measurement. The content validity of the tools with their sub-sections was established through feedback from the experts.The tools and methodology have been described in brief as below:

1. QUESTIONNAIRE

A carefully planned questionnaire was used to elicit information regarding socio-demographic profile, nutritional status, dietary intake, lifestyle factors, psychosocial profile and morbidity profile.

2. SOCIO DEMOGRAPHIC STATUS

An effort was made to identify the sample from lower, middle and higher income group to maintain uniformity. A carefully planned questionnaire was designed for obtaining all detailed information about the subjects. This included information on age, education, religion, income, occupation, mother tongue, marital status and history of medical illness, mental illness and alcoholism within the family.(Appendix-I)

3. BECK'S DEPRESSION INVENTORY SCALE

A standardized tool call Beck's Depression Inventory (BDI) was selected from number of available tools as the most feasible tool for screening women for depression. A 13-item BDI developed for the purpose of the study was pilot tested on 37 women above the age of 40 years from the free-living population of Vadodara. After the pilot test it was found that certain symptoms of depression could not be identified with a 13-item abridged version of BDI. To overcome this limitation, the second edition of the 21-item BDI was used for screening for actual data collection. Based on the scores respondents were accordingly classified as having mild, moderate or severe depression.(Appendix-I)

4. LIFESTYLE FACTORS

Life style factors in terms of activity pattern and addiction pattern were mainly studied in order to understand the impact on the daily living and health of the adult and older depressed women.(Appendix-I)

a) Activity Pattern

Data on activity pattern was collected by total self reported time spent on various activities using questionnaire. Day's activity was noted and further categorized as physical, recreational/leisure, social and religious. Total time spent under each activity was then calculated.

b) Addiction Pattern

Addictions such as alcohol, cigarette/bidi, tobacco powder, tobacco paste, snuff, tea and coffee were noted.

5. DIET SURVEY

a) General dietary aspects

Main purpose was to find out the information on food habits, lifestyle, and meal pattern of adult and older women causing depression. A pre-tested questionnaire was used to obtain the data on general dietary aspects such as: water intake, meal pattern, fasting practices, skipping meals, intake of special health foods, craving for special food, reduction in food consumption, changes in food consumption pattern, food causing allergy, and food preference.(Appendix-II)

b) Dietary intake

The Dietary intake of the women was assessed through 24-hour dietary recall method and frequency of consumption of foods, using the food frequency questionnaire.

Twenty-four hour dietary recall method was used to measure the dietary intake of all the subjects. The details of the food consumed on previous day was asked in the terms of household measures and then in the terms of standard cups and spoons. The subjects reported intake of cooked foods, which was converted into raw amounts. Information on the subject's intake of energy, protein, fats, folic acid, vitamin B12, vitamin C, isoflavone, amino acid, b-complex vitamin, mineral and choline was calculated. Nutritive value of foods consumed was calculated using food composition tables given in the Nutritive value of Indian foods (Gopalan,

1997). The average daily nutrient intake for each subject was obtained from data collected.(Appendix-II)

The food frequency list for all food groups was introduced to know their habits and frequency. For food frequency a list of important foods having role in mental health foods were prepared. Frequency in terms of frequently and non-frequently was noted.

6. NUTRITIONAL STATUS:

a) Anthropometric measurements:

These included heights, weights and mid upper arm circumferences, the most common and important parameters used for assessing the nutritional status of the individual. BMI was calculated as per the standard formula.(Appendix-II)

i) Weight Measurements:

Weight measurements were taken for all the subjects using the bathroom scale. It is portable and can be conveniently used in the field. The subjects were asked to stand erect on the scale without touching anything with no heavy clothing or footwear and looking straight ahead. The weights were taken twice to ensure accuracy and recorded to the nearest 0.25 kg .The scale was recalibrated to zero before each measurement. The weight measurements were taken for the all subjects enrolled for the study.

ii) Height Measurements:

Height measurements were made on all the subjects using a flexible, non-stretchable fiberglass tape. The subjects were asked to stand erect without touching anything, with no footwear, heels touching the wall and looking straight, ahead. Scale was kept on the subject's head and marks were made on the wall using a pen indicating the height of the subjects and then with the help of measuring tape the height of the subjects were taken. The height was taken twice to ensure accuracy recorded to the nearest of 0.1 cm.

iii) Body Mass Index (BMI):

BMI was calculated by the formula:

$$\text{BMI} = \frac{\text{Weight (kg)}}{[\text{Height (m)}]^2}$$

BMI is a good indicator for nutritional status of adults. The calculated BMI of subjects was compared with the standard suggested by WHO (1995).

BMI Class	Presumptive Diagnosis
<18.5	Underweight
18.5 – 24.9	Normal
25.0 – 29.9	Overweight
30.0 – 34.9	Obese class I
35.0 – 39.9	Obese class II
≥40.0	Obese class III

iv) Mid Upper Arm Circumference:

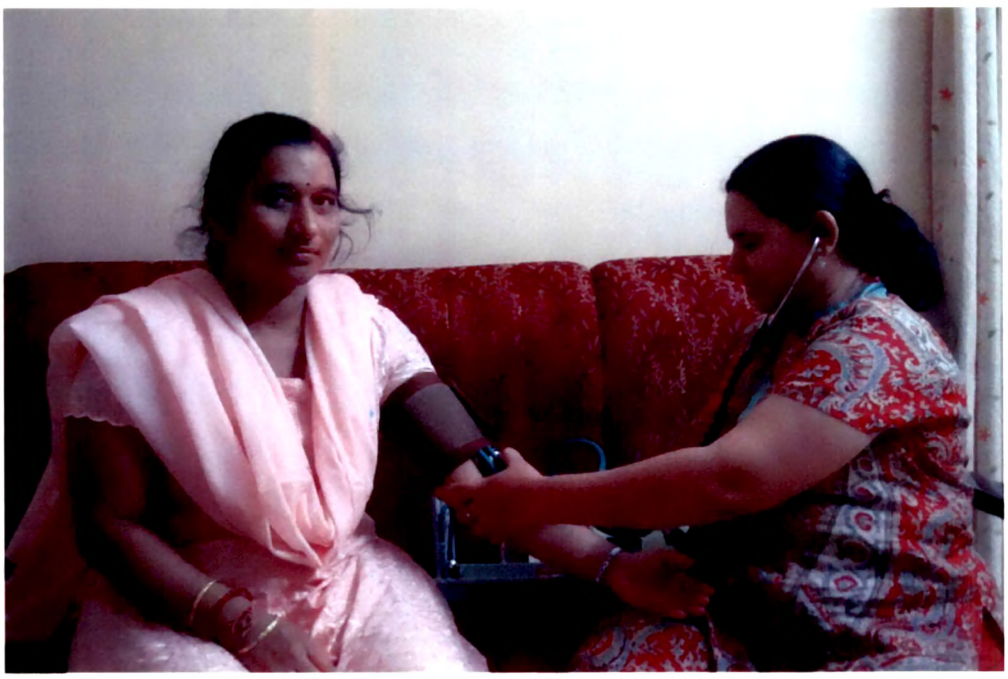
The mid point of the left upper arm was marked by asking the subjects to keep their left arm in right angle with the upper arm .The MUAC was then measured using glass tape.

b) Clinical parameters:

i) Blood pressure measurements:

Blood pressure was measured using sphygmomanometer. The main purpose of checking the blood pressure was to estimate the prevalence of hypertension in women (≥40 years). (Appendix-II). Picture below depicts the blood pressure measurement of the adult depressed women during in-depth interview session.

Figure 3.4: Blood pressure measurement of adult depressed women during in-depth interview session.



7. MORBIDITY PROFILE:

a) Major health problems

A Checklist was prepared of major health complaints for collecting information on disease profile from the subjects. A detailed checklist included:-

1. Problems of oral cavity.
2. Problems of digestive system.
3. Problems of hepato-biliary system.
4. Problems of pancreatic system.
5. Problems of respiratory system.
6. Problems of cardio vascular system.
7. Problems of genito-urinary system.
8. Problems of locomotor system.
9. Problems of neurological system.
10. Problems of endocrine system.
11. Miscellaneous problems.

Prevalence of the health problems was confirmed by the reports available with the subjects for respective complaints (Appendix-II).

b) Minor health problems

Using an exhaustive checklist and free listing method information was collected from women for identifying the frequency of minor illnesses. Data were collected by the investigation through personal interviews. A pre-tested Performa was used for recording the morbidity. The list included the commonly encountered ailments in general and elderly (Appendix-II)

c) Menopausal problems

A pre tested questionnaire was used for collecting information on impact of menopause in depressed adult and older women. Questionnaire included data on experience of women on cessation of menstrual cycle and perceptions. Detailed checklist was used including information on menopausal health symptoms with respect to vasomotor, physiological and psychosocial problems (Appendix-II).

8. PSYCHOSOCIAL PROFILE

a) Power Structure

Power structure in different roles was studied in order to understand women's position in their existing family. Questionnaire was used to measure the existing power structure, the main parameters of collecting data were: a) households headed by women; b) their involvement in household responsibilities and their perceptions regarding the same; c) their involvement in decision-making on various important aspects (e.g. health, education, marriage of children and finance besides purchasing groceries, preparing meals and fulfilling other social obligations); d) their access to and control over the family's finances(Appendix-III).

b) Loneliness and Isolation

In order to collect the information on support network parameters of loneliness and isolation were collected using shortlisted questionnaire with aspects related to when do they feel lonely and isolated, coping strategies and their experience (Appendix-III).

c) Stressful events

Information on occurrence of the life stressful event amongst the respondents were obtained using the "Gurmeet Singh's Presumptive Stressful Life Event Scale" for the same purpose.(Appendix-III)

d) Self esteem

In order to examine the association between women's satisfaction with their support network and their level of self-esteem "Self Esteem rating scale" was used with free listed questions on perception of individual subject (Appendix-III).

9. MENTAL HEALTH STATUS

Mental health status was assessed using the standardized scales used in psychiatry as mentioned below for pre and post data collection after intervention of respective supplementation given (Appendix-IV).

a) Beck's Depression Inventory (BDI): As discussed earlier,this scale was used for diagnosis of depression. The BDI questionnaire is a scale having 21 questions with 4 options each as an answer. Each answer has a score from 0-3. The total of all answers gives the extent of depression. Those who had obtained the score in the range of 20-28 were selected as the subjects having moderate depression (Amin et al, 1998). This scale was used in phase II and III respectively.

b) Mini-Mental State Examination (MMSE): This test was used to assess orientation, registration, attention, calculation, memory, language and visuospatial abilities of the subjects. The questionnaire contained different sections and the maximum score was 30. The total of all the answers gives the extent of mental health. A cut-off was 23 - 25 and those who scored above 26 were considered normal and those below 26 were considered having a mental impairment (Folstein et al, 1975). This scale was used in Phase III.

c) Cognitive Impairment Test: This test was used to assess the cognitive function of the selected subjects. Here the score of 1 was given for each incorrect response; maximum weighted error score was 28. Score of 0-11 indicates normal

and 11-28 indicates abnormality (Katzman et al, 1983). This scale was used in Phase III.

d) Self Esteem Rating Scale: This questionnaire contained 40 questions. Each question had 5 options and each option had a score of 1-5. Out of the 40 questions, 20 are positive and 20 are negative. The total of all the answers gives the extent of psychological well-being. For the results, it is considered as If the score is positive than the subject has high self-esteem and if the score is negative than the subject has low self-esteem.This scale was used in Phase III.

10. BIOCHEMICAL ESTIMATION

a) Hemoglobin levels

Hemoglobin level estimation was carried out by Cyanmethemoglobin method (INACG, 1985). This parameter was tested in Phase II and Phase III (Appendix-I)

Based on principle of treating hemoglobin (blood) with Drabkins reagent, hemoglobin presents in blood reacts with potassium ferricyanide to give hemoglobin, a rust colored compound,the intensity of which can be measured Spectrophotometrically at 540 nm. Standardization was done withcyanmethemoglobin reference standard obtained from GlaxoPvt Ltd. The calibration of spectrophotometer was done using this reference standard.

Suitable aliquots were taken in separate test tubes each containing 2.5ml, 2.0 ml, 1.5 ml and 1.0 ml respectively. The volume was made and aliquot remained undisturbed and was the top standard. The optical density was estimated spectrophotometrically at 540 mm, within 30 min, after adjusting the instrument to zero with the blank solution (Drabkins reagent). A factor for estimation of hemoglobin was calculated from the calibration.

Hemoglobin was estimated according to the following steps:

The finger was wiped with a cotton swab dipped in ethanol and allowed to dry. A bold prick was made using a sterile disposable lancet. The first drop of blood was wiped off. A big drop was allowed to form on the finger and then 20ml of blood was pipetted in duplicates using a micropipette. This was allowed to stand for minimum of 15minutes before taking the reading at 540 mm. Duplicate samples were collected from all the subjects for the estimation of hemoglobin levels.

Concentration of hemoglobin in the blood was calculated using the factor and optical density (O.D) of the samples as:

O.D x factor = Hemoglobin Concentration

b) Serum Folic Acid Estimation

Kit Used: Elecsys 2007,Cobas

Test Principle

Competition principle: Total duration of assay: 27 minutes

1st incubation: By incubating 25µL of sample with the folate pretreatment reagents 1and 2, bound folate is released from endogenous folate binding proteins.

2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample.

3rd incubation: After addition of streptavidin –coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied with formation of a ruthenium labeled folate binding –protein –folate biotin complex. Rhe entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument-specifically generated by 2- point calibration and a master curve provided via the reagent barcode.

Reagents- working solutions

PT1Pretreatment reagent 1(white cap), 1 bottle, 4 mL:Sodium 2-mercaptoethanesulfonate (MESNA) 40 g /L, pH5.5.

PT2 Pretreatment reagent 2(gray cap), 1 bottle, 5 mL: Sodium hydroxide 25 g/L.

M Streptavidin –coated micro particles (transparent cap), 1 bottle, 6.5mL: Streptavidin –coated micro particles 0.72 mg /mL ;Preservative. 2+

R1 Folatebinding protein –Ru(bpy)3 (gray cap), 1 bottle ,9 mL: Ruthenium labeled floate binding protein 75 µgL; human serum albumin (stabilizer) ; borate/ phosphate /citrate buffer 70m/mol/L, pH 5.5; preservative.

R2 Folate-biotin (black cap), 1 bottle, 8 mL: Biotinylatedfolate 17 µg/L; biotin 120 µg/L; human serum albumin (stabilizer); borate buffer 100 mmol/L,pH 9.0; preservative.

SUPPLEMENTATION

1. FOLIC ACID TABLETS

Folic acid was given in the form of tablet. The dosage of Folic acid tablet was 400micrograms manufactured by Aristo Pharmaceuticals Pvt ltd, Mumbai. Instructions were given to take folic acid with a full glass of water. The appearance was Flat, yellow, bisected tablet with beveled edges. The storage conditions were maintained at below 25°C. One tablet twice a day was given to subjects for 8 weeks. Figure below depicts the older depressed women taking supplementation during intervention period.

Figure 3.5: Depressed older women taking supplementation during the intervention period

2. BRAHMI CAPSULES

Brahmi was given in the form of a soft gel capsules (supplied by Soft Health Creations Pvt. Ltd.) to a group of 15 elderly female subjects having depression along with impaired cognitive function. One capsule contained 250 mg of Brahmi extract (20% bacoside content) in them. Three such capsules per day were given to the subjects for 6 weeks. The effect of Brahmi was then studied on mental health and nutritional status. The capsules were supplied by Soft Health Creations Pvt. Ltd., Vadodara. The proximate chemical compositions of Bacopa Monniera (Brahmi) are presented below:

Figure 3.6: Brahmi Plant

Proximate composition of the leaves of Bacopa monniera

Component	Analytical results
Moisture	88.4 g/100g
Protein	2.1 g/100g
Fat	0.6 g/100g
Carbohydrate	5.0 g/100g
Crude fiber	1.05 g/100g
Ash	1.9 g/100g
Calcium	202 mg/100g
Phosphorus	16 mg/100g
Iron	7.8 mg/100g
Ascorbic acid	63 mg/100g
Nicotinic acid	0.3 mg/100g
Energy	38 cal/100g

Statistical tests applied to analyze the data collected for obtaining the results of the study are discussed below.

STATISTICAL ANALYSIS

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS). Data was checked and entered into excel sheets. The baseline data collected was subjected to various statistical tests. Data were analyzed using bivariate tables with frequencies and percentages were prepared. Necessary statistical tests were applied wherever required. Besides this, verbatim were used for interpreting the data. Statistical analysis was also carried out using paired't' test. Statistical analysis was carried out using the following test:

PHASE I:

1. SOCIO-DEMOGRAPHIC ATTRIBUTES:

Percent responses were calculated for the different socio-demographic variables.

2. SCREENING FOR DEPRESSION

Score method was used i.e Subject's responses were calculated and its average was scored at the end and further categorized into mild, moderate and severe levels.

3. LIFESTYLE FACTORS

a. Activity pattern

Total time spent on each activity was calculated in terms of Minutes.

b. Addiction Pattern

Percent responses were calculated for the different addictions.

4. DIET SURVEY

a. General dietary aspects

Percent responses were calculated for the different food related activities, difficulties, diet related information and food frequency.

Percent responses for general diet related information was calculated for subjects belonging to different income and age groups.

b. Dietary intake:

For twenty-four hour dietary recall, mean and standard deviation was calculated for the nutrients such as energy, protein, fats, folic acid, vitamin B12, vitamin C, isoflavone, amino acid, b-complex vitamin, mineral and choline. F-test was applied to note the difference in the food intake between the groups belonging to different age group and Income.

Percent responses for food frequency were calculated for the subjects.

5. NUTRITIONAL STATUS

a. Anthropometric measurements:

Mean and standard deviation was calculated for the anthropometric measurements such as height, weight, MUAC and BMI.

b. Clinical parameters: Blood pressure measurements

Mean and standard deviation for systolic and diastolic levels were calculated for both the groups of subjects.

6. MORBIDITY PROFILE

The percent of subjects suffering from various diseases with frequency were calculated for major and minor illnesses as well as for menopausal complaints for both the groups.

7. PSYCHOSOCIAL PROFILE

a. Power Structure

Percent responses were calculated for the individual aspects.

b. Loneliness/ Isolation

Percent responses were calculated for each parameter. Further coping strategies as stated by the subjects were divided into loneliness and isolation section separately and percent responses for the same were calculated.

c. Stressful Events

Chi square test was applied on the scale by categorizing scale into three levels of low, moderate and high. Percent responses were calculated for life events.

d. Self Esteem

Percent responses were calculated for each parameter of the scale in terms of high and low levels.

PHASE II:

1. MENTAL HEALTH STATUS:

Mean and standard deviation were calculated for the score obtained from the beck depression inventory scale of the subjects. Independent "t" test was used to compare differences between the mean score in pre and post data.

2. NUTRITIONAL STATUS

a. Anthropometric measurements

Mean and standard deviation were calculated for the anthropometric measurements such as height, weight, MUAC and BMI. Independent "F" test was used to compare differences between the mean score in pre and post data obtained after intervention period.

b. Biochemical Parameters

i. Hemoglobin levels:

Mean and SD were calculated for the parameter that were expressed numerically. Paired "t" test was used to assess the difference between the mean hemoglobin of the same group before and after the intervention period.

ii. Folic Acid levels:

Mean and SD were calculated for the parameter that were expressed numerically. Paired "t" test was used to assess the difference between the mean folic acid of the same group before and after the intervention period.

c. Clinical parameter

i. Blood pressure measurement

Mean and standard deviation for systolic and diastolic levels were calculated for both the groups of subjects. Paired "t" test was used to assess the difference between the mean folic acid of the same group before and after the intervention period.

3. DIET PROFILE

a. 24-hr Dietary recall method

For twenty-four hour dietary recall, mean was calculated for the nutrients such as energy, protein, fats, iron, folic acid, vitamin B12, vitamin B6, Vitamin C, isoflavone, Calcium, tryptophan, methionine, phenylalanine, potassium, selenium and choline. Probability for $p \leq 0.05$ was calculated to note the significant difference in the mean food intake of the same group before and after the intervention period.

4. MORBIDITY PROFILE

Mean were calculated for the types of symptoms expressed for the minor health complaints. Chi square test was applied to assess the difference between the mean complaints of the same group before and after the intervention period.

PHASE III:

1. MENTAL HEALTH STATUS

Percent responses were calculated to assess the mental health status by using Beck's Depression Inventory scale, Mini-Mental State Examination scale, Cognitive impairment test scale and Self-esteem rating scale. Paired t-test was applied to note the difference in the mental health status before and after intervention.

2. NUTRITIONAL STATUS

Mean and standard deviation was calculated for the anthropometric measurements such as height, weight, MUAC and BMI, and the biochemical parameters such as hemoglobin and blood pressure measurements. The above parameters were also subjected to paired t-test to assess the difference in the nutritional status before and after a period of six weeks.

3. DIET PROFILE

Mean and standard deviation was calculated for the nutrients such as energy, protein, fat, choline, tryptophan, methionine, folic acid, vitamin B-12, Vitamin B-6 and Vitamin C. Paired t-test was applied to note the difference in the food intake before and after intervention of six weeks.

4. MORBIDITY PROFILE

Percent responses were calculated to assess the disease profile for major and minor complaints. Paired t-test was applied to note the difference in the number of major and minor health problems before and after intervention.

Based on the above mentioned methodology the data obtained was analyzed and results are presented and discussed in the next chapter.