3. METHODOLOGY

The aim of the study was to focus on the obese Non–Insulin dependent Diabetes mellitus (NIDDM) patients and the role of Herbal Supplementation capsules (with herbal products) from the traditional system of medicine development and validation of these formulations on disease specific cell in human beings and therapeutic use in relation to herbal biotechnology. The study also intends to examine the herbal capsules with other components to enhance the activity of HS capsules.

The following parameters were selected to achieve these objectives of the present study tools and methods used are detailed in this section.

3.1. Selection of the Sample

The work was carried out on patients selected from two hospitals, i.e. Government General Hospital and Sai Hospital in Guntur city, Andhra Pradesh, India. The experimental design selected to carry out the study and presented.

The patients consulting the two hospitals in Guntur City, Andhra Pradesh Government General Hospital (GGH) and Sai Hospital for regular check-ups, who are willing to cooperate throughout the study period, were selected. For the present study 1000 patients from GGH and 750 patients from Sai Hospital were selected. Out of these patients, 620 each from one hospital together 1240 were screened. Out of these 1240 patients, 250 patients were surveyed. Again from these 250 patients, among both men and women, 120 obese NIDDM patients were purposively selected (Fig 2). Obese patients were selected by using body mass index (BMI) and waist-to-hips girth ratio (WHR). The categories of obesity were described. The age of the patients was 35-70 years. These were divided into four groups as Group 1, Group II, Group III and Control group (35-70y). In each group 30 patients were taken. A questionnaire was used to obtain information on eating habits, food frequency, socio-demographic background and clinical symptoms (Refer schedule I in appendix i). The experimental work and the results were recorded. The design of the experiments planned and carried out in the study is presented in Figure 2.
Basing on the parameters, the selected 120 patients were divided into 4 groups each comprising 30 patients. Each group was supplemented with one separate treatment as described below

Group I- HS capsules (9 capsules i.e 4.5g/day) supplemented obese NIDDM patients.

Group II- HS capsules (9 capsules i.e 4.5g/day) and physical activity prescribed to obese NIDDM patients.

Group III- HS capsules (9 capsules i.e 4.5g/day), physical activity and nutritional counseling prescribed to obese NIDDM patients.

Control Group - No treatment was given to obese NIDDM patients.

For the above patients anthropometric measurement such as height and weight were taken before and after the experiment for assessing a) body mass index b) waist hip ratio by using (Willett et al., 1997).

Anthropometric characteristics such as triceps, biceps sub scapular and suprailliac were also taken before and after the experiment for analyzing a) Fat mass b) Fat free mass c) lean body mass (Durnin and Womersley, 1974).

For the above patients serological samples were collected before and after the experiment for analyzing a) Fasting blood glucose level b) Postprandial blood glucose level c) Glycosylated hemoglobin for the three serological parameters and data analysis is carried by (Carl et al., 1996).

Lipid profile of the patients were collected before and after the experiment for analyzing a) Total cholesterol b) High density lipo protein c) Low density lipo protein d) Very Low Density Lipo Protein and Triglycerides by (Trinder, 1969 and Allian et al., 1974).

Clinical assessments of the patients were collected before and after the experiment.

In India, it is allowed and permitted to perform experiment with herbal products on human beings. So, the researcher has taken up the task of conducting experiment on
selected obese NIDDM patients, under the careful observation and supervision of physician. The patients were familiarized with the experimental procedures by careful explanation and demonstration of the methods. Patients gave their formal and informal consents.

3.2. Selection of variables

In the present study patients were categorized into different groups for the purpose of the experimental study. The major objective of the project being a study on formulated capsules and their impact on patients, while focusing on the weight reduction, physiological, biochemical changes, lipid profile, clinical symptoms, dietary changes and health complications. HS Capsules along with physical activity and nutritional counseling. Further Income and disease were treated as independent (influencing) variables.

3.3. Herbal supplementation capsules

Herbal supplementation powder and capsules were prepared from *Murraya Koengii, Moringa Oleifera, Mentha Aervenses*, soy, barley, Oats and tomato etc. After pilot study patients reported their opinion on Herbal supplementation in powder form and capsule form. Herbal Supplementation powder hard to accept orally due to bitter and pungent flavor, irritating and hard to swallow, though the Herbal Supplementation powder consumed with butter milk or directly. Patients may not be able to consume whole, whereas Herbal Supplementation capsules easily digestible in Gut and their main advantages are easy to swallow characteristics and their ability to break down quickly in the stomach. Obese NIDDM Patients reported that the capsules were good, absorbing normally, No stomach pain, irritation and wastage found in supplementation consumption.
PRELIMINARY SURVEY IN 69 DIABETIC CLINICS IN GUNTUR DISTRICT

G.G.H
500-IN PATIENTS
+ 500-OUTPATIENTS

Screened

SAI HOSPITALS
300-IN PATIENTS
+ 450-OUTPATIENTS

Screened

620+620

250 SURVEYED
(PATIENTS)

SELECTED 120 PATIENTS

EXPERIMENTAL GROUP

GROUP I
AGE (35-70 YRS)
N=30
MALE FEMALE
N=13 N=17

GROUP II
AGE (35-70 YRS)
N=30
MALE FEMALE
N=14 N=16

GROUP III
AGE (35-70 YRS)
N=30
MALE FEMALE
N=14 N=16

CONTROL GROUP

AGE (35-70YRS)
N=30
MALE FEMALE
N=14 N=16

Data Recorded: - N=120
Socio-demographic background, recording of blood pressure
Anthropometric Measurements – Ht, Wt, BMI, Waist Hip Ratio
Three day diet survey (Food habits and food consumption patterns)
Bio Chemical Analysis (FBG, PPBG, HBAIC, LIPID PROFILE, TOTAL CHOLESTEROL LEVEL,
LDL, HDL, VLDL AND TRIGLYCERIDE Levels)

Before treatment

Treatment for 6 months

GROUP I
Supplementation with HS
Capsules
(9 Caps/Day=4.5g/Day)

GROUP II
SUPPLEMENTATION
WITH PHYSICAL
ACTIVITY

GROUP III
Supplementation
With Physical
Activity & Diet
Counseling

After treatment Tabulation of all data were collected.

Fig. 2: The Experimental Design of the Study
3.3.1. Preparation of capsules and supplementation

In view of the increasing demand by patients using herbal preparations with antidiabetic effects in the management of diabetes mellitus worldwide, several herbal medicines are being produced. In the present study the investigator planned to formulate Herbal Supplementation capsules (HS) from herbal plants (*Merraya Koeinigii*, *Moringa Oleifera* and *Mentha Aervensis*) and fortified the supplementation with other food items as given below because Herbal Supplementation capsules are nutritious, not only for controlling the blood sugar levels but also useful in weight reduction, to control hypertension, to reduce LDL-Cholesterol and to increase HDL-cholesterol to prevent Micro and macro complications, clinical symptoms and over all metabolic disorders in
diabetic patients. After pursuing many experimental studies which were conducted worldwide, The Investigator planned for this experimental study, by following standards of (India is famous for herbal medicine and ayurveda system). The process of the new experimental work is as mentioned below.

(1) Identification of active ingredients in leaves (2) to observe the toxic effect In leaves (3) standardisation of herbal drugs (4) Nutrient analysis in supplementation with special reference to diabetic patients (4) powdered the ingredients and encapsulated.

Curry leaves, (Murraya koenigii), Drumstick leaves, (Moringa oleifera) and mint leaves (Mentha aervensis) were procured in bulk from locally available sources. The three types of leaves were separately washed to remove dirt. After washing, leaves were shade and dried, followed by hot air oven drying at 40°C for 7 to 8 hours. Powder was made from these dried leaves and stored in air-tight containers. Nutrient and anti-nutrient composition, antioxidant activity and antimicrobial analysis were done on these leaf powders. The powders of these leaves with their respective quantities that is to say curry leaves (20%), drumstick leaves (10%), mint leaves (10%), soy bean (5%), barley (5%), oats (2.5%) and dried tomato (2%) were mixed and powdered and encapsulated, 500mg capacity of each capsule (Table-2). These capsules were treated as natural food supplementation capsules. After analyzing blood samples of 120 selected patients, no treatment was given for 6 months and treated as self control period. Fasting blood glucose and post prandial blood glucose samples were collected and analyzed at monthly intervals for 6 months and Herbal supplementation capsules were administered to three experimental group patients who were given 4.5gms of food supplementation in the form of 9 capsules per day, 3 each with breakfast, lunch and dinner. After supplementation anthropometric measurements, biochemical, clinical and dietary parameters were selected for analysis. During treatment no harmful side effects were observed, this might be because of the ingredients related to natural food products. The experiment was carried out under doctor’s supervision.
Fig 4: Food products used in supplementation
**Fig 5: Herbal Supplementation capsules**

**Table 2: Composition of Ingredients in Herbal supplementation capsules:**

<table>
<thead>
<tr>
<th>S. NO</th>
<th>POWDERS</th>
<th>AMOUNT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Curry Leaves</td>
<td>20.0</td>
</tr>
<tr>
<td>2</td>
<td>Mint Leaves</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>Drumstick leaves</td>
<td>10.0</td>
</tr>
<tr>
<td>4</td>
<td>Dried Tomato</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>Soy Bean</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>Barley</td>
<td>5.0</td>
</tr>
<tr>
<td>7</td>
<td>Oats</td>
<td>2.5</td>
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Table 3: Herbal supplementation capsules
Nutritional information (approximate value per/10g)

<table>
<thead>
<tr>
<th>NUTRIENTS</th>
<th>VALUE</th>
</tr>
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<tbody>
<tr>
<td>ENERGY (KCal)</td>
<td>14.66</td>
</tr>
<tr>
<td>CARBOHYDRATES (g)</td>
<td>1.99</td>
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<tr>
<td>PROTEIN (g)</td>
<td>1.04</td>
</tr>
<tr>
<td>FAT (g)</td>
<td>0.22</td>
</tr>
<tr>
<td>FIBRE (g)</td>
<td>0.40</td>
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</table>

<table>
<thead>
<tr>
<th>ESSENTIAL VITAMINS</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITAMIN A (IU)</td>
<td>418.28</td>
</tr>
<tr>
<td>VITAMIN B₁ (mg)</td>
<td>0.01</td>
</tr>
<tr>
<td>VITAMIN B₂ (mg)</td>
<td>0.01</td>
</tr>
<tr>
<td>VITAMIN B₃ (mg)</td>
<td>0.20</td>
</tr>
<tr>
<td>VITAMIN C (mg)</td>
<td>4.86</td>
</tr>
<tr>
<td>FOLIC ACID (µg)</td>
<td>6.69</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ESSENTIAL MINERALS</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRON (mg)</td>
<td>0.48</td>
</tr>
<tr>
<td>PHOSPHOROUS (mg)</td>
<td>12.51</td>
</tr>
<tr>
<td>CALCIUM (mg)</td>
<td>45.24</td>
</tr>
<tr>
<td>MAGNESIUM (mg)</td>
<td>6.36</td>
</tr>
<tr>
<td>SODIUM (mg)</td>
<td>0.02</td>
</tr>
<tr>
<td>POTASSIUM (mg)</td>
<td>10.04</td>
</tr>
<tr>
<td>ZINC (mg)</td>
<td>0.07</td>
</tr>
<tr>
<td>SELENIUM (mg)</td>
<td>11.05</td>
</tr>
<tr>
<td>CHROMIUM (mg)</td>
<td>0.043</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ESSENTIAL MINERALS</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRON (mg)</td>
<td>0.48</td>
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<tr>
<td>PHOSPHOROUS (mg)</td>
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<tr>
<td>MAGNESIUM (mg)</td>
<td>6.36</td>
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<tr>
<td>SODIUM (mg)</td>
<td>0.02</td>
</tr>
<tr>
<td>POTASSIUM (mg)</td>
<td>10.04</td>
</tr>
<tr>
<td>ZINC (mg)</td>
<td>0.07</td>
</tr>
<tr>
<td>SELENIUM (mg)</td>
<td>11.05</td>
</tr>
<tr>
<td>CHROMIUM (mg)</td>
<td>0.043</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMINO ACIDS</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARGININE (mg)</td>
<td>14.32</td>
</tr>
<tr>
<td>HISTIDINE (mg)</td>
<td>5.75</td>
</tr>
<tr>
<td>LYSINE (mg)</td>
<td>11.98</td>
</tr>
<tr>
<td>TRYPTOPHANE (mg)</td>
<td>3.58</td>
</tr>
<tr>
<td>PHYNALANINE (mg)</td>
<td>11.22</td>
</tr>
<tr>
<td>TYROSINE (mg)</td>
<td>3.67</td>
</tr>
<tr>
<td>METHIONINE (mg)</td>
<td>3.77</td>
</tr>
<tr>
<td>CYSTINE (mg)</td>
<td>4.33</td>
</tr>
<tr>
<td>THREONINE (mg)</td>
<td>8.86</td>
</tr>
<tr>
<td>LEUCINE (mg)</td>
<td>17.16</td>
</tr>
<tr>
<td>ISOLEUCINE (mg)</td>
<td>10.56</td>
</tr>
<tr>
<td>VALINE (mg)</td>
<td>12.54</td>
</tr>
</tbody>
</table>

Source: Nutritive value of Indian Foods, 2004
3.4. Microbial tests for leaf powders used in supplementation

3.4.1. Antimicrobial activity

About 10g of dried leaf powder (*Merraya Koeinigii*, *Moringa Oleifera* and *Mentha aervensis*) was accurately weighed and 100 ml of methanol was added. The contents were mixed thoroughly and allowed to stand for 5 hours and filtered through what Mann no. 1 filter paper. The obtained methanol leaf extract was used to evaluate the antimicrobial activity. Antimicrobial activity was evaluated by the disc diffusion method on nutrient agar medium (3g Beef extract, 3g Yeast extract, 5g Peptone, 5g sodium chloride and 1g agar in 1000ml distilled water pH adjusted to 7.0) for bacteria and Potato dextrose agar medium (4g Potato starch, 20g Dextrose and 15g Agar pH adjusted to 5.4) for fungi.

About 20ml of sterile medium was uniformly smeared in Petri dishes by using sterile cotton swabs with test pure cultures of bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sonnei* and fungi *Aspergillus niger*, *Candida albicans*. The 5mm diameter discs were impregnated with 1mg/ml, 0.1mg/ml and 0.01mg/ml leaf powder extract was placed on seeded agar plates. A disc loaded without leaf powder was kept as control. After incubation the zone of inhibition was measured (Bauer *et al.*, 1966).

3.5. Tools for collection of data

Complete data collection was made by the investigator only. However, the two hospitals selected for the study are in close proximity to the patients under study and educated patients themselves was obtained food intake data and the physical activities Relevant schedules were prepared and the data was derived through questionnaire. As the investigator hails from the region and familiar with the language used by the patients, the investigator did not encounter with any problems while collecting the information.

3.6. Assessment of standard of living

The summary of household measure called the Standard of Living Index (SLI) used in the National Family Health Survey-II (2000) was used in the present study. The SLI was calculated by adding the scores obtained for type of house, toilet facility, source
of lighting, main fuel for cooking, source of drinking water, separate room for cooking, ownership of house, agricultural land, and irrigated land, live stock and durable goods. Based on the total index scores the households were classified into three SLI categories as follows (Table 4).

**Table 4: The Index Scores for Standard of Living of Households**

<table>
<thead>
<tr>
<th>Range of Index score</th>
<th>SLI category</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>Low</td>
</tr>
<tr>
<td>15-24</td>
<td>Medium</td>
</tr>
<tr>
<td>25-27</td>
<td>High</td>
</tr>
</tbody>
</table>

The women belonging to low and medium SLI categories were chosen in the present study. The research conducted will be of value when it caters to the needs of the community. It is well known that while nutritional deprivation and its consequences are prevalent mostly among the low and middle SL groups, the observations made on these groups reveal that the risk of obesity also is on the increase (Refer schedule 2 in appendix ii).

3.7. **Socio-demographic data of obese NIDDM patients**

A suitable schedule was formulated with specific and direct questions to obtain information regarding the following aspects.

- Bio-data of the patients including Name, Address, Age, Income, Education and Occupation.
- Details regarding family history of diabetes and habits such as cigarette smoking, drinking alcohol etc.
- Specific dietary habits.

All the patients were requested to give true facts and were assured that the given information would be kept strictly confidential.
3.8. Anthropometric Measurements

3.8.1. Height (m)

The height of the patients was measured using the method described by Jelliffe (1966). The subject (patient) was made to stand on an even floor with back touching the wall, the feet parallel with heels, buttocks and shoulders and back of head touching upright. The head was made erect with the lower border of the orbit measures and the arms hanging at the side in a natural manner.

3.8.2. Weight (Kg)

The weight was measured by using procedure described by Jelliffe (1966) using bathroom scales. The balance was placed on an even floor. The subject (patient) with light clothes (0.4-0.6 kg) was requested to stand on the center of the balance (foot rest plate) with head erect. The weight was recorded avoiding parallax error. The weight was expressed in kilograms. To minimize the error, the same balance was used throughout the study and the balance was checked periodically with standard weight adjusting to read zero in the resting position.

Weight/Height Indexes – Body Mass Index (BMI)

As a measure of body composition, in fact body fat, a weight/height index has to have both high correlations with the amount of body fat, as well as a low correlation with body height, or else in short and tall people body composition would be systematically over or under estimated.

The most frequently used index today is the Quetlet or body mass index (BMI). The correlation of body mass index with body fat is relatively high (ranging from 0.6 to 0.8, depending on age) and the correlation with body height is generally low (Khosla and Lowe, 1967, Keys et al., 1972, Womersley and Durnin, 1977; Garrow and Webster, 1985 and Deurenberg et al., 1991).

For predicting body composition in the general population e.g., in epidemiological studies this method is as good as other methods, which also have their limitations. Several studies have been published in which a good relationship between the
Quetlet index and the amount of body fat were demonstrated, provided that the age-sex-specific prediction equations are used with such age-and-sex-specific prediction equations. The percentage of body fat can be predicted with an error of 3-5% (Womersley and Durnin, 1977; Deurenberg et al. 1991; Norgan and Ferro-Luzzi, 1982).

The BMI is a simple but objective anthropometric indicator of the nutritional status of the adult population and seems to be closely related to their food consumption level. It is relatively inexpensive, easy to collect and to analyze. The BMI is sensitive to socio-economic status and to seasonal fluctuations in food consumption relative to the level of physical activity.

Body mass index was calculated from weight (kg) and height (cm). It was calculated from the equation $\text{wt in (Kg)/ht (m)}^2$. Khosla. (1967) explained that this index gives a measure of weight for height that is highly independent of actual height (FAO/WHO/UNU, 1985). The BMI provides an estimate of present nutritional status. The justification for using this index is two fold and except in the very young and elderly, height appears to have little effect on energy requirements independently of its relation to weight. This index can also be used to assess the magnitude of potential health risks associated with chronic energy deficiency (underweight) or over-weight and as a guide to therapy.

3.8.3. Body Mass Index (BMI)

The BMI was calculated using the formula: $\text{BMI} = \text{Weight (Kg)/height (m}^2\text{)}$. The adults were classified into different nutritional grades based on BMI (kg/m$^2$) as suggested by Simone Lemieux et al., 2004.

Table 5: Anthropometric Classifications for Adults

<table>
<thead>
<tr>
<th>BMI CLASS</th>
<th>Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18.5</td>
<td>Under weight</td>
</tr>
<tr>
<td>20.0 – 24.9</td>
<td>Normal</td>
</tr>
<tr>
<td>25.0 – 29.9</td>
<td>Over weight</td>
</tr>
<tr>
<td>30.0 – 34.9</td>
<td>Obese, Class I</td>
</tr>
<tr>
<td>35.0 – 39.9</td>
<td>Obese, Class II</td>
</tr>
<tr>
<td>≥40</td>
<td>Obese, Class III</td>
</tr>
</tbody>
</table>
3.8.4. Waist-Hip Ratio (WHR)

The waist hip circumference ratio is a simple method for describing the distribution of both subcutaneous and intra abdominal adipose tissue (Larsson et al., 1984; Jones et al. 1986). The reading of waist and hip circumferences were taken for the calculation of waist to hip ratio. Willet et al. (1999) recommended a WHR >0.8 as indicative of abdominal/central obesity.

Waist circumference was measured around the narrowest point between ribs and hips when viewed from the front after exhaling. Hip circumference was measured at the point where the buttocks extended the maximum, when viewed from the side (Boyle et al., 1993). The recordings were made for each site to the nearest one cm using a metal tape on a horizontal plane without compression of skin.

Waist circumference has been proven in studies to predict cardiovascular disease and metabolic syndrome. Recent evidence suggests that central obesity judged by the waist to hip is important in diabetes and should be taken into consideration along with BMI. A waist circumference of greater than 88 cm in women and greater than 102 cm in men was having a very high association with the above said problems. The waist - hip ratio greater than 0.85 in women and greater than 0.95 in men is a predictive of metabolic syndrome.

Assessment of Body Fat Using Skin fold Thickness Measurement

Body fat is located both internally and subcutaneously. There is a constant relationship between subcutaneous fat and total body fat. Total body fat can be estimated by measuring the amount the subcutaneous adipose tissue. The amount of subcutaneous fat can be estimated by measuring the thickness of the subcutaneous fat layer at different sites of the body with a skinfold caliper (Durnin and Womersley, 1974). The relationship between subcutaneous fat and total fat is found to be relatively constant. It differs, however, between the two sexes (Lohman, 1981, Durnin and Womersley 1974). In adults the most frequently used formulas are those of Durnin and Womersley (1974) and Jackson and Pollok, (1978).
In the present study the sum of four SFTs viz. Triceps, Biceps, Subscapular and suprailiac was used to calculate body density and body fat and other indices following the formulas and reference values proposed by Durnin and Womersley (1974).

3.8.5. Skin fold thickness measurements and techniques

A simpler procedure for estimating body fat is the skin fold thickness, particularly in adults. A pinch of skin is precisely measured by Harpenden skin fold calipers at several standardized points on the body to determine the subcutaneous fat layer thickness. These measurements are converted to an estimated body fat percentage by an equation. Skin fold thickness provides the best field information about the level of fatness of an individual. As persons suffering from chronic energy deficiency would have minimal quantities of fat in the body, very small amounts of subcutaneous fat is seen. However, a very large amount of subcutaneous fat is seen in obese persons. Since the fat is stored (Durnin and Womersley, 1974).

In the present study four such measurements at biceps, triceps, sub scapular and supra iliac were taken. These four sites were found to be the best established sites, as proposed by Durnin and Rahman (1967) and developed later by Durnin and Womersley (1974). The skin fold measurements were measured with Harpenden skin fold caliper with an accuracy of ±0.5 mm.

3.8.6. Biceps skin fold

The measurement was taken over the center of the biceps of the muscle of the left upper arm. The arm of the subject was in a relaxed state and loosely hung. The skin fold was lifted about a cm below the mid point along the long access of the muscles. The caliper, in a horizontal position was allowed to compress the skin fold about the point where the thumb and finger grasps the skin folds. Biceps skin fold is measured as the thickness of a vertical fold in the front of the upper left arm, directly above the center of the cubical at the same level at the triceps skin fold (Weiner and Lourie, 1969).
3.8.7. Triceps skin fold

As the fat deposition in the upper arm is not uniform in thickness, the site selected was the left mid upper arm between the tip of acromial process of the scapula and the olecranon process of the ulna. The measurement was made with the elbow slightly fixed and the site of the triceps was marked. The thickness of the fat fold was measured with the hand hanging freely at the side. The fat fold thickness was noted to the nearest 0.5 mm. Triceps skinfold was measured in millimeters at the mid point of the back of the upper left arm (Weiner and Lourie, 1969).

3.8.8. Sub scapular skin fold

The skin fold was taken on the diagonal line coming from the vertebral border to between 1 and 2 cm from the inferior angle of the scapulae (A Diagonal fold about 1-2 cm below the point of the shoulder blade and 1-2 cm towards the arm). The caliper was used about 1 cm laterally downwards from this point. Sub scapular skin fold was measured just below, with the shoulder and left arm relaxed. The skin fold was angled 45° from horizontal, in the same direction as in the inner border of the scapula in medically upward and latterly downward according to Jette (1981) and Lohman et al. (1988). It was recorded in mm.

3.8.9. Supra-iliac skin fold

The skin fold was lifted just above the crest of the ilium. The fold was lifted to follow the natural diagonal line at this point. Supra iliac skin fold measured in the mid auxiliary line immediately superior to the iliac crest. The skin fold was picked up obliquely just posterior to the mid auxiliary line and parallel to the cleavage lines of the skin (Lohman et al., 1988). It was recorded in mm.

\[
\text{Sum of skin fold thickness (SSFT) mm} = \text{skin fold thickness of biceps} + \text{triceps} + \text{sub scapular} + \text{supra iliac skin folds}
\]
3.8.10. Body density

Behnke (1969) pioneered the measurement of the body density as an index of obesity. The body density can be calculated with the help of age and sex matched regression equation (Durnin and Womersley, 1974) using SSFT.

\[
\text{Body density (kg/m}^3\text{)}: c - (m \times \log \text{ of sum of SFTs})
\]

Where the values of ‘c’ and ‘m’ were taken from the tables of linear regression equation for the estimation of body density is presented below.

Linear regression equation for the estimation of body density

\[X \times 10^3 (\text{kg/m}^3) \text{ from the logarithm of the sum of skin fold thickness: Density} = c - m \times \log \text{ skin fold.}\]

**Table 6: Age and specific matched regression equation for the calculation of body density**

<table>
<thead>
<tr>
<th>Age (Yrs)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c</td>
<td>m</td>
</tr>
<tr>
<td>17-19</td>
<td>1.1620</td>
<td>0.0630</td>
</tr>
<tr>
<td>20-29</td>
<td>1.1631</td>
<td>0.0632</td>
</tr>
<tr>
<td>30-39</td>
<td>1.1422</td>
<td>0.0544</td>
</tr>
<tr>
<td>40-49</td>
<td>1.1620</td>
<td>0.0700</td>
</tr>
<tr>
<td>50+</td>
<td>1.1715</td>
<td>0.0779</td>
</tr>
</tbody>
</table>

**Source:** Durnin and Womersley, 1974
3.8.11. Body fat

Body fat was calculated as body fat percent and later computed to body fat in kilograms. These two are calculated as follows:

Calculation of body fat

The amount of fat present in the body was calculated from the fat percent. The formula used to calculate body fat in kilograms is:

\[
\text{Body fat (Kg)} = \frac{\text{Body weight (kg)} \times \text{Fat percent}}{100}
\]

Calculation of body fat percent

Although the densitometric method has been used as the most accurate method of determining percent body fat, the formulae that translate body density to percent body fat assume a constant value for the density of lean tissue for all individuals.

Calculations for the percent body fat were based on the equation given by Siri (1956).

\[
\text{Fat percent} = \left(\frac{4.95}{\text{Body density}} - 4.5\right) \times 100
\]

3.8.12. Lean body mass (LBM)

The lean body mass is composed of approximately 72 percent water, 20 percent protein, 1 percent carbohydrate and 7 percent minerals. The variability is less when compared to body fat. Neutral fat does not bind water or electrolytes; consequently the measurement of total body water or total body potassium offers a means for estimating non-fat component of the body. With the body weight of the subject and the body fat worked out earlier, the Durnin and Rahman formula is used for the calculation of LBM.

\[
\text{LBM (Kg)}: \text{Body weight (Kg)} - \text{Body fat (Kg)}
\]
3.9. Biochemical Assessment

3.9.1. Estimation of Blood glucose

Blood glucose levels in different samples were estimated by the GOD-POD method described by Carl et al. (1996). The glucose oxidase catalyzes the oxidation of glucose to glucuronic acid with the liberation of hydrogen peroxide. The released hydrogen peroxide combines with 4-aminoantipyrine and phenol yield a pink chromogen (Quinonemine) which catalyzed by peroxidase. The intensity of the color produced is directly proportional to glucose concentration in the sample. To 10μl of sample, 1.0 ml of enzyme reagent was added, mixed well and kept at 37°C for 15 minutes. 10μl of glucose standard and distilled water (blank) were also processed similarly. The absorbance of the sample was measured at 546 nm against blank by using automated merck analyzer (plate-8). The glucose concentration was expressed as mg/dl.

Fig 6: *Invitro* Biochemical analysis
3.9.2. Glycosylated hemoglobin levels (HbA\(_{1c}\))

Glycosylated hemoglobin in the blood was estimated by the method Bry et al., 2001 and Roberts et al., 2002. Hemosylate sample was prepared by adding 20ml of hemoreagent to 50ml of blood sample and was kept at room temperature for 10 to 15 min.

Commercially available resin micro-column equilibrated with 72 mm phosphate buffer (pH-6.5) was taken. The upper cap and bottom tip of the column was removed and rinsed with column buffer and let the column drain to waste completely. 50 μl of hemolysate was loaded to the upper disc with 200 μl of column buffer. Again 2ml of column buffer was loaded to run the column and let the column drain to waste. Now the resin bounded HbA\(_{1c}\) was eluted with 4ml of elution buffer. The collected HbA\(_{1c}\) fraction was read at 415 nm against distilled water by using automated merck analyzer.

3.9.3. Estimation of Total Cholesterol

Total cholesterol in the sample was estimated by the enzymatic method described by Trinder, 1969 and Allian et al., 1974. Cholesterol esters were hydrolyzed by cholesterol esterase to free cholesterol and free fatty acids. The free cholesterol produced and pre-existing one was oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide formed reacted with 4-aminoantipyrine and phenol in presence of peroxidase to produce red colored quinoneimine dye. The intensity of color produced was proportional to the cholesterol concentration. To 10 ml of sample, 1 ml of enzyme reagent was added, mixed well and incubated for 15 minutes at 37°C. 10μl of cholesterol standard and distilled water (blank) were also processed similarly. The absorbance was measured at 510 nm against blank by using merck analyzer. The concentration of cholesterol in the sample was expressed as mg/dl.

3.9.4. Estimation of HDL-Cholesterol

HDL-Cholesterol sample was estimated by the enzymatic method described by IZZO et al. (1981).
3.9.5. Estimation of VLDL and LDL Cholesterol

VLDL and LDL cholesterol was estimated and calculated by Friedwald et al., (1972) method.

3.9.6. Estimation of Triglycerides

Triglycerides in the sample were estimated by using the diagnostic kit band on the enzymic method described by Jacobs et al., 1960; Schetler et al., 1975.

The sample was hydrolyzed by lipase to glycerol and fatty acids. Glycerol was converted to glycerol-3-phosphate which was catalyzed by glycerol kinase. The glycerol-3-phosphate was oxidized by glycerol phosphate oxidase to dihydroxy acetone phosphate and hydrogen peroxide. In this reaction hydrogen peroxide was produced in equimolar concentration to the level of triacylglycerol phosphate in the sample. The hydrogen peroxide reacts with 4-aminoantipyrine and 3,5 dichloro-2-hydroxy benzene sulfonic acid in the presence of peroxidase to produce red quinoneimine. The intensity of color is proportional to the concentration of triglycerides in the sample. To 10 ml of sample, 1 ml of enzyme reagent was added, mixed well and incubated for 10 minutes at 37°C. 10μl of Triglycerides standard and distilled water (blank) were also processed similarly. The absorbance was measured at 505 nm against blank by using merck analyzer. The concentration of Triglycerides in the sample was expressed as mg/dl.

3.9.7. Estimation of creatinine

The Creatinine procedure is a kinetic modification of the Jaffé procedure (Tietz, 1986) in which creatinine reacts with picric acid at alkaline pH to form a yellow orange complex. However, this reaction is not completely specific for creatinine since other reducing substances such as glucose, pyruvate, ascorbic acid, and acetoacetates will react with picrate to form a similar color (Soldin, 1987). Fabiny and Ertingshausen.(1971) found that alkaline creatinine picrate reaches maximum color development at a different rate than pseudo-creatinine materialutilized different reaction rates of alkaline picrate positive substances to obtain greater specificity with the Jaffé reaction.
The rate of change in absorbance at 520/800nm is proportional to the creatinine concentration in the sample.

Creatinine + Alkaline Picrate Yellow-Orange Complex

Serum creatinine varies with the subject’s age, body weight, and sex. It is sometimes low in subjects with relatively small muscle mass, cachetic.

3.9.8. Estimation of Blood Urea Nitrogen

The blood urea nitrogen estimated by Beckmansynchron (2001), separated serum or plasma should not remain at +15 to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2 to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at –15 to –20°C. Frozen samples should be thawed only once. Analytic deterioration may occur in samples that are repeatedly frozen and thawed. Fasting is not required. A minimum of 0.6 mL serum is needed for the Multi-Analyte Panel. Sample volume for individual test is 10 μl added to 765 μl of reagent.

Note: Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the BUN determination is 1 mg/dL.

3.9.9. Estimation of Antioxidants

Plasma Ascorbic acid levels were estimated by using dinitrophenyl hydrazine method by Lowry et al. (1945).

β - Carotene and vitamin E estimated by Vuillemier et al. (1983).

3.10. Physiological assessment

3.10.1. Physical activity and energy consumption

Various physical activities are performed by obese NIDDM patients were assessed by following Bouchard et al. (1983) and Satyanarayana et al. (1986). Table of
activities, corresponding code values and energy costs (K.cal/min for 60kg person) were adopted from Bouchard et al. (1983). (Refer schedule 3 in appendix iii).

3.10.2. Assessment of Physical Activity and Energy Expenditure Pattern of the Subjects

Several methods of assessment of energy expenditure in free-living human populations covering periods extending from one day to several days are in vogue. Self-recording of various types of activities by the subject for 1440 min in a day in a record booklet is one of the popular methods used in the developed countries. Keeping in view the educational level and the nature of Indian subjects Satyanarayana et al. (1988) proposed a method suitable to rural women groups. In the present context this method was followed with the following modifications.

1. The physical activity was assessed part by self recording and recall and part by observation by the researcher.
2. Time spent for each activity was noted ignoring description related to intensity of the activity.

The subjects were explained regarding the importance of the information on physical activity. The schedule was divided into 96 periods of 15 Mnts intervals in each day. The various physical activities were grouped into nine activity zones. The subject was familiarized with this grouping and the respective activity codes. Later they were asked to enter the corresponding activity code in the 15 mt blocks from the time they awaken in the morning till they go to bed at night. Illiterate women were assisted by others to record the information. For each subject for a period of 18 hours the researcher herself observed the activities at randomized sessions of 6 hour duration. The remaining 12 wake hours (approximately) were either recalled or recorded by the subjects themselves.

The physical activity of the subjects was obtained for a period of three days viz., two week days and one weekend day to cover the variations that may exist in the activity pattern of rural women. The physical activity assessment was done on the same days when diet survey was conducted to facilitate the calculation of energy balance of subjects.
The activity cost of energy is given for 60 kg person (Bouchard et al., 1983). Therefore, a correction for body weight was made for the energy cost of each activity zone. The correction was obtained by dividing the Subjects actual weight by 60. This factor was multiplied with the energy cost of activity.

After recording the data the total time spent by the subject under each activity zone was obtained. This was multiplied with the energy cost of that category of activity to obtain the energy expenditure. The sum of the energy expended for each category of activity is the energy expended by the subject for a period of 24 hrs. In the present study the mean of energy expenditure of three days is presented as the Subjects energy expenditure per day.

3.10.3. Energy Balance

This is an important indicator, which can explain the nutritional state and also can throw light on the existing body composition of a subject. In the present study the energy intake and energy expenditure data is collected for a period of 3 days in a week. For each day the energy balance is computed by subtracting the energy expenditure from energy intake. Later the mean of 3 days is presented as the energy balance (±) of the subjects.

3.11. Blood Pressure (BP)

Mercury sphygmomanometers provide the most accurate measurement of BP. BK1002-sized cuff is used, the bladder should encircle and cover two third of the length of the arm; if not, place the bladder over the brachial artery to prevent high readings from bladder that is too small. When the BP is taken, the cuff should be inflated to a pressure approximately 30 mmHg greater than systolic, as estimated from the disappearance of the pulse in the brachial artery by palpation. Initial estimation of the systolic pressure by palpation avoids potential problems with an auscultatory gap. Korotkoff sounds transiently disappear as the cuff is deflated. Once the cuff is adequately inflated, the following steps should be followed:

The stethoscope should be placed lightly over the brachial artery, since the use of excessive pressure can increase turbulence and delay the disappearance of sound. The net
effect is that the diastolic pressure reading may be artifactually reduced by upto 10 to 15 mmHg.

The BP should always be taken with patient’s arm supported at the level of the heart. Allowing the arm to hang down when the patient was sitting or standing will result in the brachial artery being 15cm below the heart. As a result, the measured BP will be elevated by 10-15 mmHg due to the added hydrostatic pressure induced by gravity.

The cuff should deflate slowly at the rate of 2 to 3 mmHg per heartbeat. The systolic pressure is equal to the pressure at which the brachial pulse can first be palpated as blood flow is restored through the previously compressed vessel; the systolic pressure is also equal to the pressure at which the pulse is first heard by auscultation. As the cuff is deflated below the systolic pressure, the pulse continues to be heard until there is abrupt muffling (phase 4) and, approximately, 8 to 10 mmHg later, disappearance of sound (phase 5), although the point of muffling sound should be used in those patients in whom there is more than 10 mmHg difference between phase 4 and 5. The BP should be measured initially in both arms. If there is a disparity due to a unilateral arterial lesion, the arm with higher pressure should be used (WHO, 1978).

3.12. Dietary Survey

Diet surveys are essential part of all nutrition surveys. They provide useful information to interpret the existing nutritional situations in any community.

To elicit information pertaining to the quality and quantity of diets consumed by the obese NIDDM patients a schedule were formulated. The diet survey was conducted for 3 days in a week, selecting two week days and one weekend day. The data was collected in the combination of 24 hour recall and weighment method. The researcher herself measured the cooked food consumed by the subjects in randomized meal sessions, so that for each patient during the three day period all the meal sessions that would occur in a day were covered by the researcher.

Prior to the investigation the patients were provided with a set of standardized cups and the use was demonstrated. The patients were instructed to record the amount of
food consumed by them at all the meals, which were not attended by the researcher. Both the subjects and the investigator measured the food using the standardized cups.

The data collected on the cooked foods were converted to raw foods. Later the nutrient intake of the subjects was calculated using the nutritive value of Indian foods (ICMR, 1996).

3.12.1. Collection of Dietary data

Foods play a central role in reaffirming the cultural heritage thus food habits are an integral part of person’s life style and also one of the important determinants of nutritional status of an individual. Dietary survey was undertaken with the help of pre formulated survey schedule (Appendix-I) elucidating information pertaining to food habits, dietary intake, number of meals per day, foods consumed, away from home, exercise pattern and food consumption record. A standard cup is provided to measure the food they consume during the week period.

Each subject was asked to provide information pertaining to the size, number and thickness of breakfast recipes (Idly, dosa, puri, chapathi, etc), the amount and the type of oil used, the amount of sugar and salt consumed per day, frequency of intake of sweets and fried foods etc. The patients were assured that the information provided by them would be kept confidential.

The raw weight equivalents of cooked weight of recipes were determined in the laboratory and nutrients like total calories, carbohydrates, proteins, fats, cholesterol, dietary fiber, calcium, iron, vitamin A, B-Complex vitamins, B1, B2, B3, B6, B12, folic acid, sodium and the average nutrient composition of the three days were calculated separately. The average nutrient composition of the three days diet for each subject was calculated by using tables of food consumption (ICMR, 1991).

3.13. Clinical examination survey

Clinical examination has always been and remains an important practical method for assessing the diseased condition. Essentially the method is based on examination for changes, believed to be related to inadequate nutrition, that can be seen or felt in
superficial epithelial tissues, especially skin, eyes, hair and buccal mucosa, or in organs near the surface of the body, such as the parotids and thyroid glands, especially poly urea, poly dipsia and poly phasia.

The clinical signs were observed and noted as per the guidelines provided by Jelliffe (1966). The checklist on clinical symptoms grouped according to the deficiency. The results are expressed as percentage of the subjects showing the deficiency symptoms.


- Education about the relationship between nutrition and health
- Dietary and menu recommendations individualized for patient tastes and preferences
- Identifying food and meal choices to address both basic nutritional needs and control of weight, dyslipidemia and hyperglycemia
- Addressing and dispelling common myths about diet, nutrition and weight loss
- Guidance for the patient’s family and/or caregivers in meal planning, grocery shopping and low-fat methods of food preparation
- Identifying additional resources that may help improve the patient’s ability and willingness to observe dietary strategies.

3.14.1. Effective weight-loss programme

Effective weight-loss programmes should (NICE, 2008)

- Address the reasons why someone might find it difficult to lose weight
- Be tailored to individual needs and choices
- Be sensitive to the person’s weight concerns
- Be based on a balanced health diet
- Encourage regular physical activity
- Expect people to lose no more than 0.5-1 kg (1-2 lb) a week
- Identify and address barriers to change.
3.14.2. Counseling of Physical activity

The national recommendations are:

- To achieve general health benefits: accumulate at least 30 minutes of at least moderate-intensity physical activity on 5 or more days of the week (NICE, 2008).
- To lose weight: most people may need to do 45-60 minutes of moderate-intensity activity a day, particularly if they do not reduce their energy intake.
- People who have been obese and have lost weight may need to do 60-90 minutes of activity a day to avoid regaining weight.

The researcher has conducted counseling sessions by following the above instructions. Food pyramids and teaching charts prepared for the purpose of counseling. Nutrition counseling is given only one group as per the Research design. Obese NIDDM patients are willingly participated and gave their consent forms. The patients were counseled regarding Diabetic complications, Nutrition and health care, diabetic diets including Foods allowed and avoided, cooking methods, low fat usage, life style modification, physical activity, foot care, eye care, personal hygiene and self care etc. The patients were counseled in the presence of concern physician. At the time of counseling also provided information pamphlets. The patients were asked to come back for follow-up once in a month, for a period of 6 months. Feedback questions were asked to assess patients understanding of what was taught.

3.15. Statistical analysis

- The data obtained was analyzed using different statistical methods such as percentage, Per cent of parenthesis was used, where it required.
- Mean and standard deviation were calculated for all groups and for all the chosen parameters.
- ANOVA to find the significance of difference between groups after treatment. Logistic regression analysis was conducted between the age was associated with different complications.
For all these, SPSS, 2010 version software was used in the local computer center. The results were discussed and summarized in this treatise.

3.16. Interpretation of the data

The mean values of all parameters were compared with the Indian standards, International standards and available research data.

As the purpose of the study is to focus on the Herbal Supplementation capsules comparison with other parameters were discussed.