

CYTOTOXICITY AND INHIBITORY EFFECT OF PARA-AMINO PHENYL MERCURY(II) ACETATE AGAINST GROWTH OF SOME BACTERIA (IN VITRO)

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ABSTRACT

It was found that 0.1gm of para-aminophenyl mercuric acetate PAPMA dissolved in 10ml of distilled water added in to,Muller-Hinton agar, inhibited the growth of four standard strains bacteria [*E.coli* ATCC25922, *S.aureus* ATCC 25923, *P.aeruginosa* ATCC27853 and *S.aureus* NCTC6571] and four clinical strains bacteria positive and negative to gram stain [*Klebsiella sp.*,from blood isolate , *E.coli*, from stool isolate, *Staphylococcus sp.*, from blood isolate, and *Proteus sp.*,from urine isolate]. Higher concentrations of PAPMA solution in to the medium inhibited growth of bacteria under study more strongly. The minimal inhibitory concentration (MIC) and cytotoxicity of PAPMA were studied against human blood and it was found that it has no hemolytic in RBCs for human in 1– 5µg/ml. The acute toxicity LD₅₀ of PAPMA was studied and it was about 11.3mg/Kg.

INTRODUCTION

Mercury is a natural element found in minute quantities in air, water and all living things, mercury can find its way in to food sources through a number of channels including natural recycling, burning of fossil fuels and pollution⁽¹⁾. Mercury can be found chemically as elemental Hg⁰, inorganic as [Hg^(I) and Hg^(II)] and organic [mostly as Hg^(II)]⁽²⁾, metallic mercury is poorly absorbed in the digestive track, but enters the body via inhalation, most mercury released in to the environment was in the form of elemental or inorganic mercury, it is organic mercury- in particular, methyl mercury- that poses the greatest threat to people and wild life⁽³⁾. Mercury was used in consumer products such as batteries, paints, thermometers, pigments, dental amalgams, fungicides, laboratory reagents, medicines, pesticides and cosmetics^(4,5). Because of the above important uses of mercury , we try in this study to use PAPMA which classified as organic mercury compound to evaluate the biological activity of PAPMA against growth of some standard and clinical strains bacteria, the study which had not been investigated for this compound.

MATERIALS AND METHODS

Materials :-

Para aminophenyl mercuric acetate (PAPMA) was prepared according to the literature⁽⁶⁾ , stock solution of this compound was prepared by dissolving 0.1g in 10ml of distilled water , and different media [Nuterient agar (Difco), Nutrient broth (Difco) and Muller-Hinton agar (Difco)] were used too.Standard isolate obtained from college of medicine,and clinical isolate obtained from Abn-gazwan hospital.

Methods :-

Antibacterial activity

Four standard strains bacteria [*E.coli* ATCC25922, *S.aureus* ATCC 25923, *P.aeruginosa* ATCC27853 and *S.aureus* NCTC6571] and four clinical strains bacteria positive and negative to Gram stain [*Klebsiella sp.*, from blood isolate, *E.coli*, from stool isolate, *Staphylococcus sp.*, from blood isolate and *Proteus sp.*, from urine isolate] were used with microbial concentration 10^6 CFU/ml and the antibacterial activity of PAPMA were tested using disc diffusion method⁽⁷⁾ and the inhibition zones were measured in millimeter (mm). Eight Petri dishes were used as an experimental unit and the trial was repeated twice. Petri dishes incubate in 37°C for 24h.

Minimal Inhibitory Concentration (MIC)

Broth dilution method⁽⁹⁾ was used to detect the (MIC) using different concentrations [5, 10, 25, 50, 100, 250, 400, and 500 $\mu\text{g/ml}$] of PAPMA against four standard strains bacteria [*E.coli* ATCC25922, *S.aureus* ATCC 25923, *P.aeruginosa* ATCC27853 and *S.aureus* NCTC6571] .

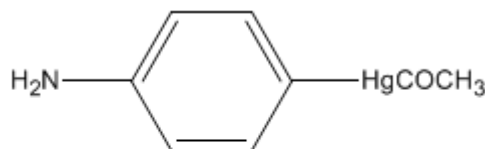
Cytotoxicity assay

The cytotoxicity of PAPMA was tested against red blood cells RBC_s for human using 2ml of blood mixed with 19ml of Ringer solution, from this mixture, 2ml was put in sterilizer test tubes to which different concentrations of PAPMA were prepared in seven test tubes [1, 5, 8, 10, 30, 50 and 100 $\mu\text{g/ml}$] respectively. After incubated at 37°C for 8 hours each one hour the test tubes were noticed and examined if there is any hemolytic for the RBC_s and the results were recorded⁽¹⁰⁾.

Median Lethal Dose (LD₅₀)

In this experiment Albino mice were used, BALB/C strain (male), 36 mice were used and divided to six groups each group contain 6 mice, the first group considered as control group which injected with 0.5ml distilled water interperitoneal while the other groups were injected with 0.5ml distilled water contain different concentrations of PAPMA, [2, 6, 9, 12 and 16 mg/Kg], the mice were watched for 72 hours and the Mortality percent were recorded. The results analyze using Probit-analysis method⁽¹¹⁾ to detect the LD₅₀ value.

RESULTS AND DISCUSSION



Paraaminophenylmercury(II)acetate [PAPMA]

The PAPMA as shown above was prepared and identified according to the literature⁽⁶⁾ and it characterized by elemental analysis which gave good result to confirm the above structure.

Elemental analysis :- calculated C : 28.62 , H : 2.70 , N : 4.17
Found C : 28.59 , H : 2.71 , N : 4.20

Generally the data given in (Table-1) confirm very high inhibitory active of PAPMA against clinical and standard strains positive and negative to Gram stain (Table-1) and Fig.-1,2

Table-1- The inhibitory effect of PAPMA against clinical and standard strains through incubation period (24 hour) in 37C°.

Standard strains	Inhibition zones (mm)
<i>S.aureus</i> NCTC6571	38
<i>E.coli</i> ATCC25922	27
<i>P.aeruginosa</i> ATCC27853	33
<i>S.aureus</i> ATCC 25923	35
Clinical strains	
<i>Staphylococcus</i> sp. (blood isolate)	35
<i>E.coli</i> (stool isolate)	23
<i>Proteus</i> sp. (urine isolate)	20
<i>Klebsiella</i> sp.(blood isolate)	25



P.aeruginosa ATCC27853

E.coli ATCC25922



S.aureus NCTC6571

S.aureus ATCC25923

Fig.1; Antibacterial activity of PAPMA against standard isolates



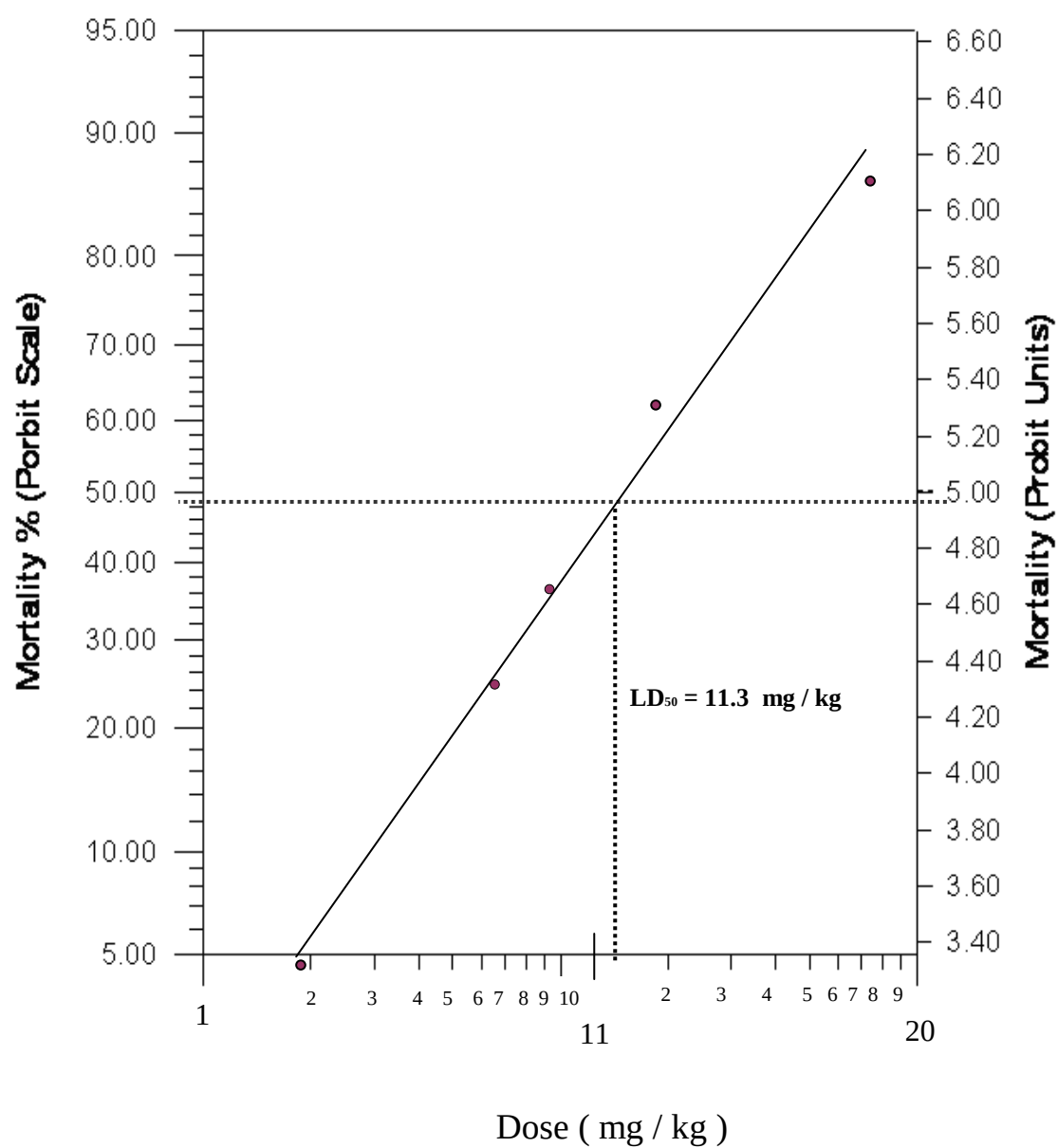
Clinical isolate:
Staphylococcus sp.
Klebsiella sp.
Proteus sp.
E. coli

Fig.2;Antibacterial activity of PAPMA against clinical isolate

Table-2- Minimal inhibitory concentration of PAPMA against standard strains positive and negative to Gram stain.

Standard strains	MIC ($\mu\text{g/ml}$)
<i>S.aureus</i> NCTC6571	50
<i>E.coli</i> ATCC25922	10
<i>P.aeruginosa</i> ATCC27853	05
<i>S.aureus</i> ATCC 25923	10

According to (Table 2) ,the minimal inhibitory concentrations level of PAPMA against standard strains, *S.aureus* NTCC6571, *E. coli* ATCC25922, *P.aeruginosa* ATCC 27853 and *S. aureus* ATCC25923 was 50, 10, 05, and 10 $\mu\text{g/ml}$ respectively. The mechanism of antimicrobial action of PAPMA was not known, but one can suggest that the PAPMA at bactericidal level disrupt cell metabolism by binding through hydrogen bond with amino acids and proteins including enzymes or by coordination of amino acids and proteins including enzymes with mercury atom through available vacant orbital, also may be by destruction of the pathogen cell wall⁽¹²⁾. The PAPMA shows high antibacterial action (Table-1-), Fig.1,2 against gram positive bacteria than Gram negative bacteria and that could be attributed to the fact that Gram negative bacteria are protected against most of the antibiotics, detergents and chemicals by their outer cell-wall, the outer layer of Gram negative bacteria cell-wall was made of lipopolysaccharide and protein, it covers a very few layers of peptidoglycan as compared with gram positive bacteria in which the outer layer cell-wall was made of peptidoglycan only and does not contain lipoproteins⁽¹³⁾. A widely employed mechanism of bacterial resistance to mercurial compounds is the reduction of Hg^{2+} to the volatile form, Hg^0 , 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⁽¹⁴⁾. The mercury resistance gene Mer was found to be closely associated with multiple antibiotic resistance gene in Gram negative bacteria from the intestines of monkey and other primate⁽¹⁵⁾. Generally mercuric compounds have more bactericidal effect among other antimicrobial agents, such as penicillin, sulfa, tetracycline ...etc⁽¹⁶⁾. The results of cytotoxicity test shows that the toxicity of PAPMA like all mercuric compounds, the researches indicates, that nervous system is very sensitive to all forms of mercury but the methyl mercury and metallic mercury vapors are more harmful than others forms because more mercury in these forms reaches the brain⁽¹⁷⁾. In spite of that in this study there are some concentrations arrange between 1 to 5 $\mu\text{g/ml}$ at which PAPMA have no toxic this results is the first step to continues study and work with this compound to produce new drug and antiseptic which may be used in future. The study of LD_{50} for compounds is very important step to detect the harmful effect of compounds⁽¹⁸⁾, LD_{50} for PAPMA was found 11.3mg/Kg, Fig.3 which is similar to other organic mercuric compounds, for example methyl mercury has LD_{50} 5.5mg/Kg and 16.5mg/Kg with several animals which began to display signs of neurotoxicity⁽¹⁹⁾. This value is toxic, all forms of mercury are toxic and each form produces different health effect in human⁽²⁰⁾. Additional studies and experiments required to prove the importance of this compound PAPMA before used as drug in future.



السمية الخلوية والتأثير المثبط للمركب بارا- أمينو فنييل خلات الزئبق ضد نمو بعض انواع البكتيريا (مختبريا).

شاكِر عبد السالم نعمه الجدعان ، سبأ علي محمد الفضل ورفيف عامر عبد الجبار السامرائي
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الخلاصة

وجد ان 1،. من المركب بارا-أمينوفنييل خلات الزئبق (PAPMA) المذاب في 10ملليلتر من الماء المقطر قد
ثبط نمو أربعة سلالات مرجعية:

S.aureus NCTC6571

E.coli ATCC25922

P.aeruginosa ATCC27853

S.aureus ATCC25923

Staphylococcus sp.

بالاضافه الى أربعة عزلات سريرية:

Klebsiella sp.

Proteus sp.

E.coli

كما وجد ان التراكيز العالية من المركب المضاف الى الوسط الزرعى يؤدي الى تثبيط النمو بشكل اكثر.
تمت دراسة التركيز المثبط الادنى وكذلك السمية الخلوية لكريات دم الانسان ووجد انه لا يوجد تحلل لكريات الدم
عند تراكيز تراوحت بين 1 مايكروغرام/ملليلتر 5 مايكروغرام/ملليلتر ويبدأ التحلل عند تراكيز اعلى، كما تمت
11.3 ملغم/كغم وتعتبر هذه الدراسة هي الخطوة الاولى دراسة الجرعة القاتلة الوسطى من المركب فوجد انه
لدراسة هذا المركب الجديد والذي ممكن الاستفاده منه في المستقبل كدواء.....

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