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Antimicrobial Effect of Chloroformic Garlic Extract on Mycobacterium Tuberculosis

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Abstract: This study assesses the antimicrobial effect of chloroformic extract of garlic on strains of mycobacterium tuberculosis. A standard H37RV isolate and isolates from patients with drug resistant pulmonary tuberculosis were used. A strain sensitive to 4 drugs (rifampin, isoniazid, ethambutol and streptomycin), resistant to the four drugs, resistant to two drugs and a strain resistant to one drug, were used. The antimicrobial effect was tested *in vitro* with minimum inhibitory concentrations of the chloroformic extract of garlic on the mycobacteria. Middel Broke 7H10 agar medium with 1:128 dilution or ($167 \pm SD \mu\text{g mL}^{-1}$) of chloroformic extract of garlic were used. Our study showed that garlic extract is effective in inhibiting growth of not only drug sensitive, but also drug resistant isolates of mycobacterium tuberculosis.

Key words: Mycobacterium tuberculosis, drug resistance, garlic extract

INTRODUCTION

Although the prevalence of tuberculosis (TB) has drastically decreased in recent decades, it is still an important health concern to specialists and centers of disease control in different societies. Control efforts are top priorities of health ministries in most countries. TB comprises one-fourth of all adult mortalities in developed countries (Dolin *et al.*, 1994). It is known that 95% of TB cases occur in the developing world where adequate first-line drugs may simply not be readily available to all patients (Virginia, 1999). At the end of the 20th century, TB remains a major public health issue. In developing countries, it is a leading cause of morbidity and mortality. The spread of the increasing HIV epidemics contributes significantly to this dire situation especially with the regard to its association with TB. Some 90 million new cases of TB and some 30 million deaths are expected to occur world-wide during the next decade (Gerd and Ttaelman, 1999).

According to WHO statistics, outbreak rates and new cases of TB have been rising world-wide since 1980. The increasing number of patients and growing mortality rate resulting from TB in recent years, especially HIV contaminated individuals is a warning that combat programs against this disease have not been adequate and needs for new efforts are lacking. This may be

attributed to reduced welfare, social-economic status, inadequate health services availability, increasing HIV patients, immigration and lack of education (WHO, 1992). One of the most important problems contributing to this issue is the appearance of drug resistant strains of TB. Reports of resistant TB are numerous and reports demonstrating cases resistant to 11 antituberculosis drugs are also at hand (Rahbar, 1996). Drug resistance has become a medical crisis in some countries and it is increasing daily (Dolin *et al.*, 1994).

Effective control of TB not only seeks the development of new antituberculosis drugs, but also perception of mechanism by which to combat development of drug resistance. Our study evaluates the effect of garlic extract on TB and drug resistant strains of TB based on the increasing trends of patients throughout the world turning towards herbal drug therapy (Moore and Atkins, 1977). Although, garlic extract has previously been tested on TB organisms with success (Rajahunandana *et al.*, 1947) we sought to assess its effect on drug-resistant strains of pulmonary TB.

MATERIALS AND METHODS

To prepare garlic extract, 500 g of peeled garlic bolls were grinded with 150 mL distilled water in a blender then,

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Table 1: Characteristics of selected strains of mycobacterium tuberculosis

Strain code	Drug resistance characteristics	RIF	INH	ETB	SM
A	H37 RV	S	S	S	S
B	The patient's isolate	R	R	R	R
C	The patient's isolate	S	S	S	S
D	The patient's isolate	S	S	R	R
E	The patient's isolate	S	S	S	R

passed through a cheese cloth and filtrated. Afterwards, we centrifuged this solution at 2000 rpm for 20 min and isolated the supernatant. We then added 50 mL chloroform to 100 mL aqueous extract. Then, we slowly the mixed this solution in a decontor funnel until completely mixed. Three phases were formed. Regarding of chemical characteristics of chloroform, we isolated the lower phase that contained chloroformic extract of garlic and repeated the extraction (nine times, each time with 50 mL chloroform). In order to omit the solvent (chloroform), we placed the chloroformic solution in a distiller vacuum at a temperature of 35°C and the chloroform was removed within 4 h yielding a yellow, concentrated, jelly substance with a strong garlic odor and was maintained at 45°C.

The prepared 1:16 (1339±SD µg mL⁻¹) to 1:128 (167±SD µg mL⁻¹) dilutions of garlic were placed in Middle Broke culture 7 H10 agar medium. To survey the effect of garlic extract on growing mycobacterium tuberculosis *in vitro*, standard dilutions from four antibiotics (rifampin, isoniazid, ethambutol and streptomycin) and dilutions of 1:16 to 1:128 of garlic extract were prepared and added to Middel Broke 7 H10 agar medium. Then, bacteria were inoculated in dilutions of 10⁻¹, 10⁻³, 10⁻⁵ to the medium and antibiogram results were read after 28 and 44 days. The antibiogram results were obtained an average of 3 times. The isolates were isolated from referred patients with pulmonary TB. Their characteristics of drug resistance were compared with the standard H37RV strain as shown in Table 1.

RESULTS

In this study, the effects of chloroformic extract of garlic on resistant and sensitive strains of mycobacterium tuberculosis were evaluated. Drug resistance of the isolated strains cultured in Middel Broke 7H10 agar medium is shown in Table 2. Growth of strains A and C (sensitive to rifampin, isoniazid, ethambutol and streptomycin) were inhibited via garlic extract at 1:128 dilution (167±SD µg mL⁻¹). Strains of B, D and E (resistant to 4, 2 and 1 drug, respectively) were completely inhibited also using garlic

Table 2: Inhibitory effect of garlic extract on resistant and sensitive strains of mycobacterium tuberculosis

Antibiotic strain code	1339±SD (µg mL ⁻¹)				669.5±SD (µg mL ⁻¹)		335±SD (µg mL ⁻¹)	167±SD (µg mL ⁻¹)
	SM	ETB	INH	RIF	RIF	INH	RIF	INH
A	S	S	S	S	S	S	S	S
B	R	R	R	R	S	S	S	S
C	S	S	S	S	S	S	S	S
D	R	R	S	S	S	S	S	S
E	R	S	S	S	S	S	S	S

* S= Sensitive, * R= Resistance, *SD= Standard Deviation

extract at 1:128 dilution, but growth of these strains were not inhibited using more dilute garlic extracts.

DISCUSSION

Tuberculosis an ancient human disease discovered in 1882 by Robert Koch, is still a major health problem today, not only the third world countries, but also in the industrialized world. This disease has resurfaced again after a period of reduction (WHO, 1992). Today, drug resistance has become a major medical crisis in some countries and is increasing the number of individuals afflicted (Dolin *et al.*, 1994). Research now aims to discover new antituberculosis drugs to combat drug-resistant strains of TB. Herbal drugs are continually tested and have been used to treat different diseases world wide (Moore and Atkins, 1977). Rajahunandana *et al.* (1947) evaluated antimicrobial effects of garlic extract and concluded that garlic extract has a inhibition effect on growing mycobacterium tuberculosis. In 1975, Delaha and Garagus (1985) investigated the effect of garlic extract on atypic mycobacteria. According to their study, 1.67 mg mL⁻¹ concentration of chloroformic extract garlic had an inhibitory effect on mycobacterium tuberculosis.

Absence of synergismic effects of this extract with antituberculosis drugs has been investigated (Abbruzzese *et al.*, 1987). The effect of aqueous garlic extract on yeasts (Moore and Atkins, 1977). Rajashri and Mahfuz (1993) investigated inhibitory effects of garlic extract on isolated mycobacterium avium from AIDS patients and they concluded that aqueous garlic extract was effective. In our study a 1:128 dilution of garlic extract (167±SD µg mL⁻¹ concentration) had an inhibitory effect on not only drug-sensitive TB but also, drug resistant strains.

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