Cytotoxicity and Antibacterial Effect of 2-(2-hydroxy naphthylazo) phenyl mercuric chloride and 4-(2-hydroxy naphthylazo) phenyl mercuric chloride against some bacterial isolates *in vitro*

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Abstract

The 2-(2-hydroxynaphthylazo) phenyl mercuric chloride and 4-(2-hydroxynaphthylazo) phenyl mercuric chloride were evaluated for their biological activity against standard strains of *Staphylococcus aureus* ATCC25923 and *Ecsherichia coli* ATCC 25922. The results showed that there is a potent antibacterial activity for these compounds. Minimal inhibitory concentration was determined for two compounds, it was 20µg/ml, 40µg/ml for *Staph. aureus*, and 50µg/ml, 60µg/m for *E. coli* respectively. Cytotoxicity assay was carried out against human red blood corpuscles, the two compounds exhibited a toxic effect in all used concentrations.

Key words: Cytotoxicity, Inhibitory effect, Toxic effect, Antibacterial activity

Introduction

Mercury is called quicksilver or hydrargyrum which is a chemical element with the symbol Hg (Latinized Greek: *hydrargyrum*, meaning watery or liquid silver) and of atomic number 80. Mercury is an extremely rare element in the Earth's crust, having an average crustal abundance by mass of only 0.08 parts per million **[1]**. Mercury enters the environment as the result of the normal breakdown of minerals in rocks and soil from exposure to wind and water, and from volcanic activity **[2]**. It was released from natural sources that have remained relatively constant in recent history, resulting in a steady rise in environmental mercury, human activities since the start of the industrial age (e.g., mining, burning of fossil fuels) have resulted in additional release of mercury to the environment **[3]**.

It was found naturally in the environment and exists in several forms, these forms can be organized under three headings, metallic mercury (also known as elemental mercury), inorganic mercury and organic mercury. All forms of mercury can enter the body and are potentially toxic. Some microorganisms such as bacteria, fungi and natural processes can change the mercury in the environment from one form to another **[4]**. The most common organic mercury compound that microorganisms and natural processes generate from other forms is methyl mercury **[5]**. Some inorganic mercury compounds are used as fungicides, inorganic salts of mercury, including ammoniated mercuric chloride and mercuric iodide, have been used in skin-lightening creams **[6]**. Mercuric chloride is a topical antiseptic or disinfectant agent. In the earlier studies, mercurous chloride was widely used in medicinal products including laxatives, worming medications and teething powders **[7]**.

Another mercury compounds Merbromin (Mercurochrome contains a small amount of mercury, 2%),) is a topical antiseptic used for minor cuts and scrapes is used in some countries **[8]** also, thimerosal and phenyl mercuric nitrate which are used in small amounts as preservatives in some prescription and over-the-counter medicines **[9]**.

Aim of this investigation: was try to use the mercuric compounds as topical antiseptics or disinfectants for medicinal tools and surgery.

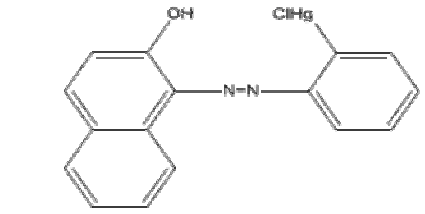
Materials and methods

Bacterial isolates

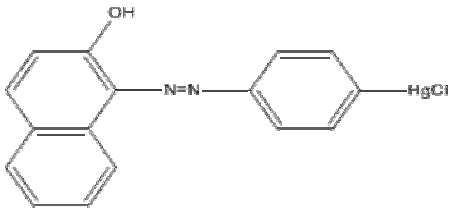
Two standard strains of *Staph. aureus* ATCC 25923 and *E. coli* ATCC 25922 were obtained from College of Medicine/University of Basrah, used in this investigation.

Synthesis:-

Mercuration of aniline gave ortho and para isomers which can be isolated from hot water **[10]**. The diazonium salts of these mercurated isomers (8mmol.) reacts with 2-Naphthol (1.15g, 8mmol.) at 0°C by using ice bath to give 2-(2-Hydroxynaphthylazo) phenyl mercuric chloride and 4-(2-Hydroxynaphthylazo) phenyl mercuric chloride as a yellowish brown solid, which were washed several times with distilled water then diethyl ether, after that dried in vacuum at 50°C. The yields were 77% and 82% respectively, as well as the m. p. were 188-190 °C and 212-214°C.



2-(2-Hydroxynaphthylazo)phenylmercury(II)chloride



4-(2-Hydroxynaphthylazo)phenylmercury(II)chloride

Antibacterial activity

The *in vitro* antibacterial activity of the 2-(2-hydroxynaphthylazo) phenyl mercuric chloride and 4-(2-hydroxynaphthylazo) phenyl mercuric chloride was tested against two standard strains of *Staph. aureus* and *E. coli*, by using disc diffusion method **(11).** Nutrient agar plates were inoculated with 24hr. growth (containing 10^6CFU/ml) of both two standard strains, after that discs that impregnated with two mercuric compounds in different concentrations that ranged between 1000µg/ml to 1µg/ml were placed on inoculated nutrient agar plates and incubated at 37°C for 24hr., then the inhibition zones for each concentration were measured. The experiment was repeated three times under the same conditions for each compound.

Minimal Inhibitory Concentrations (MICs)

By using broth micro dilution method **[12]**, MICs were detected for both two mercuric compounds under investigation. Nutrient broth test tubes that contain different concentrations of mercuric compounds that ranged between 4µg/ml to 1000µg/ml which were inoculated with 24hr. growth of standard strains of *Staph. aureus* and *E. coli*, then incubated at 37°C for 24hr., the results were examined visually and by re-growth at the same conditions of MIC test tubes contents that poured on nutrient agar plates, control plates were carried out for each bacterial isolate without adding the mercuric compounds.

Cytotoxicity

The cytotoxicity of 2-(2-hydroxynaphthylazo) phenyl mercuric chloride and 4-(2-hydroxynaphthylazo) phenyl mercuric chloride was assayed against human red blood corpuscles (RBCs) **[13]**. 2ml of human RBCs (with EDTA as anti-coagulant) were mixed with 38ml of Ringer solution, the mixture dispensed in 2ml dry clean test tubes. Different concentrations of mercuric compounds were prepared (1, 10, 30, 50, 100, 200,

300, 400, 500 µg/ml) respectively, after that added to the suspension of RBCs in Ringer solution, and incubated for 8hrs. at 37°C, then the results were recorded.

Results and Discussion

Elemental analysis

The results of elemental analysis of the studied compounds showed in Table (1).

Table (1). Elemental analysis of the mercuric compounds

Compounds	C (cal.)	H (cal.)	N (cal.)
2-(2-hydroxy naphthylazo) phenyl mercuric chloride	39.69 (38.76)	2.33 (2.29)	5.78 (5.80)
4-(2-hydroxy naphthylazo) phenyl mercuric chloride	39.79 (39.76)	2.31 (2.29)	5.84 (5.80)

Cal. = calculated

Antibacterial activity / MIC

The results exhibited a potent antibacterial activity for mercuric compounds under investigation against all tested bacteria represented by inhibition zones diameters recorded after bacterial growth. The minimal inhibitory concentration values of 2-(2-hydroxynaphthylazo) phenyl mercuric chloride equaled 20 µg/ml, 50 µg/ml for standard strains of *Staph. aureus* and *E. coli* respectively and 40 µg/ml , 60 µg/ml for the 4-(2-hydroxynaphthylazo) phenyl mercuric chloride, the data are presented in Table(2).

Table (2). MICs values applied on standard strains of *Staph. aureus* and *E. coli*

The compounds	MIC (µg/ml)	Inhibition zone (mm)	Standard strains
2-(2-hydroxy naphthylazo) phenyl mercuric chloride	20 µg/ml	5mm	*Staph. aureus*
	50 µg/ml	4mm	*E. coli*
4-(2-hydroxy naphthylazo) phenyl mercuric chloride	40 µg/ml	8mm	*Staph. aureus*
	60 µg/ml	5mm	*E. coli*

mm = millimeter , µg/ml = microgram/milliliter

The MICs for 2-(2-hydroxy naphthylazo) phenyl mercuric chloride were 20µg/ml for *Staph. aureus* and 50µg/ml for *E. coli* while they were 40µg/ml for *Staph. aureus*, 60µg/ml for *E. coli* for 4-(2-hydroxynaphthylazo) phenyl mercuric chloride. The results showed high activity of both two mercuric compounds in low concentrations confirmed by the re-growth of MICs test tubes contents on nutrient agar plates which showed no growth on these plates in comparison with control plates which exhibited a heavy bacterial growth. Also, the results showed differences in the inhibition zones diameters between G +ve and G –ve bacteria, *since Staph. aureus* was more sensitive to the action of these compounds which were indicated by wide, clear inhibition zones in comparison with *E. coli*, and this may ascribe to the differences in the nature of the cell wall components of G +ve and G –ve bacteria **[14]**, so these organisms differ in the organization of the structure outside the plasma membrane but below the capsule, most G +ve bacteria have a thick (about 20 to 80 nm) continuous cell wall, which is composed largely of loose layer of peptidoglycan **[15]**.

In contrast the cell walls of G -ve bacteria are more chemically complex, thinner and less compact, peptidoglycan makes up only 5-20% of the cell wall, and is not the outermost layer, but lies between the plasma membrane and an outer membrane **[16]**. This outer membrane is similar to the plasma membrane, but is less permeable and composed of lipopolysaccharides (LPS) and face into the external environment **[17]** LPS is a harmful substance classified as an endotoxin, the space between the cell

wall and the plasma membrane is called the periplasm, which controls molecular traffic entering and leaving the cell, so these features of G –ve cell wall made the bacteria belong to this group more resistant to the action of antibiotics or chemical compounds **[18].**

The results showed differences in MICs for both strains, generally 2-(2-hydroxynaphthylazo) phenyl mercuric chloride which shows more effect and low MIC than 4-(2-hydroxynaphthylazo) phenyl mercuric chloride and that can be attributed to the chemical structure of both compounds, both hydroxyl group and mercuric chloride moiety are fit in position requirement for chelating with hydroxyl and amino acids in the cell wall of bacteria, in addition to the coordination which may occur between mercury atom and amino acids in addition to hydrogen bonding which occurs between hydroxyl group of mercurial compound with amino acids of the cell wall of bacteria. At the same time in 2-(2-hydroxynaphthylazo) phenyl mercuric chloride which are easier than that for 4-(2-hydroxynaphthylazo) phenyl mercuric chloride due to the position of mercuric chloride in this compound as shown in the above chemical structures, i. e. both hydroxyl group and mercuric atom work together in chelating and coordinating in 2-(2-hydroxynaphthylazo) phenyl mercuric chloride and work individually in 4-(2-hydroxynaphthylazo) phenyl mercuric chloride that give different MICs.

Cytotoxicity

The results of cytotoxicity assay of 2-(2-hydroxynaphthylazo) phenyl mercuric chloride and 4-(2-hydroxynaphthylazo) phenyl mercuric chloride against human RBCs revealed that these compounds have a toxic effect on RBCs in the used concentrations (1, 10, 30, 50, 100, 200, 300, 400, 500 µg/ml), the microscopic examination under 40X power to the RBCs showed a haemolysis and destruction of the RBCs in all concentrations after incubation for 1 hour**,** these results agreed with all researches that related to mercuric compounds which have lethal effects on human body **[19, 20, 21]**, but on the other side, these compounds in highly diluted concentration may overcome its toxicity, then can be used as anti-septic or disinfectant. Wells, confirmed that mercuric chloride is sometimes used in dilute solution as an antiseptic for inanimate objects and as fungicide **[22].**

Recommendation

Further studies must be done to evaluate the efficacy of mercuric compounds in medical fields.

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السمية الخلوية والتأثيرضد الجرثومي لمركبي 2- (2- نفثايل آزو) فينايل كلوريد الزئبق و 4- (2- هايدروكسي نفثايل آزو) فينايل كلوريد الزئبق ضد بعض العزلات الجرثومية خارج الجسم الحي.

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الخلاصة

قدرت الفعالية البايولوجية لمركبي 2- (2- نفثايل آزو) فينايل كلوريد الزئبق و4- (2- هايدروكسي نفثايل آزو) فينايل كلوريد الزئبق ضد السلالات المرجعية لجرثومتي المكورات العنقودية الذهبية والأشريكية القولونية وأظهرت النتائج الفعالية ضد الجرثومية العالية للمركبين. حدد التركيز المثبط الأدنى للمركبين وكان 20مايكروغرام/مل ،40مايكروغرام/مل لجرثومة المكورات العنقودية الذهبية و50مايكروغرام/مل ،60مايكروغرام/مل لجرثومة الأشريكية القولونية على التوالي ولكلا المركبين. أختبرت السمية الخلوية للمركبين المحضرين ضد كريات الدم الحمراء للأنسان، وأظهر المركبان تأثيراً ساماً في كافة التراكيز المستخدمة.