STUDIES ON THE ANTITUBERCULAR ACTIVITY OF SESAMIN

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Summary

1. The antibacterial and the antitubercular properties of sesamin have been studied. While sesamin has little antibacterial properties against the common pathogenic gram positive and gram negative bacteria, it has exhibited a very high antitubercular activity.

2. The stability of sesamin at various temperatures and hydrogen-ion concentrations has been studied.

3. The antitubercular activity of sesamin has been studied, in presence of serum, cysteine and nucleic acid.

In spite of the spectacular achievements of the synthetic antitubercular drugs, it can safely be said that no single compound can claim the distinction of causing radical cure of the disease. The search for more efficient and easily available alternate remedies must go on, not only as a supplement for the synthetic compounds, but also for the treatment of cases, where the organisms develop resistance to the drug.

Recently several compounds possessing antitubercular activity have been isolated from algæ, lichens and higher plants. Out of the several compounds known, mention may be made of usnic acid[1] from *Ramalina reticulata*, roccelic acid[2] from *Laconora sordida*, cepharanthine[3] from *Stephania cepharantha*, allicin[4] from *Allium sativum* and pterygospermin[5] from *Moringæ pterygosperma*.

It is well known that our indigenous systems of medicine like the Ayurveda and Yunani contain drugs useful in the treatment of tuberculosis. Centuries of successful clinical practice with these drugs should warrant us for a study of the isolation of these drugs in pure form and to assess their usefulness on a firm scientific basis.

Since long, *Cucurbita pepo* has been employed in the Ayurveda[6] system of medicine for treatment of tuberculosis. Recently its antitubercular activity has been confirmed[7,8] by systematic studies employing modern methods.

The details of our studies on the *in vitro* antitubercular activity[9] of another drug sesamin.[10] isolated from the unsaponifiable fraction of sesame oil (*Sesamum indicum*. N. O. Pedaliaceæ) are presented in this paper. Sesamin content of sesame oil varies from 0·2 to 0·5 per cent. on the weight of the oil.

The Ayurveda system[11] of medicine advocates the use of the oil as a whole as a therapeutic agent against tuberculosis. In the Yunani system of medicine,[12] the oil is mentioned to be useful for many ailments including dry cough, asthma and diseases of the lungs.

In the present study, while sesamin has shown little activity against the common pathogenic bacteria like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bact. coli* and *Bact. typhosum*, it has indicated activity against *Mycobacterium tuberculosis* even in 1 : 10,00,000 dilution and the results are presented in Tables I and II.

Sesamin has been found to be stable at higher temperatures and at hydrogen-ion concentrations (Table III) varying from pH 2–10, thus indicating that it does not require extraordinary precautions for storage. It is also found to be stable in presence of cysteine and nucleic acid (Tables V and VI). A slight diminution of activity, however, has been found in presence of bovine serum (Table IV).

EXPERIMENTAL

Preparation of Sesamin

Sesamin can be prepared from the oil by several procedures.[10,13] In the present experiments, the method of Tocher[10] has been followed, the procedure in brief being:

The oil (1 lb.) is shaken with glacial acetic acid (400 c.c.) for 40 hours at room temperature, and the acid layer separated and distilled under reduced pressure. The thick residue is treated with warm dilute potassium hydroxide, shaken from time to time and set aside for twelve hours in a conical flask. The supernatant liquid is siphoned off, and the deposited layer of sesamin washed several times with distilled water. It is then boiled with dilute hydrochloric acid, filtered, washed with water and dried. Sesamin is crystallised from alcohol in needle-shaped crystals. The yield is 1 gm. It melts at 121° C. and has an optical rotation of + 68·19° in chloroform solution.

Sesamin is found to be very soluble in acetone, carbon tetrachloride and chloroform, moderately soluble in alcohol and insoluble in petroleum-ether, ether and water.

Antibacterial Activity of Sesamin

The antibacterial activity of sesamin has been tested against the common gram positive and gram negative pathogenic bacteria like the *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bact. coli* and *Bact. typhosum* using the cup plate and the filter-disc methods.

Sesamin has been found to have negligible activity against these pathogenic organisms.

Antitubercular Activity of Sesamin

The antitubercular activity of sesamin has been studied using two virulent strains, $H_{37}R_v$ and D_{13} of *Mycobacterium tuberculosis* as follows:

(*a*) *With $H_{37}R_v$ Strain.*—Sesamin in acetone solution has been tested for its *in vitro* tuberculostatic activity in Youmans[14] synthetic liquid media using the virulent $H_{37}R_v$ strain (3–4 weeks old) of *Mycobacterium tuberculosis* by the usual surface growth method.[15] This strain was first obtained from the National Institute of Type Cultures, Kasauli, and has been maintained in our laboratories by subculturing every three weeks. The inhibition of growth has been studied at the end of each week upto three weeks. The results are presented in Table I.

TABLE I

Antitubercular Activity of Sesamin ($H_{37}R_v$ Strain)

Week	Dilution of sesamin in the media								
	1/10,000	1/50,000	1/100,000	1/500,000	1/1 mil.	1/5 mil.	1/10 mil.	Control with acetone	Control
1st ..	—	—	—	—	—	—	—	±	+
2nd ..	—	—	—	—	—	—	±	+	+
3rd ..	—	—	—	—	—	—	+	+	+

— No growth. + Growth. ± Slight growth.

(*b*) *With D_{13} Strain.*—Similar experiment has been carried out using the virulent strain D_{13}, isolated locally from an active case of pulmonary tuberculosis.[16] The results are presented in Table II.

TABLE II

Antitubercular Activity of Sesamin (D_{13} Strain)

Week	Dilution of sesamin in the media								
	1/10,000	1/50,000	1/100,000	1/500,000	1/1 mil.	1/5 mil.	1/10 mil.	Control with acetone	Control
1st ..	−	−	−	−	−	−	−	±	+
2nd ..	−	−	−	−	−	−	−	+	+
3rd ..	−	−	−	−	−	−	±	+	+

− No growth. + Growth. ± Slight growth.

Similar results have been obtained when tested with an alcoholic solution of sesamin in the above methods.

The above results indicate that sesamin is a powerful antitubercular drug, having an *in vitro* activity comparable to *para*-amino-salicylic acid[17] (P. A. S.) and *iso*-nicotinic acid-hydrazide.[18] Its negligible activity against the common pathogenic bacteria in contrast to its high activity against *Mycobacterium tuberculosis*, indicates that it may have a specific action against the acid-fast bacteria. Similar results are also reported in the case of P. A. S.[17] and *iso*-nicotinic acid-hydrazide.[18]

Stability of Sesamin

Effect of Temperature.—The two series of antibacterial and antitubercular tests have been carried out using (i) sesamin solution, sterilised by autoclaving under 15 lb. pressure for 20 minutes and (ii) by filtering sesamin solution through Seitz filter, and they both have yielded similar results indicating that sesamin is stable to high temperatures.

Effect of Hydrogen-Ion Concentration.—The effect of pH on the stability of the drug is next studied, since this is known to affect the activity of antibiotics like penicillin and streptomycin to a very great extent.

Equal volumes of acetone solutions containing known amounts of sesamin and the buffers[19] of various hydrogen-ion concentrations (pH 2–10) are mixed, and the emulsions so obtained are incubated for 24 hours at 37 °C. At the end of this period, the pH of each solution is adjusted to 7 and the

antitubercular activity of each solution is assayed by the usual method. The results at the end of three weeks are presented in Table III.

It can be seen from Table III that pH variation does not bring considerable change in the activity of sesamin. It can also be inferred that sesamin is more stable in acid range than in the alkaline range.

TABLE III

Stability of Sesamin at Various H-ion Concentrations (After Three Weeks)

Dilution	pH								
	2	3	4	5	6	7	8	9	10
1/10,000 ..	—	—	—	—	—	—	—	—	—
1/50,000 ..	—	—	—	—	—	—	—	—	—
1/100,000 ..	—	—	—	—	—	—	—	—	—
1/500,000 ..	—	—	—	—	—	—	—	—	±
1/1 million ..	—	—	—	—	—	—	—	±	+
1/5 million ..	—	—	—	—	—	—	±	+	+
1/10 million ..	—	—	±	+	+	+	+	+	+

— No growth. + Growth. ± Slight growth.

Effect of Serum on Sesamin.—Abraham, *et al.*,[20] have recorded that incubation of penicillin for 3 hours at 37° C. with blood, slices of liver, spleen, kidney, brain, muscles, lymph gland, lungs and intestines cause no detectable destruction of the activity. Chain and Florey[21] have reported that pus, tissue autolysates and serum have no inhibitory effect on the activity of penicillin. Bigger[22] has however shown that penicillin is inactivated by contact with human blood serum, the degree of inactivation varying greatly with the species of sera and is much greater at body temperatures than at lower temperatures. Further the stability of an antibiotic in the presence of blood or serum is of great importance from the point of estimation of blood levels of the antibiotic and its rate of excretion by the kidney.

The effect of serum on the activity of sesamin is therefore studied by incorporating sterile bovine serum (kindly supplied by the Serum Institute, Hebbal, Bangalore) in a concentration of 10% of the total volume of the

media containing various amounts of sesamin in each tube. The antitubercular activities of these solutions are studied by the usual methods. The results observed at the end of each week upto three weeks are given in Table IV.

TABLE IV

Stability of Sesamin in Presence of Serum

Week	Dilution of sesamin in the media containing 10% serum								
	1/10,000	1/50,000	1/100,000	1/500,000	1/1 mil.	1/5 mil.	1/10 mil.	Control with acetone	Control
1st ..	−	−	−	−	−	±	+	+	+
2nd ..	−	−	−	−	±	+	+	+	+
3rd ..	−	−	−	±	+	+	+	+	+

− No growth. + Growth. ± Slight growth.

Effect of Sulphydryl Compounds.—It has been observed[23] that a number of antibiotic substances of heterogeneous chemical nature are inactivated by cysteine and other compounds containing sulphydryl groups of enzymes. Several instances of sulphydryl groups inactivating antimicrobial agents are recorded in literature.[24-27] Recently Cavallito, *et al.*,[28] have studied in detail the inactivation of a number of antibacterial agents by thiol compounds. Based on their experimental findings, they concluded that the majority of antibacterial agents act by reacting with the sulphydryl groups of enzymes. The differences in antibacterial action of various agents are dependent upon the ability of these agents to come into contact with sulphydryl groups.

The effect of cysteine on the activity of sesamin is therefore studied, by incorporating 5 mg. of cysteine in each tube of Youmans media containing various amounts of sesamin, and carrying out the test for activity as usual. The results upto the end of three weeks are given in Table V.

It can be seen from Table V that cysteine does not interfere with the activity of sesamin.

Effect of Nucleic Acid on the Activity of Sesamin.—Nucleic acid and related compounds have been found to antagonize the antibacterial activity of different antibiotics.[29] Based mainly on these observations, a theory has been suggested to explain the mode of action of antibiotics.[30]

TABLE V

Stability of Sesamin in Presence of Cysteine

Week	Dilution of sesamin in the media containing 5 mg. cysteine								
	1/10,000	1/50,000	1/100,000	1/500,000	1/1 mil.	1/5 mil.	1/10 mil.	Control with cysteine	Control
st ..	—	—	—	—	—	—	±	+	+
nd ..	—	—	—	—	—	—	+	+	+
3rd ..	—	—	—	—	—	—	+	+	+

-- No growth. + Growth. ± Slight growth.

The effect of nucleic acid on the activity of sesamin is therefore studied by incorporating 0·5 mg. of yeast nucleic acid into each tube containing various amounts of sesamin, and testing the activity by the usual methods. The results upto the end of three weeks are presented in Table VI.

TABLE VI

Stability of Sesamin in Presence of Nucleic Acid

Week	Dilution of sesamin in the media containing 0·5 mg. nucleic acid								
	1/10,000	1/50,000	1/100,000	1/50,0000	1/1 mil.	1/5 mil.	1/10 mil.	Control with nucleic acid	Control
1st ..	—	—	—	—	—	—	—	±	+
2nd ..	—	—	—	—	—	—	±	+	+
3rd ..	—	—	—	—	—	±	+	+	+

— No growth. + Growth. ± Slight growth.

It can be seen from Table VI that nucleic acid does not reduce the activity of sesamin.

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