

SHORT COMMUNICATION

Evaluation of antimicrobial activities of Harmine, Harmaline, Nicotine and their complexes

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Abstract: Harmine, Harmaline, Nicotine and its various complexes synthesized have been characterized by physical, spectral and analytical methods and curtailed for in-vitro antimicrobial activity against different bacterial and fungal species at two different concentrations i.e. 100 µg/100 µl and 200 µg/100 µl dose level respectively. Analysis showed that Nicotine, Zinc-Nico, Cd-Nico, Hg-Nico, Ni-Nico, Cu-Nico, Co-Nico, Harmine, and Harmaline having conc. of 100 µg/100 µl had antibacterial activity on zero, 5, 4, 10, zero, 5, 7, zero, zero strain of bacteria having an average of zero (SD=0.0000), 15.2000 (SD=1.30384), 18.2500 (SD=3.30404), 20.2000 (SD=1.39841), zero (SD=0.0000), 14.6000 (SD=0.89443), 15.8571 (SD=1.34519), zero (SD=0.0000), zero (SD=0.0000) respectively. Zinc (II) chloride, Cadmium (II) Iodide, Mercury (II) chloride, Nickel (II) chloride, Copper (II) chloride, Cobalt (II) chloride, Mercury (II) chloride, Mercury (II) harmine, Mercury (II) harmaline at 100 µg/100 µl is valid for 7, 8, 9, 2, 7, 8, 9, 10, 8 strains of bacteria with an average of 7.1429 (SD=1.06904), 10.0000 (SD=5.01427), 14.8889 (SD=6.00925), 6.0000 (SD=0.0000), 8.5714 (SD=4.27618), 8.2500 (SD=0.88641), 14.8889 (SD=6.00925), 18.6000 (SD=2.45855), 18.5000 (SD=1.85164) respectively. The above given compounds at the conc. of 200 µg/100 µl is valid for 10, 9, 10, 8, 8, 10, 10, 10, 10 strains of bacteria with an average of 8.1 (SD=1.66333), 11.7778 (SD=5.28625), 16.1000 (SD=6.36745), 6.5000 (SD=0.92582), 9.7500 (SD=4.43203), 9.9000 (SD=2.76687), 16.1000 (SD=6.36745), 22.0000 (SD=2.44949), 20.4000 (SD=2.75681) respectively. The above given compounds at conc. of 200 µg/100 µl showed antibacterial action on 3, 8, 8, 10, 3, 9, 8, zero, 3 strains of bacteria with an average of 14 (SD=0.000), 16.8750 (SD=1.35620), 18.2500 (SD=3.45378), 22.7000 (SD=1.82878), 14.3333 (SD=0.57735), 16.7778 (SD=1.71594), zero (SD=0.000), 12.0000 (SD=1.00000) respectively. Hence according to the average value of the zone of inhibition, maximum antibacterial activity at 100-200 µg/100 µl is of Hg-Nico and Mercury salt; Mercury (II) harmine having an average of 20.2000 (SD=1.39841)-22.7000 (SD=1.82878) and 18.6000 (SD=2.45855)-22.0000 (SD=2.44949). Minimum antibacterial activity at 100-200 µg/100 µl is Nicotine 100, Nicotine-Nico 100, Harmine 100, Harmaline 100, Harmine 200 having zero average (SD=0.000).

Keywords: Nicotine, Harmaline, Harmine, zone of inhibition, anti-microbial activity, complexes.

INTRODUCTION

Harmine and its metal complexes were isolated from seeds of *Peganum harmala*. Inhalation of its smoke relieves pain in the liver. It is used for the treatment of jaundice, asthma and colic as well. (Niroumand *et al.*, 2015) Usually powder of the seeds and watery infusion are given for treatment of these disease. The active alkaloids of Harmal seeds are the MAOI-A (monoamine oxidase inhibitor A) compounds. *Pegnum harmala* extract have antifungal and antimicrobial effects (Sassoui *et al.*, 2015). Psychoactive effects of *Esfand* seeds are rooted in inhibitory effect of monoamine oxidase (MAO) of betacarboline alkaloids effects the cardiovascular system (Zhang *et al.*, 2014). Harmine alone was ineffective

towards all the selected species of fungi except *Fusarium moniliforme* but its metal complexes showed good antimicrobial properties (Chen *et al.*, 2015). One study showed that harmaline inhibited the dioxin-mediated induction of CYP1A1 at the transcriptional and posttranslational levels (Gendy *et al.*, 2012) and showed effective antimicrobial activity against suspensions and biofilm cultures of *S. aureus*. It also shows anti-HIV and anti-parasitic properties (Khan *et al.*, 2013).

Nicotine, 3-(1-methyl-2-pyrrolidinyl) pyridine is present in the leaves of *Nicotiana tabacum*. Nicotine is an important bioligand and has good chelating sites for coordination with numerous metals (Rajesh Arora *et al.*, 2010, Zaidi *et al.*, 2012). The novel copper complexes with nicotinic acid and carboxylic acids were shown to exhibit super oxide scavenging activity as well as

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antimicrobial activities against *B. subtilis* (Malczewska-Jaskóla *et al.*, 2015).

Nicotinic acid derived Schiff bases and their transition metal complexes showed antibacterial activity against pathogenic strains of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Patel *et al.*, 2012, Xing *et al.*, 2012). Siverbidentateligands based on oligomers of polyethylene glycol, functionalized at both ends with either nicotinic or iso-nicotinic acid showed good antimicrobial properties (Suksrichavalit *et al.*, 2008).

In our study: Nicotine, Zinc-Nico, Cd-Nico, Hg-Nico, Ni-Nico, Cu-Nico, Co-Nico, Harmine and Harmaline, Zinc (II) chloride, Cadmium (II) Iodide, Mercury (II) chloride, Nickel (II) chloride, Copper (II) chloride, Cobalt (II) chloride, Mercury (II) chloride, Mercury (II) harmine and Mercury (II) harmaline were prepared and analyzed at different concentrations for antimicrobial properties.

MATERIALS AND METHODS

The study was conducted at HEJ Karachi, Pakistan. The antimicrobial sensitivity tests of the compounds were tested against ten different species of gram positive and gram negative bacteria including *Escherichia coli*, *Streptococcus pyogenes*, *Klebsiellapneumoniae*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Shigelladysenteriae*, *Salmonella typhi*, *Staphylococcus aureus* and *Corynebacterium diphtheriae* bacterial strains and for *in vitro* antifungal activity against *Candida albicans*, *Epidermophyton floccosum*, *Microsporumcanis*, *Fusariummoniliformi*, *Alternariainfectoria*, *Fusariumsolani*, *Aspergillusnigar*, *Pseudallescherisboydii*, *Candida solani*, *Penicillium*, *Penicilliumnotatum*, *Saccharomyces ceravaceae*, *Candia tropicalis*, *Candia lusitaniae*. The agar medium for testing of both antibacterial and anti-fungal activities is given (table 1). The compounds were used in two concentrations i.e. 100µg/100µl (first dose level) and 200µg/100µl, the second dose level.

RESULTS

Analysis of the zone of inhibition of Harmine, Harmaline, Nicotine and their complexes in millimeters At 100ug/100ul

Analysis showed that Nicotine, Zinc-Nico, Cd-Nico, Hg-Nico, Ni-Nico, Cu-Nico, Co-Nico, Harmine, and Harmaline having conc. of 100ug/100ul had antibacterial activity on zero, 5, 4, 10, zero, 5, 7, zero, zero strain of bacteria having an average of zero (SD=0.0000), 15.2000 (SD=1.30384), 18.2500 (SD=3.30404), 20.2000 (SD=1.39841), zero (SD=0.0000), 14.6000 (SD=0.89443), 15.8571 (SD=1.34519), zero (SD=0.0000), zero (SD=0.0000) respectively.

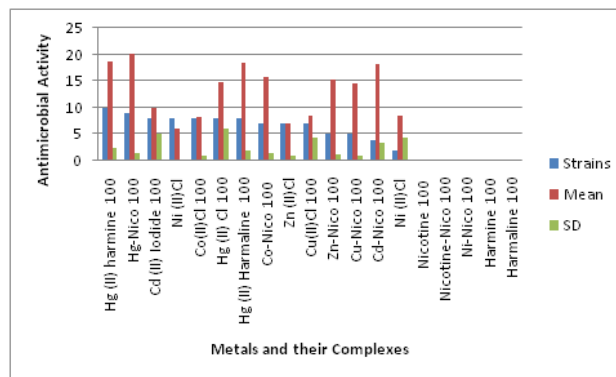


Fig 3: Activity of Harmine, Harmaline, Nicotine and its various complexes at 100 µg/100µl.

Zinc (II) chloride, Cadmium (II) Iodide, Mercury (II) chloride, Nickel (II) chloride, Copper (II) chloride, Cobalt (II) chloride, Mercury (II) chloride, Mercury (II) harmine, Mercury (II) harmaline at 100ug/100ul is valid for 7, 8, 9, 2, 7, 8, 9, 10, 8 strains of bacteria with an average of 7.1429 (SD=1.06904), 10.0000 (SD=5.01427), 14.8889 (SD=6.00925), 6.0000 (SD=0.0000), 8.5714 (SD=4.27618), 8.2500 (SD=0.88641), 14.8889 (SD=6.00925), 18.6000 (SD=2.45855), 18.5000 (SD=1.85164) respectively.

At 200ug/100ul

5. The above given compounds at the conc. of 200 ug/100ul was valid for 10, 9, 10, 8, 8, 10, 10, 10, 10 strains of bacteria with an average of 8.1 (SD=1.66333), 11.7778 (SD=5.28625), 16.1000 (SD=6.36745), 6.5000 (SD=0.92582), 9.7500 (SD=4.43203), 9.9000 (SD=2.76687), 16.1000 (SD=6.36745), 22.0000 (SD=2.44949), 20.4000 (SD=2.75681) respectively. The above given compounds at conc. of 200 ug/100ul showed antibacterial action on 3, 8, 8, 10, 3, 9, 8, zero, 3 strains of bacteria with an average of 14 (SD=0.000), 16.8750 (SD=1.35620), 18.2500 (SD=3.45378), 22.7000 (SD=1.82878), 14.3333 (SD=0.57735), 16.7778 (SD=1.71594), zero (SD=0.000), 12.0000 (SD=1.00000) respectively.

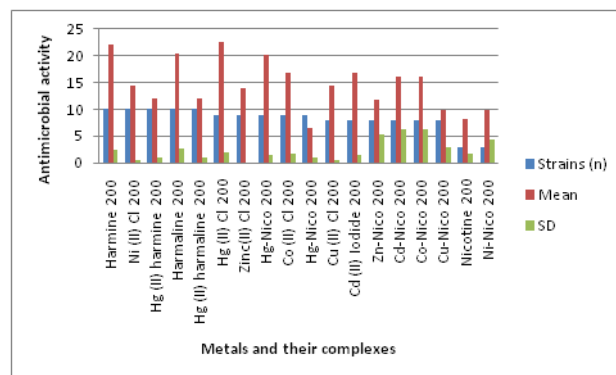


Fig 4: Activity of Harmine, Harmaline, Nicotine and its various complexes at 200µg/200µl.

Table 1: Ingredients of the medium and preparation of the stock solutions.

Preparation of Mueller-Hinton Medium (DIFCO laboratories, 1984)		Sabouraud Dextrose Agar (Oxoid Manual, 1976, Sabouraud, 1910)	
Constituents	Strength, g/l	Ingredients	Strength, g/l
Beef Infusion	300.0	Mycological peptone ¹	10g
Casamino acids	17.5 g/l	Dextrose	40
Starch	1.5	Agar No.1 ²	15
Bacto Agar	17.0		
pH at 25°C	7.3± 0.1	pH at 25°C	5.6
Stock solution	1mg/ml DMSO*	Stock solution	5mg/ml DMSO**

¹(oxoid L40) *diluted to 100µg and 200µg/ml dimethylsulphoxide (DMSO) ²(oxoid LII)**diluted to 300 µg/ml

Table 2: Anti-microbial activity of Harmine, Harmaline, Nicotine and their complexes.

Anti-micro bial activity at 100 µg/100ul				Anti-microbial activity at 200µg/100ul			
Complexes	Strains	Mean	SD	Complexes	Strains	Mean	SD
Mercury (II) harmine	10	18.6000	2.45855	Mercury (II) harmaline	10	12.0000	1.0000
Hg-Nico	7	6.5000	0.92582	Hg-Nico	9	20.2000	1.39841
Cadmium (II) Iodide	8	10.0000	5.01427	Cadmium (II) Iodide	8	16.8750	1.35620
Cobalt (II) Chloride	8	8.2500	0.88641	Cobalt (II) Chloride	9	16.7778	1.71594
Mercury (II) Chloride	8	14.8889	6.00925	Mercury (II) Chloride	9	22.7000	1.82878
Mercury (II) Harmaline	8	18.5000	1.85164	Mercury (II) harmine	10	12.0000	1.0000
Co-Nico	7	15.8571	1.34519	Co-Nico	8	16.1000	6.36745
Zinc (II) Chloride	7	7.1429	1.06904	Zinc (II) Chloride	9	14	0.000
Copper (II) Chloride	7	8.5714	4.27618	Copper (II) Chloride	8	14.3333	0.57735
Zn-Nico	5	15.2000	1.30384	Zn-Nico	8	11.7778	5.28625
Cu-Nico	5	14.6000	0.89443	Cu-Nico	8	9.9000	2.76687
Cd-Nico	4	18.2500	3.30404	Cd-Nico	8	16.1000	6.36745
Ni-Nico	0	0	0.000	Ni-Nico	3	9.7500	4.43203
Nickel (II) Chloride	2	8.5714	4.27618	Ni (II) Chloride	10	14.3333	0.57735
Nicotine	0	0	0.000	Nicotine	3	8.1	1.66333
Harmine	0	0	0.000	Harmine	10	22.0000	2.44949
Harmaline	0	0	0.000	Harmaline	10	20.4000	2.75681

DISCUSSION

The compounds that showed minimum or low activities were: (1) Compounds presenting zero activity: Nicotine 100, Nicotine-Nico 100, Harmine 100, Harmine 200, Harmaline 100. (2) Compound displaying activity on 1 strain of bacteria: Nil. (3) Compounds demonstrating activity on 2 strains of bacteria was just Nickel (II) Chloride. (4) Compound exhibiting activity on 3 strains of bacteria were: Nicotine 200, Ni-Nico 200 and Harmaline 200.

Compounds that displayed moderate antibacterial activity were: (1) Compounds exhibiting activity on 4 strains of bacteria: Cd-Nico 100. (2) Compounds showing activity on 5 strains of bacteria: Zn-Nico 100, Cu-Nico 100. (3) Compounds displaying activity on 6 strains of bacteria: Nil. (4) Compounds showing activity on 7 strains of bacteria: Zinc (II) Chloride 100, Copper (II) Chloride, 100 and Co-Nico 100.

Compounds that showed high antibacterial activity were:

(1) Compounds showing activity on 8 strains of bacteria: Zn-Nico 200, Cadmium (II) Iodide 100, Cd-Nico 200, Nickel (II) Chloride, Cu (II) Cl 200, Co (II) Cl 100, Co-Nico 200, Mercury (II) Harmaline 100, Cadmium (II) Iodide 200, Mercury (II) Chloride 100, Cu-Nico 200. (2) Compounds showing activity on 9 strains of bacteria: Zinc (II) Chloride 200, Mercury (II) Chloride 200, Hg-Nico 100, Hg-Nico 200, Cobalt (II) Chloride 200. (3) Compounds showing activity on 10 strains of bacteria: Mercury (II) harmine 100, Mercury (II) harmine 200 and Mercury (II) harmaline.

Further research is underway on the antimicrobial mechanism of this nicotine, harmaline and harmine metal complexes that either this is cell wall inhibitors or bactericidal or bacteriostatic. However, some researchers studied considerable changes in the bacterial cell membranes upon metal ion treatment, which might be one of the cause or consequence of cell death (Zaidi *et al.*, 2012).

CONCLUSION

According to the average value of the zone of inhibition, maximum antimicrobial activity at 100-200 μ g/100 μ l is of Hg-Nico and Mercury (II) harmine. Minimum antibacterial activity at 100-200 μ g/100 μ l is Harmine 100, Harmaline 100 and Harmine 200. As compared to nicotine alone; the nicotine (II) complexes are able to inhibit majority of the studied gram positive and gram-negative organisms at the higher dose level. Therefore, these complexes are broad-spectrum anti-microbial agents, active against the variety of gram positive and gram negative bacteria, with minimal antifungal activity. According to the average value of the zone of inhibition, maximum antimicrobial activity at 100 μ g/100 μ l was showed by Hg-Nico and Cd-Nico. At 200 μ g/100 μ l: Cd-Nico and Co-Nico showed maximum activity. Minimum anti-microbial activity at 100-200 μ g/100 μ l was showed by Nicotine100 and Nicotine-Nico100.

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