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Anti Tuberculosis Effect of *Ocimum sanctum* Extracts in *in vitro* and Macrophage Culture

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This study was conducted to evaluate the anti *Mycobacterium tuberculosis* effects of *Ocimum sanctum* directly and in macrophage culture which were infected with *Mycobacterium tuberculosis* before treatment with different concentrations of this plant. Suspensions of bacteria were prepared in 7H9 broth and after Macrophage culture, cell suspensions of the *M. tuberculosis* were added to the attached macrophages. Adherent monolayers was disrupted and bacterial suspensions were serially diluted and plated onto Middle brook 7H10 agar plates. Colonies were counted under a dissecting microscope and reported as CFU. For each culture dilution, six replicate samples were plated and the mean number of colonies was calculated and then Intracellular and extra cellular killing of *Ocimum sanctum* extracts were measured by colony counting. Present findings showed that in a defined laboratory and macrophage culture, *Ocimum sanctum* has a potent anti-*Mycobacterium tuberculosis* effects both directly and in infected macrophage culture. In this study we confirmed anti-tuberculosis effects of different concentration of *O. sanctum* extracts *in vitro* and in Macrophage culture but key components of anti tuberculosis action of these extracts and their mechanisms of actions must be discovered in future researches.

Key words: *Ocimum sanctum*, *Mycobacterium tuberculosis*, extracts

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INTRODUCTION

We are in the beginning of the new millennium with tuberculosis being an even greater global problem than it was at the beginning of the twentieth century. Tuberculosis continues to be a major cause of morbidity and mortality throughout the world. Five decades of tuberculosis control programs using potentially efficacious drugs and the availability of BCG vaccine, have failed to reduce the prevalence of infection in most parts of the world (O'Brien and Nunn, 2001; Chopra *et al*., 2003) and tuberculosis continues to kill young and middle-aged adults faster than any other disease apart from Acquired Immune Deficiency Syndrome (AIDS). It is estimated that there were approximately eight million new tuberculosis cases and nearly two million deaths due to the disease (Dye *et al*., 1999). The situation has exacerbated because of the presence of some complicating factors like, emergence of multi-drug-resistant TB (Culliton, 1992), HIV co-infection (Butler, 2000), lack of patient compliance with chemotherapy and variable efficacy of Bacille-Calmette Guerin (BCG) vaccine.

In spite of the new advances in understanding the biology of *Mycobacterium tuberculosis* and availability of functional genomic tools, such as micro array and proteomics, in combination with modern approaches, no new drug has been developed in the past 30 years. Therefore, there is an urgent need to identify new drug targets in mycobacteria and eventually, develop new drugs (Chopra *et al*., 1999).

The recent rise in TB cases and especially the increase of drug resistant mycobacteria indicate an urgent need to develop new anti-TB drugs. The long duration of TB therapy is a consequence of persistent *M. tuberculosis*, not effectively killed by current anti-TB agents.

Holy basil (*Ocimum sanctum*) is an herb native to India, where it is known as Tulsi. It is sacred in the Hindu religious tradition and is regarded as one of the most important plants used in Ayurvedic medicine (Wagner *et al*., 1994). Holy basil grows in profusion around Hindu temples. It comes in red and green varieties, both with a strong, pleasant aroma. More clove-like than that of culinary basil, holy basil has been used for centuries to treat a variety of medical conditions (Godhwani *et al*., 1987) including heart problems, asthma, bronchitis, arthritis and eye disorders (Maity *et al*., 2000; Agrawal *et al*., 1996). In the past decade or so a number of scientific studies have looked at holy basil for various treatment purposes (Mandal *et al*., 1993; Sembulingam *et al*., 1997; Ganasoundari *et al*., 1997a, b,1998). Also previous studies showed that the juice of this plant exhibited potent anti-viral activity and aqueous leaf extract exhibited a complete inhibition of the growth of all the three tested *Mycobacterium tuberculosis* strains (Joshi and Magar, 1952; Grover and Rao, 1977).

This study was conducted to evaluate anti *Mycobacterium tuberculosis* effects of *Ocimum sanctum* directly and in macrophage culture which were infected with *Mycobacterium tuberculosis* before treatment with different concentrations of this plant.

MATERIALS AND METHODS

This study was done in Research Center for Infectious Disease in Zahedan University of Medical Sciences in April 2004-April 2005.

Bacterial cell association and replication: Suspensions of bacteria, homogenization, Plate culture and measurement of viability were done according to previous works (Birkness *et al*., 1999; Ramachandra *et al*., 2001). Viability ranged from 70 to 84% in these experiments.

Human peripheral blood mononuclear cells: Mononuclear cell preparation, counting and culture were done according to previous published procedure (Clements *et al*., 2000). The participation of normal human blood donors in our research was approved by the Zahedan University Medical Ethics Review Board.

Infection of macrophages for viable counts: Virulent *M. tuberculosis* was originally obtained from Iranian National Research Center for Tuberculosis and Pulmonary disease. Cell suspensions of the *M. tuberculosis* were added to the attached macrophages at a Multiplicity of Infection (MOI) of 1:10 (1 bacterium per 10 host cells). Each day, the infected macrophages were washed twice with HBSS and overlaid with fresh IMDM.

CFU assay: Adherent monolayers were disrupted with a solution of water containing 0.016% Digitonin and 0.25% Tween 80 (Sigma Chemical Co.). Bacterial suspensions were serially diluted and plated onto Middlebrook 7H10 agar plates supplemented with oleic acid-albumin-dextrose-catalase enrichment (Difco). Plates were incubated for 14 to 21 days at 37°C. Colonies were counted under a dissecting microscope and reported as CFU. For each culture dilution, six replicate samples were plated and the mean number of colonies was calculated (Manca *et al*., 1999).

Intracellular and extracellular killing assays: To determine intracellular killing of added sacred extract,

4-day-infected monocytes were treated for 6 h different concentrations (50, 75 and 100 mg mL^{-1}) of sacred basil extracts. The cells were then disrupted and the culture was processed for the CFU assay as described above. For determination of extracellular killing by sacred extract, the cell monolayers were disrupted before the addition of sacred extract. Sacred extract was then added for 6 h and the cultures were processed for the CFU assay as described above. Results for the killing assays represent the mean±standard error of the mean (SEM) of three to six independent experiments (Manca *et al*., 1999).

RESULTS AND DISCUSSION

To study the anti tuberculosis effects of different concentrations of sacred basil extract on survival of *Mycobacterium tuberculosis* directly and indirectly, we tested the sensitivity of *Mycobacterium tuberculosis* strain which was 0, 11±2 and 3±20 to 50, 75 and 100 mg mL^{-1} concentrations, respectively of sacred basil extract in Middlebrook 7H10 medium directly and in macrophage cell culture (Table 1). Previous studies of clinical isolates have demonstrated significant killing *in vitro* (Joshi and Magar, 1952; Grover and Rao, 1977) but did not mentioned any specific concentration for that.

We next compared killing of mycobacteria following treatment with Sacred basil extracts with controls. Fresh human monocytes were infected with *M. tuberculosis* and after lyses different concentrations of *Ocimum sanctum* extracts (50, 75 and 100 mg mL^{-1}) was added to infected monolayer. After 6 h of treatment, numbers of CFU were determined (extra cellular killing) which were 20, 11 and 0, respectively (Table 2).

The infected monocytes were disrupted after 6 h of treatment with different concentrations of Ocimum sanctum extracts (50, 75 and 100 mg mL^{-1}). Cultures were then harvested for the CFU assay (intracellular killing) and the results of colony counting were 33, 30 and 28, respectively.

Table 1: Effect of different concentrations of *Ocimum sanctum* on mycobacterial colony counts in middle brook 7H10 medium

Concentration	50 (mg mL^{-1})	75 (mg mL^{-1})	100 (mg mL^{-1})
Colony count	3±20	2±11	0

Mean±SD, SD = Standard Deviation for 6 independent experiments

Table 2: Mean of colony counts of *Mycobacterium tuberculosis* After treatment with different concentrations of *Ocimum sanctum* in the macrophage culture

Concentration	50 (mg mL^{-1})	75 (mg mL^{-1})	100 (mg mL^{-1})
Colony counts	4±33	2±30	2±28

Mean±SD, SD = Standard Deviation for 6 independent experiments

As anti-tuberculosis effects of *O. sanctum* extracts in a well defined laboratory condition has not been studied before, direct comparison of our findings especially in macrophage culture with previous works is not possible. Direct treatment of *M. tuberculosis* with *O. sanctum* extracts, as shown in results, significantly decreased colony counts of *M. tuberculosis* which is compatible with aged previous reported studies (Joshi and Mgar, 1952; Grover and Rao, 1977).

On the other hand, comparison of colony counts of *M. tuberculosis* culture after extracellular treatment with different concentrations of *O. sanctum* extracts with colony counts of *M. tuberculosis* culture resulted from lysis of the infected Macrophage culture and has incubated with different concentration of *O. sanctum* extracts showed that the colony count decreased in the latter and even in this case, the colony count of extracellular treatment is the same as colony count of *M. tuberculosis* culture in agar dilution with different concentrations of *O. sanctum* extracts.

Although in this study we confirmed anti-tuberculosis effects of different concentration of *O. sanctum* extracts *in vitro* and in macrophage culture, key components of anti tuberculosis action of these extracts and their mechanism of actions must be discovered in future researches.

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