Original Article

Combinatorial Combination Synergism Of Augmentin And Diclofenac Dosage Forms Against Community And Methicillin Resistant Staphylococcus Aureus

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Abstract

One of the approaches of combinatorial chemistry is to get new derivatives help in human health well-being. Treatment of the infectious disease via fighting the responsible pathogenic bacteria is one of combinatorial chemistry targets. The rate of resistance of Staphylococcus aureus bacterial strains to the most common antibiotics is increasing massively. This study aimed to assess the synergistic effect of a combination between the most common used non-steroidal anti-inflammatory agents and antibiotic in Al-Bayda city against pathogenic Gram-negative bacteria. In this study, Diclofenac Sodium 50mg and Diclofenac Potassium 50mg separately and in combination with Amoxicillin & Clavulanic Acid 625mg were tested for if they have any synergistic effect against both community Staphylococcus aureus; Methicillin sensitive Staphylococcus aureus and Methicillin resistant Staphylococcus aureus (MRSA). The Well Diffusion Method was used to assess the antibacterial activity. The results showed that the the different combination between Amoxicillin & Clavulanic Acid 625mg + Diclofenac Potassium 50mg + Diclofenac Sodium 50mg exhibited more inhibitory potency against both tested bacteria. Diclofenac Sodium 50mg showed higher synergistic effect against Methicillin resistant Staphylococcus aureus (MRSA). In conclusion, this study proved that combinations of Augmentin with the tested non-steroidal drugs showed clear inhibitory activity on the tested community Staphylococcus aureus and Methicillin resistant Staphylococcus aureus compared to the inhibition activity showed from the tested antibiotics alone.

Keywords: Combinatorial combination, NSAIDs, Augmentin, MRSA, Synergism

INTRODUCTION

Combinatorial chemistry is concerned with the synthesis of multiple compounds at same time zone via a collection of methods and techniques. In the last few years, more new compounds have been produced by this new technology compared with that have been produced by the traditional pharmaceutical industry since its starting date. This make the use of the traditional chemistry goes back, where the chemists have been directed by this technology to avoid thinking in direction of synthesizing a well identified single drugs, but directed them in term of simultaneously producing large scales of compounds. One of the approaches of using this technique is to get new derivatives help in human health wellbeing, such as in treatment of the infectious disease via fighting the responsible pathogens, such as bacteria either by direct inhibition or by synergistic action added to another combined drug.

Although, non-steroidal anti-inflammatory drugs (NSAIDs) are not antibiotics, but they are reported to have antibacterial potential (Hersch et al., 1991). Diclofenac is a drug belong to NSAIDs group. It is widely used as analgesic and anti-inflammatory agent, and act by inhibiting the cyclooxygenase (COX) enzymes which are essential substances in the process of prostaglandin biosynthesis (Vane et. al., 1998, Ricciotti et.al., 2011). This drug has been

documented to possess antibacterial properties (Annadurai et.al., 1998), where it has been reported to showed antibacterial performance against some bacterial species belong to both Gram-positive and Gram-negative bacteria (Mazumdar et. al., 2009). The NSAIDs activity is relied mainly on its ability to reduce the production of pro-inflammatory mediators, the action which is thought to be stand behind their therapeutic actions in treatment of infections caused by multi-drug resistant (MDR) bacteria. The problem of the massive increasing rate of bacterial resistance to antibiotics could be addressed a combination treatment between NSAIDs and antibiotics, in order to enhance the antibiotics activity (E. Farouk et.,, al 2017, Nakka et, al., 2011).

Materials and Methods

This study aimed to evaluate the effectiveness of NSAIDs in combination with antibiotics against two strains of Gram-positive bacteria; In order to find out the most prescribed drugs of the targeted medications, this study was supported by a questionnaire form, designed by the study researchers which was distributed to most of private pharmacies in Al-Bayda city, Libya

Tested Drugs

Antibiotic used in this study was Augmentin 625 (AUGMENTIN BID 625 mg Film Tablet), while the NSAIDs used were Diclofenac Sodium under the brand name of DIKLORON 50mg Enteric Film Coated Tablet, and Diclofenac Potassium under the brand name of RONFENAC-P 50mg Film Coated Tablet.

Tested Bacteria:

Two isolates of Gram stain positive bacteria were included in this study; Methicillin resistant Staphylococcus aureus (MRSA), and community Staphylococcus aureus (Non-Methicillin Staphylococcus aureus). Both bacteria were obtained from the laboratory of microbiology at Faculty of Pharmacy, University of Omar Al-Mukhtar, Libya.

Preparation of drug sample:

On the day of assay on tablet of each of the tested Augmentin (625mg), Diclofenac sodium (50mg), and of Diclofenac potassium was dissolved in 1 ml of sterilized distilled water, agitated well until completely dissolved.

Assay of combinational effect of NSAIDs with Amoxicillin + Clavulanic acid dosage:

25 ul of the tested Augmentin dosage form was mixed with 25 ul of Diclofenac sodium solution and poured into duplicate wells of two Muller-Hinton medium plates, which their surfaces were previously streaked separately with both tested bacteria. The same procedures were done for Diclofenac potassium. All plates were incubated at 37C for 24 hours. After 24 hours the inhibition zones were measured in (mm) unit. Same protocol was used for the combination of the three tested drugs (Augmentin + Diclofenac sodium + Diclofenac potassium), and for a combination between the two NSAIDs used in this study (Diclofenac sodium + Diclofenac potassium).

Results:

This study via its questionnaire revealed that the concentration of 625 mg is the most frequently prescribed and used one of the Amoxicillin + Clavulanic acid dosage form in Al-Bayda city, Libya. Also, the questionnaire's output showed that among the used NSAIDs, Diclofenac sodium (50mg), and of Diclofenac potassium(50mg) appeared the most commonly used ones (Fig. 1-3).

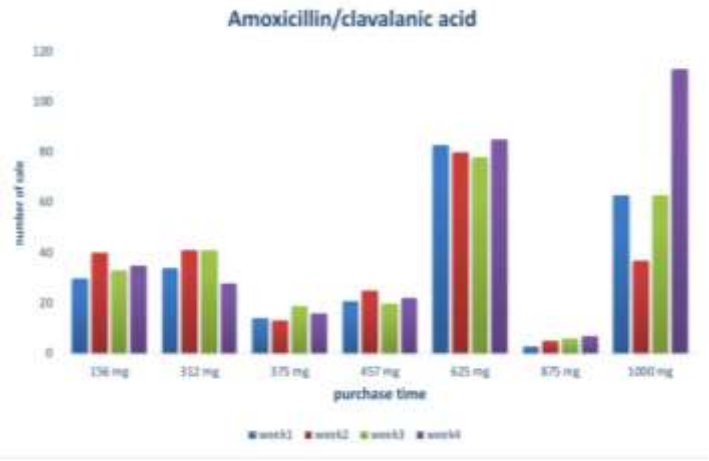

Fig .1. Number of Sale of Amoxicillin + Clavulanic acid.

Fig .2. Number of Sale of Diclofenac sodium

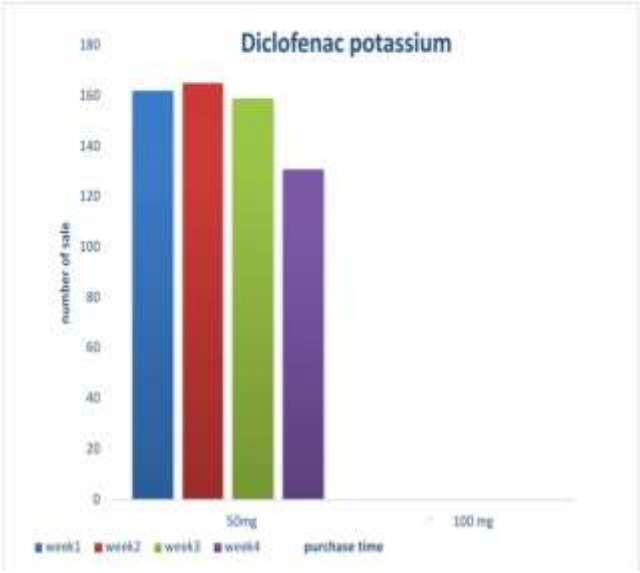

Fig .3. Number of Sale of Diclofenac potassium.

The antibacterial assay done in this study targeted to investigate the antibacterial activity of the tested antibiotic and NSAIDs against the tested Gram-positive bacteria, and the results showed that the tested Amoxicillin + Clavulanic acid 625mg showed very active performance against both Community Staphylococcus aureus and Methicillin resistant Staphylococcus aureus with mean of inhibition zones of means of 40mm and 31mm, respectively (Table 1, Photo 1 & 2).

Photo (1): Antibacterial Activity of Augmentin 625mg against community Staphylococcus aureus.

Photo (2): Antibacterial Augmentin 625mg against Methicillin resistant Staphylococcus aureus (MRSA).

Table 1: Antibacterial Activity of Tested Drugs onSensitive staphylococcus aureus and Methicillin resistant Staphylococcus aureus (MRSA).

Tested Drugs	Mean of Diameter of Inhibition Zones (IZ) in Millimeter Against Tested Bacteria	
	Sensitive staphylococcus aureu	Methicillin resistant Staphylococcusaureus (MRSA)

Amoxicillin& Clavulanic Acid 625mg	40mm	31mm
Diclofenac Potassium50mg+Amoxicillin & Clavulanic Acid 625mg	41mm	34mm
Diclofenac Sodium 50mg +Amoxicillin & Clavulanic Acid 625mg	38 mm	44 mm
Diclofenac Sodium 50mg +Amoxicillin & Clavulanic Acid 625mg +Diclofenac Potassium 50mg	42mm	37 mm
Diclofenac Sodium 50mg +Potassium Diclofenac 50mg	34 mm	32 mm

In a next step the antibacterial activity of a combination between both Amoxicillin + Clavulanic acid 625mg and Diclofenac potassium against both tested bacteria was investigated and the results showed growth inhibition zones of means of 41mm and 38mm against Community Staphylococcus aureus and Methicillin resistant Staphylococcus aureus, respectively (Table 1, Photo 3 &4).

Photo (3): Antibacterial Activity of Diclofenac Potassium 50mg + Augmentin 625mg against community Staphylococcus aureus

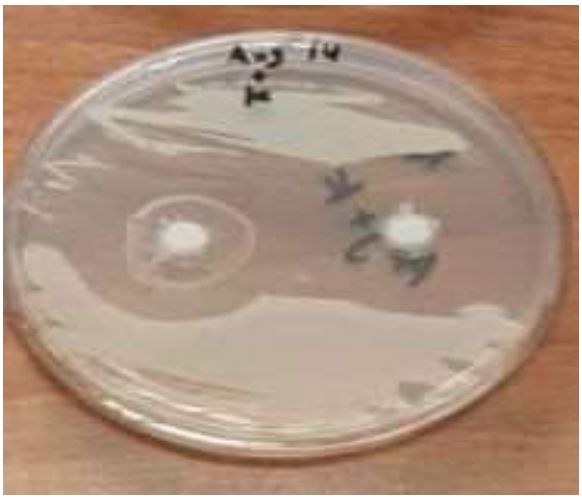

Photo (4): Antibacterial Activity of Diclofenac Potassium 50mg + Augmentin 625mg against Methicillin resistant Staphylococcus aureus (MRSA).

Furthermore, this study results proved that the combination between both Amoxicillin + Clavulanic acid 625mg and Diclofenac sodium has good activity against both tested bacteria which revealed growth of inhibition zones of means of 38mm against Community Staphylococcus aureus and 44mm against Methicillin resistant Staphylococcus aureus (Table 1, Photo 5 & 6).

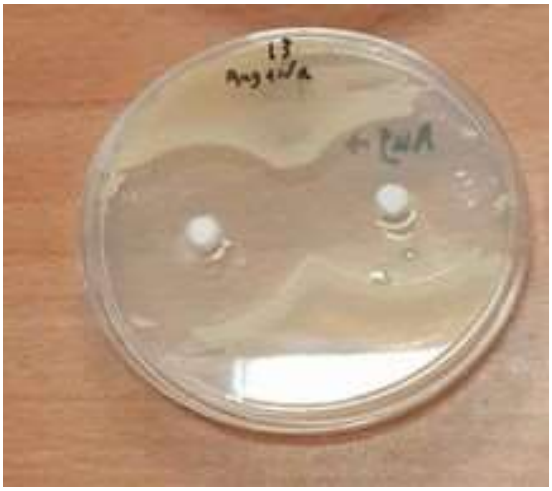

Photo (5): Antibacterial Activity of Diclofenac Sodium 50mg + Augmentin 625mg against sensitive Staphylococcus aureus.

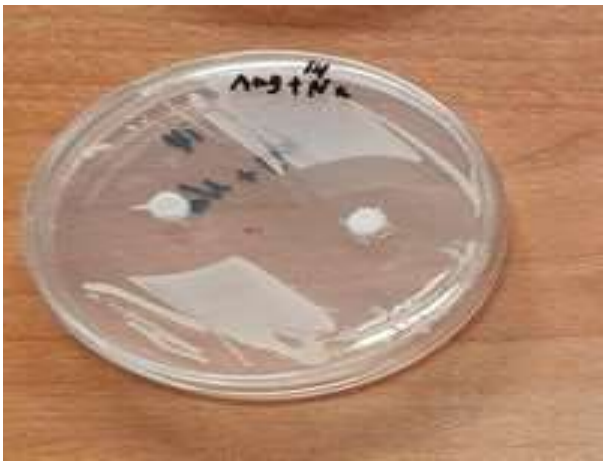

Photo (6): Antibacterial Activity of Diclofenac Sodium 50mg + Augmentin 625mg against Methicillin resistant Staphylococcus aureus (MRSA).

When this study tested the antibacterial performance of combination of the three tested drugs; Amoxicillin + Clavulanic acid 625mg, Diclofenac potassium, and Diclofenac sodium together, the results showed that this combination revealed inhibition zones of means of 42mm against Community Staphylococcus aureus and 37mm against Methicillin resistant Staphylococcus aureus (Table 1, Photo 7&8).

Photo (7): Antibacterial Activity of Augmentin 625mg + Diclofenac Sodium 50mg + Diclofenac Potassium 50mg against community Staphylococcus aureus

Photo (8): Antibacterial Activity of Augmentin 625mg + Diclofenac Sodium 50mg + Diclofenac Potassium 50mg against Methicillin resistant Staphylococcus aureus (MRSA).

In addition, and in sense of the good activity shown by the above different combinations between the tested antibiotic and two tested NSAIDs, this study assessed the antibacterial activity of the combination of both used non-steroidal anti-inflammatory agents; Diclofenac potassium, and Diclofenac sodium, and the results cleared that this combination revealed inhibition zones of means of 34mm against Community Staphylococcus aureus and 32mm against Methicillin resistant Staphylococcus aureus (Table 1, Photo 9 & 10).

Photo (9): Antibacterial Activity of Diclofenac Sodium 50mg + Diclofenac Potassium 50mg against community staphylococcus aureus

Photo (10): Antibacterial Activity of Diclofenac Sodium 50mg + Diclofenac Potassium 50mg against Methicillin resistant Staphylococcus aureus (MRSA).

Discussion:

Up to date of this study, no study found done to determine the synergistic effects of combinatorial different combinations of Diclofenac Sodium 50mg and Potassium Diclofenac 50mg with Amoxicillin & Clavulanic Acid 625mg against Staphylococcus aureus and Methicillin resistant Staphylococcus aureus.

The combinatorial approach to different drugs is considered a new area of research methodology that targets the improvement of the available drugs and discovering of new ones. It is well documented that Augmentin (amoxicillin/Clavulanate potassium), Diclofenac sodium, and Diclofenac potassium are belong to different pharmaceutical chemical groups, where the first is an antibiotic while the second and third are belong to the non-steroidal anti-inflammatory drugs. On other hand, with concept of drug improvement, it is well illustrated that the combinatorial chemistry is a protocol used in the pharmaceutical industries in purpose to get a new compounds derivatives have better properties and higher good activity.

Since the combinatorial approach to different drugs is considered a new area of research methodology that targets the improvement of the available drugs and discovering of new ones, this study was interested to know if there is any synergistic effect between the above mentioned drugs, and it revealed an interesting evidence where it proved that the combination of Amoxicillin & Clavulanic Acid 625mg and Diclofenac Sodium 50mg and Potassium Diclofenac 50mg have a good synergistic effects on Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus, where it clarified that the antibacterial activity of antibiotic + NSAIDs combination is stronger than that given from Amoxicillin & Clavulanic Acid 625m alone .These findings point to the possible adjuvant use of antibiotics and NSAIDs in the treatment of MRSA infections.

The Augmentin (amoxicillin /clavulanate potassium), which is a combination between an antibiotic (Amoxicillin), and an inhibitor of the resistance B-lactamase enzyme (clavulanate potassium), is a successful example of the antibiotic-adjuvant method. This combination made it possible to treat illnesses caused by bacteria that had become resistant to -lactam antibiotics with amoxicillin (Z Yin et, al 2014).

There is a pressing medical need for the achievement of new antibacterial agents that have the ability to treat infections caused by bacteria that resist the commonly used antibiotics. The Methicillin-resistant Staphylococcus aureus (MRSA) type of the Staphylococcus aureus bacteria strains that considered a multi-drug resistant bacterium that exhibited high level of resistance to the common β-lactam antibiotics group, and recently started developing resistance to other antibiotics groups in addition to the beta-lactam ones. This bacterium; MRSA, becomes a serious problem in hospitals, and nowadays it becomes an alarming community pathogen and the rate of occurrence of infectious disease caused by this bacterial species are becoming a serious problem (Hersch et al., 2010; Wang et, al 2011).

The good antibacterial activities showed in this study from both Augmentin and NSAIDs resulted in a result in agreement with that reported by Jamil and his team, 2017. However, in sense of Gram-positive bacteria, and regarding the good activity revealed in this study by diclofenac sodium against tested bacteria, Salem-Milani et al., 2013, documented that a pronounced antibacterial activity had shown from diclofenac sodium against tested Gram-positive Entero faecalis bacterium, while this study has tested Community Staphylococcus aureus and MRSA instead. Even though both studies investigated different bacterial genera, both were belonging to Gram-positive bacteria and proved good activity. The results that pointed to a hypothesis says that this drug; Diclofenac sodium has a specific selective activity against Gram-positive bacteria.

With the work in the concept of combinatorial chemistry in this study via the investigation of the different drug combinations, the enhancement antibacterial activity revealed against both Community Staphylococcus aureus and MRSA bacteria was clear. With this revealed good antibacterial performance with the combination of tested drugs, despite the difference in the chemical structures of NSAIDs; Diclofenac Sodium and Potassium Diclofenac in front of the tested beat-lactam antibiotic; Augmentin, the pathways these NSAIDs path to reveal this good activity upon combination push this study to suggest that there is a probability of arising of a novel structure have good synergistic activity against tested MRSA bacterium. However, the molecular basis of the synergism of Diclofenac Sodium and Potassium Diclofenac requires further studies.

In the same context with different medications used, Chan, 2017, proved that the combination of the NSAIDs; Ibuprofen/Aspirin with Chloramphenicol provided synergistic action against MRSA and displayed an additive effect against Community Staphylococcus aureus.

Even though the mechanism of action is unclear, but this result is considered as a promising output, where it introduces a regimen help to treat infections caused by Staphylococcus aureus species, particularly Methicillin resistant Staphylococcus aureus, that considered as a concern in hospitals, where it can attack those most vulnerable older adults and people with weakened immune systems and may cause serious infection.

Conclusion:

This study conclude that the combinatorial combination of both Diclofenac sodium and Diclofenac potassium has a good synergistic effect with Augmentin against the Gram-positive community Staphylococcus aureus and Methicillin resistant Staphylococcus aureus, and it suggests that this combination could be considered as a good regimen to treat infection caused by these pathogenic bacteria. In addition, this study recommends further studies to figure out the exact chemical reaction happened between the tested antibiotic and NSAIDs agents that stand behind this promising activity.

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