ABSTRACT

Fungal infections have been inflicting human beings from time immemorial. Fungi were recognised as causative agents of human diseases earlier than bacteria. Unfortunately the study of pathogenic fungi recieved only a little attention in comparision with the advances made in bacteriology.

Today, it is reported that fungal diseases occur widely throughout the world and an estimate of 17% of world population is affected by various fungal infections. Further, fungal infections have become one of the major causes of death among immunocompromised patients particularly with AIDS, Cancer, organ transplant, undergoing chemotherapy and haematologic disorders. It is also claimed that indiscriminate usage of broad spectrum antibiotics seem to have resulted in a real increase in the incidence of certain intercurrent fungal infections. The fungal infections in man can be divided into a) superficial mycosis involving skin, hair and nails. b) Systemic mycosis involving internal organs, which are of two kinds i) Caused by primary pathogens ii) Caused by opportunistic pathogens.

The current estimate of world wide antifungal market is 3 billion U.S. Dollars and represent 6% of the total antiinfective agents. Various antifungals like, Ketoconazole, Griseofulvin, Candidin etc. are commercially available. Great majority of synthetic antifungal agents like the imidazole derivatives are effective only in superficial mycosis preferably
Synthetic drugs like aromatic diamidines are effective by oral and parenteral routes in systemic blastomycosis; but resistance develops rapidly. Hence they become less effective after constant use. The natural polyenes are absorbed poorly through the G.I. tract. The toxic side effects also limit the clinical use of polyene antifungals.

Hence there is a regular thrust for an ideal, potent and effective antifungal antibiotic. In the present study, efforts were directed to explore the possibility of developing a novel ideal antifungal antibiotic.

In the research and development division of Hindustan Antibiotics Ltd., a soil screening programme was undertaken in which soils were collected from different parts of the country. Cultures were also obtained from different research Institutions. One of the culture strain obtained from Central Drug Research Institute (CDRI), Lucknow, had shown promising antimicrobial activity during initial screening. The culture belonged to *streptomyces* species and was named as *streptomyces CDRIL-312*. The main metabolite produced from *streptomyces CDRIL-312* was found to be a polyene macrolide antibiotic and was named as HA-1-92.

The present study deals with strain identification, fermentation optimization, isolation and purification, structure elucidation, pharmacological and pharmaceutical evaluation of HA-1-92. The other minor metabolites, pimprinine and thiolutin produced along with HA-1-92 during fermentation were also identified.
Fermentation studies were initially carried out in shake flasks. Media composition consisted of various carbon, nitrogen sources and trace metals. The associated environmental conditions viz. pH, dissolved oxygen, temperature, agitation, viscosity, aeration, utilization of various nutrients during fermentation were monitored and optimised to obtain maximum yield of HA-1-92.

A production media containing W/V of :- corn steep liquor (1.0 %), peanut meal (2.5 %), Cornmeal (2.5 %), Sodium Chloride (0.25 %), Ammonium sulphate (0.5 %), Dextrose (2.5 %) and Calcium Carbonate (0.75 %) was used for final fermentation. The fermentation cycle was optimised for 120 hrs at pH 6.5, temperature 28 C agitation 200 rpm, Airflow 20,000 ltrs per min and back pressure 0.7 Kg/Cm². The productivity titre of HA-1-92 obtained in pilot plant fermenter (chemap 41 L) was 1400 units/ml at 120 hrs under optimised conditions. The mycelial biomass was separated and HA-1-92 was extracted from biomass using butanol. The final purification was carried out on a silica gel column using a series of solvent-gradient. The final product HA-1-92 thus obtained was subsequently used for physicochemical studies.

The purity of polyene macrolide isolated was confirmed by high pressure liquid chromatography and thin layer chromatography. The pure component was subsequently characterised based on its physicochemical properties like physical appearance, solubility, colour reactions, melting point, optical rotation, elemental analysis, U.V. visible...
Based on the above studies HA-1-92 was assigned a tentative mol wt. 560 & tentative molecular formula C_{31}H_{44}O_9. A tentative structure was also elucidated. After chemical characterization, pharmacological screening of HA-1-92 was carried out. Acute toxicity studies were conducted by different routes in rats and mice and LD₅₀ was determined.

Since some polyene antibiotics have shown hypolipidaemic activity, experiments were carried out to study hypolipidaemic and hypoprostatic activity of HA-1-92 in old obese rats. The blood samples were analysed for total cholesterol, low density lipoproteins (LDL), Very low density lipoproteins (VLDL), high density lipoproteins (HDL) and triglycerides. The treatment of HA-1-92 for 15 days reduced triglyceride and total cholesterol levels. The weight of prostate gland was also reduced significantly, suggesting a promising hypolipidemic and hypoprostatic activity.

HA-1-92 was tested on normotensive anaesthetised cats, isolated muscle preparations viz. smooth muscles (Rat colon, guinea pig ileum) Skeletal muscle (frog rectus abdominus) and cardiac muscle (isolated perfused heart). HA-1-92 did not elicit any significant effect on these preparations even at high concentrations.

Antimicrobial activity of HA-1-92 was tested both in vitro and in vivo experiments. The in vitro antimicrobial activity was determined by minimum inhibitory concentration (MIC) by turbidimetric method and also by measuring the zone of inhibition by agar plate diffusion method. The organisms tested
E. coli, S. cerveseae, B. subtilis, S. aureus, and S. leutea. Similarly, various plant pathogens viz. F. oxysporium, M. phaeolina, A. macrosporum, C. gloesporides, C. lunata, Helminthosporum sp. and Sclerotium sp. were also tested.

The results of in vitro experiments indicated that HA-1-92 was highly effective against fungi, yeast and plant pathogens and was devoid of antibacterial activity.

A liposomal interchalated HA-1-92 was prepared by the method of Moonis et al. (1992) with necessary modifications. The liposomal incorporated HA-1-92 preparations were evaluated for toxicity, absorption and antifungal activity. The results indicated that liposomal preparation increased absorption and also reduced toxicity.

In vivo antimicrobial activity of HA-1-92 of both free and liposomal incorporated HA-1-92 was evaluated on mice model of systemic fungal infections by administering the drug via intravenous route. The infection was induced by administering clinical isolates viz. Cryptococcus neoformans, Candida albicans, Aspergillus fumigatus (Lopez-Berestein et al). The efficacy of HA-1-92 was determined by observing the percentage mortality of treated animals alongwith the number of Colony Forming Units (CFUs) of the pathogen in vital organs after sacrificing the animals. The results were compared with that of vehicle treated control animals. The in vivo experimental studies clearly suggest that liposomal incorporated HA-1-92 was more effective than free form of HA-1-92 on animal models of systemic mycosis. The order of potency of liposomal preparation of HA-1-92 were C. albicans > A. fumigatus > C. neoformans.
Serum concentration of free HA-1-92 as well as liposomal HA-1-92 was determined after intravenous administration in rats. Here too, liposomal preparation treated rats showed significantly higher serum concentration which may be due to increased absorption. The enhanced absorption and higher blood levels resulted in improved therapeutic efficacy of liposomal HA-1-92 against systemic mycosis.

In conclusion it is stated that HA-1-92 is a new microbial metabolite produced by streptomyces CDRIL-312. It is identified as polyene oxohexaene macrolide with molecular wt 560 and molecular formula C_{31}H_{44}O_{9} exhibiting significant antifungal, hypolipidemic and hypoprostatic activity in animal experiments. HA-1-92 is comparatively less toxic. Furthermore, liposomal incorporated HA-1-92 preparation showed significant antifungal activity on animal models of systemic mycosis. These studies warrant further investigations to find out the underlying mechanism(s) and the site of action. Long term toxicological and clinical studies need to be undertaken to develop HA-1-92 as a probable potential antifungal agent for systemic use.