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The Effect of Different Concentration of Silver Nanoparticles in Inhibiting the Growth of Pathogenic Bacteria Isolated From the Diarrhea Patients

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ABSTRACT:
The study was conducted in the laboratories of the department of Biology - Faculty of Science, which cope with the effect of different concentrations of silver nanoparticles in inhibiting the growth of pathogenic bacteria species isolated from thechildren with diarrhea aged (2-4) years. The results of this study proved the efficiency in inhibiting growth of E. coli by silver nanoparticles where diameters of the inhibition zone (12.21, 16.24, 23.11, 26.38 mm) at concentrations 25, 50, 75and 100 (µl)respectively, the inhibiting efficacy of the silver nanoparticles in inhibition of the growth of Proteus mirabilis(10.76,13.32,17.44, 20,33 mm) , the inhibiting ability on the Shigella flexnerigrowth (8.32, 10.32, 11.07, 13.58) mmat the same concentrations , the inhibiting efficacy of the silver nanoparticles in inhibition of the growth of Staphylococcus aureus wherediameters of the inhibition zone (11.72, 13.42, 15.31, 17,12 mm) at the same concentration , also The results exposed that silver nanoparticles were able to inhibit Enterobacter cloacae growth wherediameters of the inhibition zone (10.31, 12.43, 14.71, 15.68 mm) at concentrations 25, 50, 75and 100 (µl)respectively .
Key words:nanoparticles , inhibition , silver.

I. INTRODUCTION

Diarrhea is state of having at least three loose or liquid bowel movements each day, it frequently lasts for a rare days and can result in dehydration due to fluid loss ,symptoms of thirst begin with loss of skin stretchiness, this can progress to declined urination, loss of skin color, and a decrease in responsiveness as it becomes more severe, the source of an intestines infection because virus,bacteriaor parasite , these infections are often come from water or food that has been polluted from infected person or by feces, the three kinds of diarrhea include: short periodbloody diarrhea, short periodwatery diarrhea, and persistent diarrhea, the smallperiod watery diarrhea perhaps because an infection by cholera, this is unusual in the developed world , if blood is present it is also well-called dysentery(1) .

Nanoparticles is particleamong 1 to 100 nanometers in size with a close interfacial layer. The interfacial layer is an vital part of Nano scale matter, basically affecting all of its characterizes. The interfacial layer normallycontainsorganic and inorganicmolecules also ions. Organic molecules covering inorganic nanoparticles are called stabilizers, capping and surface ligands, or passivizing agents , The field of nanotechnology is one of the greatest active areas of research in modern material science. Silver has long been familiar as one of the nanoparticles having inhibitory result on microbes present in industrial and medical route, Metals nanoparticles such as gold andsilver are receiving abundant interest due to their uses in various field such as medicine field (15), coatings, packaging ,electronics, cosmetics, and biotechnology. The production of metal nanoparticles is a more significant research branch in nanotechnology. Chemical ways are the most broadly used approaches for synthesis of metallic nanoparticles ,in the past few years, the possible of numerous plants for the synthesis of silver nanoparticles (SNPs) was explored (2). In this current work, silver nanoparticles was used toinhibitthe growth of E.coli, Proteus mirabilis,Shigella flexneri,Staphylococcusaureusand Enterobacter cloacae that isolate from the diarrhea patients.

II. MATERIALS AND METHODS :

1.Bacterial species that associated with the stool samples

30 stool samples collected from the children with diarrhea aged (2-4) years from Zahra Hospital in Najaf governorate, after the end of incubation period of bacteria isolates , identification of these isolates were based on morphological and biochemical tests explain by (3) .Then the percentage of appearance of each bacteria isolates calculated by the next equation :

The number of samples in which the bacteria

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The percentage of appearance =---------- ---------- x 100
The total number of samples

2. preparation of silver nanoparticles

silver nanoparticles powder with size (50nm) was used , the concentrations of silver nanoparticles were required in this study prepared by dissolved 100 mg of silver nanoparticles in 100 ml of the distal water this solution act as the stock solution, then other concentrations were prepared 25, 50, 75and 100 (µl)

3: Evaluation of the silver nanoparticles efficiency in the inhibition of the bacteria species growth

The preparation of Muller Hinton Agar, it sterilized in the autoclave and poured in 30 petri dishes , then 30 petri dishes spliced into 5groups, each comprising 6 dishes , the first group were inoculated with E.coli , the second group were inoculated with Proteus mirabilisthe third group were inoculated with Shigella flexneri, the fourth group inoculated with Staphylococcusaureus and the last group inoculated with Enterobacter cloacae . Antibacterial silver nanoparticles activitywas detected using the agar well diffusion assay method, all the dishes were incubated at a temperature 37 ° C for 24 h, The plates were investigated for evidence ofinhibition zone, which seem as a clear zone around the wells . Then measured diameter of inhibition zone using a meter ruler.

III. RESULTS AND DISCUSSION :

1:Isolate and Identification of bacterial species that associated with the stool samples :

9 bacterialspecies were isolated from the stool samples collected from the children with diarrhea aged (2-4) years , they have been elected 5 bacteria species E.coli , Proteus mirabilis and Shigella flexneri, Staphylococcusaureus and Enterobacter cloacae which the most frequently than the rest bacteria that neglected recurrence rates (Figure 1) .This results agree with other researches showed the most common organisms responsible for most cases of diarrhea include, ETEC, Vibrio cholerae , shigella, spp., , and non-typhoidal salmonella, Hospital- and community-based etiology studies exposed the predominance of enterotoxigenic E. coli as causes of diarrhea(4 , 5), also (6,7) they showing the chief enteric pathogens include, Shigella spp. , Escherichia coli, Enterobacterspp , Vibrio parahaemolyticus , nontyphoidal Salmonella, Vibrio cholera, Aeromonas spp., Plesiomonas spp. and Campylobacter spp.

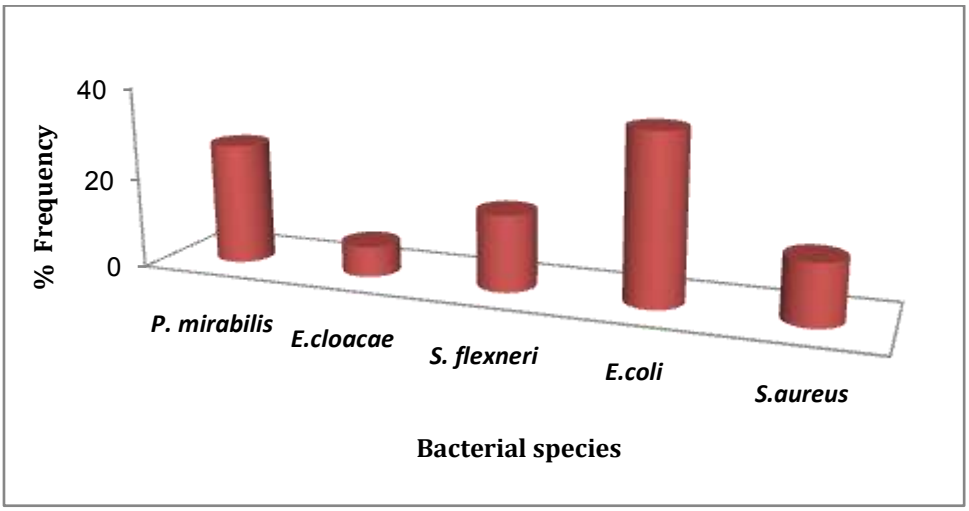

Figure (1) :Percentage of frequency of bacterial species that isolated from stool samples from the children with diarrhea

The Table (1) displayedthe results of biochemical tests for identification ofbacterial speciesfrom stool samples of children, these results showed all bacterial species are positive for catalase test, moreover all the bacterial species are negative for oxidase test (3) .

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Table (1) : Biochemical tests for bacterial species

bacterial species	Catalase	Voges-Proskauer	Indole Production	Methyl Red	Urease	Citrate	Motility	Oxidase
E.coli	+	-	+	+	-	-	+	-
Proteus mirabilis	+	-	-	+	+	+	+	-
Shigella flexneri	+	-	Variable	+	-	-	-	-
Staphylococcusaureus	+	+	-	+	+	+	-	_
Klebsiella pneumoniae	+	+	-	-	+	+	-	-
Staphylococcus epidermidis	+	+	-	-	+	+	-	-
Enterobacter cloacae	+	+	-	-	-	+	+	-
Pantoeaagglomerans	+	+	-	+	-	+	+	-
Serratia marcescens	+	+	-	-	+	+	+	-

2.Efficiency of silver nanoparticles in the inhibition of the bacteria growth :

The results indicated that silver nanoparticles were able to inhibit E.coligrowth where the diameters of the inhibition zone (12.21, 16.24, 23.11, 26.38 mm) at concentrations 25, 50, 75and 100 (µl) respectively, the silver nanoparticles were capable to inhibit Proteus mirabilis growth where diameters of the inhibition zone (10.76,13.32,17.44, 20,33 mm) at the same concentration , the silver nanoparticles were able to inhibit Shigella flexneri growth where the diameters of the inhibition zone (8.32, 11.32, 14.42, 17.58) at the same concentration , the silver nanoparticles were capable to inhibit Staphylococcusaureus growth where the diameters of the inhibition zone (11.72,13.42,15.31, 17,12 mm) at the same concentration , also the results exposed that silver nanoparticles were able to inhibit Enterobacter cloacae growth where the diameters of the inhibition zone (10.31, 12.43, 14.71, 15.68 mm) at concentrations 25, 50, 75and 100 (µl)respectively (figure 2).

The antimicrobial properties of silver ions arerecognized ,the silver ions interfere with enzymes participating in the respiratory processes of the bacteria, produce failure in the ATPcreation(8). Moreover, silver ionslink tocarrier ofenzymes and proteins located in theplasma membrane of bacteria. This chain of proteins and enzymes transport electrons and at the same time transport protons from the cytoplasm into periplasmic space generating gradient in concentration called PMF. In bacteria, this electron transport system in cell membrane is producer of ATP during aerobic respiration, and this process called chemiosmosis. The only place protons can move inside cytoplasm again by ATP synthase complex, formation of ATP by a redox reaction between ADP and inorganic phosphate, and the electrons achieve the final site of electron acceptor, dissolved oxygen is final electron acceptor in the case of aerobic respiration, and primarily results in water or in small concentrations of cytotoxic ROSs, which specialized enzymes process (9).

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The attachment of silver ions to bearerof enzymes and protein, cause proton leakage inducing a failure of the PMF then prevent ATP synthesis lead to lysis of bacteria (10) . Moreover, it was displayed that the cell membrane sometimes completely dissolves, which leads to intracellular leakage. alsosilver ions bind with DNA molecules in the bacteria cell, thereforeproducing mutation (11) .This study was decided with (12) when they displayed the Biological tests showed that the silver nanoparticles showed antibacterial activity against E. coli and S. aureus, also (13) they exposed antimicrobial activity of silver nanoparticles against S.typhi, S.epidermidis, K.pneumoniae,P.aeruginosa, P.vulgaris, E.coli. While the study by (14) showed the nanosynthesis titanium nanoparticles displayed antibacterial and antibiofilm activity against both multidrugresistantKlebsiella pneumonia and Staphylococcus aureus.

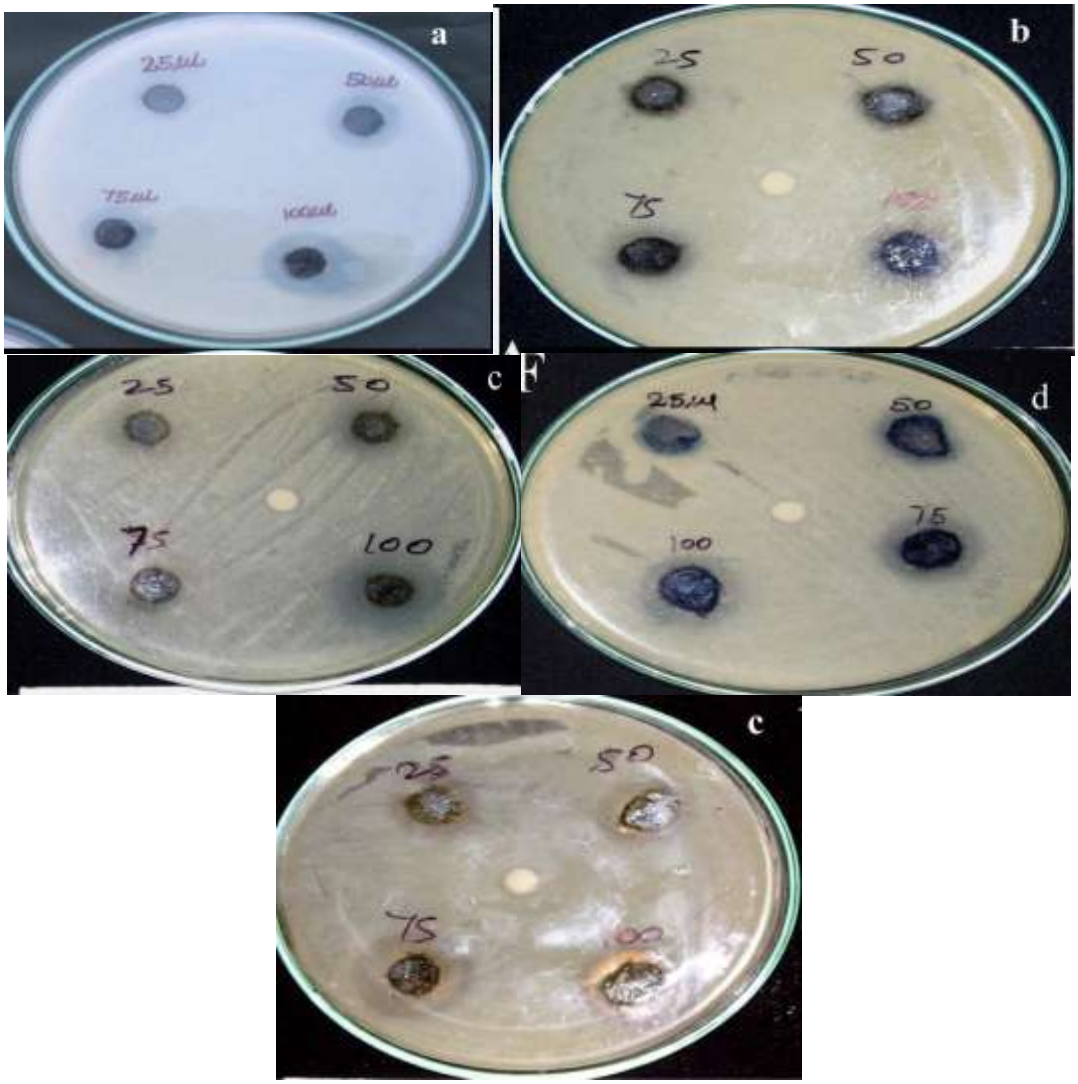

Figure (1): Effect of Different Concentrations of silver nanoparticles in Bacterial species growth a = S. saureus , b = S. flexneri, c = E.coli, d = P. mirabilis , e=E. cloacae

REFERENCES

[1]. BasemAbdelmalak, John Doyle, eds. (2013). Anesthesia for otolaryngologic surgery. Cambridge University Press. pp. 282–287 .

[2]. Jose R M, Jose C L, Alejandra E, Katherine Holt, Juan B K, Jose Tapia R, Miguel Jose Y. Nanotechnol, 2005,16,2346.

[3]. Macfaddin ,J.F . (2000) . Biochemical test for identification of medical bacteria . 2 ed . William and Wilkins . Comp. Baltimore , p.490 .

[4]. Platts-Mills JA, Babji S, Bodhidatta L, Gratz J, Haque R, Havt A, McCormick BJ, McGrath M, Olortegui MP, Samie A, et al.(2015). Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health.3(9):e564–75.

[5]. Liu J, Kabir F, Manneh J, Lertsethtakarn P, Begum S, Gratz J, Becker SM, Operario DJ, Taniuchi M, Janaki L, et al. (2014).Development and assessment of molecular diagnostic tests for 15 enteropathogens causing childhood diarrhoea: a multicentre study. Lancet Infect Dis.;14(8):716–24.

[6]. Hao R, Li P, Wang Y, Qiu S, Wang L, Li Z, Xie J, Wu Z, Lin R, Liu N, et al. (2013) .Diversity of pathogens responsible for acute diarrheal disease in China. Clin Infect Dis.;57(12):1788–90.

[7]. Zhang SX, Zhou YM, Xu W, Tian LG, Chen JX, Chen SH, Dang ZS, Gu WP, Yin JW, Serrano E, et al. (2016) . Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in southwest China. Infect Dis Poverty.;5(1):64.

[8]. Katherine B Holt and Allen J Bard.(2005) .Interaction of silver (i) ions with the respiratory chain of escherichia coli: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar ag+. Biochemistry, 44(39):13214–13223,

[9]. Kathleen Park Talaro. (2009) .Foundations in Microbiology. McGraw-Hill 10.Woo Kyung Jung, Hye Cheong Koo, Ki Woo Kim, Sook Shin, So Hyun Kim, and Yong Ho Park. (2008) .Antibacterial activity and mechanism of action of the silver ion in staphylococcus aureus and escherichia coli. Applied and environmental microbiology, 74(7):2171–2178.

[10]. Arakawa, H., JF Neault, and HA Tajmir-Riahi. (2001) . Silver (i) complexes with dna and rna studied by fourier transform infrared spectroscopy and capillary electrophoresis. Biophysical journal, 81(3):1580–1587.

[11]. Ngoc Duong Trinh, Thi Thanh Binh Nguyen and Thanh Hai Nguyen (2015). Preparation and characterization of silver chloride nanoparticles as an antibacterial agent Adv. Nat. Sci.: Nanosci. Nanotechnol. 6 (045011) .

[12]. Nithya.G , N. Hema shepangam and S. Balaji . (2011) . Biosynthesis of silver nanoparticle and its antibacterial activity. Archives of Applied Science Research . 3 (2):377-380.

[13]. Aldujaili, N H And Banoon, S. R.(2020) Antibacterial Characterization of Titanium Nanoparticles Nanosynthesized by Streptococcus Thermophilus. PeriódicoTchêQuímica. 17(34):311-320.

[14]. Hassan, B. A., Lawi, Z. K. K., Banoon, S. R. (2020) detecting the activity of silver nanoparticles, pseudomonas fluorescens and bacillus circulans on inhibition of aspergillus niger growth isolated from moldy orange fruits. PeriódicoTchêQuímica. 17(35):678-690.