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Demonstration of Antimicrobial Activity of Azithromycin with Antacids, Metal and Investigation of In-vitro Interaction and Complexation

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Abstract

This research work comprises of interaction studies of Azithromycin with different essential metals and investigation of antimicrobial activity of Azithromycin both with and without interactions. Azithromycin is anew macrolide antibiotic with a better activity against intracellular gram negative bacteria comparison with Erythromycin. Macrolides are one of the most commonly used families of antibiotics. Since the presence of complexation may affect the bioavailability of the drugs and other essential elements, therefore in order to study the probable interaction of Azithromycin with essential and trace elements present in the body. Azithromycin has been interacted with metal Zn, antacids $CaCO_3$ and $Mg(OH)_2$ in vitro. All the reaction conditions were simulated to natural environments. Also, the antimicrobial activity of drug and the complexes were determined. The studies were carried out in buffer at pH 7.4 in variable ratios of drug and metal both at room temperature conditions. In this study, it is observed that almost all the portion of antibiotic is complexed with antacid metal. It has also been observed that Azithromycin interacts with metal at pH 7.4. In order to investigate the numbers of metal ions involved in the complexation with Azithromycin complexes were elucidated by exploiting various spectrophotometric methods. The UV-spectrophotometric studies of these complexes were carried out and compared.By antimicrobial investigation it was observed that the zone of inhibition of the drug Azithromycin with Mg Zn and Ca reduced from 14mm to 9mm, 8mm and 4mm respectively.

Keywords: *Antimicrobial activity, azithromycin, staphylococcus aureus, interaction, job's plot*

INTRODUCTION

Drug interaction is a common phenomenon. These unwanted and unsought-for interactions are adverse and undesirable but there are other interactions that can be beneficial and valuable, such as the deliberate co-prescription of antihypertensive drugs and diuretics in order to achieve antihypertensive effects possibly not obtainable with either drug alone. The mechanisms of both types of interaction, whether adverse or beneficial, are often very similar [1]. There are different types of drug interactions: Drug-drug interact ions, Drug-herbal interact ions, Food-drug interact ions etc. [2]. Drug interactions are complex and chiefly unpredictable. A known interaction may not occur in every individual. This can be explained because there are several factors that effects the likelihood that a known interaction will occur [3]. Zinc is an important co-factor for several enzymatic reactions in the human body, vitamin B_{12} has cobalt atom and its core, and hemoglobin contains iron. Likewise Cu, Mn, Se, Cr, Mo are all trace elements, which are important in the human diet. Another subset of metals includes those used in therapeutically in medicine, Al, bi,

Au, Ga, Li and Ag are all part of medical armamentarium [4]. Humans need a certain amount of certain metals to function normally. Most metals are used as cofactors or prosthetics in enzymes, catalyzing specific reactions and serving essential roles. Anemia symptoms are caused by lack of a certain essential metal. Anemia can be associated with malnourishment or faulty metabolic processes, usually caused by a genetic defect [5].The metal complexes can be utilized for the transport of selected organic chemotherapeutic drugs to target organs, or for the decorporation of those toxic organic compounds which are able, before or after metabolic activation of reacting with metals or 1:1 complexes [6]. It is emphasized that degree to which metal ions interact in vivo should employ the conditional constants which take into account competition from other ions specially Ca^{2+}, H^{+} and OH^{-} [7]. In the previous research work, it was reported that the genotoxic consequences of the different chemical factors influenced in chelation such as kinetics, stabilization, oxidation state etc [8]. Azithromycin is an antibiotic useful for the treatment of a number of bacterial infections [9]. This includes middle ear infections, strep throat, pneumonia, traveler's diarrhea, and certain other intestinal infections [10]. It may also be used for a number of sexually transmitted infections including chlamydia and gonorrhea infections [11]. Along with other medications, it may also be used for malaria [12]. It can be taken by mouth or intravenously with doses once per day [13].

MATERIALS AND METHODS

All the chemicals used here were analytical grade and sorted under optimum storage conditions. The experimental mixtures and solutions were prepared in standard volumetric flasks about one hour prior to obtain and recording the data.

Preparations of buffer solution:

1.76 gm of Disodium hydrogen phosphate was dissolved in demineralized water with 2.43gm of Sodium di-hydrogen phosphate and pH was adjusted to 7.4 and the volume was made up to 1000 ml with the same solution.

Preparations of stock solution:

Azithromycin solution 250 ml of 1×10^{-2}M was prepared by dissolving 1.962gm of Azithromycin standard in 250 ml of demineralized water in a 250 ml volumetric flask. Then 1ml was withdrawn from the prepared 250 ml solution and diluted by adding 99 ml of buffer solution and the concentration would be0.00001 or 1×10^{-5} M.

Preparations of metal solution:

For the preparation of metal solution 0.16144 gm zinc sulfate was weighed accurately and introduced with the help of funnel in 100 ml volumetric flask, dissolved in demineralized water and make up to the mark with the same solvent. This solution was diluted ten folds in the same solvent and the final concentration of the solution was 0.01M.

Preparations of Antacids solutions:

For the preparation of antacids solutions 0.0151 gm of calcium carbonate and 0.0585 gm magnesium hydroxide were weighed accurately and introduced with the help of funnel in 100 ml volumetric flasks separately, dissolved in demineralized water and make up to the mark with the same solvent. These solutions were diluted ten folds in the same solvent and the final concentration of the solutions was 0.01M.

Preparation of standard curve of Azithromycin:

Azithromycin stock solution at pH 7.4 and concentration of 1×10^{-5} M was added in different concentrations to ten test tubes, to have the following concentrations: 9×10^{-5} M, 8×10^{-5} M, 7×10^{-5} M, 6×10^{-5} M, 5×10^{-5} M, 4×10^{-5}M, 3×10^{-5} M, 2×10^{-5} M, 1×10^{-5} M. The solutions were then properly mixed. The absorbance values were determined at 235nm by UV spectrophotometer. As a control of reference sample, phosphate of pH 7.4 was used. The standard curve was obtained by plotting the absorbance values against the corresponding concentrations.

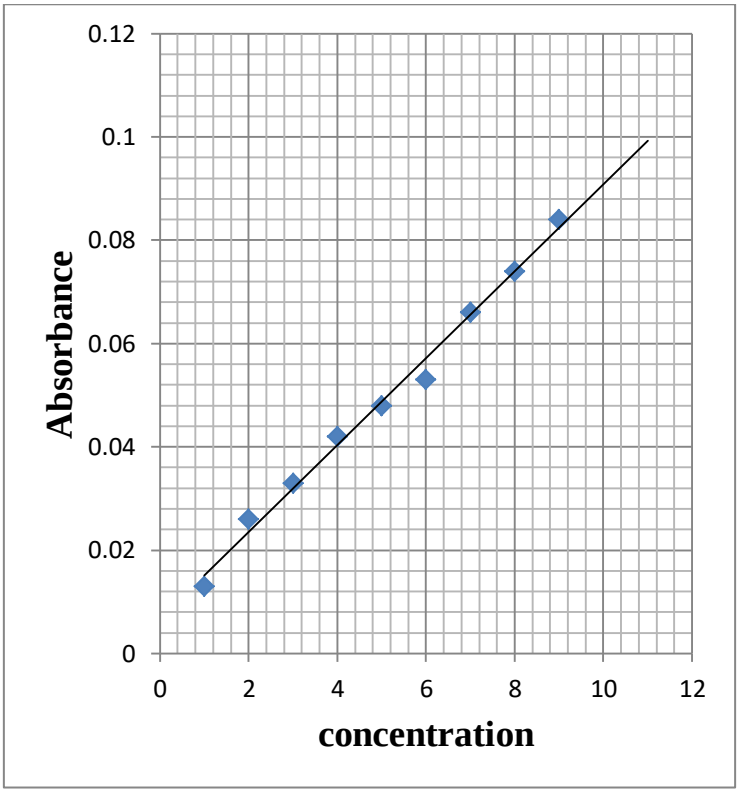

Figure 1: *Standard curve of Azithromycin.*

From the above Fig. 1, we can observe that the absorbance of Azithromycin increases with increasing concentration which follows the Beer Lambert's law.

RESULTS AND DISCUSSION

Combined spectral analysis of Azithromycin with both antacids:

It is observed that the absorbance of Azithromycin is different when it interacts with both antacids at different wavelengths from 200 to 400nm.

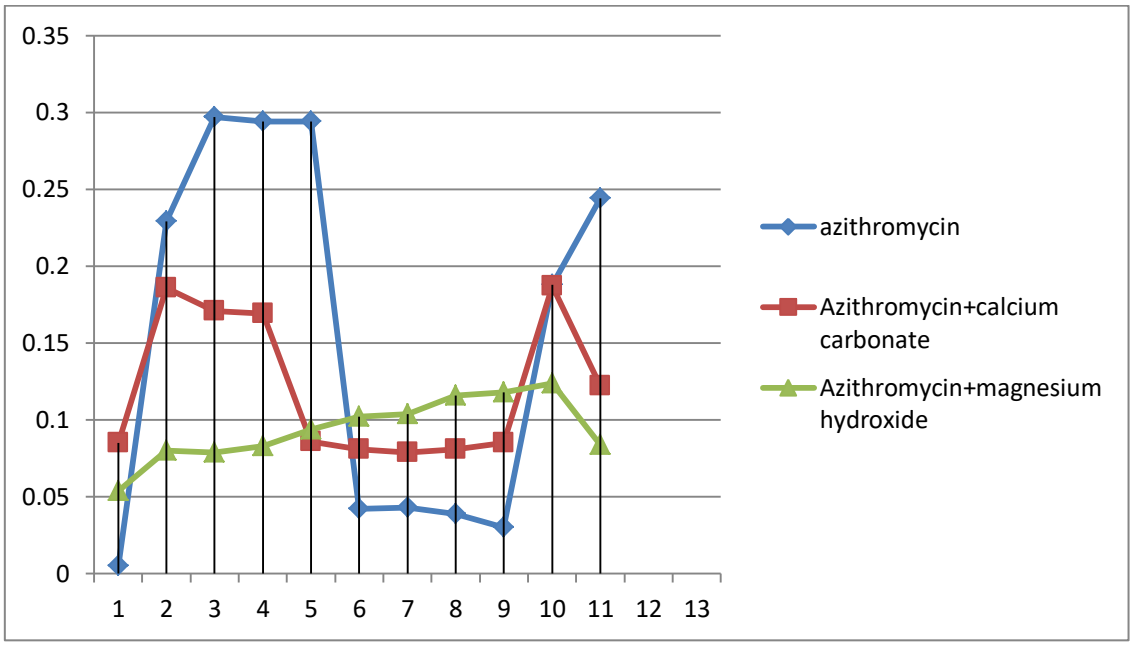

Figure 2: *Combined spectral analysis of azithromycin and azithromycin with both antacids.*

Table 1: *Values of job's plot of azithromycin and metal, zinc sulfate.*

Concentration of Azithromycin	Absorbance of Azithromycin (A)	Concentration of zinc sulfate	Absorbance of zinc sulfate (B)	Absorbance of mixture C	Absorbance difference D=(A+B)-C
1×10^{-5} M	0.013	9×10^{-5} M	0.093	0.061	0.049
2×10^{-5} M	0.026	8×10^{-5} M	0.073	0.055	0.044
3×10^{-5} M	0.033	7×10^{-5} M	0.079	0.051	0.061
4×10^{-5} M	0.042	6×10^{-5} M	0.105	0.099	0.048
5×10^{-5} M	0.048	5×10^{-5} M	0.115	0.089	0.071
6×10^{-5} M	0.053	4×10^{-5} M	0.106	0.065	0.091
7×10^{-5} M	0.066	3×10^{-5} M	0.099	0.085	0.08
8×10^{-5} M	0.074	2×10^{-5} M	0.094	0.138	0.03
9×10^{-5} M	0.084	1×10^{-5} M	0.089	0.141	0.037

The molar ratios (1:1) of the complexes of azithromycin with metal salt were estimated by job's method of continuous variation. The observed absorbance values were measured in pH 7.4 at various concentration of drug and metal salt at 235nm. The job's plots were obtained by plotting absorbance difference against the mole fraction of drug which is presented.

Table 2: *Values of job's plot of Azithromycin and Mg (OH)$_2$.*

Concentration of Azithromycin	Absorbance of Azithromycin (A)	Concentration of Mg(OH)2:	Absorbance of Mg(OH)2: (B)	Absorbace of mixture C	Ansorbance difference D=(A+B)-C
1×10^{-5} M	0.013 0,0013	9×10^{-5} M $\times 10$	0.156156	0.117117	0.052
2×10^{-5} M	0.026	8×10^{-5} M	0.158	0.111	0.073
3×10^{-5} M	0.033	7×10^{-5} M	0.162	0.108	0.087
4×10^{-5} M	0.042	6×10^{-5} M	0.165	0.111	0.096
5×10^{-5} M	0.048	5×10^{-5} M	0.168	0.103	0.113
6×10^{-5} M	0.053	4×10^{-5} M	0.163	0.121	0.095
7×10^{-5} M	0.066	3×10^{-5} M	0.149	0.127	0.088
8×10^{-5} M	0.074	2×10^{-5} M	0.145	0.137	0.082
9×10^{-5} M	0.084	1×10^{-5} M	0.113	0.145	0.52

Table 3: *Values of job's plot of azithromycin and calcium carbonate.*

Concentration of Azithromycin	Absorbance of Azithromycin (A)	Concentration of calcium carbonate	Absorbance of calcium carbonate (B)	Absorbance of mixture C	Absorbance difference D=(A+B)-C
1×10^{-5} M	0.013	9×10^{-5} M	0.173	0.159	0.027
2×10^{-5} M	0.026	8×10^{-5} M	0.177	0.153	0.053
3×10^{-5} M	0.033	7×10^{-5} M	0.186	0.146	0.073
4×10^{-5} M	0.042	6×10^{-5} M	0.209	0.140	0.109
5×10^{-5} M	0.048	5×10^{-5} M	0.231	0.154	0.125
6×10^{-5} M	0.053	4×10^{-5} M	0.216	0.197	0.09
7×10^{-5} M	0.066	3×10^{-5} M	0.213	0.191	0.088
8×10^{-5} M	0.074	2×10^{-5} M	0.204	0.196	0.082
9×10^{-5} M	0.084	1×10^{-5} M	0.169	0.191	0.062

Combined absorbance difference of Azithromycin with different antacids:

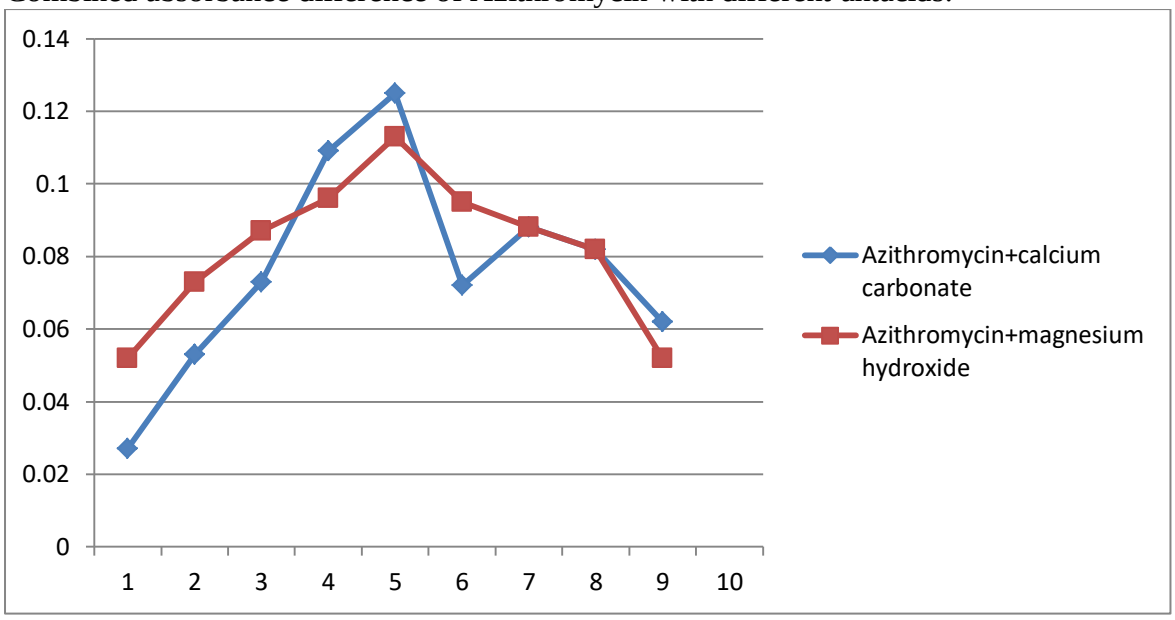

Figure 3: *Combined absorbance difference of azithromycin with different antacids.*

ANTIMICROBIAL STUDY

Disc Diffusion Method

Solution of known concentration (3µg/ml) of the test samples are made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6mm diameter) are then impregnated with known amounts of the test substances using micropipette. Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganism, Standard antibiotic discs and blank discs (impregnated with solvent) are used as positive and negative control. These plates are then kept at low temperature (4°C) for 24 hours to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and then the test materials dissolve and diffuse out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel. As a result there is a gradual change of test materials concentrations in the media surrounding the disc. The plates are then incubated at 37°C for 24 hours to allow maximum growth of the organism. If the test materials have any antimicrobial activity, it will inhibit the growth of the microorganisms and a clear, distinct zone of inhibition will be visualized surrounding the medium. The antimicrobial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out more than once and the mean of the readings is required [14].The antibacterial potency of the test agents were measured by their activity to prevent the growth of microorganisms surrounding the discs which gives clear zone of inhibition. After incubation diameter of zone of inhibition were measured with a transparent millimeter scale.

Standard disk (zone of inhibition)	Sample disk (zone of inhibition)
Azithromycin standard =14mm	Azithromycin +magnesium hydroxide =9mmhroamycin +magnesium hydroxide =9mm
	Azithromycin +zinc sulfate =8mm
	Azithromycin+calcium carbonate =4 mm

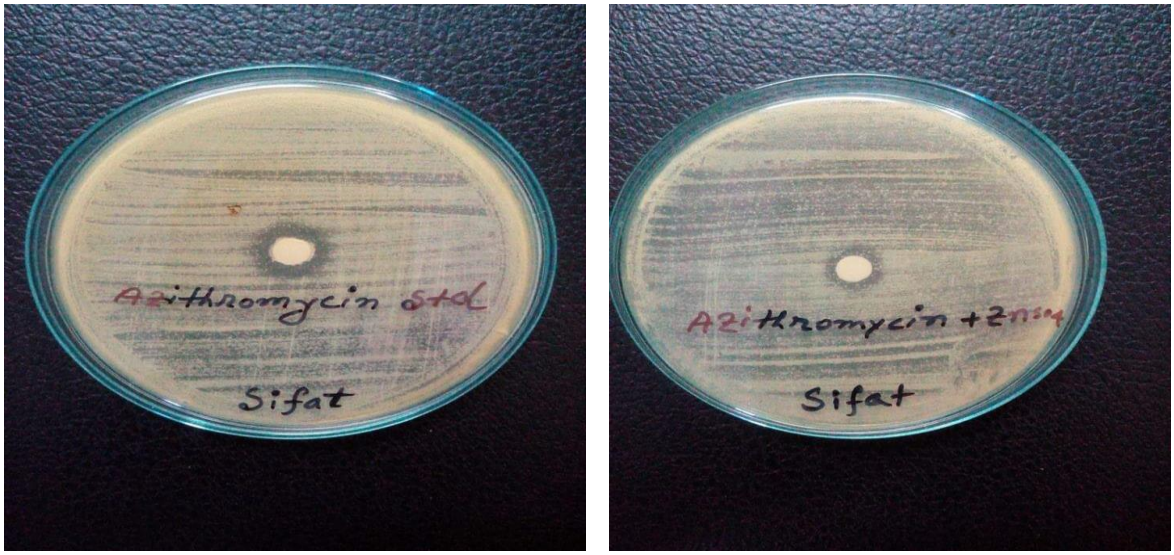

Figure 4: *Comparative study by zone of inhibition between Azithromycin standard and zinc sulfate.*

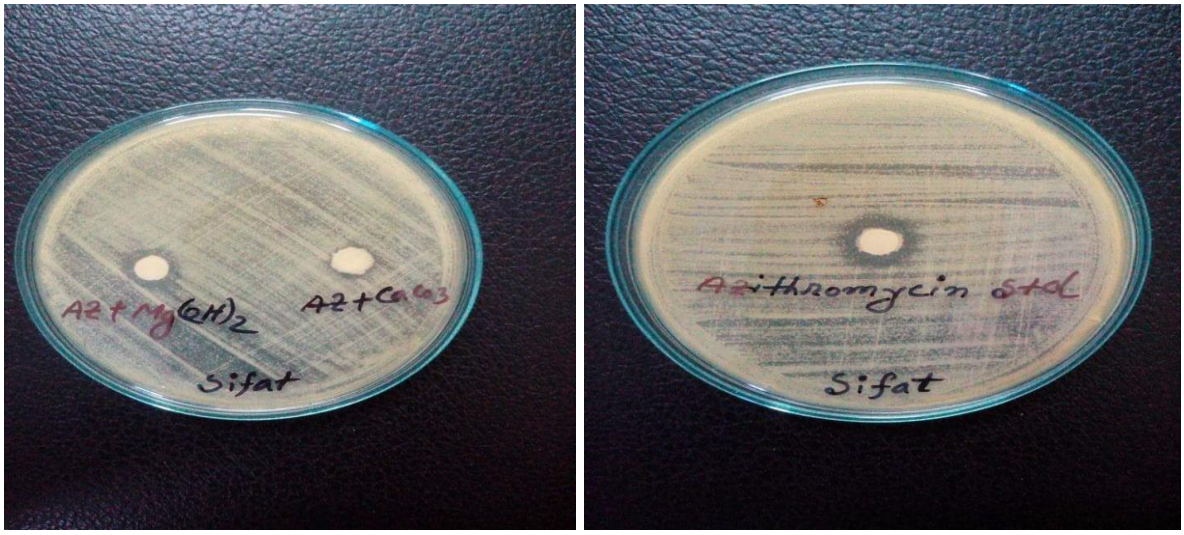

Figure 5: *Comparative study by zone of inhibition of Azithromycin with different antacids.*

CONCLUSION

It has been observed that Azithromycin gives a sharp peak at 235nm. When zinc sulfate, magnesium hydroxide and calcium carbonate were mixed with azithromycin at ratio 1:1, the intensity of the peak changes remarkably. Absorption characteristics altered due to the interaction but the position of the compound do not shift. Job's plot has given the molar ratio of complexes of azithromycin with antacids and metal. At pH 7.4 Azithromycin forms strong complexes with zinc sulfate, magnesium hydroxide and calcium carbonate and '∧' shape curved was obtained. The antibacterial screening of were done by measuring the zone of inhibition. In this study the zone of inhibition decreased remarkably. Which indicated the interaction of Azithromycin with metal and antacids. By antimicrobial investigation it was observed that the zone of inhibition of the drug Azithromycin with Mg Zn and Ca reduced from 14 mm to 9 mm, 8 mm and 4 mm respectively.

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