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"THE ROLE OF NUTRITIONAL VALUE IN CAUSING TUBERCULOSIS AND GROWTH OF TB MICROBES IN THE PRESENCE OF SOME HERBAL PLANT EXTRACT"

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ABSTRACT

Mycobacterium tuberculosis is a leading cause of morbidity and mortality worldwide. *Mycobacterium tuberculosis* infects about one third of world population and tuberculosis remains the most frequent cause of *death* due to a single infectious disease. Anciently, herbal plants have established home remedies for many of common alignments, diagnostic procedures and preventive measures. Using this strategy, this study was proposed to find out anti-tuberculosis activity of medicinal plants viz. *Neem, Ginger, Tulsi, Garlic* against *M. tuberculosis*. *Mycobacterium tuberculosis* activity was tested using minimal inhibitory concentration method (MIC). Aqueous extract of *Neem* (0.01-1.0 mg/mL) had significant effect against *M. tuberculosis* whereas, *Garlic* in higher concentration showed no inhibition.

KEYWORDS: *Mycobacterium tuberculosis,* anti-tuberculosis activity, *Neem, Ginger, Tulsi, Garlic.*

INTRODUCTION

According to the World Health Organization (1998), tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis*. It was noted that *Mycobacterium tuberculosis* mainly affects the lungs, causing lung tuberculosis (pulmonary tuberculosis).[1] However, in some cases other parts of the body may also be affected leading to extra-pulmonary tuberculosis.[2] Tuberculosis spreads easily in overcrowded settings and in conditions of malnutrition and poverty. It is mainly transmitted by exposure to *Tubercle*

bacilli in airborne droplets from coughing or sneezing. The common symptoms of TB are coughing, fever, hemoptysis, chest pain, fatigue and weight loss. In South Africa, patients with one or more of these signs or symptoms are considered "TB suspect" and must be further investigated for active TB disease according to the national TB guidelines (Department of Health, 2010).[3]

Every day 4000 people develop Tuberculosis (TB) in India and 1000 people die from the disease. It means that in India, three deaths occur every two minutes due to TB despite the fact that TB is preventable and curable.[4] India accounts for one-fifth of the global TB cases. On an average, TB leads to loss of annual family income by as much as 20 per cent. It has a direct and indirect cost to the country in the region of 23.7 billion USD.[5] About 300,000 people died from TB in India in year 2012. Men, women and children- all were those amongst these deaths.[6] Across the world, every year 1 million children are detected with TB which accounts for 10-15% of total TB cases. It accounts for 10 high probability causes of child mortality.[7] According to the estimates of the World Health Organization, this year 10 to 15% of the 9 million TB patients will belong to the age of 14 years and below who will need the TB treatment.[8] This data is apprehended to rise should we fail to check the infection amongst the children.[9] Since TB in children is largely owed to the TB of adults, it is very important that family members and care givers are educated about safeguarding the children through prevention of TB infection.

TB (Tuberculosis?)

TB is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It is a rod-shaped bacterium. It typically affects the lungs (pulmonary TB) but can also affect other sites (bones and joints, kidneys, genitourinary tract, spine, lymph gland etc.). When TB affects organs other than the lungs, it is referred as extra pulmonary TB.

This disease is spread in the air when people who are sick with pulmonary TB expel bacteria by coughing or sneezing. When the healthy people are exposed to these bacteria, they too get infected with this disease without being aware of it. It is important to recognize here that whilst one may inhale the bacteria by being in the proximity to the exposure, she has been infected by TB bacteria but is not (yet) ill with the disease. However, it is crucial to detect and mobilise symptomatic cases earlier so as to reduce transmission.

Scenario

In 1993, the World Health Organization (WHO) declared TB a global emergency because it killed more adults each year than any other infectious disease (The South African Tuberculosis Control Programed, 1998). Approximately one third of the world's population harbors TB infection.[10] An estimated 8.3 million new cases and 1.8 million deaths were attributed to this disease in 2000. Developing countries have much higher incidences of TB than developed countries. A prevalence of 9.2% and fatality rate of 12% have been recorded in Nigeria. Mozambique was ranked among the 20 highest TB burden countries in the world, with an estimated 81000 cases and an incidence rate of 436 per 100 000 people in 2002 (WHO, 2004).[11-14]

Control programs

Tuberculosis control programmes currently emphasize the Directly Observed Treatment Short Course (DOTS) strategy, promoted by the World Health Organization and the International Union against TB and lung disease. South Africa adopted the WHO's DOTS strategy in all nine provinces (Department of Health, 2011). Key tenets of plan are standardized treatment of 68 months for all infectious patients; with directly observed therapy for at least the initial two months (WHO, 2005). However, previous studies by Needham et al. (1998) and Russell (2004) noted that rural patients often delay TB treatment, as they cannot afford to travel to treatment centers (DOTS clinics) daily to have a health worker watch them take their drugs. Kandel (2008) found that of the 255 TB patients who came for treatment at Mbekweni Health Centre in the King Sabata Dalidyebo (KSD) district in the Eastern Cape Province, South Africa, 121 had interrupted their treatment.[15-16]

Symptoms

- Cough lasting for more than 2 weeks
- Fever in the evenings
- Chest pain
- Loss of appetite
- Loss of weight
- Heavy breathing

Interrelationship between Nutrition - Malnutrition and TB

Children suffering from TB are generally found to be malnourished and anemic. Malnutrition and TB are deeply intertwined. Malnutrition primarily affects the Cell Medicated Immunity16 (CMI) and it is the CMI which plays an important role in preventing TB. Risk becomes multifold for the malnourished children who are suffering from TB.[17] Being able to combat TB, one needs to have diet rich in fats, Vitamins, minerals and proteins. And it is difficult for the poor families to be able to mobilize such a diet. Hence, TB is also referred as the illness of the poor. TB, in tandem with malnutrition reinforces the vicious cycle poor health and poverty. However, what people generally do not know is that TB by itself is a major risk factor and that it can be one of the potential causes of malnutrition.[18]

In patients with tuberculosis, malnutrition leads to reduction in appetite, nutrient malabsorption, micronutrient malabsorption, and altered metabolism leading to wasting, muscles become atrophic and the patient becomes revulsive towards food and shows signs of general weakness.[19] Alongside, the TB patient has to expend more energy in coping with the infection leading to higher requirements for energy and nutritional intake. In children, these requirements are further pronounced because of their growth as well.[20]

Nutrition and TB have a very complex association with each other and it keeps up the vicious cycle of malnutrition, poverty and disease. We all are aware that malnutrition harboring determinant of TB. We also know it well that inadequacy of nutrition delays in restoration of health because it affects the efficacy of antibiotic medicines used for the treatment of TB.[21] Malnutrition is a significant risk factor in transforming the latent TB in to active TB, particularly in children and HIV-affected persons because their immune system is already weak. Status of malnutrition in India is worse than that in many other poor countries. Our country is amongst those where the number of malnourished children is the highest. Here, more than 40% children under 5 years are malnourished. The adults are a little better off in this regard. According to the NFHS-3 Survey in 2005-06, 36% women and 34% men in the age-group of 15-49 years were malnourished.[22]

MATERIAL AND METHODS

Mycobacterium tuberculosis (MTB) used in the current study were isolated from the clinical samples of the patients sent to the laboratory for diagnosis using 12B (Mycobacterial Middle brook 7H 12 medium) medium vials for Bactec 460 (Becton, Dickinson and Co., Towson,

MD). The samples were cultured by inoculating 100µl of stored pathogenic culture into fresh 12B medium vials prior to the study.

Plants samples used

All the plant samples were dissolved primarily in ethanol (70%) and double distill water.

S.No	Plants used	Parts used
1	*Azadirachta indica* (Neem)	leaves and thin green stems
2	*Ocimum sanctum* (Tulsi)	leaves
3	*Zingiber officinale* (Zinger)	root
4	*Allium sativum* (Garlic)	clove

Preparation of aqueous extracts

Different plant was used in the current study as described above. All the plant parts were cut into pieces, air dried. Then the samples were grinded with DDW to make the stock solution of 1 g plant/ 10ml of solvent. The plant extracts were stored at 4˚C. Control was taken to set up each set of experiments with distill water.

***Mycobacterium* cultures**

Mycobacterium cultures used in the current study were previously isolated from the clinical samples sent to the laboratory for diagnosis. The samples were inoculated in 12B medium vials and incubated at 37˚C. The growths of microorganisms in the vials were tested through Basters 460 TB system (Becton, Dickinson and Co., Towson, MD) every week. The positive cultures isolates were confirmed by *Ziehl-Neelsen* staining (*Ellis and Zabrowarny, 1993*). The positive cultures were tested for sensitivity against 08 different first and second line drugs to confirm M.TB (Table 1). Inoculum of 0.05 mL *M.TB* was mixed with 4 mL of 12B medium broth and equally transferred to the vials using MIC method. Each vial was treated with different concentration of 0.0(control), 0.01, 0.1 and 1.0 mg/mL plant aqueous extracts and correspondingly labeled Neem (N1,N2,N3,N4), Tulsi (T1,T2,T3,T4), Zinger (Z1,Z2,Z3,Z4), Garlic (G1,G2,G3,G4). The aliquot dilutions of *M.TB* treated with medicinal extracts plated on a petri dish containing agar medium in the basis of 0, 3 and 7 day were incubated at 37°C for 15 day. At the end of 15th day, each plate of 0, 3 and 7 day cultures were counted for the colony developed in the control and plant extracts and colony forming units (CFU) in log Units/mL were calculated.

Table 1: Drugs used in following study.

S.No.	Primary line drugs used	Secondary line drugs used
1	Rifampicin	Capreomycin
2	Pyrazinamide	Pyrazinamide
3	Isoniazid	Cycloserine
4	Ethambutol	ρ-aminosalicylic acid (PAS)

RESULTS

The dose effects of labeled Neem (N1,N2,N3.N4), Tulsi(T1,T2,T3,T4), Zinger(Z1,Z2,Z3,Z4), Garlic(G1,G2,G3,G4) on the growth of *M.TB* are shown in Table 2. The effects of aqueous extracts (leaves and roots) on inhibition of *M.TB* were compared with control. When tested by MIC method, aqueous extracts of different concentrations of *neem, zinger showed* consistent reduction in activity against *M.TB*. The increase in anti-mycobacterial activity by *Neem* was dose dependent and the increase in anti-mycobacterial activity was significant in 7th d and remained increasing. Aqueous extract of *Zinger showed* in significant activity against *M.TB* even in higher concentration and longer period of treatment. Likewise in higher dilutions of *M.TB* also shows no potential activity against *M.TB* in *Zinger* tested. *Neem extract* showed substantial inhibitory antibacterial activity against *M.TB*. Extracts of 0.0 to 1.0 mg/mL concentrations treated with *M.TB* were tabulated using colony forming units (CFU) in log Units/mL. Hence, the colony counts were equated by nth dilution of *M.TB* and CFU were calculated. Percent inhibitions of medicinal extracts were calculated using control CFU values. The obtained results of CFU in log units for *Neem* showed a potential significant activity in CFU observed when analyzed with *M.TB*. At the same time, *Garlic* didn't show any definite reductions in CFU when compared to control values. Table 2 shows the percentage inhibition of different concentrations of *Neem and Zinger* extracts. The highest antibacterial activity of *M.TB* was found in N1 on 7^{th} day. Therefore, it could be deduced that *Zinger* has a coherent inhibitory effects.

Table 2: Anti-mycobacterial activity of 0.01-1.0 mg/mL concentrations of plant extracts against *M. tuberculosis.*

Plants extract (mg/ml)		0 day(CFU/ml)	3 day(CFU/ml)	7 day(CFU/ml)
Neem	N1(0.0)	30+2.1	238+12.7	169+14.1
	N2(0.01)	30+4.2	193+10.9	144+1.1
	N3(0.1)	29+2.8	166+16.2	121+19.7
	N4(1.0)	29+1.4	131+1.13	101+16.9
Tulsi	T1(0.0)	27+2.1	231+11.3	155+5.6
	T2(0.01)	29+2.8	204+4.20	198+4.2
	T3(0.1)	29+1.4	191+14.2	161+5.6
	T4(1.0)	28+2.1	152+1.13	144+16.9
Zinger	Z1(0.0)	29+2.8	239+11.3	203+12.0
	Z2(0.01)	29+1.4	200+1.13	184+16.9
	Z3(0.1)	30+4.4	176+16.2	160+2.1
	Z4(1.0)	29+0.4	144+4.20	138+4.2
Garlic	G1(0.0)	30+2.1	211+11.3	188+4.2
	G2(0.01)	29+2.8	186+16.2	171+5.6
	G3(0.1)	29+1.6	139+11.3	134+3.1
	G4(1.0)	30+1.1	134+4.20	127+4.2

DISCUSSION

The view of the inhibition for aqueous extracts showed that *Neem* has exposed substantial reduction in the growth of *Mycobacterium* activity and is a prospect for future curative intrusions. Simultaneously, *Garlic* has clearly indicated that there was some satisfactorily reduction in *M.TB* growth. Proposed study doesn't confirm antibacterial activity in *Zinger* may be because of aqueous based studies or else may be due to species specificity. Moreover there may be many other components in the extracts, which may have an inhibitory or activating effect on the bacilli and a solid method of isolation of components has to be established using gel electrophoresis or chromatographic technique to purify them. Thus, by this means it might suggest that the high polar molecule in the isolated components of soluble extracts may be responsible for anti *M.TB* activity. Appropriate doses of the extracts may act against the most deadly disease in the humankind can be battled in near future.

CONCLUSION

From the examined results, it could be seen that *Neem* has shown considerable activity against *M.TB* and no change in *garlic treated M.TB.* Aqueous extract of *Neem* (0.01-1.0 mg/mL) had significant effect against *M. tuberculosis* whereas, *Zinger* in higher concentration showed no inhibition. Main disadvantage potent anti-tuberculosis component

of the plant could be water insoluble and so other ether extract of the plant could give better results.

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