4. STUDY DESIGN

(MATERIAL AND METHODS)

4.1 Locus

The present study was undertaken in Acharya Vinoba Bhave Rural Hospital, teaching hospital of Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha and Civil Hospital, Wardha. All the Biochemical procedures were carried out in Central Research Laboratory, DMIMS, Sawangi (M), Wardha.

4.2 Duration of Study

The data was collected from Jul. 2006 to Apr. 2010.

4.3 Approval from Institutional Ethics Committee (IEC)

The approval of Institutional Ethical Committee was obtained vide their letter no. DMIMS/IEC/20056-07/109 dated 28/07/2006. (Copy enclosed as Appendix –Page no. 340).
4.4 Methodology:

4.4.1 Study design (Type of study)

i) Block Randomization Open Study: (321)

ii) Sample size: (321)

Assistance of statistician was sought for determination of sample size and the block randomization. The details of the procedure are as under-

Factors considered for calculation of sample size:

a) Pilot study:

1. In thirty (30) patients pilot study was carried out. Oxidative stress parameters, MDA and SOD were estimated on Day-0 and Day-60. The average values of these parameters in the Control and group treated with Hemidesmus indicus were summarized in the following table. The percent variation was calculated from Day-0 to Day-60. This percentage variation was considered as an event rate for calculation of sample size.

<table>
<thead>
<tr>
<th>Group</th>
<th>Average MDA in n Mol/ml</th>
<th>Average SOD U / gm Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment*</td>
</tr>
<tr>
<td>0 days</td>
<td>32.528</td>
<td>29.718</td>
</tr>
<tr>
<td>60 days</td>
<td>38.944</td>
<td>28.285</td>
</tr>
<tr>
<td>% of variation</td>
<td>+ 19.724</td>
<td>-4.823</td>
</tr>
<tr>
<td>Remarks</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
</tbody>
</table>

Treatment* - Hemidesmus Indicus
From the table, it is observed that treatment with *Hemidesmus indicus* reduced the levels of MDA and increased the levels of SOD significantly by 19% and 4% respectively as compared to control from Day-0 to Day-60. These approximate values were considered as an event rate and were denoted by $\pi_1 = 19.00\%$ and $\pi_2 = 4.00\%$ for further calculation.

It is aimed to detect the smallest treatment effect.

The significance level at which the null hypothesis is to be rejected.

The power with which an effect is detected.

The design of the study (parallel or crossover, etc.)

The expected dropout rate of subjects during the study.

**Level of significance**

The significance level is 5% at which we will reject the null hypothesis that (there is no difference in the treatment effects).

**Power of a study**

The power of a study is the ability to find a significant difference if it exists and it was $100\% - \beta$, which is $100\% - 20\% = 80\%$. 
The following formula was used to calculate the sample size

\[ n = \frac{\left[ z \left(\frac{\alpha}{2}\right) \sqrt{2\pi \left(1 - \pi\right)} + z \left(\beta\right) \sqrt{\pi_1 \left(1 - \pi_1\right) + \pi_2 \left(1 - \pi_2\right)} \right]^2}{\delta^2} \]

where

\( \alpha \) = the Type I error rate

\( \beta \) = the Type II error rate

\( \pi_1 \) = the expected event rate in the control group

\( \pi_2 \) = the expected event rate in the treatment group

\( \pi = (\pi_1 + \pi_2) / 2 \)

\( \delta = \pi_1 - \pi_2 \)

\( z \left(\frac{\alpha}{2}\right) \) = constant from the standard normal distribution depending on the value of \( \alpha \)

\( z \left(\beta\right) \) = constant from the normal distribution depending on the value of \( \beta \)

For the given study:

\( \alpha = 5\% = 0.05 \)

\( \beta = 20\% = 0.20 \)

\( \pi_1 = 19.00\% = 0.1900 \)
\[ \pi_2 = 4.00\% = 0.0400 \]

\[ \pi = (\pi_1 + \pi_2) / 2 = (19.00\% + 4.00\%) / 2 = 23.00\% / 2 = 0.11500 \]

\[ \delta = \pi_1 - \pi_2 = 19.00\% - 4.00\% = 15.00\% = 0.1500 \]

\[ z(\alpha / 2) = 1.96 \]

\[ z(\beta) = 0.842 \]

Therefore

\[ n = \frac{[1.96 \times (\sqrt{0.1150 (1 - 0.1150)}) + 0.842 \times (\sqrt{0.1900 (1 - 0.1900) + 0.0400 (1 - 0.0400)})]^2}{(0.1515)^2} \]

\[ = 91.39 \approx 91 \]

Therefore for our Randomized Control Trial (RTC), a sample size of 91 subjects is required for each group.

**Accounting for drop-out:**

Considering 20% dropout rate (a ratio of 0.20) within the trial, the sample size per group adjusted accordingly as \[ 91 / (1 - 0.20) = 113.75 \approx 114 \] subjects are required in each treatment group. For our Randomized Clinical Trial (RCT),

No. of subjects required = 342 (=114*3).
b) Block Randomization

Procedure for Block randomization:

Total 270 patients suffering from Tuberculosis DOT (90) and Hypertension (CCB (90) and OA (90)) with 4 treatment groups each having two dose levels (single and double) were randomized in 27 groups of 10 patients each with control groups for category of treatment. The allocation of patients for treatment with different levels of doses was done with permuted-block randomization. A block randomization method was used periodically to enforce a balance in the number of patients assigned to each treatment. The 10 patients was the size of each block of allocations of treatment groups with 4 treatment strategies at each dose level and control. A block randomization was implemented in three steps:

Step 1: Calculated block size and the number of blocks needed to cover the number of patients in the study.

Step 2: List all possible permutations of treatments in a block.

Step 3: Generate a randomization code for the order in which to select each block.
Block Randomization:

**Table -4.2 Block Randomization Chart.**

<table>
<thead>
<tr>
<th>Block</th>
<th>Treatment with dose level to be assessed in patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COX₁  H₁  H₂  E₁  E₂  C₁  C₂  C₁E₁  C₂E₂</td>
</tr>
<tr>
<td>2</td>
<td>H₁X₂  H₂  E₁  E₂  C₁  C₂  C₁E₁  C₂E₂  CO</td>
</tr>
<tr>
<td>3</td>
<td>H₂X₃  E₁  E₂  C₁  C₂  C₁E₁  C₂E₂  CO  H₁</td>
</tr>
<tr>
<td>4</td>
<td>E₁X₄  E₂  C₁  C₂  C₁E₁  C₂E₂  CO  H₁  H₂</td>
</tr>
<tr>
<td>5</td>
<td>E₂X₅  C₁  C₂  C₁E₁  C₂E₂  CO  H₁  H₂  E₁</td>
</tr>
<tr>
<td>6</td>
<td>C₁X₆  C₂  C₁E₁  C₂E₂  CO  H₁  H₂  E₁  E₂</td>
</tr>
<tr>
<td>7</td>
<td>C₂X₇  C₁E₁  C₂E₂  CO  H₁  H₂  E₁  E₂  C₁</td>
</tr>
<tr>
<td>8</td>
<td>C₁E₁X₈  C₂E₂  CO  H₁  H₂  E₁  E₂  C₁  C₂</td>
</tr>
<tr>
<td>9</td>
<td>C₂E₂X₉  CO  H₁  H₂  E₁  E₂  C₁  C₂  C₁E₁</td>
</tr>
</tbody>
</table>

X- indicates the extra patient considering the chances drop out

**iii) Statistical Model (Statistical technique used)**

Considering the nature and type of data to be generated in the present study following statistical measures were used.

- **Mean and Standard deviation**: Data relating to various characteristics of patients under study were represented in form of frequency and percentage. For that same basic statistical tools like mean and standard deviation were used.

  (Mean ± S.D).
- **95% Confidence Interval**: The exact value of parameter as per patient’s data for different doses of treatment, point estimates were used with 95% of confidence interval.

- **One Way ANOVA and Post –Hoc Least square technique**: To test the treatment effect at various doses of treatment, One Way ANOVA technique was used and significant dose effect was traced out by Post – Hoc Least square technique.

- **Coefficient of variation**: For identifying the effective dose from the non significant difference, Coefficient of variation was used to identify the consistent dose effect for the given patients.

- **F- value**: To test the mean significance difference between the different doses and treatments F- value was calculated for testing the variation between groups and within groups was tested at $P < 0.05$ and $P < 0.01$.

- **Statistical software SPSS13.0 and SYSTAT 12.0**: To handle the large data and reduce the error for several variables, Statistical software SPSS13.0 and SYSTAT 12.0 and statistical functions in MS-Excel 2007 were used.

### 4.4.2 Types of oxidative stress:

It is an open study without blinding. However treatment subgroups were assigned by random allocation. This study was carried in oxidative stress caused by two different diseases.
i. Oxidative stress caused by chronic infection - Tuberculosis

ii. Oxidative stress caused by chronic degenerative condition - Hypertension.

4.4.3 Estimation of specific and non-specific markers of oxidative stress.

i) The specific markers of oxidative stress are—

   i.a). Malondialdehyde (MDA)

   i.b). Superoxide dismutase (SOD)

ii) The non-specific markers of oxidative stress are

   ii.a). Total Cholesterol

   ii.b). Triglyceride (Tg)

   ii.c). High Density Lipids (HDL)

   ii.d). Low Density Lipids (LDL)

   ii.e). Very Low Density Lipids (VLDL)

iii) Other investigations

   iii.a). Haemoglobin (Hb)

   iii.b). Serum Sodium (Na+)

   iii.c). Serum Potassium (K+)
Collected blood sample was further processed and prepared for specific and non-specific markers of oxidative stress.

4.4.4 Preparation of sample

i). Blood sample from plane bulb was centrifuged to separate serum & this serum was used as sample for estimation of –

   Malondialdehyde (MDA)

   Total Cholesterol

   Triglyceride (Tg)

   High Density Lipids (HDL)

   Low Density Lipids (LDL)

   Very Low Density Lipids (VLDL)

   Serum Sodium (Na$^+$)

   Serum Potassium (K$^+$)

ii) Blood samples from EDTA bulbs were used for estimation of Hemoglobin (Hb) and same were also used for preparation of haemolysate. Haemolysate was prepared according to procedure mentioned by Ramnik (322). Haemolysate was used for estimation of Superoxide dismutase (SOD).
### 4.4.5 Procedures:

i) The specific markers of oxidative stress –

1) Estimation of Malondialdehyde (MDA) in serum –(323)

Principle: Thio-barbituric acid reacts with malonaldehyde, one of the aldehyde products of lipid peroxidation to give a colored product which is extracted in butanol and absorbance measured spectrophotometrically at 530 nM.

![Thiobarbituric acid reaction](image)

**Fig-4.1. Thiobarbituric acid reaction**

Standard solution: Malonaldehyde bis (diethyl-acetal) procured from Merck Schuchardt OHG 85662 Hohenbrum, Germany, was dissolved in 0.05M sulphuric acid to prepare 10 uM solution. This 10uM solution was further diluted to obtain standard MDA of different concentration like 1 nmole/ml, 2 nmole/ml, 3 nmole/ml, and 4nmole/ml ---------- upto 10 nmole/ml.

Before starting with the samples a standard graph of concentration against absorbance was plotted. A straight line graph indicates persistency and accuracy of the procedure.
Procedure:

Table – 4.3 Addition of reagents for estimation of MDA.

<table>
<thead>
<tr>
<th></th>
<th>Standard (ml.)</th>
<th>Test (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard sol.</td>
<td>0.200</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>0.200</td>
</tr>
<tr>
<td>TCA (20%)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>TBA (0.67%)</td>
<td>0.800</td>
<td>0.800</td>
</tr>
</tbody>
</table>

2.00 ml of TCA was added to each test tube, (0.200) ml of standard or sample was added to standard and test tubes respectively. 0.800ml of TBA (0.67%) was also added to each test tube. It was mixed well and then kept in boiling water bath for 30 min. After 30 min. the test tubes were cooled under tap water. 4.00ml of n-Butyl alcohol was added to each test tube. All the tubes were centrifuged at 3000 rpm for 10 min. The absorbance of supernatant was read at 530nM using n-Butyl alcohol as blank.

Calculations:

MDA

Conc. (nMol/ml) test = Abs. of test/Abs. of std. × Conc of std. (nMol).

**Principle:**

This method utilizes the inhibition of autoxidation of pyrogallol by Superoxide dismutase enzyme.

**Procedure:**

The assay mixture in a 3.00 ml volume consisted of 300 ul of Pyrogallol (0.2 mM),

<table>
<thead>
<tr>
<th>Table – 4.4 Addition of reagents for estimation of SOD.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagents</strong></td>
</tr>
<tr>
<td>Tris buffer</td>
</tr>
<tr>
<td>Standard/Haemolysate</td>
</tr>
<tr>
<td>Working Pyrogallol</td>
</tr>
<tr>
<td>Total volume</td>
</tr>
</tbody>
</table>

The reaction mixture prepared in three sets of test tubes according to table –

a) Control – Neither test nor standard was added to assay mixture to obtain uninhibited auto oxidation of pyrogallol.

b) Standard – Known amount of SOD units in different concentrations were added to assay mixture to achieve inhibition of pyrogallol auto-oxidation.
c) Test – The haemolysate of individual samples were added in place of standard SOD units.

Pyrogallol was added after the addition of all other reagents to reaction. Initial 90 sec. period was considered as induction period of enzyme. So after 90 sec. change in absorbance at 420 nm at 20 sec. interval was recorded for a period of 270 sec (4.5 min.). Change in absorbance per min. was calculated and percentage of inhibition in standard and test was calculated using the formula:

\[
\% \text{ of inhibition} = 100 - \frac{\text{Diff. Blank} - \text{Diff. STD}}{\text{Diff. Sample}} \times 100
\]

For standard graph in place of sample different concentrations of standard were added and absorbance was read at 420nM.

Before starting with the samples a standard graph of concentration against absorbance was plotted. A straight line graph indicates persistency and accuracy of the procedure (Next page).
**Study Design (Material and Methods)**

**Fig- 4.2 Standard graph- MDA**

**Fig-4.3 Standard graph- SOD**
ii) Estimation of Non-specific Markers

1) Total Cholesterol (Tot.Chol),

2) Triglyceride (Tg) and

3) High density lipoprotein cholesterol (HDL) were carried out by the Kit Method.

4) Low lipoprotein cholesterol (LDL),

5) Very low density lipoprotein cholesterol (VLDL) were calculated from the estimated values of (Tot.Chol), (Tg) and (HDL)

iii) Other Investigations

1) Estimation of Hemoglobin: (325)

2) Estimation of Sodium and Potassium: (326)

*Fig– 4.4 Flamephotometer.*
4.5 **Material**

4.5.1 **Drugs:**

   a) Antitubercular Drugs   
   b) Antihypertensive Drugs

   c) Antioxidants

4.5.2 **Selection of doses**

4.5.3 **Patients**

4.5.4 **Equipments and Instruments**

4.5.5 **Chemicals**

4.5.1 **Drugs:**

   a) Antitubercular Drugs:

   Directly Observed Treatment- short course (DOTs) strategy as prescribed in Revised National Tuberculosis Control Programme (RNTCP).

   b) Antihypertensive Drugs:

   i) Calcium Channel Blockers (CCB):

   ii) Antihypertensive drugs other than Calcium Channel Blockers (OA):

   c) Antioxidants:

   Types of antioxidants used –
i) *Hemidesmus indicus* - Herbal antioxidant

ii) Vitamin E - Lipid soluble antioxidant

iii) Vitamin C - Water soluble antioxidant

iv) Combination of Vitamin C and Vitamin E - Both lipid soluble and water soluble antioxidant.

**Preparations used:**

i) *Hemidesmus indicus* root powder.

ii) Vitamine E Capsules USP (Cap. Evion 400)

iii) Vitamin C Tablet IP 500 mg. (Tab. Celin 500 mg.)

**Sources of antioxidants:**

Drugs (Antioxidants) were obtained from following sources:

i) *Hemidesmus indicus* root powder –

Drug was procured from Department of Dravyaguna, MGACH&RC, Salod (H), Wardha.

The experts of Department of Dravyaguna, MGACH&RC identified the dried roots prepared fine powder in their Ayurvedic Pharmacy Laboratory. The authenticated powder was procured from above source for this study.
Fig-4.5  Photograph of herberium of *Hemidesmus indicus*. 
Study Design (Material and Methods)

Fig-4.6 Photograph of *Hemidesmus indicus*.

Fig-4.7 Photograph of *Hemidesmus indicus*. 
ii) Vitamine E – Capsules USP (Merck Limited) 1 Cap. Evion 400

Manufactured specially for JNMC, Sawangi (M), Wardha.

Manufactured by- Merck Limited, Usgaon, Ponda, Goa. 403407. The provided sample was - Cap. Evion 400. Batch no. 60382608

Date of manufacturing: Feb. 2008

Date of expiry: Apr. 2010

iii) Vitamin C Tablet IP 500 mg.

Tab. Celin 500 mg. (GSK)

Manufactured by- GlaxoSmithKline (gsk), 10, MIDC, Ambad, Pathari block, Nashik. 422010

The provided sample was - Tab. Celin 500 mg. LOT NA 549


Date of expiry: Feb. 2010.

4.5.2 Selection of dose: Single and double dose -

As reported by Robert F. Cathcart (1985) (27), the responses of the treatment are variable depending on the doses. Massive doses of Ascorbate help in improvement of the infectious diseases. In our study antioxidants act as scavenger so if we use different doses, patients may be benefited with higher doses and we may get some idea about additional benefits provided with higher
doses of antioxidants. It was decided to use two dose levels of each antioxidant treatment were selected for this study.

Single dose– Conventional therapeutic dose or prescribed dose and

Double dose– Two times Conventional therapeutic dose.

**Doses of Hemidesmus indicus :**

Conventional therapeutic dose of *Hemidesmus indicus* is

1.00gm - 6.00gm of root powder with milk according to home remedies guide (327).Following were the doses selected for this study-

Single dose– Conventional therapeutic dose -3.00gm o.d.

Double dose– Two times Conventional therapeutic dose-3.00gm b.d.
4.5.3 **Patients:**

Oxidative stress (patients)

I) Oxidative stress caused by chronic infection

II) Oxidative stress caused by degenerative condition

- Tuberculosis
- Hypertension

Tuberculosis Calcium Channel Blockers Antihypertensives

(T) (90 patients) (CCB) (90 patients) (OA) (90 patients)

*Fig -4.8 Distribution of patients.*

**a) Selection of the Patients**

**i) Oxidative stress caused by chronic infection - Tuberculosis**

Once diagnosis was confirmed by the Physicians of Department of T.B.
& Chest Medicine, A.V.B.R. Hospital, Sawangi or DOT Centre, Civil Hospital,
Wardha, the patients were randomly selected for the study. Written informed consent (annexure I) was obtained from each patient before commencement.

**Inclusion Criteria:**

- Patients diagnosed as a case of Pulmonary/ Extra Pulmonary Tuberculosis.
- Ambulatory patients of either sex.
- Age 18 years to 70 years.

**Exclusion Criteria:**

- Patients suffering from other infections along with Tuberculosis (HIV).
- Patients having history of relapse for more than two times or labeled as Multidrug Resistant cases.
- Patients below the age of 18 years and diagnosed as the patient of Tuberculosis.
- Patients with liver failure.
- Patients with kidney failure.

ii) Oxidative stress caused by chronic degenerative condition - Hypertension
Inclusion Criteria:

➢ The patients with mild to moderate essential hypertension, attending the out-patient department of Medicine, A.V.B.R. Hospital, Sawangi (M) were selected for the study. Written informed consent (annexure I) was obtained from each patient before selection into the study.

➢ Patients diagnosed with Stage I (SBP 140-159; DBP 90-99 ) and Stage II (SBP 160-179; DBP 100-109) uncomplicated essential hypertension as defined by “The Sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure”.

➢ Ambulatory patients of either sex.

➢ Age 20 years to 65 years.

➢ Patient who gave written consent.

Exclusion Criteria (328)

Patient suffering from following condition were excluded from the study:

➢ Addicted to tobacco and alcohol.

➢ Receiving lipid lowering agents.

➢ Any of the complication of hypertension such as heart failure, stroke, and renal failure.
Study Design (Material and Methods)

- Pregnancy and lactation.
- Female patients who were on any oral contraceptives.
- Patient who did not consent for participation in study.

b) Grouping of Patients

After taking written consent the subjects were randomly distributed in three groups. Primary group (A, B and C) were assigned by Block randomization.

The primary groups and subgroups were as per details given below:

Group A: Patients suffering from Tuberculosis under DOT Therapy (T)

Group B: Hypertensive patients under treatment with Calcium Channel Blocker (CCB)

Group C: Hypertensive patients under treatment with other antihypertensive drugs than Calcium Channel Blockers (OA)
Each primary group consisted of nine (9) sub-groups as under:

**I. Oxidative stress caused by chronic infection -**

**A) Tuberculosis**

Ninety patients taking treatment for Tuberculosis from DOTs center were randomly selected and divided in nine sub-groups, each sub-group consisting ten patients. The sub-groups were labeled according to the antioxidants and the dose they received.

**Tuberculosis (T)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1)</td>
<td>T-CO</td>
</tr>
<tr>
<td>A2)</td>
<td>T-H1</td>
</tr>
<tr>
<td>A3)</td>
<td>T-H2</td>
</tr>
<tr>
<td>A4)</td>
<td>T-E1</td>
</tr>
<tr>
<td>A5)</td>
<td>T-E2</td>
</tr>
<tr>
<td>A6)</td>
<td>T-C1</td>
</tr>
<tr>
<td>A7)</td>
<td>T-C2</td>
</tr>
<tr>
<td>A8)</td>
<td>T-C1E1</td>
</tr>
<tr>
<td>A9)</td>
<td>T-C2E2</td>
</tr>
</tbody>
</table>
iii) Oxidative stress caused by chronic degenerative condition - Hypertension

B) Patients taking Calcium Channel Blockers as antihypertensive

**Hypertension (CCB)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1) CCB-CO</td>
<td>CCB control (pt. receiving Calcium Channel Blockers Antihypertensive drugs)</td>
</tr>
<tr>
<td>B2) CCB-H1</td>
<td>CCB + <em>Hemidesmus indicus</em> (R.P) 3.00gm o.d.</td>
</tr>
<tr>
<td>B3) CCB-H2</td>
<td>CCB + <em>Hemidesmus indicus</em> (R.P) 3.00gm b.d.</td>
</tr>
<tr>
<td>B4) CCB-E1</td>
<td>CCB + Vit.E 400mg o.d.</td>
</tr>
<tr>
<td>B5) CCB-E2</td>
<td>CCB + Vit.E 400mg b.d.</td>
</tr>
<tr>
<td>B6) CCB-C1</td>
<td>CCB + Vit.C 500mg o.d.</td>
</tr>
<tr>
<td>B7) CCB-C2</td>
<td>CCB + Vit.C 500mg b.d.</td>
</tr>
<tr>
<td>B8) CCB-C1E1</td>
<td>CCB + Vit.C 500mg o.d. + Vit.E 400mg o.d.</td>
</tr>
</tbody>
</table>
C) Patients taking Antihypertensives Other than Calcium Channel Blockers (OA)

**Hypertension (OA)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1) OA-CO</td>
<td>(OA) control (pt. receiving Antihypertensive other than Calcium Channel Blockers)</td>
</tr>
<tr>
<td>C2) OA-H1</td>
<td>(OA) + <em>Hemidesmus indicus</em> (R.P.) 3.00gm o.d.</td>
</tr>
<tr>
<td>C3) OA-H2</td>
<td>(OA) + <em>Hemidesmus indicus</em> (R.P.) 3.00gm b.d.</td>
</tr>
<tr>
<td>C4) OA-E1</td>
<td>(OA) + Vit.E 400mg o.d</td>
</tr>
<tr>
<td>C5) OA-E2</td>
<td>(OA) + Vit.E 400mg b.d</td>
</tr>
<tr>
<td>C6) OA-C1</td>
<td>(OA) + Vit.C 500mg o.d</td>
</tr>
<tr>
<td>C7) OA-C2</td>
<td>(OA) + Vit.C 500mg b.d</td>
</tr>
<tr>
<td>C8) OA-C1E1</td>
<td>(OA) + Vit.C 500mg o.d + Vit.E 400mg o.d</td>
</tr>
</tbody>
</table>
4.5.4 **Equipments and Instruments**:

b) **Equipments**

1. Single pan Electrical balance

2. Cyclomixer

3. Centrifuge machine

4. Flame photometer

5. Spectrophotometer

*Fig – 4.9 Centrifuge Machine.*
Study Design (Material and Methods)

Fig – 4.10 Spectrophotometer.

b) **Instruments**  
   1. Micro-pipettes  
   2. Boiling water bath

Fig- 4.11 Working place-CRL
4.5.5 **Chemicals**

a) **For Specific Parameters**-

i) For estimation of Malondialdehyde (MDA) in serum –

**Chemicals used:**

- Malonaldehyde bis (diethyl-acetal): (Merck Schuchardt OHG 85662 Hohenbrum, Germany)
- Trichloroacetic acid: (Merck Specialities pvt. Ltd.)
- Thiobarbituric acid: (Loba Chemie pvt.Ltd.)
- Sulphuric acid:
- Sodium sulphate solution:
- n-Butyl alcohol:

**Reagents:**

- Trichloroacetic acid (TCA) – 20%
- Thiobarbituric acid (TBA) -0.67 gm%
- Sulphuric acid - 0.05 M
- Sodium sulphate solution – 2 M
- n- Butyl alcohol
ii) For estimation of Superoxide dismutase (SOD)

**Chemicals used:**

- Superoxide dismutase: (Sigma Cemie pvt. Ltd).
- Triss buffer: (Loba Chemie pvt. Ltd.)
- Sod.Cacodylate: (HiMedia laboratory pvt. Ltd.)
- Dethyl triamine penta acetic acid (DTPA): (S.D. fine chem. Ltd)
- Pyrogallol: (S.D. fine chem. Limited)

**Reagents:**

- Tris cacodylic acid buffer (pH 8.2)

  Tris - 3.025 gm.

  Sodium cacodylate - 4.00 gm.

  Dethyl triamine penta acetic acid (DTPA) – 0.200gm.

  All dissolved in 450.00 ml of distilled water. The pH of the solution was adjusted to 8.2. The volume was made upto 500.00 ml with distilled water.
Study Design (Material and Methods)

- Pyrogallol reagent (2.5 mM)

  Stock: 250.00 mg. of pyrogallol was dissolved in 1.00 ml of 0.5 mol/L HCl in dark colored bottle. This stock pyrogallol is stable for 2 months at 2-8°C.

- Working solution: Pyrogallol

  0.001ml stock Pyrogallol was diluted to 5.00 ml with dist. water.

b) Nonspecific Parameters

- Estimation of

- Total Cholesterol (Tot.Chol)

- Triglyceride (Tg)

- High density lipoprotein cholesterol (HDL) was carried out by the Kit Method.

- Low lipoprotein cholesterol (LDL)

- Very low density lipoprotein cholesterol (VLDL) were calculated from the estimated values of (Tot.Chol), (Tg) and (HDL).

Diagnostic Kits are marketed by:

Euro Diagnostic Systems Pvt. Ltd.,

Euro House, No.3/808, Rajiv Garden, Thuraipakkam, Chennai – 600096.
C) **Other Investigations**

- **Estimation of Hemoglobin**

  Chemicals:

  i) Drabkin’s reagent:

  ii) Cyanmethemoglobin standard:

  Both were manufactured by - Pathozyme Diagnostics,


- **Estimation of Sodium and Potassium**

  Reagents:

  Standard solutions: 120/2 mmol/L, 140/4 mmol/L Marketed by

  BioLab Diagnostics (I) Pvt. Ltd.
**Study Design**

**Material and Methods**

**PLAN OF STUDY**

- **Tuberculosis** (DOTs) (90 patients)
  - Treatment: DOTs - 10 No.
  - Treatment + Antioxidant: DOTs + H.I.-1 (10 No.)
  - 2) DOTs + H.I.-2 (10 No.)
  - 3) DOTs + Vit C-1 (10 No.)
  - 4) DOTs + Vit C2 (10 No.)
  - 5) DOTs + Vit E1 (10 No.)
  - 6) DOTs + Vit E2 (10 No.)
  - 7) DOTs + Vit C1 + Vit E1 (10 No.)
  - 8) DOTs + Vit C2 + Vit E2 (10 No.)

- **Hypertension** 180 patients
  - Treatment: Calcium Channel Blockers (C.C.B.) (90 Pt)
  - Treatment + Antioxidant: CCB + H.I.-1 (10 No.)
  - 2) CCB + H.I.-2 (10 No.)
  - 3) CCB + Vit C-1 (10 No.)
  - 4) CCB + Vit C2 (10 No.)
  - 5) CCB + Vit E1 (10 No.)
  - 6) CCB + Vit E2 (10 No.)
  - 7) CCB + Vit C1 + Vit E1 (10 No.)
  - 8) CCB + Vit C2 + Vit E2 (10 No.)

- **Other Anti hypertensive agents** (O.A.) (90 Pt)
  - Treatment: O.A. + H.I.-1 (10 No.)
  - 2) O.A. + H.I.-2 (10 No.)
  - 3) O.A. + Vit C-1 (10 No.)
  - 4) O.A. + Vit C2 (10 No.)
  - 5) O.A. + Vit E1 (10 No.)
  - 6) O.A. + Vit E2 (10 No.)
  - 7) O.A. + Vit C1 + Vit E1 (10 No.)
  - 8) O.A. + Vit C2 + Vit E2 (10 No.)

**Note:**

DOTs – DOT Therapy. H.I.-1 - Hemidesmus idicus 300mg. o.d., Vit E1 – Vitamin E 400mg. o.d., Vit C1 – Vitamin C 500mg. o.d.,

CCB – Calcium Channel Blocker, H.I.-2 - Hemidesmus idicus 300mg. b.d., Vit E2 – Vitamin E 400mg. b.d., Vit C2 – Vitamin C 500mg. b.d.,

O.A. -- Other antioxidants