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EFFECT OF *ALOE VERA* EXTRACT AND ISONIAZID - RIFAMPICIN DRUG ON *MYCOBACTERIUM TUBERCULOSIS* BACTERIAL (MTB) STRAIN -$H_{37}Rv$

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ABSTRACT Many researchers have performed the experiments on several plants to investigate the effect of plant extract against different *Mycobacterium* strains. In view of the above cited claims, the present study was undertaken to screen the *Aloe vera* plant extract for its antitubercular activity in vitro. This experimental work would serve as baseline model system to establish new templates in the bioprospection and development of new and more effective pant – based antibiotics. This study was conducted to find the direct effect of drugs RIF- INH and *Aloe vera* extract individually as well as in combination with drugs and *Aloe vera* on micobacterial strain H37Rv and we found that while using *Aloe vera* extract, it does not affect the activity of antitubercular drug RIF and INH either individually or in combination. It was also found that *Aloe vera* extract alone seems to have antimicobacterial activity in some extent.

KEYWORDS : *Aloe vera,* Mycobacterium Tuberculosis strain H37Rv, Drug, Isoniazid, Rifampicin

INTRODUCTION

Tubrculosis (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 lung 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 causing 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 15th countries with the highest estimated TB incidence rates 13th 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 is 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).

b) Indian Scenario:

Why is TB such a big problem in India, despite the success of DOTS program? TB is a disease of poverty, with several known social determinants such as malnutrition and tobacco smoking. These problems have not been addressed properly DOTS strategy. Furthermore there is no awareness in the society about early detection and successful treatment of patients with TB is the cornerstone of TB control. In India, since the very inception of the National TB Program (NTP), TB treatment services do not exist as a specialized service but are integrated with the general health services. The main driver of TB mismanagement in the NTP was the low priority given to healthcare in general, especially primary health care services, and the very low priority given to TB among various healthcare programs. In 1982, the total budget of the NTP was less than $0.5 million (Rupees 20 million) for an estimated burden of 2 million patients per year (Mukherjee 1995).

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 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 100000 and 323 per 100000 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., 1987).

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 months without break; otherwise, the risk of drug-resistant tuberculosis is greatly increased (Long and James .1991). Rifampicin is typically used to treat *Mycobacterium* infections, including tuberculosis and leprosy. It also has a role in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) in combination with fusidic acid. It is used in prophylactic therapy against *Neisseria meningitidis* (meningococcal) infection. It is also used to treat *Listeria* species, *Neisseria gonorrhoeae*, *Haemophilus influenzae* and *Legionella pneumophila*. For these non-standard indications, sensitivity testing should be done before starting Rifampicin therapy. Rifampicin resistance develops quickly during treatment and rifampicin monotherapy should not be used to treat these infections (Long and James .1991).

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 speciality 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 antidiabetic 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 $H_{37}Rv$ 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 :

1. 2% Malachite green
2. Mineral salt solution
3. Distilled Water

B. Other Requirements:

1. 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.
2. Sterile Mc Cartney Bottles ……20 nos.
3. Mortar and pestle.
4. Sterile Conical flask 200 ml capacity.
5. Measuring cylinder ….200ml capacity.
6. Conical Flask …… 500ml capacity.
7. Conical Flask with broken glass …… 1litre capacity.
8. Sterile measuring cylinder ……250 ml capacity.
9. Sterile beakers 2 nos.
10. Sterile funnel with double layer' of gauze fixed over mouth.
11. Soap solution.

12. Methylated spirit……200 ml.
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. I – 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 :*Avoe 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 µ 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 isoniazid 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 oncentration 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^{6} 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:

Drug Media	Concentration	Critical proportion to determine resistance
Isoniazid	0.2 µg/ml	1%
Rifampicin	40 µg/ml	1%

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 rotatory 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^{-2} and 10^{-4} dilutions as given below: The dilution 10^{-2} 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^{-2} into a small tube containing 2 ml of distilled water, and shaking.

RESULTS AND DISCUSSION

Figure:-1 Inoculums of $H_{37}RV$ Concentration

S2 Inoculum of H37RV

1) LJ medium inoculated with S_2 Inoculum of $H_{37}RV$ we inoculate duplicate slants of S_2& S_4
2) LJ medium inoculated with S_2 Inoculum of $H_{37}RV$
3) LJ medium containing only *Aloe vera* - inoculated with S_2 Inoculum of $H_{37}RV$

4) LJ medium containing Isoniazid inoculated with S_2 Inoculum of $H_{37}RV$
5) LJ medium containing Isoniazide, Rifampicin and *Aloe vera* - inoculated with S_2 Inoculum of $H_{37}RV$
6) LJ medium containing Rifampicin inoculated with S_2 Inoculum of $H_{37}RV$
7) LJ medium containing Rifampicin and *Aloe vera* - inoculated with S_2 Inoculum of $H_{37}RV$
8) LJ medium containing Isoniazid and *Aloe vera* - inoculated with S_2 Inoculum of $H_{37}RV$

Figure:-2 Inoculum of H37RV S_4 concentration S4 Inoculum of H37RV

1) LJ medium inoculated with S4 Inoculum of $H_{37}R_V$ we inoculate duplicate slants of S_2 & S_4
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 S_4 Inoculum of $H_{37}Rv$
4) LJ medium containing Rifampicin inoculated with S_4Inoculum of $H_{37}Rv$
5) LJ medium containing Rifampicin and *Aloe vera* extract - inoculated with S_4Inoculum of $H_{37}Rv$
6) LJ medium containing Isoniazid – inoculated with S_4Inoculum of $H_{37}Rv$
7) LJ medium containing Isoniazid and *Aloe vera* extract - inoculated with S_4 Inoculum of $H_{37}Rv$

Table No. 1. Showing the effect of *Aloe vera* extract and isoniazid - rifampicin drug on *Mycobacterium tuberculosis* bacterial (MTB) standard PAN sensitive strain -$H_{37}Rv$.

	H37 Rv	LJ Extract (5µg/ml)	INH	Rif	INH+A.V. Extract (5µg/ml)	RIF+A.V. Extract (5µg/ml)	INH+ RIF+ A.V. Extract (5µg/ml)
S1	3+	XX	XX	XX	XX	XX	XX
S2	2+	Tiny 1+colonies	NG	NG	NG	NG	NG
S2	2+	XX					
S4	1+	>20 colonies	NG	NG	NG	NG	NG
S4	1+	XX					

S1= Neat inoculum (1 x 10^7 -1 x 10^8CFU/ml) Inoculated single LJ with standard strain

S2= 10^{-2} and S4 = 10^{-4} Inoculated standard strain in duplicates and one slant of LJ with extract

XX=Not Done
NG= No Growth (Indicate Sensitive)
1+= 20-100 colonies
2+=>100 colonies
3+= confluent growths

Many researchers have performed the experiments on several plants to investigate the effect of plant extract against different *Mycobacterium* strains. A Number of plant derived compounds have been synthesized and isolated the purified compounds and tested their ability to inhibit the particular stain. These Chemistry- based compounds were optimized to design the chemical structure of these plant based molecules to improve potency. In view of the above cited claims, the present study was undertaken to screen the plant extracts for its antitubercular activity in vitro. This experimental basis would serve as baseline model system – new templates in the bioprospection and development of new and more effective plant – based antibiotics.

Aloe vera is used in the traditional native medicines to treat many diseases. In the present study *Aloe vera* extract was screened against *M. tuberculosis* strains $H_{37}Rv$ and it was found that the *Aloe vera* extract effective against mycobacteria only at higher concentrations. The drug free control slants without *Aloe vera* extracts (Positive control) and vehicle control showed growth of *M. tuberculosis* strains $H_{37}Rv$ as shown in the Fig. No 1 and 2. Table No. 1, represents the antitubercular activity data of *Aloe vera* extract and concentrations tested for the *M. tuberculosis* $H_{37}Rv$ strain.

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 vara* extract against toxicity induced by isoniazid and rifampicin drugs by reversal of biocamical 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 hepatoprotection against INH+RIF induced hepatotoxicity, as evidenced by the partial reversal of increased serum transaminases showed trend towards returning to normal (but partially) by supplementation of *Aloe vera* indicating partial hepatoprotective effect. Therefore we have also carried out a study on *M. tuberculosis* $H_{37}Rv$ strain to find the effect of RIF, INH and *Aloe vera* extract. This kind of study was conducted to find the direct effect of antitubercular drug RIF, INH and *Aloe vera* extract on micobacterial strain $H_{37}Rv$ and we found that while using *Aloe vera* extract, it does not affect the activity of antitubercular drug RIF and INH either individually or in combination. It was also found that *Aloe vera* extract alone seems to have antimicobacterial activity in some extent.

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REFERENCES:

1. Bhise P.P and Zodape G.V. (2016). Effect of Aloe vera juice on the toxicity induced by isoniazid and rifampicin drugs on complete blood count in male wistar rats. ijpsr, vol. 7(9): 3728-3733.
2. Chadha VK. (2005). Tuberculosis epidemiology in India: a review. International Journal of Tuberculosis and Lung Disease.;9(10):1072–82.
3. Eshun, K.; He, Q. (2004). Aloe Vera : A valuable ingredient of the food, Pharmaceutical and Cosmetic industries- A review. Crit. Rev. Food Sci. Nutr; 44, 91-96 Ethnopharmacology. vol.; 68. p 3-37.
4. Gilhotra R (1987). Int J. Clin Pharmacol Ther Toxicol; 25 (5); 259-261
5. Gomez G., Gunther G., Lange C., Rupert S. and Leth F. (2015). Availability, price and affordability of anti – tuberculosis drug in Europe: a TBNET survey. Eur Respir J., 45: 1081 – 1088.
6. Gopi PG, Subramani R, Santha T, Chandrasekaran K, Kolappan C and Selvakumar N (2005). Estimation of burden of tuberculosis in India for the year 2000. Indian Journal of Med-ical Research; 122:243–8.
7. Gurnani A (1992). Anaesthesia; 47 (9): 781-783
8. He,Q.; Changhong, L.;Kojo, E.; Tiah,Z (2005). Quality and safety assurance in the processing of aloe vera gel juice. Food Control;16, 95-104.
9. Hirsh A. E, Tsolaki A. G., DeRiemer K. Enciso J. A., Wong M. Z., Hannan M., Goguetde la Salmoniere YO, Aman K., Kato-Maeda M., and Small P. M.. (2004). Functional and evolutionary genomics of Mycobacterium tuberculosis: insights from genomic deletions in 100 strains. Proc.Natl.Acad.Sci.U.S.A 101: 4865-4870.
10. Khatri GR, Frieden TR (2002). Controlling tuberculosis in India. N Engl J Med; 347:1420-5.
11. Krinsky NI, Mathews-Roth MM, Welankiwar S, Sehgal PK, Lausen NCG and Russett M (1990). The metabolism of [14 C] β- carotene and the presence of other carotenoids in rats and monkeys. J Nutr.; 120: 81-87.
12. Long and James W (1991). Essential Guide to Prescription Drugs 1992. New York: HarperCollins Publishers; pp. 925–929 (From my research papaer)
13. Mokrousov (2013). Molecular snapshot of Mycobacterium tuberculosis population structure and drug-resistance in Kyrgyzstan. 93 (5), 501-507.
14. Mukherjee AK (1995). Tuberculosis Control Programme in India : Progress and Prospects. Indian Journal of Tuberculosis.;42(2): 75–85.
15. RNTCP (2009). RNTCP Status Report, 2009.
16. Rubin SA (1995) Tuberculosis. Captain of all these men of death. Radiologic Clinics of North America 33: 619– 39
17. Vinson, J.A.; Al Kharrat, H.; Andreoli, L (2005). Effect of aloe vera preparations on the human bioavailability of vitamins C and E. phytomedicine,; 12, 760-765.
18. WHO (1995). Global Tuberculosis Programme. Tuber-culosis Programme Review,

India, Geneva: World Health Organisation,.
19. WHO Report 2010. Global Tuberculosis Control. Epidemiology, Stratetgy and Financing. Geneva: World Health Organisation, Geneva, 2010.
20. WHO, (2014). Global tuberculosis control: surveillance, planning and financing. WHO report. World Health Organization, Geneva. WHO/HTM/TB/2014.7
21. WHO,(2013). Global Tuberculosis Report 2013; WHO/HTM/TB/2013.11: Geneva, Switzerland.
22. World Health Organization (WHO) Report (2009). Global Tuberculosis Control.
23. Zodape G. V. and Bhise P. P. (2016). Effect of aloe vera extract on the hepatotoxicity induced by isoniazid and rifampicin drug in male wistar rats. IJPSR; Vol. 7(3): 1314-1320.