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CASE REPORT

DISTINCT ANTIMICROBIAL ACTIVITY OF DIFFERENT POTENCIES OF HOMEOPATHIC MEDICINE ZINCUM OXYDATUM

BidishaGhosh[1], PapiyaNandy[2], Sukhen Das[1] and Sujata G. Dastidar[3]*

[1]Department of Physics, Jadavpur University, Kolkata 700032, India
[2]Centre for Interdisciplinary Research and Education, 404B, Jodhpur Park, Kolkata-700068, India
[3]Department of Microbiology, Herbicure Healthcare Bio-Herbal Research Foundation, SaralDighi (E), Boral, Kolkata 700154, India.

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ABSTRACT

From ancient times zinc and its different compounds have been used extensively in various fields of medicine and industry. Since nanoparticles of zinc oxide are used topically in skin ointments homeopathic medicine zincumoxydatum was selected to determine its antimicrobial action. Several homeopathic practitioners claim that this drug is active in treating various abdominal disorders. Dilutions of zincumoxidatum were tested for their *in vitro* antimicrobial properties against 14 bacteria and two fungi. The strains were of human origin and obtained from international centres. Agar dilution technique was followed to determine the potentiality of the drug.

The Gram positive bacteria were *Bacillus*spp, *Staphylococcus* spp. The Gram negative organisms included *E.coli,* species of several members of *Enterobacteriaceae* and the notoriously virulent *Pseudomonas aeruginosa.* All the test bacteria grew luxuriantly in the 6C dilution of zincumoxydatum. However, all these organisms except *Pseudomonas aeruginosa* BVC 2 were totally inhibited at higher dilutions of the drug, 30C and 200C. A greater inhibitory action of the drug was noted with fungi since both of them failed to grow even at the dilution of 6C.

Zincumoxydatum proves to be a truly potent antimicrobial agent and the concept of nanoparticles in homeopathic medicine is corroborated in this investigation as higher dilutions provided greater antimicrobial action of the compound.

INTRODUCTION

Zinc and its compounds are well known since ages in encompassing a variety of contributions in the fields of industry and medicine. Compounds of zinc find their use in notable applications, like in paint industry (zinc sulphide) (Coombs PG *et al*., 2004), deodrants (zinc chloride), in making antidandruff shampoos zincpyrithione have been of great importance (Faergemann J, 2000). Zinc is given as a dietary supplement as in zinc gluconate (Suxi HX *et al*.,1999; Sazawal S *et al*.,1998). The deficiency of this active mineral has been found to cause a number of diseases and health related issues like diarrhea and also retardation of prenatal and post natal growth and development (Benjamin CW *et al*., 2012).

Among all other compounds, the oxide of the metals a wide band gap semiconductor that has excellent uses of industrial and medicinal importance (Takahashi K *et al*., 2007). Zinc oxide has fungicidal properties that employ its use in paints and varnishes (Dusan Z *et al*.,2011;Baskar G *et al*.,2013). Zinc oxide also been used in skin conditioning treatments like in ointments, in soaps and in dental inlays (AydinSevinc B *et al*.,2010).

Such activities of zinc oxide have provided us with an insight to work with the homeopathic medicine, zincumoxydatum which is made from the same compound. Homeopathic medicines have widely been used as alternatives for their major role of having acceptable taste and no side effects or toxicity. The effectiveness of homeopathic medicine increases with increase in dilution followed by succussion and potentiation. In a recent study it has been shown that with the increase in potentiation the size of the constituent particles in the homeopathic preparation reaches nanoscale dimensions, which corroborates with the result obtained by Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS) techniques. The medicines show extreme activity at the dilution of $10^{400}$and even higher (Nandy P, 2015).The above properties of zinc oxide have also been tested to judge its efficacy in the different potencies of the homeopathic medicine zincumoxydatum. The medicine has been employed to generate thermo voltage in an electrochemical cell. The present study elaborates the antimicrobial and the antifungal

properties of the compound against 14 different strains of bacteria and in 2 fungi with the help of international standard guidelines.

MATERIALS AND METHODS

Bacteria

A total of 14 pathogenic bacteria belonging to 8 genera comprising Gram negative and Gram positive strains were taken in the study. These were of human origin, identified as described by Barrow and Feltham (Barrow GI and Feltham RKA,1993) and preserved in freeze dried state.

Chemical compounds

Homeopathic medicine zincumoxydatum in three dilutions,6C, 30C, 200C were purchased from Hahnemann Publishing Company (Hapco) All the three samples were diluted with distilled water to determine their antibacterial action.

Media

Liquid media used for the study of bacteria were nutrient broth (NB, Oxoid) and Mueller Hinton Broth (MHB, Oxoid), and the solid media were nutrient agar (NA, Oxoid) and Mueller Hinton agar (MHA, Oxoid). For the two fungi the solid medium was Sabouraud's Dextrose Agar (SDA, Oxoid).

Minimum Inhibitory Concentration (MIC) of zincumoxydatum

The Gram negative bacteria were grown in MHB and the Gram positive ones in NB for 18 hr to obtain optimum growth. Different potencies of zincumoxydatum was added to molten NA at 50ºC, thoroughly mixed, final pH adjusted to 7.2 to 7.4 and poured into sterile Petri dishes .The inoculums consisted of suitably diluted 18h broth culture of a bacterium. The MIC of each medicine was determined by spot inoculating one 2mm loopful of a culture containing ca.10^5 colony forming units (CFU), on the plates following the guidelines of Clinical and Laboratory Standard Institute 2012(CLSI) (CLSI,2012). The control used was one which was untreated cells and the vehicle control being succussed solvent obtained from the supplier. The plates were incubated at 37 °C. Growth was recorded after 18h as well as at 72h.

Antifungal activity of zincumoxydatum

Antifungal activity of the three different concentrations of zincumoxydatum was studied against two fungal strains *Candida albicans* ATCC 2091, and *Aspergillusbrasiliensis* ATCC 16404. SDAslants containing different potencies (6C, 30C, 200C) of the homeopathic medicine zincumoxydatum were prepared. These were inoculated with both the fungi, incubated at 25-26°C in a BOD incubator. Growth of *C.albicans* ATCC 2091was recorded first after 48 hr and again after 96 hr, while growth of *A.brasiliensis* ATCC 16404 was noticed after 5 days and incubation was continued for 2 weeks.

RESULTS

Determination of Minimum Inhibitory Concentration (MIC) of bacteria

In a total of 14 bacteria, 4 strains were Gram positive and the remaining 10 strains were Gram negative.
All the Gram positive bacteria were able to grow in dilution of 6C while they were unable to grow in the dilutions of 30C and 200C. Among 10 different Gram negative bacteria, 9 strains revealed no growth in the dilutions of 30C and 200C. However, *Pseudomonas aeruginosa* BVC 2 could not be inhibited in any of the dilutions of the compounds 6C, 30C and 200C. (Table 1)

Table 1 Antibacterial activity of zincumoxydatum

Bacteria	Control	Action of different potencies of zincumoxydatum		
		6C	30C	200C
*Bacillus subtilis*6633	+	+	-	-
*B.pumilus*8241	+	+	-	-
Staphylococcus aureus ATCC 6538	+	+	-	-
*S.aureus*NCTC 6571	+	+	-	-
Escherichia coli R122	+	+	-	-
*E.coli*K12 Row	+	+	-	-
*Salmonella.typhi*59	+	+	-	-
*S.uganda*101	+	+	-	-
*Shigellaflexneri*2a 33220	+	+	-	-
*Klebsiellapneumoniae*1	+	+	-	-
*K.pneumoniae*R114	+	+	-	-
*Vibrio cholerae*2080	+	+	-	-
*V.cholerae*752	+	+	-	-
*P.aeruginosa*BVC 2	+	+	+	+

Control, plain NA ;"+" Growth; "-" No growth

MIC of different dilutions of zincumoxydatum in fungi

Two different fungal strains were tested, *Aspergillusbrasiliensis* ATCC 16404 and *Candida albicans* ATCC 2091. None of these fungi were able to grow in any of the dilutions of zincumoxydatum. (Table 2).

Table 1 Antibacterial activity of zincumoxydatum

Action of different potencies of zincumoxydatum				
Fungi	Control	6C	30C	200C
Aspergillusbrasiliensis ATCC 16404	+	-	-	-
Candida albicans ATCC 2091	+	-	-	-

Control , SDA; '+', Growth ; '-' No growth

DISCUSSION

In pharmaceutical industries various types of compounds of the metal zinc are synthesized since most of them have definite applications in various pathological conditions.

Zinc has gained predominance since ancient times as an ingredient in skin ointments as was revealed in the Egyptian papyrus from 2000B.C and during the time of the Romans (Frederickson CJ*et al*., 2005).Though it may appear that zinc is a late addition to the myriads of effective ions in the field of biology and medicine, its ubiquitous biological and medicinal contributions indicate that it might well be considered as the "calcium of the twenty first century" (Frederickson CJ *et al*.,2005).

Interests on nanoparticles have generated in recent years in view of their simple structures, chemical physical and biological properties that are often distinctly different from their respective bulk materials. In view of the information gained from the antimicrobial action of zinc oxide with the help of a simple procedure, Ganguly *et al* have repeatedly shown the potentiality of ZnS nanoparticles as not only effective antimicrobials but also possess anticancer properties (Ganguly S *et al*.,2013).

While determining the antibacterial effects of ZnO nanoparticles Sidra Sabir *et al* found that the nanoparticles

have direct interaction with cell surface permeability, which subsequently induces oxidative stress in bacterial cells, thereby inhibiting cell growth and eventually cell death (Sabir S *et al*.,2014). These authors were able to provide evidences that their nanoparticles were active on *S.aureus* and *E.coli.* YanpingXie *et al* showed that nanoparticles of ZnO were sensitive against the intestinal bacterium, *Campylobacter jejuni*, which could be inhibited at the minimal concentration of 0.05 to 0.025mg/ml, that was 8 to 16 fold lower than that of *Salmonella enterica*serovar Enteriditis and the food borne pathogen *E.coli O157:H7* (0.4mg/ml) (Xie Y *et al*., 2011) and reduces skin infection when used topically in mice (Pati R *et al*.,2014). Moreover, the concept that at higher dilutions the particles are said to attain nanostructures are supported by the facts put forth by Roy R *et al* that during succession process the pounding of solutions against a rubber stop creates nanobubbles (Roy R *et al*.,2005). High dilutions of the homeopathic medicine may raise doubts regarding the physical presence of the medicine, it has been seen the nanoparticle nanobubble complex rises to the surface and can be within a monolayer once the metal under question has reached concentration below 1ppm. (Chikramane PS *et al*.,2010).

On the basis of such information we selected the homeopathic medicine zincumoxydatum to determine its antimicrobial properties. Our studies have shown that 13 out of 14 different bacteria were unable to grow at higher dilutions of zincumoxydatum. It may be pointed out here that the highly multiply drug resistant *P.aeruginosa*BVC2 failed to exhibit inhibition in any concentration of the homeopathic medicinal compound. Further the distinctly different fungi were completely inhibited by any of the dilutions of this particular medicine. Therefore this study is likely to generate interests and open new avenues among homeopathic practitioners to apply zincumoxydatum in varieties of human diseases including those of pulmonary, gastrointestinal and nasopharyngeal infections.

CONCLUSION

This study has shown that 13 out of 14 different bacteria were unable to grow at higher dilutions of zincumoxydatum. It may be pointed out here that highly multiply drug resistant *P.aeruginosa* BVC 2 could not be inhibited by any of the concentrations of this homeopathic medicine. Moreover, the two pathogenic fungal strains were completely refractory to the same medicine. Therefore this study is likely to generate interests and open new avenues among homeopathic practitioners to apply zincumoxydatum to alleviate various human pathogenic ailments.

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References

1. Coombs PG, Zieba J, Bradley RA *et al*. Luminescent pigments and foils with color-shifting properties, 2004.US 6572784 B1.
2. Faergemann J. Management of Seborrheic Dermatitis and PityriasisVersicolor. *Am J Clin Dermatol*. 2000; 1:75-80.
3. Suxi HX, Margaret M, Artur CC, Roy KN. Effect of dietary zinc supplementation on *Escherichia coli* septicemia in weaned pigs. *Swine Health Prod*.1999; 7:109–111.
4. Sazawal S, Black RE, Jalla S, Mazumdar S *et al*. Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children: a double-blind, controlled trial. *Pediatrics*.1998;102: 1-5.
5. Benjamin CW, Janet C, Kinga B. Effect of Zinc Supplementation on Pregnancy and Infant Outcomes: A Systematic Review. *Paediatr Perinat Epidemiol*.2012; 26: 118–137.
6. Takahashi K, Yoshikawa A, Sandhu A.Wide bandgap semiconductors: fundamental properties and modern photonic and electronic devices*: Springer*, 2007
7. Dušan Z, Vladimir SV, MajaKA, Milan MN . Antimicrobial properties of ZnO nanoparticles incorporated in polyurethane varnish. *Processing and Application of Ceramics,* 2011; 5: 41–45.
8. Baskar G, Chandhuru J, SherazFahad K, Praveen AS . Mycological Synthesis, Characterization and Antifungal Activity of Zinc Oxide Nanoparticles. *Asian J. Pharm. Tech*.2013; 3: 142-146.
9. AydinSevinç B, Hanley L. Antibacterial activity of dental composites containing zinc oxide nanoparticles. *J Biomed Mater Res B ApplBiomater*.2010; 94 (1): 22-31.
10. Nandy P. A Review of Basic research on Homeopathy from a physicist's point of view. *Indian J Res Homeopathy*.2015; 9:141-151.
11. Barrow GI and Feltham RKA. Cowan and Steels Manual for the identification of medical bacteria, Cambridge University Press, Cambridge UK,1993:329.
12. Clinical and Laboratory Standard Institute (CLSI).Methods for dilution Antimicrobial Susceptibility, testing for bacteria that grow aerobically, Approved standard- Ninth edition MO7 A9, Clinical and Laboratory standards Institute, *Wayne Pa.* 2012.Vol 32 No.2
13. Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. *Nat Rev Neurosci*.2005; 6:449-462.
14. Ganguly S, Sultana P, Das S, DastidarSG. Characterization of newly synthesized Zinc sulphide nanoparticles by a simple aqueous chemical method and determination of its antimicrobial activity against various pathogenic bacterial strains. *Int J Biol Pharm Res*.2013; 4:377-381.
15. Sabir S, Arshad M, Chaudhuri SB. Zinc oxide nanoparticles for Revolutioning Agriculture: Synthesis and application. *The Scientific World J*.2014; 1-9
16. Xie Y, He Y, Irwin PL, Jin T, Shi X.Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*: *Appl Environ Microbiol*.2011; 77(7):2325-2331.
17. Pati R, Mehta RK, Mohanty S *et al*. Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits antibacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages. *Nanomed Nanotech Biol Med*.2014; 10:1195-1208.
18. Roy R, Tiller WA, Bell I, Hoover MR. The structure of liquid water: novel insights from material research; potential relevance to homeopathy. *Mater Res.Innov*.2005; 9:577-608.
19. Chikramane PS, Suresh AK, Bellare JR, Kane SG: Extreme homeopathic dilutions retain starting materials: A nanoparticulate perspective. Homeopathy.2010; 99(4):231-242.