# Chapter IV

# Materials & Methods

## 4.1. Study Design:

This is open label; parallel study was conducted over a six months period. Patients were randomized in to six groups and each group enrolled fifty patients and patients were advised to have diet and exercise prescribed by physician. FBS, PBS were determined with semi autoanalyser and HbA1c by nycocard reader.

- ✓ The blood glucose level reading of each group compared.
- ✓ The weight of patients receiving drugs is monitored at each visit.
- ✓ The patients were assessed at base line, three months and six months for weight, HbA1c, FBS and PBS.

## 4.2. Duration of study:

The expected duration for participation of each subject enrolled in the study was 6 months.

## 4.3. Methodology:

### 4.3.1. Study Site:

Government Medical College and Hospital Aurangabad,

Deogiri Diabetic Research Center Aurangabad.

### 4.3.2. Study Design:

This is open label; parallel study.

### 4.3.3. Study Period:

The study was conducted for a period of six months from June 2010 to February 2011.

### 4.3.4. Source of Data:

Data was collected from

1. Patient Case sheet and medications chart.
2. Laboratory test reports.

### 4.3.5. Study criteria:

#### 4.3.5.1. Inclusion criteria:

1. Prediabetic or diabetic type 2 patients not controlled on diet and exercise.
2. HbA1c value between 5.7 to 7.5.
3. FBS more than 110 and PBS more than 140.
4. Readiness to give written informed consent.

#### 4.3.5.2. Exclusion criteria:

1. Patients with type-1 diabetes.
2. Patients requiring insulin for diabetic control.
3. Patients known allergic to study drugs.
4. Patients who has taken insulin in the past.
5. Patients with deranged liver function tests or kidney function tests.
6. Patients with rapidly progressive retinopathy, neuropathy, nephropathy.
7. Patients with myocardial infarction or anemia.
8. Patients not willing to give informed consent.
9. Pregnant and lactating female.
10. Presence of significant disease or condition including emotional disorders and substance abuse likely to alter the course of diabetes or patients ability to complete the study .
11. Presence of gastrointestinal diseases
    - Inflammatory bowel disease
    - Large hernias
    - Intestinal obstruction
    - Active ulcers
    - Chronic pancreatitis.
12. Concomitant medication effecting glucose homeostasis such as glucocorticoids within 8 weeks.
13. Any infections likely to affect glucose metabolism.

14. Uncontrolled thyroid function.
15. Any other investigational drug or participating in clinical study within 8 weeks before screening was allowed to participate.

### 4.3.6. Study Procedure:

Study protocol subject to approval by the Ethical committee of Govt. Medical College and Hospital, Aurangabad.

1. Four hundred (n=400) Type-2 diabetes patients were enrolled in the study.
2. These patients divided into eight groups and each group enrolled fifty (50) patients.
3. Patients were explained about the study pattern and related hazards.
4. Informed written consent was obtained from the patients.
5. A detailed clinical history will be taken and clinical examination will be performed.
6. Those included will undergo all baseline investigations like complete blood count, liver function tests, kidney function tests, blood sugar level, fundoscopy and Glycosylated Hb, triglyceride levels and TSH at the **start** of the study and at the **end** of the study.
7. Enrolled patients will be divided into two groups of 30 each according to random number table.
8. Each patient in respective group will be provided free samples for fifteen days and asked to visit the diabetic clinic for follow up and for collection of drugs. At each follow up visit, patients will be assessed for glycemic control (blood sugar level) once in a month (4 weekly), history pertaining to adverse drug effect will be asked. All patients will be given advice about diet and exercise.
9. The study population will not allow taking antidiabetic drugs other than study drugs.

**Table No. 14. Patients Group:**

| | |
|---|---|
| Group I | Acarbose |
| Group II | Miglitol |
| Group III- | Acarbose+S. Oblonga |
| Group IV | Acarbose+ S. Reticulata |
| Group V | Miglitol+ S. Oblonga |
| Group VI | Miglitol+S. Reticulata |
| Group VII | S. Oblonga |
| Group VIII- | S. Reticulata |

### 4.3.7. Efficacy Measures:

- Change in Fasting blood glucose level from baseline to end of study (6 Months)
- Change in Post meal blood glucose level from baseline to end of study (6 Months)
- Change in Glycosylated haemoglobin (HbA1C) from baseline to end of study (6 Months).
- Change in weight from baseline to end of study (6 Months)

#### 4.3.7.1. Collection of Blood Samples:

Patients were asked to come fasting in diabetic clinic, 2 ml of venous blood sample were collected in fluoride bulb to estimate the fasting blood sugar levels, followed by assessment of Postprandial blood sugar values after 2 hours.

#### 4.3.7.2. Sample Analysis:

Estimation of blood Glucose Level and Glycosylated Hb

### 4.3.8. Blood Glucose estimation:

#### 4.3.8.1. Methodology:

##### 4.3.8.1.1. Trinder's Method

**Principle:**

$$Glucose + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} Gluconic\ acid + H_2O_2$$

$$H_2O_2 + 4\ HBA + 4\ AAP \xrightarrow{\text{Peroxidase}} Quinoneimine\ Dye + 2H_2O$$

4AAP: 4 – Aminoantipyrine

4HBA: 4 – Hydroxy benzoic acid

The intensity of the pink color formed is proportional to the glucose concentration and can be measured photometrically between 490 to 540nm.

**Table 15: Reagent 1:**

| | |
|---|---|
| Glucose oxidase | 20000 IU/L |
| Peroxidase | 3250 IU/L |
| 4-Aminoantipyrine | 0.52 mmol/L |
| 4 – Hydroxybenzoic acid | 10 mmol/L |
| Phosphate buffer | 110 mmol/L |

Also contains non reactive fillers and stabilizers pH 7.0 + 0.2 at 25$^{o}$C.

**Table 16: Reagent 2: Glucose Standard**

| | |
|---|---|
| Glucose Standard | 100 /dl (5.55 mmol/L) |

**Table 17: Assay Parameters:**

| **Mode** | **End Point** |
|---|---|
| Wavelength 1 (nm) | 510 |
| Wavelength 2 (nm) | 630 |
| Sample volume (µl) | 5/10 |
| Reagent Volume (µl) | 500 /1000 |
| Incubation time (Min.) | 15 |
| Incubation Temperature (0C) | 37 |
| Normal Low (mg/dl) | 70 |
| Normal High (mg/dl) | 110 |
| Linearity Low (mg/dl) | 0 |
| Linearity High (mg/dl) | 500 |
| Concentration of Standard (mg/dl) | 100 |
| Blank with | Reagent |
| Absorbance Limit (Maximum) | 0.3 |
| Units | mg/dl |

**Table 18: Assay Procedure:**

| **Pipette into tubes marked** | **Blank** | **Standard** | **Test** |
|---|---|---|---|
| Working Reagent | 1000 µl | 1000 µl | 1000 µl |
| Distilled Water | 10 µl | -- | -- |
| Standard | -- | 10µl | -- |
| Sample | -- | -- | 10µl |

### 4.3.9. Calculation:

**Glucose (mg/dl) = [Absorbance of Sample / Absorbance of standard] x Concentration of standard.**

#### 4.3.9.1. Estimation of HbA1C (Glycosylated Hb):

It is done by NYCOCARD HbA1C KH method.

- It is rapid in Vitro Method.

##### 4.3.9.1.1. Test Principle:

- Nycocard HbA1C is a baronate affinity assay.
- The kit contains test devices with a porous membrane filter, test tubes pre-filled with reagent and a washing solution.
- The reagent contains agents that lyses Erythrocytes and precipitate Hb specifically as well as a blue boronic acid conjugate that binds cis-diols of glycated Hb.
- The precipitate is evaluated by measuring the blue (glycated haemoglobin) and the red (total Hb) colour intensity with the NycoCard.

Reader II the ratio between them being proportional to the percentage of HbA1C in the sample.

##### 4.3.9.1.2. Kit Contents, (24 test kit):

1. TD/Test Device (1 x 24 units)
2. Plastic device containing a membrane filter.
3. R1/ Reagent (1 x 24 units x 0.2 mL)
4. Glycinamide buffer containing Zn ions, dye bound boronic acid and detergents.
5. R2/ Washing Solution (1 x 2.0 mL) .
6. Morpholine buffered NaCl solution and deterents.
7. Measuring range: 3 – 18% HbA1C .
8. Measuring interval: 0.1% HbA1C

##### 4.3.9.1.3. Stability and Storage:

- The expiry date of kit applies to storage at 2-80C or humidity above 70% should be avoided.
- Do no freeze.

**4.3.10. Sample Material:**

Blood samples can be stored up to 10 days at 2-80C before analysis. Avoid measuring haemolysed sample.

**4.3.10.1. Test Procedure:**

1. To precipitation of haemoglobin add 5µL whole blood to the test tube prefilled with R1Reagent. Mix well. Leave the tube for minimum 2 minutes, maximum 3 minutes.
2. Application of sample: Remix to obtain a homogenous suspension. Apply 25µL of the reaction mixture to a TD/ Test Device by holding the pipette approx 0.5cm above the test well. Empty the pipette into the membrane (approx. 10 seconds).
3. Application of R2/Washing Solution: Apply 25µL R2/Washing Solution to the TD/Test device. Allow the washing solution to soak completely into the membrane. Wait for minimum 10 seconds.
4. Test result measurement: Read the test result within 5 minutes using NycoCard Reader II.

Note: Further instructions are given in the NycoCard Reader II instruction manual.

**4.3.10.2. Important Procedural Notes:**

Do not interchange components from different kits or kit lots

- Equilibrate the R1/Reagent to room temperature (20–250C) before use.
- Do not touch the membrane with the pipette tip.
- Change the pipette tip between each pipetting step.

**4.3.11. Sample material:**

Capillary blood and venous blood with or without anticoagulant (EDTA, heparin and NaF) can be used.

**4.4. Statistical Analysis:**

- For comparing the effect of Acarbose Miglitol, and in combination with Salacia species on blood sugar level and HbA1c before and after therapy, statistical

analysis was done by descriptive statistics as mean, SD, percentage, proportions etc.

- Data were presented in the form of Tables and graphs. Student's Paired and Unpaired 't' tests were applied to observe performance before and after stage, and comparisons of all groups under study..
- The probability values were considered as (p, 0.05) and (p, 0.01).
- At $p<0.05$ results were considered as significant and $p<0.01$, considered as highly significant.
- Two ways ANOVA were performed for repeated measures and Tukey– Kramer test (F test) were applied to analyze the group's data.
- Statistical analysis software namely SYSTAT version 12 by Cranes Software, Bangalore (a licensed copy) were used to analyze the data in the study.

**4.5. Informed Consent:**

Patients willing to participate and eligible in the view of investigator were given the Patient Information Sheet in his/her vernacular language and all study related tests and procedures were explained to him/her in simple yet detailed manner. After imparting sufficient information, if the patient desired to be a part of the study then his consent (signature or thumb impression) was taken in the informed consent form as in Appendix. The informed consent form was agreed by IRB/IERC

**4.6. Ethical Committee Approval:**

Intuitional Ethical Committee Clearance was obtained from the Ethical Committee of Government Medical College and Hospital Aurangabad.

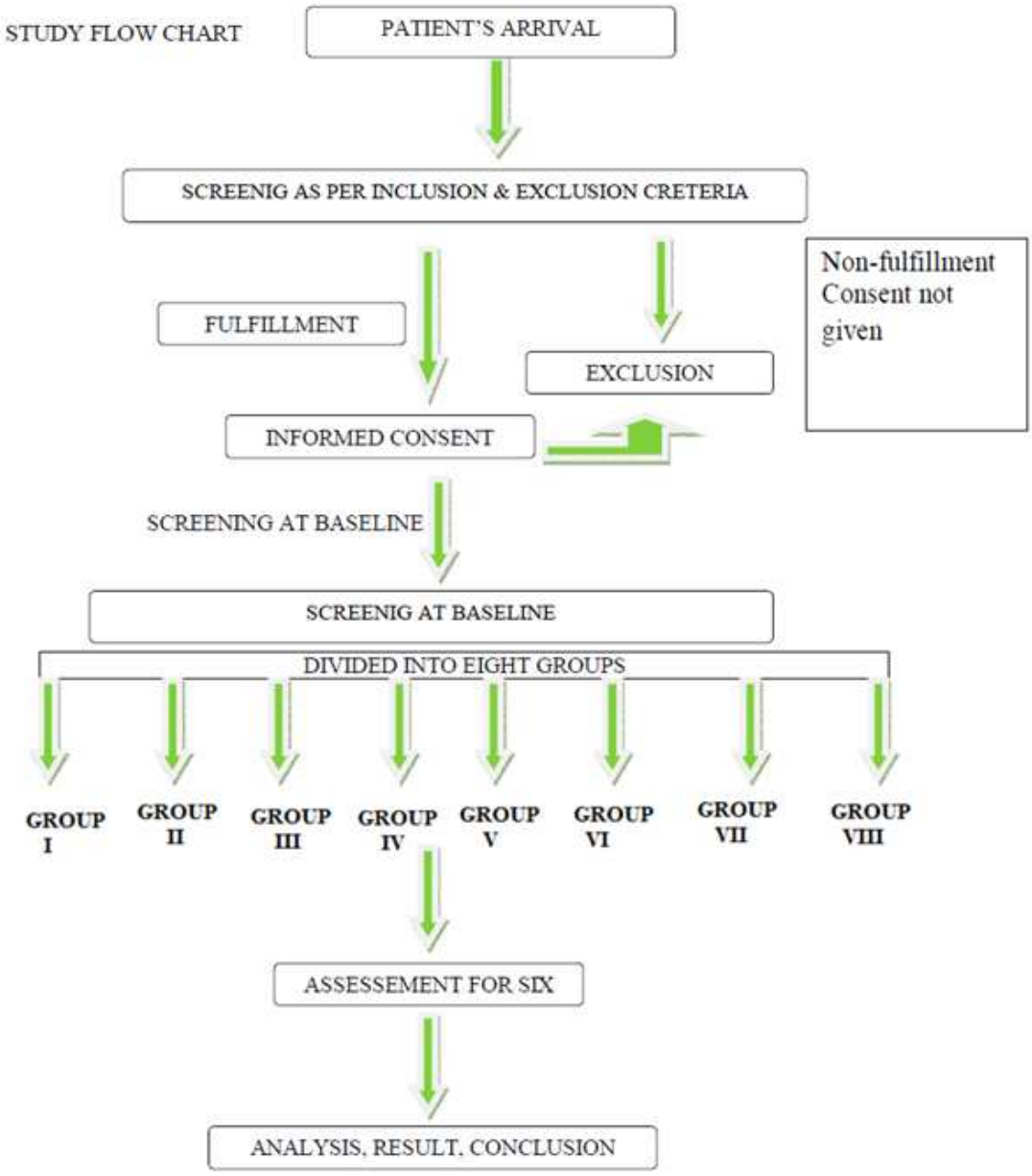
**4.6. Dropouts:**

07 patients left study from acarbose group before completion of study.

**4.7. Adverse Drug Reaction:**

In some patients from acarbose and miglitol group GI disturbances and epigastric distress observed.

**4.8. Flow chart of study:**

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