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ANTITUBERCULAR ACTIVITY OF GARLIC (*ALLIUM SATIVUM*) EXTRACT IN COMBINATION WITH CONVENTIONAL ANTITUBERCULAR DRUGS IN TUBERCULAR LYMPHADENITIS

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ABSTRACT

Based on our demonstration earlier that ethanol extract, water extract and a compound purified from garlic possessed *in vitro* antitubercular activity against drug resistant and susceptible *Mycobacterium tuberculosis*, we tried the effect of garlic extract in 30 patients of tubercular lymphadenitis. For ethical considerations, two groups of patients, 30 each, were given antitubercular therapy (ATT) consisting of isoniazid, rifampicin, ethambutol and pyrazinamide for 30 days. For the next 15 days (31 to 45 days) group 1 patients received 3-6 garlic pearls per day in addition to ATT while group 2 patients received ATT only. From 46th day onwards both the groups received ATT only for 6-8 months. Antitubercular activity of the serum samples collected on 45th day was assessed by its effect on the growth of *M.tuberculois*. The serum of group 1 patients showed significantly much higher antitubercular activity than that of group 2 patients. Further, there was relief of dyspeptic symptoms caused by ATT therapy in patients of group 1 with garlic plus ATT therapy but no change in group 2 patients with ATT only. Liver function and hematological tests were normal in both the groups after 6 months of therapy. Garlic extracts or compounds have a good potential as antitubercular(s) drug if given as a supplement to ATT.

KEY WORDS: Garlic, *Allium sativum*, tubercular lymphadenitis, chemotherapy, *Mycobacterium tuberculosis*.

INTRODUCTION

Garlic, modified garlic, juice and oil of garlic and allicin from garlic are known all over the world for their hypocholesterolemic, fibrinolytic, antithrombotic, antibacterial, antiprotozoal, anticancer and insecticidal effects (1-3). Rao et. al. (2) and Delaha and Garagusi (4) studied the antitubercular activity of allicin and chloroform extract of garlic. In view of the very high minimum inhibitory concentration (MIC) of both the preparations, there was no interest on the antitubercular activity of garlic till Murthy and his colleague (5) obtained a preparation called CEF-allicin from garlic. They demonstrated that it inhibited the growth of not only susceptible laboratory strains but also isoniazid resistant clinical isolate of *Mycobacterium tuberculosis*. Its MIC of 25 μg/ml when tried alone was the

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lowest reported so far for any garlic compound. They also investigated its mechanism of action and found that it inhibited the synthesis of lipids from ^{14}C-acetate (99%) and uptake of ^{3}H-thymidine i.e. DNA synthesis and ^{14}C-glycine i.e. proteins and glycine derived carbohydrates (76%) by whole cells in less than 6 hours of exposure of the tubercle bacilli to it (5). Ratnakar and Murthy (5) stressed that since multi drug therapy is used for tuberculosis, CEF - allicin or any garlic preparation must be tried in combination with other antitubercular drugs. However, CEF - allicin can be tried only after pharmacology and toxicity studies necessary for a new drug are carried out. We, therefore, tried the antitubercular effect of a garlic preparation already in the market in combination with other antitubercular drugs in 30 patients of tubercular lymphadenitis. The results of the antitubercular activity of the serum of these patients are reported here.

MATERIALS AND METHODS

Sixty patietns of the surgical out patients department in University College of Medical Sciences and GTB Hospital, Delhi of different age groups, who were diagnosed to have tubercular lymphadenitis were chosen for this study. Due permission from the local ethical committee of our institution and informed consent from each patient (parents also in the case of children) were taken.

Investigations

In addition to general checkup, chest x-ray, complete hemogram, ESR, platelets count, liver function tests [serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin, albumin, A/G ratio], routine and microscopic examination of urine, fine needle aspiration cytology (FNAC) of the lymph nodes, staining of the smear of FNAC by Giemsa stain for cytology and Ziehl Neelsen stain for acid fast bacilli (AFB) were carried out as described recently by us (6). Counting of AFB on slides according to American Lung Association criteria, growth of mycobacteria in liquid Youmans and Karlson's medium and on Lowensterin - Jensen (LJ) solid medium were also according to the methods described (6). Biopsy of the lymph nodes was done only in patients who gave consent, 16 in group 1 and 10 in group 2.

Treatment of patients

Two groups of 30 patients each were included in the study. For ethical considerations, patients of both the groups were given daily antitubercular therapy (ATT) for one month. ATT consisted of isoniazid (5-10mg/kg, maximum 300 mg), rifampicin (10 mg/kg, maximum 600 mg), ethambutol (25 mg/kg) and pyrazinamide (30 mg/kg in two divided doses). In this preliminary study, garlic extract was given as a supplement to ATT for 15 days only (from 31 to 45 days) to group 1 patients while group 2 patients received only ATT during the same period. From 46th day upto 2 months, both the groups were given the same four drugs. Afterwards isoniazid plus rifampicin therapy (same for all the patients) was given upto 6 to 8 months depending on the need of the patients. Garlic pearls (each pearl 250 mg, 0.25%w/w) of M/s Ranbaxy Ltd., Delhi, was used as garlic extract. Three to six pearls were administered in divided doses according to the tolerance of the patients.

Assesement of antitubercular activity of the serum of patients treated with either ATT plus garlic extract or ATT alone.

Patients with overnight fasting were allowed light breakfast with low fat content to minimize lipemia. Two blood samples were collected from the patients of both the groups on day 30 and day 45 i.e. before and after garlic extract therapy and serum was separated. Antitubercular activity of the serum of the treated patients (group 1 with ATT plus garlic extract and group 2 with ATT only) was tested by adding 0.5 ml of the serum to 4.5 ml of Youmans and Karlson's medium with Tween 80 in which *M.tuberculosis* H_{37} R_v was inoculated. Control tubes contained 0.5 ml of serum of healthy volunteers (three). After 14 days of growth, optical density (O.D.) at 540 nm was taken. Details were described earlier (6,7).

RESULTS AND DISCUSSION

Sixty fresh untreated patients of tubercular lymphadenitis of 4 to 60 (mean 23.3) years of age were part of this study. Out of these 18% were below 10 years, 23% of 11-20 years, 35% of 21-30 years, 12% of 31-40 years, 10% of 41-50 years and 2% of more than 50 years; while 64% were females and 36% males.

Lymph nodes involved were most frequently cervical (77%) followed by inguinal (10%), axillary (8%) and cervical plus axillary (5%). The disease at the beginning of the study was of 1-36 (mean 5.2) months with about 74% of the cases of 1-5 months, 18% of 5 months to 1 year and 8% of 1 to 3 years. Involvement of multiple nodes was in 87% of the patients and single node in 13%. X-ray chest was normal in 90% of the cases at the time of diagnosis. FNAC was conclusive of tuberculosis in 85% of the cases while in the remaining, histopathological examination confirmed tuberculosis. Lymph nodes biopsy material (smear and growth on LJ medium) also indicated the presence of *M.tuberculosis*.

Assessment of Improvement

General

Increase in ESR and decrease in body weight and hemoglobin seen in about 80% of the patients at the beginning returned to normal after treatment with no statistical difference between the two groups. In both the groups lymph nodes disappeared completely in early cases if no fibrosis occured and in the cases where fibrosis / calcification occured, there was reduction in the size of the nodes after treatment.

Relief of dyspepsia by garlic extract

Many patients in both the groups developed dyspeptic symptoms after one month of taking regular ATT of four drugs. Addition of garlic to ATT to group 1 patients for 15 days relieved dyspepsia in all the patients of this group while there was no change in group 2 patients.

Antitubercular activity of the serum of treated patients

As explained above for ethical reasons. the duration of supplementation of garlic pearls to group 1 patients was for a short period of 15 days only from 31 to 45 days. The treatment was the same to patients of both the groups 1 and 2 before and after garlic supplementation. Therefore, the assessement of antitubercular

activity of the serum of treated patients was with blood samples collected on day 30 and day 45 i.e. before and end of garlic supplementation. 0.5 ml of the serum of patients of group 1, group 2 and of three healthy volunteers was added to Youmans and Karlson's liquid medium inoculated with *M.tuberculosis*.

Since the serum of the treated patients contains the antitubercular drugs used for treatment or their metabolites, there will be less growth of *M.tuberculosis* i.e. lower O.D. values in the tubes containing the patient's serum than in those containing the serum of healthy persons.

Table 1 shows the O.D. values obtained with the serum of patients of groups 1 and 2 and of healthy persons. It can be seen that the mean O.D. values in groups 1 and 2 were less than those with control (healthy) serum. However, the decrease in O.D. was much more with the serum of group 1 than the serum of group 2 patients. ATT plus garlic treatment reduced the mean O.D. by 75% while ATT alone decreased it by 40%. Further, the number of samples with much less growth i.e. a lower O.D. range of 0.0 to 0.3 was much higher 21/25 (84%) in garlic plus ATT treated patients than in group 2 (ATT alone) patients 14/26 (54%). Likewise the number of samples with more growth i.e. higher O.D. range of 0.3 to 0.5 was much less (16%) in ATT plus garlic group than in ATT alone group (46%). This significant ($P<0.05$) difference is interesting. It indicates higher concentration of antitubercular drugs or their metabolites in the serum of patients treated with garlic plus ATT than with ATT alone.

Table 1. Antitubercular activity of the serum of tubercular lymphadenitis patients treated with either ATT plus garlic or ATT alone.

Treatment	Mean O.D.* ± S.D.	% reduction in O.D.	No. of serum samples with O.D. 540 nm		AFB count in smears of culture
			0.0 - 0.3	0.3-0.5	
Group 1	0.16 ± 0.10	75	21(84%)	4(16%)	0(19)
ATT 30 days					1+(6)
ATT+garlic 15 days					
(25 patients)**					
Group 2	0.28 ± 0.09	40	14(54%)	12(46%)	0(2)
ATT 45 days					1+(19)
(26 patients)**					2+(5)
Healthy	0.47 ± 0.01				3+(all)
Controls (3)					

* O.D. at 540 nm of 0.3 corresponds to about $2x10^8$ mycobacteria for 5 ml medium under our experimental conditions.

** 5 samples of group 1 and 4 samples of group 2 were contaminated and excluded from data in this table. Numbers in brackets in last column indicate the number of samples with particular O.D.

Five and four samples from group 1 and 2 respectively were contaminated and excluded from the data in table 1. To make sure that the O.D. of the medium was due to growth of *M.tuberculosis* but not any contaminant, a smear of the grown culture (after taking O.D.) was also made on a slide and stained with Ziehl Neelsen stain. All the bacilli were found to be acid fast bacilli (AFB) i.e. mycobacteria without any contamination. Furthermore, AFB on the slide were counted. AFB count of 0 was seen in 19/25 (76%) in ATT plus garlic treated but only 2/26 (8%) in the group treated with ATT alone. There were no samples with a higher AFB count of 2+ in ATT plus garlic group but 5 in ATT alone group.

The above results clearly point out that the serum of ATT plus garlic treated patients possessed significantly much higher antitubercular activity than that of the serum of patiens treated with ATT alone.

Although Imada reported some effects like edema, reduction in body weight in rats treated with garlic extracts (8), none of the 30 patients treated with ATT plus garlic complained of any untoward side effects attributable to garlic. On the other hand garlic supplementation helped in relieving dyspeptic symptoms. Serum total protein, albumin, A/G ratio (Table 2), transaminase (SGPT) and alkaline phosphatase (ALP) and total bilirubin (Table 3) were all in normal range even in the patients supplemented with garlic extract to ATT.

Table 2. Effect of antitubercular therapy in tubercular lymphadenitis patients on liver function tests: Serum proteins

Treatment	Total Proteins g/100 ml.		Albumin g/100 ml.		A/G ratio	
	0M	6M	0M	6M	0M	6M
Group I (garlic days 31-45, ATT remaining period)	7.56 ± 0.85	7.89 ± 0.60	4.04 ± 0.59	4.45 ± 0.43	1.17 ± 0.26	1.31 ± 0.21
Group 2 ATT only	7.87 ± 0.84	7.95 ± 0.83	4.30 ± 0.59	4.56 ± 0.67	1.22 ± 0.23	1.33 ± 0.27

Values represent mean ± S.D. at the beginning (OM) and end of six months(6M) treatment.

Supplementation of garlic extract to regular ATT for just 15 days after one month's ATT provided additional benefit as indicated by higher serum antitubercular activiy during the period of garlic supplementation and relief of dyspepsia. It also indicates that garlic compounds have probably *in vivo* antitubercular activity and there is synergistic effect between garlic extract and other drugs of ATT used in this study. This is the first *in vivo* demonstration of antitubercular activity of garlic extract. The ability of garlic extract (CEF-allicin) to inhibit the synthesis of the

Table 3. Effect of antitubercular therapy in tubercular lymphadenitis patients on liver function tests: serum enzymes SGPT, ALP and total bilirubin.

Treatment	SGPT		ALP		Bilirubin	
	0M	6M	0M	6M	0M	6M
Group I	38.0 ±	26.7 ±	312 ±	323 ±	0.83 ±	0.84 ±
(garlic 31-45 days,	35.9	12.3	104	90	0.36	0.36
ATT remaining period)						
Group 2	34.8 ±	25.9 ±	326 ±	339 ±	0.71 ±	0.70 ±
ATT only	34.0	12.4	125	95	0.36	0.36

Values represent mean ± S.D. at the beginning (OM) and end of six months(6M) treatment.

three macromolecules, lipids, DNA and proteins and of the growth of isoniazid resistant tubercle bacilli are added advantages. The structure of allicin, sulphoxide of diallyl disulphide is different from that of the existing antitubercular drugs (9). These encouraging results justify further studies in pulmonary and other forms of tuberculosis.

In view of the above, it is worth while conducting further detailed studies on the antitubercular activity of CEF-allicin purified by Ratnakar and Murthy (4) from garlic in combination with other known antitubercular drugs required for multi drug therapy. We would also like to emphasize that it would be unrealistic to consider higher MICs of garlic compound as a draw-back. The yard stick of lower MIC applicable to synthetic or other drugs which are not items of human diet need not necessarily apply in the case of garlic (or its constituents) consumed as article of diet and tolerated well at higher doses.

REFERENCES

1. Lau, B.H.S., Lam, F. and Cheng, W.R. (1987) Effect of odor modified garlic preparation on blood lipids. Nutr. Res. 7, 139-149.

2. Rao, R.R., Rao, S.S., Natarajan, S. and Venkataraman, P.R. (1946) Investigation on plant antibiotics. I. Studies on allicin, the antibacterial principle of *Allium sativum*, (garlic) J. Sci. Industr. Res. 18, 31-33.

3. Cavallito, C.J. and Bailey, J.H. (1944) Allicin, the antibacterial principle of *Allium sativum* I. Isolation, physical properties and antimicrobial action. J. Amer. Chem. Soc. 66, 1950-1951.

4. Delaha, E.C. and Garagusi, V.F. (1985) Inhibition of mycobacteria by garlic extract (*Allium sativum*) Antimicrob. Agents and Chemother. 27, 485-486.

5. Ratnakar, P. and Murthy, P.S. (1995) Purification and mechanism of action of antitubercular principle from garlic (*Allium sativum*) active against isoniazid susceptible and resistant *Mycobacterium tuberculosis* $H_{37}R_v$. Ind. J. Clin. Biochem. 10, 34-38.

6. Gupta, R.L., Sundeep Jain, Talwar, V., Gupta, H.C. and Murthy, P.S. (1998) Studies on a new antitubercular drug, trifluoperazine: Its effect in combination with conventional antitubercular drugs in tubercular lymphadenitis. Ind. J. Clin. Biochem. 13(2), 92-97.

7. Ratnakar, P. and Murthy, P.S. (1992) Antitubercular activity of trifluoperazine, a calmodulin antagonist. FEMS Microbiol. Letts. 97, 73-76.

8. Imada, O. (1990) Toxicity aspects of garlic. Proc first World Congress on the health significance of garlic and garlic constituents. Washington, D.C., U.S.A.

9. Cavallito, C.J., Buck, J.S. and Suter, C.M. (1944) Allicin the antimicrobial principal of *Allium sativum* II. Determination of the chemical structure. J. Amer. Chem. Soc. 66, 1952-1953.