INTRODUCTION

Tuberculosis (TB)

Tuberculosis commonly called as TB is one of the most transmittable diseases known to the mankind for several millions of years (Rubin, 1995). TB was not identified as a single disease until J. L. Schonlein named it as derivative of the Latin word “Tubercula” meaning a small hump and the Greek suffix ‘osis’, which signifies an abnormal or diseased condition, action or process. TB is a devastating public death problem with grave socioeconomic consequences and causes an enormous burden of morbidity and mortality around the world (Rubin, 1995).

The principal cause of human TB is due to Mycobacterium tuberculosis bacteria (MTB). TB is an infection caused by slow growing bacteria that grow best in areas of the lung that have high supply of blood and oxygen and is called as Pulmonary TB (PTB). TB can also spread in other parts of the body where it is called as Extra Pulmonary TB (EPTB). The pathogenic species are able to survive and grow within macrophages which enable them to evade the lost process. It usually takes about 6 to 9 months to treat TB.

TB is of two types, (1) Latent TB and (2) Active TB. Latent TB is asymptomatic meaning that TB bacteria are present in the body but the lungs defences (immune system) are keeping it from turning into Active TB. Latent TB does not have symptoms unless the disease becomes active.

Active TB means that TB bacteria are growing and causing symptoms. If the lungs are infected with active TB it can easily spread to other organs. It is highly contagious. TB spreads when a person who has active TB disease exhales air containing TB bacteria and another person inhales the bacteria from the air (WHO 2010, WHO 2014). These bacteria can float in the air for several hours. Coughing, sneezing, laughing or singing releases more bacteria than breathing. TB germs present in organs other than lungs (EPTB) it does not spread easily.

Current status of TB

a) Global Scenario:

Despite the availability of affordable and effective treatment, the annual toll of a million new TB cases and two million TB deaths worldwide represents an intolerable burden of human suffering and unacceptable barrier to socioeconomic development.

According to WHO, TB is a pandemic. Among the 15* countries with the highest estimated TB incidence rates 13 are in Africa while half of the new cases are in six Asian countries, viz., Bangladesh, China, India, Indonesia, Pakistan and Philippines. The global community woke up to this disease when 1993, WHO declared TB as a global emergency. According to WHO “Global TB report 2013” there seems to success for MDG. The present strategy is to terminate the global TB epidemic between 2015 and 2035 reducing TB deaths and new cases by 95% and 90% respectively. The strategy has also set interim milestones for 2020, with MDR-TB was 3.5% in 2013 and has not changed compared with recent years (WHO; 2013). On average an estimated 9% of patients with MDR-TB. TB remains one of the world's deadliest communicable diseases (WHO 2014; WHO 2013).

In India over 1.21 billion people, has the highest burden of tuberculosis (TB) in the world. According to global incidences India shares more than 20% of global incidences of multi-drug resistant (MDR) TB (WHO, 2010). The success of any disease is depending on the successful effort on its critical effective control. India has launched the Indian National TB Program (NTP) in 1962, but this program was ineffective due to inadequate program funding, managerial weaknesses, irregular drug supply and multiplicity of treatment regime (WHO, 1995). This was also found unsuccessful due to low rates of case detection and high rate of treatments incompletion (30%) and high rate of defaults (40-60%), thus continuing high rate of mortality (50 per). Acknowledging this reality, a Revised National Tuberculosis Control Program (RNTCP) was launched by the Government of India in 1997. The RNTCP have achieved detection rate to 70% and 85% cure rates. In 2006, 100% of the Indian population was covered by DOTS program making this scale-up one of India’s most significant public health accomplishment. The RNTCP has resulted in impressive improvements in cure rates (currently >80% in new infectious cases), substantial decline in death rates with low rates of defaults (<10%) (Khatri and Frieden, 2002 and RNTCP Report, 2009).

Despite of this success, India continues to have an estimated annual incidence of more than 2 million TB cases (Chadha, 2005). According to
to Tuberculosis Research Centre (TRC) the estimated burden of TB is substantially higher with 8.5 million cases of TB of all forms in the year 2000. As per the data collected by the TRC, the 3.8 million cases were smear-positive, 3.9 million cases were smear-negative and 0.8 million cases were extra-pulmonary TB (Gopi et al., 2005). This was based on survey conducted in India, where the prevalence of culture-positive and smear-positive pulmonary TB were found to be 605 per 100,000 and 323 per 100,000 respectively, considerably higher than the WHO estimates (Gopi et al., 2005).

India ranks second in harbouring MDR-TB cases (Mokrousov, 2013). Women experience different risk factor, social and economic consequences and barriers to treatment than men. The success against TB in India was in immunizing against TB. Albert Calmette and Camille Guerin in 1906 developed bacillus of Calmette and Guerin (BCG) from attenuated bovine (Mycobacterium bovis) stain of TB. It was first used in France on humans in 1921. With the support of WHO and UNICEF, a BCG vaccine production centre was set up in Guindy (Chennai). In 1951, India started a mass BCG campaign to control TB and for the first time in the history of India, massage of health and prevention of disease was taken to the remotest parts of the country (WHO, 2013: WHO,2014).

MDR TB and current challenges

MDR-TB is caused by organisms that are resistant to at least two most effective anti-TB drugs, Isoniazid (INH) and Rifampicin (RIF) (Hirsh et. al., 2004; WHO, 2009). The emergence of MDR-TB is of great concern because it requires the use of second line of drugs that are difficult to procure and, they induce adverse drug reactions resulting in treatment noncompliance there for the detection and treatment of drug susceptible or single drug treatment. TB is an important strategy for preventing the emergence of MDR-TB (Gomes et al., 2015).

In major challenges to control TB in India include;
- Poor primary healthcare infrastructure in rural areas
- Unregulated private health care
- Irrational use of FLD and SLD anti TB drugs
- Spreading HIV infection and
- Poverty

Isoniazid drug (INH)

INH was introduced in 1952 for treatment of TB. Later it was also recommended to use for primary prophylaxis of tuberculosis infection and treatment of latent infection to prevent active TB. Isoniazid drug is used for the treatment of tuberculosis. Isoniazid induces generalized convulsions, coma and metabolic acidosis. Death may occur from acute respiratory failure or hypertension, liver and peripheral nervous and hematologic system is the main target organs of Isoniazid chronic toxicity. Overdosage of Isoniazid has produced nausea, vomiting, dizziness, slurred speech, blurred vision and visual hallucinations. Symptoms of over dosages usually occur within 30 minutes to 3 hours following ingestion of the drug (Guranan., et al., 1992.; Gilhotra et al., 1997).

Rifampicin drug (RIF)

Rifampicin or rifampin is a bacteriostatic antibiotic drug of the Rifampicin group. Rifampicin was introduced in 1967 as a major addition to the cocktail-drug treatment of tuberculosis and inactive meningitis, along with Isoniazid, ethambutol, pyrazinamide and streptomycin. It must be administered regularly daily for several addition to the cocktail-drug treatment of tuberculosis and inactive respiratory failure or hypertension, liver and peripheral nervous system is the main target organs of rifampicin chronic toxicity. Overdosage of rifampicin has produced nausea, vomiting, dizziness, slurred speech, blurred vision and visual hallucinations. Symptoms of over dosages usually occur within 30 minutes to 3 hours following ingestion of the drug (Guranan., et al., 1992.; Gilhotra et al., 1997).

Role of medicinal plants in Hepatotoxicity:

Medicinal plant play a role in the human health care system pharmacological medicinal plants and there taxonomical health era system. Herbal medicines are great demand in various diseases. Many drugs cause hepatic injury and is a great concern of the world for primary health. To overcome these effects drugs, now a day's many herbal preparations are in use to cure the disease because of their efficiency, safety, lesser side effects and narrow therapeutic window. Therefore the use of herbal drug is much safer then synthetic products available in the market. Herbal remedies support natural healing phenomena through blocking the progression of degenerative pathological process. Ayurveda has a clinical specialty called rasayana which prevents diseases and control the drug process by means of optimization of homeostasis. There are many herbal medicinal plants having antioxidant properties which show hepato protective activity (Krinsky et. al., 1990).

Aloe Vera plant and its applications:

Aloe Vera is succulent plant species of the genus Aloe. It grows wild in tropical climates around the world and is cultured for agriculture and medicinal uses. Aloe Vera contains many ingredients such as vitamins, minerals, sugars, enzymes, lignin's, antibiotics, anthraquinones, saponins, fatty acids, salicylic acid etc. which are useful for growth process and healthy functions of the all body system. Aloe vera gel has therapeutic properties such as prevention of radiation damage effect, antibacterial, antiviral and neoplastic activation and stimulation of haematoma process.

In the pharmaceutical industry, Aloe vera has been used for the manufacture of tropical products such as ointments and gel preparations, as well as in the production of tablets and capsules (Eshun K.; He Q.; 2004; He Q. et al., 2005). Important pharmaceutical properties that have recently been discovered from both the Aloe vera gel and whole leaf extracts includes the ability to improve bioavailability of co-administered vitamins in human subjects (Vinson, et al., 2005). The biological activities include promotion of wound healing, antifungal activity, hypoglycemic or antiabetic effects, anti inflammatory, anticancer, immunomodulatory and gastro protective properties.

MATERIALS AND METHODS

1) Preparation Aloe vera extract Solution

Collection and identification of Aloe vera plant: Fresh Aloe vera plant leaves were brought from botanical garden and sample was indentified and brought to laboratory in the Department of Zoology, S.S. & L.S. Patkar-Varde College, Goregaon (W), Mumbai - 62. Aloe vera leaves were rinsed 2-3 times in the tap-water. 50 grams of leaves were then grounded with 50ml of distilled water in sterilized mortar and pestle. The homogenized mixture was filtered twice through a cotton cloth and centrifuged at 5,000 rpm for 10 minutes. Supernatants were collected and diluted with 50 ml of distilled water to obtain a concentration of 50mg/70kg BW.

Weigh 500 µg aloe vera extract in a sterile container
- Add 100 ml sterile distilled water to it filter sterilize it using 0.22µm membrane filter.

Procurement of Mycobacterium strain:

For the present work, mycobacterium strain H, Rv was procured from B. J. Medical, Pune, for present investigation. The antibacterial work was carried in the laboratory at B. J. Medical College, Pune.

Drug Containing Media preparation

A. Reagents:
- 2% Malachite green
- Mineral salt solution
- Distilled Water

B. Other Requirements:
- For 20 bottles of LJ we require 200ml of media which should consist of 125ml of egg solution and 225 ml of mineral salt solution.
- means Sterile Mc Cartney Bottles
- Mortar and pestle.
- Conical flask 200 ml capacity.
- Measuring cylinder 200ml capacity.
- 50ml capacity.
- litre capacity.
- 250 ml capacity.
- 2 nos.
- 10 nos.
13. Isopropyl alcohol.........................200 ml.
14. Fresh hen’s eggs: 4 eggs (@35ml / egg = 300ml). Quality of media depends on the freshness of the hen's eggs. Eggs should be obtained always from a reliable source. The eggs should not be more than a day or two old, at the time of purchase.

15. Drugs
   Isoniazid- Sigma, Cat No. 1 – 3377.
   Rifampicin- Sigma, Cat No. R.3501.

16. Concentrations of Drug: Only one concentration per drug is used. The final concentrations in LJ medium are as follows:
   1. Isoniazid------------------------- 0.2 μg/ml.
   2. Rifampicin-------------------------- 40 μg/ml.
   3. Aloe vera extract Aloe vera extract was weighed 500microgram and added in 100ml sterile distilled water.

Preparation of Drug solutions

2) Preparation working Isoniazid (H) Drug Solution:
   Drug potency = 1g to 1g substance. Potency Factor = 1, (Preferred substance: Sigma I-3377).

Stock Solution preparation
   - Weigh out 20mg of Isoniazid powder in 40ml of sterile distilled water to obtain a concentration of 500μg/ml Isoniazid solution.
   - Label with date of preparation, as ‘H Stock solution’.

Working solution:
   1 ml of stock solution (500μg/ml) + 24ml of sterile distilled water (+25ml of 20μg/ml).
   Sterilize by filtering through a 0.22 μm membrane filter. Do not store this solution.

3) Preparation working Rifampicin ®: Drug Solution
   Drug potency = 1g to 950mg substance. Potency factor = 0.95 (Preferred substance Sigma R3501)

Stock solution preparation:
   - Weigh out 42.1 mg of rifampicin (Potency correction: Weight required divided by potency factor, i.e., 40mg/0.95) dissolve in 5 ml of absolute methanol, followed by addition of 5 ml of 99% ethanol to get 4000μg/ml of stock solution.

PROCEDURAL STEPS

1. Isoniazid containing LJ media
   Add 0.5ml working drug solution per 50ml LJ medium prepared to obtain final concentration of 0.2 μg/ml isoniazid LJ medium.

2. Rifampicin (R) containing LJ media
   Add 0.5 ml per 50ml LJ medium prepared to obtain final concentration of 40μg/ml LJ medium.

3. Aloe vera extract containing LJ media
   Add 0.5 ml per 45ml of LJ medium prepared to obtain final concentration of 0.5 μg/ml LJ medium.

4. Isoniazid + Aloe vera extract containing LJ media
   Add 0.5ml working drug solution per 50ml LJ medium prepared to obtain final concentration of 0.2 μg/ml isoniazid LJ medium. Remove 0.5ml of this LJ fluid and add 0.5ml of Aloe vera extract and mix well.

5. Rifampicin + Aloe vera extract containing LJ media
   Add 0.5ml working drug solution per 50ml LJ medium prepared to obtain final concentration of 0.2 μg/ml rifampicin LJ medium. Remove 0.5ml of this LJ fluid and add 0.5ml of Aloe vera extract and mix well.

6. Isoniazid + Rifampicin + Aloe vera extract containing LJ media
   Add 0.5ml working INH drug solution and Rifampicin per 50ml LJ medium prepared to obtain final concentration of 0.2 μg/ml isoniazid LJ medium. Remove 0.5ml of this LJ fluid and add 0.5ml of Aloe vera extract and mix well.

PROPORTION METHOD

Principle: All strains of tuberculosis contain some sub-population of bacilli that are resistant to anti-TB drugs. However, in resistant strains, the proportion of such bacilli is considerably higher than the sensitive strains. The proportion method calculates the proportion of resistant bacilli present in a strain. Two appropriate dilution of the bacilli, 10^-2 and 10^-4 dilutions (undiluted = 10^0 to 10^8 CFU/ml), are inoculated on drug-containing and drug-free media, in order to obtain countable colonies on both media. The ratio of number of colonies observed on the drug-containing media to drug-free medium indicates proportion of resistant bacilli present in the strain. Below a certain proportion (critical proportion = 1%), the strain is classified as sensitive; above, as resistant. Drug concentrations added to LJ Media and critical proportion for Interpretation for economic variant of Proportion Method are:

<table>
<thead>
<tr>
<th>Drug Media</th>
<th>Concentration</th>
<th>Critical proportion to determine resistance</th>
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<tbody>
<tr>
<td>Isoniazid</td>
<td>0.2 μg/ml</td>
<td>1%</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>40 μg/ml</td>
<td>1%</td>
</tr>
</tbody>
</table>

One set of media bottles for testing one culture consist of five LJ slope, one for neat, two for 10^-2 and two for 10^-4; eight LJ drug containing slopes, two each for drugs H, R, E & S (one each for 10^-2 and 10^-4 suspensions) and one for Para Nitro Benzoic acid (PNB) slope, total 14 LJ slopes are required.

PROCEDURAL STEPS: Method: It is a proportion method used to detect the resistance in mycobacteria.

1. Inoculum preparation:
   A. With a 3mm wire loop, a representative sample of approximately 4-5 mg (loop full) is taken from the primary culture and placed on the side wall of a McCartney bottle containing 1 ml Sterile Distilled Water (SDW) and 6 glass beads of diameter 3 mm.
   B. Emulsify the bacterial inoculum, (with a loop of water, if required), on to the side wall of McCartney bottle in round rotary movements with inoculation loop, till the bacterial mass is emulsified, (this is visible by reduction in the clumpy hydrophobic to aqueous hydrophilic nature of suspension).
   C. Let the emulsified suspension be fully dissolved in the 1ml of Sterile Distilled Water (SDW).
   D. Vortex the bottle for 20–30 seconds and add 4 ml of distilled water is added slowly.
   E. Allow the coarse particles to settle down (leave it on stand for approximately 5 min).
   F. Decant the Mycobacterium solution carefully into another clear, sterile McCartney bottle.
   G. Match the opacity/turbidity of inoculum with McFarland standard no.1, against a black background. This is the neat bacterial suspension, standardized at 1 mg/ml, equalling to 107 to 108 CFU/ml. Make sure that no clumps are taken.

H. If required, the opacity of the bacterial suspension is then adjusted by the addition of distilled water to obtain a concentration of 1 mg/ml of tubercle bacilli by matching with McFarland's standard 1

I. Make further two log dilutions to achieve 10^-3 and 10^-4 dilutions as given below. The dilution 10^-3 is produced by discharging two loopful of the neat bacterial suspension, into a small tube containing 2 ml of distilled water, and shaking.

J. Similarly, the dilution 10^-4 is produced by discharging two loopful of the dilution 10^-3 into a small tube containing 2 ml of distilled water, and shaking.

RESULTS AND DISCUSSION

Figure-1 Inoculums of H,RV Concentration

S2 Inoculum of H37RV
1) LJ medium inoculated with S, Inoculum of H RV we inoculate duplicate slants of S, & S
2) LJ medium inoculated with S, Inoculum of H RV
3) LJ medium containing only Aloe vera - inoculated with S, Inoculum of H37RV
The present study was undertaken to screen the plant extracts for its molecules to improve potency. In view of the above cited claims, the and isolated the purified compounds and tested their ability to inhibit the effect of plant extract against different strains. Inoculated standard strain in duplicates and one standard strain – H Rv. Table No. 1.

**Table No. 1.** Showing the effect of Aloe vera extract and isoniazid- rifampicin drug on Mycobacterium tuberculosis bacterial (MTB) standard PAN sensitive strain -H Rv.

<table>
<thead>
<tr>
<th>H37Rv</th>
<th>LJ Extract (5µg/ml)</th>
<th>INH</th>
<th>Rif</th>
<th>INH+A.V. Extract (5µg/ml)</th>
<th>Rif+A.V. Extract (5µg/ml)</th>
<th>INH+RIF+A.V. Extract (5µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>3+</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>S2</td>
<td>Tiny colonies</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>S3</td>
<td>2+</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>S4</td>
<td>1+</td>
<td>&gt;20 colonies</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
</tbody>
</table>

S1= Neat inoculum (1 x 10^7 - 1 x 10^8 CFU/ml) inoculated single LJ with standard strain
S2= 10^7 and S4 = 10^8  Inoculated standard strain -H Rv.

**Figure: 2 Inoculum of H37Rv**

1) LJ medium inoculated with S4 Inoculum of H Rv, we inoculate duplicate slants of S, S2
2) LJ medium containing Isoniazid, Rifampicin and Aloe vera extract inoculated with S4 Inoculum of H37Rv
3) LJ medium containing only Aloe vera extract - inoculated with S4 Inoculum of H Rv
4) LJ medium containing Rifampicin inoculated with S Inoculum of H Rv
5) LJ medium containing Rifampicin and Aloe vera extract - inoculated with S Inoculum of H Rv
6) LJ medium containing Isoniazid – inoculated with S Inoculum of H Rv
7) LJ medium containing Isoniazid and Aloe vera extract - inoculated with S Inoculum of H Rv
8) LJ medium containing Isoniazid and Aloe vera - inoculated with S Inoculum of H Rv

CONCLUSION: In our previous studies on “effect of Aloe vera extract on the hepatotoxicity induced by isoniazid and rifampicin drug in male wistar rats” and effect of Aloe vera extract on the toxicity induced by isoniazid and rifampicin drug on complete blood count in male wistar rats” (Zodape and Bhise; 2015 and Bhise and Zodape, 2016), we have found the hepatoprotective effect of Aloe vera extract against toxicity induced by isoniazid and rifampicin drugs by reversal of biocatalytic and haematological parameters. We have also found the effect of Aloe vera extract and isoniazid and rifampicin on histological architecture of liver and we found that there is partial restoration of hepatic function as evident from normalization of serum markers of liver function, and we were able to show hepatoproteaction against INH+RIF induced hepatotoxicity, as evidenced by the partial reversal of increased serum transaminases showed trend towards returning to normal (partially) by supplementation of Aloe vera indicating partial hepatoprotective effect. Therefore we have also carried out a study on *M. tuberculosis* H Rv strain to find the effect of RIF, INH and Aloe vera extract. This kind of study was conducted to find the direct effect of antimicrobial drug RIF, INH and Aloe vera extract on micobacterial strain H Rv and we found that while using Aloe vera extract, it does not affect the activity of antimicrobial drug RIF and INH either individually or in combination. It was also found that Aloe vera extract alone seems to have antimicrobial activity in some extent.

ACKNOWLEDGEMENT: Authors are thankful to the Director, B. J. Medical College, Pune, for providing the mycobacterial strain H Rv and supporting and funding facilities for carrying out antibacterial work in the laboratory of at B. J. Medical College, for present investigation. Thanks are also due to the Principal, S.S. & L.S. Patkar College of Arts and Science & V.P. Varde College of Commerce and Economics, Road, Goregaon (West), Mumbai-400 062.

REFERENCES: