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Original Articles

The Antimicrobial Activity of Different Zinc Salts

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SUMMARY

Antimicrobial activity of zinc chloride, zinc citrate, zinc sulphate and zinc gluconate was assessed against Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi and Candida albicans using hole plate diffusion method. Zinc chloride and zinc citrate showed almost identical activity against the three strains of test bacteria. However, S. pyogenes was sensitive to three salts as compared with S. aureus and S. typhi. Zinc sulphate exhibited relative less antibacterial activity while zinc gluconate was found to possess the least activity in comparison to other three zinc salts. Zinc gluconate was devoid of antifungal activity whereas the other salts showed antimycotic activity but, it was significantly lower than their respective activities against tested bacterial strains.

INTRODUCTION

Although present in minute quantities, trace elements play a vital role for optimum growth and maintenance of human health at every age and every stage of life starting with foetus and finishing with end of old age[1]. Of nine trace elements, generally accepted essential for humans, zinc is most extensively investigated and found to be one of keys to good health. Being either part of the structure or essential for biological activities of more than 200 enzymes, zinc has imperative role in human metabolism[2,3] Zinc participates in the synthesis and storage of insulin in beta cells of Islets of Langerhans[4]. Conditions like acne vulgaris[5] common cold, sickle cell anaemia have been successfully treated with zinc therapy proving that deficiency of zinc may be one of the factors for precipitation of such conditions. Zinc, in lozenges dosage form, has been suggested to significantly reduce the duration of common cold by either inhibiting viral polypeptide cleavage and/or minimizing histamine release from mast cells and basophilic[6]. The experiments in mice and rats[7] and humans[8] showed the cellular immune response to be zinc dependent.

Antimicrobial activity of zinc is well documented[9]. It has been proposed that some warning system of the body puts zinc at work to fight against infection, this probably being the reason for lower than normal zinc plasma levels in acute/chronic infections[10]. Evidence in published literature exists that beneficial effects can be achieved by using combination of zinc and antimicrobials[11]. Even the growth of erythromycin resistant strain, has been reported to be inhibited by supplementation of zinc to erythromycin[12].

An increasing number of microbes becoming resistant to antimicrobials is adding to the pre-existing problems of combating with the infectious diseases. The present project was aimed to investigate the antimicrobial potential of different zinc salts on different pathogenic micro-organisms.

MATERIALS AND METHODS

The four zinc salts i.e. zinc sulphate, zinc chloride, zinc citrate and zinc gluconate were prepared in PCSIR laboratories, Lahore and were 99-100% pure. Muller Hilton Agar media and nutrient broth were purchased from local market. The four test microorganisms, Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi and Candida albicans were obtained from the microbiology laboratory of Sheikh Zayed Hospital

Lahore. The microbes were stored in slants at room temperature. Double distilled water, cotton wool and all glass apparatus was sterilized by autoclaving at 121°C for 30 Minutes. Serial dilutions 6.25(A), 12.5 (B), 18.75 (C), 25 (D), 37.5 (E), and 50.0 (F) μg/ul of zinc sulphate and zinc chloride were prepared in water. Whereas zinc citrate and zinc gluconate were dissolved in water with the help of sulphuric acid and serial dilutions of these salts were prepared with water. These solutions were sterilized by passing through 0.2μm millipore filter paper.

Preparation of Muller Hilton agar media

Accurately weighed 38gms of media were dissolved in 1000 ml of distilled water. The solution was heated gently to boiling point and it was autoclaved for 15 minutes at 121°C

Preparation of inocculum

A standard inocculum of each test organism was prepared by adding a loop-full of organisms from a 24 hours incubated culture to 10 ml of sterilized nutrient broth and concentrating it till its turbidity matched with test tube No. 10 of McFarland nephelometer turbidity. This gave the viable count of 30 x 10^9/ml[13].

Determination of antimicrobial activity

Hole Plate diffusion or well method was used to find out the antimicrobial activity of zinc salts[14]. The whole procedure was carried out under strict sterile conditions and the laminar lamp. Standard inocculum (2ml) of a test organism was pippetted into each of sterilized petri dishes. Then Muller Hilton Agar Media was added to each petri dish. The dishes were rotated clock and anti-clock wise to mix the inocculum and media thoroughly. The petri dishes were left aside for solidification. Using well technique, seven holes were made in each petri dish, each hole measuring 8mm in diameter. The holes were seeded with 20ul of different concentrations of test salts. The petri dishes were then incubated at 37°C for 24 hours and then zone of inhibition for each dilution was measured in mm[15]. The control (seeded with vehicle) and blank (unseeded) tests were run with each set of experiments to check the activity of vehicle(s) used and sterile condition of the lab respectively. The procedure was carried out for all the four strains of microorganisms.

RESULTS

Antifungal activity of four salts of zinc is represented in Table 1. It is evident from the table that zinc gluconate was completely devoid of activity. Zinc sulphate and zinc chloride exhibited activity at 25μg/ul concentration (D). However, the activity at the highest concentration employed was higher in case of zinc citrate. Zinc citrate showed relatively less activity at concentration E but at concentration F, the value was the highest amongst the three active salts. Bactericidal activity of zinc salts against S. aureus is shown in Table 2. Zinc chloride and zinc citrate showed almost similar antibacterial activity, with concentration A presenting significant activity and reaching highest at concentration F. Zinc sulphate and zinc gluconate were found inactive up to concentration C. However, the activity appeared at concentration D in case of zinc sulphate was comparable to that of zinc chloride and zinc citrate. Zinc gluconate, after exhibiting the activity at the same concentration as zinc sulphate, remained significantly less potent than sulphate salt at highest concentration used.

Table 1: Antimicrobial activity (measured as zone of inhibition in mm) of various zinc salts against Candida albicans.

Conc. of zinc salt	*Zone of Inhibition (mm)*			
	Zinc Chloride	*Zinc Citrate*	*Zinc Sulphate*	*Zinc Gluconate*
A	0	0	0	0
B	0	0	0	0
C	0	0	0	0
D	15.2±0.4	0	12.8±0.2	0
E	16.2±0.4	13.0±0.3	14.6±0.3	0
F	18.8±0.2	19.6±0.2	16.2±0.4	0

Each value represents Mean±SEM of five observations. Control and blank tests showed zero zone of inhibition

Although S. pyogenes was found sensitive to zinc chloride and zinc citrate at concentration B, yet the maximal activity of these two salts against S. pyogenes at highest concentration was significantly greater than S. aureus (Table 3).

Table 2: Antimicrobial activity of various zinc salts against Staphylococcus aureus. Activity was recorded as zone of inhibition in mm.

Conc. of zinc salt	Zone of Inhibition (mm)			
	Zinc Chloride	Zinc Citrate	Zinc Sulphate	Zinc Gluconate
A	11.4±0.7	10.0±0.7	0	0
B	13.2±0.3	12.6±0.4	0	0
C	14.4±0.4	13.8±0.3	0	0
D	16.0±0.9	15.4±0.2	15.4±0.2	9.2±0.2
E	19.4±1.1	19.4±0.4	18.4±0.7	12.4±0.2
F	22.4±0.2	20.6±0.7	19.8±0.7	15.0±0.4

Each value represents mean ± SEM of five observations. Control and blank tests showed zero zone of inhibition

Table 3 Antimicrobial activity of different zinc salts against Streptococcus pyogenese. Activity was recorded as zone of inhibition in mm.

Conc. of zinc salt	Zone of Inhibition (mm)			
	Zinc Chloride	Zinc Citrate	Zinc Sulphate	Zinc Gluconate
A	0	0	0	0
B	14.6±0.5	11.4±0.2	0	0
C	15.7±0.2	15.8±0.3	0	0
D	24.6±0.5	22.4±0.2	18.0±0.3	0
E	26.4±0.4	26.6±0.2	22.0±0.3	13.8±0.4
F	28.0±0.3	28.2±0.2	25.0±0.3	23.8±0.4

Each value represents mean ± SEM of five observations. Control and blank tests showed zero zone of inhibition.

S. pyogenes was less sensitive to zinc sulphate and least to zinc gluconate when compared to S.aureus. However, the activity exhibited by these two salts was greater than that against S.aureus.

Table 4 presents the activity of all four zinc salts against S. typhi. Zinc chloride and zinc citrate showed same trend of activity as in case S. aureus and S. pyogenes, zinc sulphate revealed activity at concentration B but maximum effect was nearly approaching to that of zinc chloride or zinc citrate. Zinc gluconate was found least potent among four salts as was found against other tested microorganisms.

Table 4: Antimicrobial activity of different zinc salts against Salmonella typhi. Activity was recorded as zone of inhibition in mm.

Conc. of zinc salt	Zone of Inhibition (mm)			
	Zinc Chloride	Zinc Citrate	Zinc Sulphate	Zinc Gluconate
A	11.4±0.5	9.8±0.5	0	0
B	13.2±0.2	12.4±0.2	12.8±0.3	0
C	14.2±0.5	14.2±0.4	13.8±0.3	0
D	18.6±1.1	16.0±0.3	15.8±0.2	3.6±2.2
E	21.0±0.6	19.8±0.5	17.6±0.6	4.6±1.0
F	23.6±0.2	23.4±1.6	21.6±0.5	16.2±0.3

Each value represents mean ± SEM of five observations. Control and blank tests showed zero zone of inhibition.

DISCUSSION

In the present study zinc chloride and zinc citrate showed nearly identical anti microbial activity against each of the four test microbes. In contrast to these, zinc sulphate exhibited relatively less antibacterial effect against all the four microbes where as zinc gluconate did show antibacterial effect but at higher concentrations (D. E. & F) and even at these concentrations the antimicrobial effect was significantly lower as compared to the rest of the zinc salts. The present data show that zinc gluconate was absolutely devoid of antifungal activity against Candida albicans at all concentrations employed in the experiment, Antifungal activity in the case of zinc citrate was observed at relatively higher concentration as compared to the other two salts i.e., zinc chloride & zinc sulphate. However, the anti mycotic activity achieved at highest concentration employed was almost the same in all the three active zinc salts.

The mechanism of antimicrobial activity of

metal complexes still seems to be obscure. Whether the metal ion itself, ligand attached or/and both is/are responsible for the activity. Our data show that different zinc salts had varying degree of antimicrobial effect against the test bacteria meaning by that, the zinc being the common moiety, the difference in degree of potency might be attributed to the ligand attached to the zinc cation. This idea is supported by Soloman[16] that the ligand attached is responsible for the transmembrane movement of zinc salts. This concept is further supported by the observation that the bioavailability of zinc citrate in humans is found maximum when compared to other ligands attached to zinc[16].

Das describes that one of the hypotheses for antibacterial actions of zinc is the ligand and zinc complexation with ligand simply facilitating its entry into the cells. Taking this hypothesis as such it can be assumed that various complexes will provide different titre of zinc into the cell because the cell membrane would offer different permeability to different complexes. It is very likely after having access to the interior of the bacterial cell it exerts its antibacterial effect by either preventing the protein synthesis and/or inhibition of nucleic acid synthesis by complexing with apoenzyme or displacing any of the native metal ion essential for the enzymatic action needed for bacterial cell replication leading to its deactivation[17], the other likelihood is that zinc might be activating the autolytic enzymes in the cell that leads to lesions which cause bacterial death because zinc is shown to be vital component of the enzyme structure or essential for the biological activity of more than 200 enzymes[18].

The antimicrobial activity of zinc salts observed in the present study is in full agreement with the earlier findings gathered in invitro studies that zinc chloride had antibacterial effect on the streptococcal flora of the dental plaque[19]. It was also found that zinc chloride solution (0.2% or 0.4%) could complete the mechanical dental and oral hygiene in an effective way.

Zinc sulphadiazine has been shown to be effective alternative to silver sulpadiazine in treating burn wound indicating that zinc possesses antibacterial activity comparable to silver ions[20]. Gradin and Schmitz (1983) while testing the susceptibility of 18 strains of bacteroids nodosus to different antimicrobial found that of the chemicals used in footbath for the treatment of ovine footrot copper sulphate was the most effective and it was followed by zinc sulphate[21]. Thus the gathering of antimicrobial effect of zinc salts in the present investigation is in line with the findings of the earlier researches.

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