Antimicrobial Activity of Metal Cystine Complexes

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ABSTRACT: Metal cystine complexes of nickel(II), mercury(II) and cadmium(II) have been synthesized and their antimicrobial activities have been evaluated. It is found that mercury(II) cystine and cadmium(II) cystine complexes have pronounced antibacterial and antifungal activities. On the other hand nickel(II) cystine shows moderate activities. The LC_{50} values of nickel(II) cystine, mercury(II) cystine and cadmium(II) cystine are found to be 5.2, 8.0 and 7.2 μg ml^{-1}, respectively. These values were obtained from cytotoxic studies indicating that they are biologically active compounds.

Key words: Antimicrobial activity, Cytotoxic activity; Metal amino acid complexes.

INTRODUCTION

Constituents from natural resources[1-4] as well as synthetic[5,6] organic and inorganic compounds have been receiving increasing attention in biological systems. Many of these compounds are being used as chemotherapeutic agents against infectious diseases. As a matter of fact no such agents till now can able to destroy effectively pathogenic micro organism. It is mainly because these pathogenic organisms are developing resistance heriditically towards these agents. It is therefore, necessary to find out consistently new, more safe, effective and inexpensive agents for the purpose. In this connection, a few metal coordination complexes have already been appeared in the literature[7,8] as antibacterial agents. Treshchalina *et al.*[9] studied antitumour properties of some amino acid complexes of copper(II). The above informations inspired us to study the anti-microbial activity of cystine complexes of some divalent metals namely nickel(II), cadmium(II) and mercury(II). In addition cytotoxic effects of these complexes have also been studied.

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MATERIALS AND METHODS

Synthesis and characterization of the complexes. All metal complexes were prepared[10] by the direct combination of saturated aqueous solutions of metal acetates with that of L(-) cystine in metal/legand ratio of 1:1. The mixture solution was heated to about 60°C on water bath to reduce half of its volume. It took about 6-8 hours. The purity of these compounds was confirmed by measuring density and also taking melting points and IR spectral data.

Brine shrimp bioassay method.[11] For this study, we have used artificial seawater, which was prepared by dissolving 38g of sodium chloride in 1L-distilled water. Shrimp livers were added to it. The

temperature was maintained at 37°C with a thermostatic system having a light source. The medium was provided with constant oxygen supply. Two days were allowed for the shrimp to hatch and mature as nauplii.

Stock solution. Metal cystine solution was prepared by dissolving 75 mg solid in 25mL water.

Methodology. Seven clean vials were taken for the experiment (6 for different concentrations and one for the control). Exactly 5 ml seawater was given to each of the vials. Stock solution was then added to the vials with the aid of a micro pipette so as to get final concentrations of 3μg ml^{-1}, 6 μg ml^{-1}, 9 μg ml^{-1}, 12 μg ml^{-1}, 15μg ml^{-1} and 18μg ml^{-1}. No sample solution was added to that of the control. With the help of a pasteur pipette 10 living shrimps were taken to each of these seven vials. After 24 hours, the survived nauplii in each vials were counted and the results were noted.

Screening method. The antimicrobial screening was performed by disc diffusion method as described elsewhere.[12,13]

Test organisms for antibacterial activity. The following bacteria were used for the study.

1. *Bacillus subtilis*
2. *Salmonella typhi*
3. *Shigella dysenteriae*
4. *Shigella flexneriae*
5. *Staphylococcus aureus*
6. *Streptococcus*-β - *haemolyticus*

Procedure. Solutions of known concentration of the test samples were made by dissolving in distilled water. Dried and sterilized filler paper discs (5 mm in diameter) soaked with known amount of test agents were placed on the nutrient agent media solidified in petridishes (120 mm diameter) and inoculated with the test organisms. These plates were than kept at low temperature (4°C) for 24 hours to allow maximum diffusion and then in an incubator at 37°C for 24 hours to allow maximum growth of the organisms. The antibacterial activity was determined by measuring the diameter of zone of inhibition in mm.

Antifungal screening. The antifungal screening was also performed by disc diffusion method.[12,13] The following fungi were selected for this study.

1. *Aspergillus niger*
2. *Aspergillus flavus*
3. *Aspergillus fuserium*
4. *Aspergillus fumigatus*
5. *Tricodarma harzianum*
6. *Candida albicans*
7. *Penicillium* sp.

Methodology. The test samples were dissolved in distilled water to make 24 μg/ml and 12 μg/ml solutions. A solution of 12 μg/ml of standard drug *Flugal* was also prepared.

Sample discs. Sterilized metrical filter paper discs of 3 mm diameter were taken in a blank petridish. Sample solution 25 μl/discs was applied on the discs with the help of a micropipette in an aseptic condition. The discs were left for a few minutes in the aseptic condition for complete removal of the solvent.

Preparation of inoculums. Isolated spore (4-6 similar) of pure fungus was inoculated in screw capped tube containing equal amount of potato dextrose agar (PDA) media and incubated at 28°C for 5-7 days for development of new pure culture that was used as inoculum.

Determination. PDA medium was steamed to dissolve and dispersed 4 ml amount of it in to a petridish. It was then autoclaved at 121°C for 15 minutes. It was allowed to cool to 30°C until the media became solid.

Each petridish was inoculated with different types of inoculums removed from a seven days old culture of fungi. Dried and sterile sample discs and standard (*Flugal*) disc were placed on nutrient agar plates seeded with the test organism. These were then kept at low temperature (4°C) for 24 hours to allow maximum diffusion. Finally the petridishes were inoculated at 27-28°C for 5-7 days. The activity was justified by measuring the diameter of zone of inhibition in mm.

RESULTS AND DISCUSSION

All these complexes showed positive results in brine shrimp lethality bioassay. For determination of cytotoxic effect, medium lethal concentration (LC_{50}) of brine shrimp lethality was measured from the plots of percentage of mortality versus concentration of the test samples (Figure 1). The data thus obtained have been presented in Table 1. The mortality rate of the brine shrimp is found to be increased with the increase of concentration of the samples. So there is a positive correlation between brine shrimp toxicity and cytotoxicity. The very low values of LC_{50} indicate the high cytotoxic effect of all these complexes.

The antibacterial activity of Hg(II) cystine, Cd(II) cystine and Ni(II) cystine measured interms of zone of inhibition has been shown in Table 2. The complexes showed a remarkable sensitivity against a number of both gram-positive and gram-negative bacteria. The results obtained with the standard drug *Kanamycin* have also been presented here for comparison purpose.

Table 1. Physical parameters of the complexes.

Test compounds	LC_{50}, μgml^{-1}	Melting point, °C	Density,g/ cc	Half life, hour
Ni(II) cystine	5.2	300	2.51	90
Hg(II) cystine	8.0	180	3.56	60
Cd(II) cystine	7.2	80	3.25	110

Table 2. Data of antibacterial activity of the complexes.

Test bacteria	Diameter of zone of inhibition, mm.						
	Hg(II) cystine, µg/disc		Cd(II) cystine, µg/disc		Ni(II) cystine, µg/disc		Kanamycin, µg/disc
	50	80	50	80	100	200	30
Staphytococus aureus	17	28	22	35	11	17	19
Bacillus subtilis	18	31	28	39	12	15	24
Shigella dysonteriae	23	34	25	34	10	19	23
Salmonella typhi	21	32	24	36	5	11	17
Shigella flexneriae	25	31	21	28	15	20	25
Streptococcus -β-haemolyticus	-	-	-	-	7	12	21

(-) Expt. was not performed..

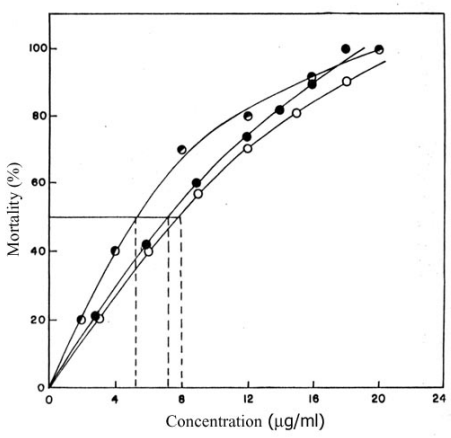

Figure 1. Effect of metal cystine complexes on brine shrimp lethality bioassay. ○–Hg(II) cystine; ●-Cd(II) cystine and ⊖-Ni(II) cystine.

The diameter of zone of inhibition of Hg(II) cystine complex at the dose of 50 µg/disc against *Staphylococcus aureus*, *Bacillus subtilis*, *Shigella dysenteriae*, *Salmonella typhi* and *Shigella flexneriae* were found to be 17, 18, 23, 21 and 25 mm, respectively. These values were quite comparable with those obtained with *Kanamycin* at dose 30 µg/disc (Table 2). However Hg(II) cystine complex at dose 80 µg/disc was found to be more sensitive as the zone of inhibition were 28, 31, 34, 32 and 31 mm, respectively for the bacteria stated before. Still better results were obtained with Cd(II) cystine complexes. However, Ni(II) cystine complex was found to be some what less sensitive. Moderate results were

obtained when tested with higher doses (200 μg/disc) of the complex.

The antifungal activity of these complexes take the antibacterial activity was measured interms of zone of inhibition against a number of fungi. The results have been presented in table 3. The results obtained with the standard antifungal agent *Flugal* have also been shown here (Table 3). Both Hg(II) cystine and Cd(II) cystine complexes showed pronounced antifungal activity and even much better than that of *Flugal*. The zone of inhibition in mm for Cd(II) cystine with dose 300 μg/disc were 33, 28, 22 and 8 for *Aspergillus niger. Aspergillus flavus, Aspergillus fuserium* and *Tricodarma harzianum,* respectively. The activity of Hg(II) cystine with the same dose were 20, 22, 21, 14 and 32 for *Asperigullus niger, Aspergillus flavus Aspergillus fumigatus, Tricodarma harzianum and Candida albicans* respectively. Moderate activity was found with Ni(II) cystine complex. It did not show any activity with the fungus *Tricodarma harzianum* at all.

From the above discussion it can be concluded that at least Hg(II) cystine and Cd(II) cystine possess fairly both antibacterial and antifungal activities. Again the positive response in bioassay study suggests that all these complexes might have antitumour activities also. However further studies in advanced level are required to explore these complexes as anticancer agents.

Table 3. Data for antifungal activity of the test compounds.

Test fungi	Diameter of zone of inhibition, mm.						
	Hg(II) cystine, μg/disc		Cd(II) cystine, μg/disc		Ni(II) cystine, μg/disc		Flugal, μg/disc
	300	600	300	600	300	600	300
Aspergillus niger	20	27	33	39	6	10	9
Aspergillus flavus	22	29	28	34	0	9	12
Aspergillus fuserium	-	-	22	27	11	24	16
Aspergillus fumigatus	21	30	-	-	-	-	0
Tricodarma harzianum	14	21	8	12	0	0	26
Candida albicans	32	37	-	-	0	11	8
Penicillium sp.	-	-	-	-	11	18	0

(-) Expt. was not performed; 0-No antifungal activity was found.

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