Chapter III
Materials and Methods
The present study entitled as “Clinical Evaluation of a Unani Adjuvant Formulation For Enhancement of Efficacy of Anti Tubercular Treatment” was conducted to evaluate the safety and efficacy of a Unani coded drug (UNIM-104) as an adjuvant with ATT and prevention of MDR-TB. The patients were enrolled from OPD in Majeedia Hospital, Jamia Hamdard, New Delhi, and DOTS Center, Lal Kuwan near chawdi Bazar, old Delhi. Before starting the study, an ethical clearance was obtained from Internal Ethical Committee. After that, this clinical study was started by enrolling the patient who fulfill the inclusion and exclusion criteria, all the patients in both test and control groups are new AFB smear positive or Culture positive. The study was double blind, randomized, bi-centric, variable time trial, with sample size of 20 patients in test group and 20 patients in placebo-control group. The duration of protocol therapy is three month.

Objectives:
1) To evaluate safety and efficacy of a Unani formulation as an adjuvant with ATT
2) To evaluate the safety and efficacy of Unani formulation as an adjuvant in the prevention of MDR Tuberculosis.

Estimated Duration of Study:
2 years

Study Design:
Randomized, single blinded, Placebo Controlled, Adjuvant study.

Study population:
20 patients in each group with a total of 40 patients in both groups.

Inclusion Criteria:
The following patients were included in the study:
- All patients presenting with history of pulmonary tuberculosis (smear positive) of the age 18-60 years.
- New cases (smear positive pulmonary tuberculosis).
- New cases (smear negative but culture positive pulmonary tuberculosis).
Patients willing to sign the informed consent.

**Exclusion Criteria:**
The following patients will be excluded in the study.

- Patients suffering from extra pulmonary or miliary TB and defaulter /Retreatment cases.
- Patients of chronic liver disease.
- Alcoholics and cirrhotic patients.
- Patients suffering from renal insufficiency,
- Diabetes Mellitus,
- Malignancies
- Cardiovascular disease.
- Pregnant or lactating women.
- Patients receiving oral contraceptives.
- Patients receiving antiepileptic drugs, corticosteroid and diuretics.

**Sample Size:**
20 patients in each group with a total of 40 patients in both groups.

**Subject Selection:**
Those patients diagnosed and confirmed for pulmonary TB were selected. New cases of positive sputum smear or culture were included and age of the patients between 18-60 year and all of them were willing to sign the informed consent.

**Monitoring the treatment response**
Patients with pulmonary TB were monitored by the standard diagnostic, radiological and biochemical parameters.

**Investigation product**
Standardized coded (UNIM-104) drugs and Placebo (UNIM-104) provided by CCRUM in the form of majoon.

**Supplies, packaging, labeling and storage**
The investigational drug UNIM-104 packed in plastic bottles and supplied to patients.
The drugs are stored in dry and cool place and the patients were advised for the same.

**Dosage and Administration**

Patients of pulmonary tuberculosis were divided into 2 groups as per simple randomization. Both the groups were given ATT as per schedule (that is 6 months). One group received the test drug (UNIM-104) and other group received placebo, 5gm orally twice daily each drug (10gm in a day) for at least 3 months. Rest of the ATT drugs were continued as per treatment protocol in the both groups.

**Study Duration.**

Three months.

**Duration of Protocol Therapy**

Three months.

**Concomitant Medication**

No other medication was allowed to the patients during the study. If drug therapy other than that specified in the protocol was required during the period of each treatment, a proper record of the same was maintained and the decision to continue or discontinue was based on the following:

a) Pharmacology and Pharmacokinetics of the non study medication.

b) The likelihood of drug-drug interaction, thereby affecting pharmacodynamic comparison of the study medication.

c) The time of administration of non-study medication.

**Precautions**

In case of any drug related adverse effects, was reported immediately. Though no adverse event was reported during the study.
Procedures and Methods

Documentation

The informed consent along with the case report form for each volunteer duly filled was maintained by the investigator and provided to the sponsor at the end of the trial.

Informed Consent and Subject Information

The patients were informed about the purpose, content and procedures to be carried out during the study in non-technical terms. Only willing patients were to be given the test medication. Their consent was taken on the form especially prepared for this test.

Assessment Periods

The patients were assessed on Day 0, 2-Wks, 4-Wks, 8-Wks 12 wks, as given in the Case Report Form.

Observations and Measurements: Assessment for Diagnosis and treatment effects

The following were evaluation parameters:

1. Microbiological: Sputum test for AFB by microscopy on overnight samples for three consecutive days and culture and sensitivity.

Collection: Sputum is collected from the suspected person. Three samples are taken preferably of early morning. An outpatient provides one sample on the spot and a container is given to bring the early morning sample next day. Third sample is collected on spot.

Staining: It is stained with Zeihl-Neelsen stain. In this, a fixed smear covered with carbol-fuchsin is heated, rinsed, decolourized with acid alcohol and counterstained with methylene blue.

Slide reporting: The number of bacilli seen in a smear reflects disease severity and patient infectivity, the table below shows the standard method of reporting using 1000X magnification.
<table>
<thead>
<tr>
<th>Number of bacilli</th>
<th>Results reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB per 100 oil immersion fields</td>
<td>0</td>
</tr>
<tr>
<td>1-9 AFB per 100 oil immersion fields</td>
<td>Scanty</td>
</tr>
<tr>
<td>10-99 AFB per 100 oil immersion fields</td>
<td>+</td>
</tr>
<tr>
<td>1-10 AFB per oil immersion field</td>
<td>++</td>
</tr>
<tr>
<td>&gt;10 AFB per oil immersion field</td>
<td>+++</td>
</tr>
</tbody>
</table>

On the above basis, patient is classified into:

1. **Positive**: When at least two smears are positive
2. **Negative**: When at least two smears are negative
3. **Intermediate**: Either one smear is examined or three examined but only one smear is +ve.

In this situation, either further sputum smears or chest X-ray (CXR) is advised. Sputum smears microscopy for tubercle bacilli are +ve when there are at least 10000 organisms present per ml of sputum. The sputum smear positivity rate in TB/HIV patient depends on the degree of immunocompromise.

**False Positive Results of Sputum Smear Microscopy:**

1. Accidental transfer of AFBs from +ve slide to –ve one.
2. Contamination of slide or smear by environment and
3. Presence of acid-fast particles.

**False Negative Results of Sputum Smear Microscopy:**

A false negative result means that the sputum smear result is negative even though the patient has smear positive PTB.

This may arise because of the problems in the following areas:

(A) **Collection**

1. Inappropriate container
2. Long time spent after the collection of sputum

(B) **Processing**

1. Faulty sample of sputum
2. Faulty smear staining

(C) Interpretation
1. Inadequate time spent
2. Inadequate attentions paid

(D) Administrative Errors
1. Misidentification of patient
2. Incorrect labelling of sample
3. Mistakes in documentation

If the patient presents symptoms but sputum smear is negative then sputum culture is done. It has secondary importance in case finding programme. It has following shortcomings:

- Difficult
- Tedious/boring
- Lengthy (at least six weeks)
- Expensive
- Needs expertise and skilled man.

Other Methods:
If cough is non productive then specimens such as gastric lavage, urine and laryngeal swab are obtained. The Bronchoscopy with alveolar lavage has the best diagnostic yield. In some cases a specimen can not be obtained by sputum culture or bronchoscopy then in these situations biopsy of tissue from a suspected part is obtained by mediastinoscopy. In pleural effusion, biopsy reveals granulomas.

2. Radiological Parameters: Chest X-ray

Radiological classification of disease extent by national tuberculosis association of USA.

Minimal: Lesion involving small part of one lung of both lungs, the total extent regardless of distribution should not exceed the volume of lung one side, which is present above the 2nd costochondral junction and the spine of the 4th or the body of the 5th thoracic vertebra.
Moderately advanced: may be present in one or both lungs but the total extent should not exceed the volume of lung on one side up to the level of lowest point of 4th costochondral junction.

Far advanced: Lesion more extensive than moderately advanced lesion.

3. Haematological: Haemoglobin, TLC, DLC and ESR. (In case of TB, ESR is raised. It is only prognostic not diagnostic test).

4. Biochemical parameters: LFT.

5. Other laboratory parameters: KFT and routine urine examination.

6. Clinical: Weight, loss of appetite, fever and general well being will be assessed by the doctor. Global efficacy and tolerance both by the doctor and the patient was assessed at the end of the therapy.

Follow up

Follow up would be done every two wks and sputum smear was repeated at every visit, rest routine investigations for disease prognosis were done after 3 months.

<table>
<thead>
<tr>
<th>Days</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit</td>
<td>Visit-1</td>
<td>Visit-2</td>
<td>Visit-3</td>
<td>Visit-4</td>
<td>Visit-5</td>
<td>Visit-6</td>
</tr>
</tbody>
</table>

Subjects Compliance

Patients not complying with the protocol therapy would be excluded from the analysis of results.

Adverse Events

Adverse Events Monitoring

Any adverse events or side effects will be elicited by recording the response to a standard question "Have you noticed any problems with the treatment".
Adverse Event Documentation

The adverse events reported by the volunteers and as observed by the physician were filled in the case report form and reported to ethics committee accordingly and maintained for the purpose of documentation.

Serious Adverse Events Reporting

In case of over dosage or any serious adverse events the investigator should notify the health authorities and sponsor immediately and taken appropriate measures to safeguard the subjects. (No Serious adverse event was reported).

Premature Withdrawal of Patients from the Study

Patients were informed that they are free to withdraw from the study at any time without stating the reason. The investigator may withdraw a subject from the study if he experiences serious adverse effects and withdrawal would be in the best interest of the subjects.

Criteria for Evaluation of Study Results

Criteria for evaluation of study efficacy
Clinical, Microbiological, Radiological, Hematological and Laboratory parameters specifically sputum smear.

Criteria for evaluation of safety
Non occurrence of any drug related adverse effect.

Statistics
Analysis was restricted to patients who complete the study. Analysis of data was done as per statistical guidelines.

Standardization:

Standardization of the formulation and the ingredients (on preliminary parameters)
Standardization on the following parameters were carried out of the ingredients in the formulation

- **Organoleptic parameters**
- **Physical Parameters**
  - Moisture content
  - Ash value
  - pH value
  - Extraction value
- **Chemical Parameters**
  - Identification of different biologically active compounds
  - Estimation of alkaloids
    - Tannins
    - Total phenolics etc

This will be followed by the standardization of the compound formulation on the same parameters.

**Medical and statistical data analysis:**

Analysis of data was done using statistical methods. The data was calculated by using ANOVA followed by Dennett's 't' est.