CYTOTOXICITY AND THE INHIBITORY EFFECT OF β-NAPHTHY MEREURIE CHLORIDE AGAINST SOME CLINICAL ISOLATES OF BACTERIA AND FUNGI(*IN VITRO*)

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Key words:- β-Naphthyl Mercuric chloride, bacterial isolates, fungal isolates.

ABSTRACT

The inhibitory effect of β-Naphthyl Mercuric chloride against some clinical isolates of bacteria and fungi was examined , It was found that when 0.1 gm of β-Naphthyl Mercuric chloride was dissolved in 10ml distilled water and then added to the Muller-Hinton agar and Sabouraud Dextrose agar was inhibited the growth of six clinical isolates of bacteria[*E.coli* from stool isolate, *P.aeruginosa* from urine isolate , *S.aureus* from blood isolate , *S. epidermidis* from urine isolate, *Klebsiella sp* from urine isolate. And other clinical isolate of *S.aureus* from urine isolate].The same solution was used to inhibit the growth of three fungal isolates [*Aspergillus flavus ,Candida albicans* and *Cryptococcus sp.]* the minimal inhibitory concentration (MIC) against these bacterial isolates were evaluated, and the Cytotoxicity of β-Naphthyl Mercuric chloride against human red blood cells was also evaluated and it was found that this compound cannot form hemolytic in RBCs in a concentrations(0.25-0.1 mg/ml).

INTRODUCTION

As a result of a highly spread of microorganisms that cause a different infectious disease for human , animals and plants, the antimicrobial agents were used these agents may be defined as a substance (drugs or chemicals) that kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). among the antimicrobial agents are antibacterial drugs ,antiviral agent ,antifungal agent and antiparasitic drugs[(1).]

Many of the Mercuric compounds consider as one of these antimicrobial agents ,some of these compounds used as skin disinfection like Mercuric oxide which is used as skin ointment[(2)].Disinfection is antimicrobial substance used on non-living objects[(1)]. And Mercuric ammonium chloride that used as ointment for treatment of chronic Eczema (parasitic skin disease) and many other compounds [(4)].

Generally the Mercuric compounds especially the metallic mercury are very toxic in their nature and have a very harmful effect in direct exposure to it on the nervous system ,kidney , lung, chromosomes and DNA[(3)].the problem is Mercury simply love Sulfur too much ,that it will compete with other molecules for Sulfur and can usually steal Sulfur out of other molecular structures, in effect killing them if it cannot steal Sulfur Mercury will band to the Sulfur atom the best it can ,this usually prevent the molecule from performing its function[(4)].

Despite that we can find that this element inter in manufacturing of some of our tools like Barometer, Thermometer, Hydrometer and in some electrical tools like lamps and fluorescent lights and in medicine as purgative because the most important mercury salt is Hg_2cl_2 Calomel , and used in dental amalgams for filling teeth[3].

MATERIALS AND METHODS

β-Naphthyl Mercuric chloride was prepared according to the literature[5],stock solution of this compound was prepared by dissolving 0.1gm in 10ml of distilled water, different media were used [Nutrient agar(Difco), Nutrient broth(Difco),Muller-Hinton agar (CDH) and Sabouraud Dextrose agar(HIMEDIA)]. Six clinical isolates of bacteria and three fungal isolates were obtained from the Pharmacy college in Basra university.

Antimicrobial activity.

Six clinical isolates of bacteria[*E.coli* from stool isolate, *P.aeruginosa* from urine isolate , *S.aureus* from blood isolate , *S. epidermidis* from urine isolate, *Klebsiella sp* from urine isolate. And other clinical isolate of *S.aureus* from urine isolate]were used with microbial concentration 10^6 CFU/ml and the antibacterial activity of the β- Naphthyl Mercuric chloride were test by using disc diffusion method [6]and the inhibition zone were measured in millimeter (mm).six Petri dishes were used as an experimental unit and the trial was repeated twice .Petri dishes incubate in 37 C° for 24 hours. . Similarly, the antifungal activity of the extracted oil was tested against three fungal isolates [*Aspergillus flavus , Candida albicans , Cryptococcus sp.*] and by using Sabouraud Dextrose agar medium ,three Petri dishes were used as an experimental unit and the trial is repeated twice. The fungal cultures were incubate at 27 C° for three days[7].

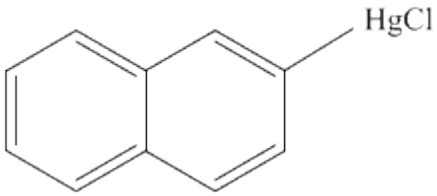
Minimal Inhibitory Concentration

Broth dilution method[8] was used to detect the (MIC) using different concentration [0.5 ,1 ,5 ,10 ,20 ,25 ,50 ,75 ,100 mg/ 10ml] against six clinical isolates of bacteria [*E.coli*, *S.aureus*, *P.aeruginosa, S.epidermidis ,Klebsiella sp.* and other clinical isolate of *S.aureus*].

Cytotoxicity assay

The cytotoxicity of β-Naphthyl Mercuric chloride was tested against human red blood cell RBCs using 2ml of blood mixed with 18ml of Ringer solution , from this mixture 2ml was put in sterilized test tube to a different concentration of β-Naphthyl Mercuric chloride were prepared in nine test tubes [0.1 ,0.25 ,0.5 ,1 ,2.5 ,5 ,10 ,25 ,50 mg/ml] respectively after incubated at 37 C °for 8 hours each one hour the test tubes were examined if there is any hemolytic for RBCs and the results were recorded[9]

RESULTS AND DISCUSSION



β-Naphthyl Mercuric chloride

The β-Naphthyl Mercuric chloride as shown above was prepared and identified according to the literature[5] and it characterized by elemental analysis which gave good result to confirm the above structure.

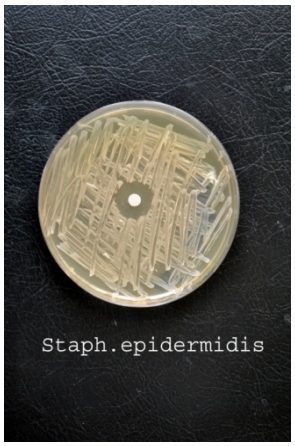
Elemental analysis :- calculated C : 33.07 , H : 1.94

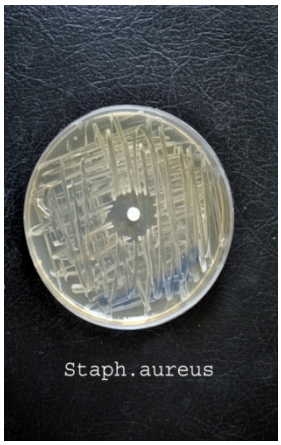
Found C : 33.19 , H : 2.01

Generally the data given in table-1-confirm very high inhibitory effect of β-Naphthyl Mercuric chloride against clinical isolates positive and negative to gram stain.

Table -1- the inhibitory effect of β-Naphthyl against the clinical isolates of bacteria.

Clinical isolates	Inhibition zone(mm)
E.coli	39
S.aureus	35
P.aeruginosa	45
S.epidermidis	20
Klebsiella sp.	37
S.aureus	22





Also the data given in table-2-confirm very high inhibitory of β-Naphthyl Mercuric chloride against Fungi isolates.

Table -2- the inhibitory effect of β-Naphthyl mercuric chloride against the fungal isolates.

Fungal isolates	Inhibition zone(mm)
Aspergillus flavus	25
Candida albicans	30
Cryptococcus sp.	30

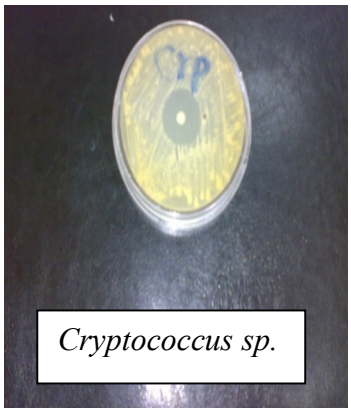


Table -3- the minimal inhibitory concentration of β-Naphthyl mercuric chloride against clinical isolates of bacteria

Clinical isolates	MIC(mg/ml)
E.coli	25
S.aureus	5
P.aeruginosa	0.5
S.epidermidis	0.5
Klebsiella sp.	0.5
S.*aureus*	1

According to the results we notice that this compound is very active against all types of bacteria and the mechanism of action of this compound is unknown but generally all Mercuric compounds shear the same features that they effect on the protein structures inside the

microbial cell as well as some of these compounds may effect on the bacterial cell wall [10] and all mercury antiseptic readily worked to stopped the microorganisms from reproduction and spreading and did not kill them [2],this compound also have a Chlorine in its structure this element have a high toxic effect when present in some chemical compound like the Chlorine or Sodium hypochlorite the mechanism of action for all Chlorine compounds are similar to somewhat ,all of them work as bactericidal to inhibit essential protein synthesis either in the protoplasm inside the bacterial cell or at the bacterial cell wall [12,13,14].

From the results of MIC(table-3-) , there are no a big difference in the minimal inhibitor concentration between Gram positive and negative bacteria and that belongs to the mechanism of action of the Mercuric compounds which do not influenced with the change in bacterial cell wall structure . the change in MIC results between *E .coli* and all other bacteria belong to the fact that some strains of *E .coli* show a resistance for Mercury ,specially that have antibiotic resistance element(plasmid) that carry antibiotic resistance gene, these bacteria work by enzymatic detoxification process of the Hg^{+2} to the less reactive formHg^{0}[15],[16],this biotransformation is mediated by an inducible NADPH-dependent Nicotinamide adenine dinucleotide phosphate and some case ,NADPH-dependent Flavin-containing disulfide Oxidoreductase enzyme, Mercuric reductase[17]. and also *E.coli* appear to be able to develop a defense mechanism against some Chlorine compounds that helps protect the bacterium, though the implications of this defense mechanism have not been fully investigated[12].that explain the high (MIC) for *E.coli* than all other bacteria. the table-2- show that the β-Naphthyl Mercuric chloride also have a high activity against fungi the mechanism of action also not very clear it may coming due to the formation of hydrogen bond through β-Naphthyl mercuric chloride with the active center of the cell constituents resulting in interference with the normal cell process [19],[18], and also the presence of chlorine element in this compound increase its activity against fungi because some chlorine compounds work to inhibit some key enzymatic reaction in the cell the inhibition of these essential cytoplasmic metabolic reactions is largely responsible for the destruction of both bacterial and fungal cell [14].

The cytotoxicity of β-Naphthyl mercuric chloride was found about (0.25 -0.1 mg\ml)and that mean this compound is toxic in all the concentration above that, and thats belong to the fact that the sulfur is part of our blood cells and exactly it inter in the structure of Hemoglobin ,and Mercury will bond to the Sulfur atom the best it can and usually prevents the molecule from performing its function[4]and at this concentration the red blood cell still unaffected may be related to the low concentration of the β-Naphthyl Mercuric chloride compound that make Mercury too little to bind with all Sulfur of Hemoglobin and other structural Sulfur .

السمية الخلوية والتأثير المثبط للمركب بيتا نفثايل كلوريد الزئبق ضد بعض العزلات البكتيرية السريرية وبعض العزلات الفطرية (مختبرياً)

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الخلاصة

تم دراسة التأثير المثبط للمركب β-Naphthyl Mercuric chloride ضد بعض العزلات السريرية الجرثومية وكذلك بعض عزلات الفطريات وقد وجد ان اذابة 0.1غم من المركب في 10مل الماء المقطر واضافته الى الاوساط الزرعية Muller-Hinton agarو Sabouraued Dextrose agarسوف يؤدي الى تثبيط نمو ستة عزلات جرثومية *S.aureus* وعزلة سريرية اخرى من *E.coli ,P.aeroginosa ,S.aureus ,S.epidermidis ,Klebsiella sp.* وثلاث عزلات فطرية وهي *Candida albicans ,Aspergillus flavus* و*Cryptococcus sp.* كما قُيم التركيز المثبط الأدنى لهذا المركب تجاه العزلات الجرثومية السريرية الستة وأختبرت السمية الخلوية للمركب تجاه كريات الدم الحمراء للأنسان وقد وجد انه عند تركيز (0.1-0.25 ملغم\مل) من هذا المركب سوف يتوقف عن تحليل كريات الدم الحمراء.

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