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Negative effect of non-steroidal anti-inflammatory drugs on the antibacterial activity of ciprofloxacin - an *in vitro* study

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Abstract
Ciprofloxacin, a second generation fluoroquinolone is often co-administered with non-steroidal anti-inflammatory drugs (NSAIDs) in life threatening situations in which *Staphylococcus aureus* infections are accompanied with pain and inflammation. This study was carried out to investigate possible *in vitro* interactions in co -administration of ciprofloxacin and some NSAIDs. The study was carried out in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, and University of Benin, Benin City in 2013. The *in vitro* evaluation was carried out by incorporating appropriate concentrations of the NSAIDs with ciprofloxacin in 20 ml final volume of Muller Hinton broth in the presence of *Staphylococcus aureus* and incubated at 37°C in a hot water-bath. Aliquots of 0.1 ml were removed every two hours; serially diluted and plated out on Mannitol Salt agar and incubated at 37ºC for 24 hours. The number of colony-forming units (CFU) of *Staphylococcus aureus* was noted. It was observed that all the NSAIDs reduced the antibacterial activity of ciprofloxacin against *Staphylococcus aureus* in *vitro* to varying degrees. The highest reduction was exhibited by acetyl salicylic acid (ASA) as it yielded the highest number of CFU. This was followed closely by piroxicam, then indomethacin and lastly paracetamol (130 cfu; 65 cfu; 43 cfu; and 33 cfu.) respectively. There is a suggestion that the studied NSAIDs and perhaps NSAIDs generally should not be co-administered with ciprofloxacin at the same time.

Keywords: Ciprofloxacin; NSAIDs; Interaction.

INTRODUCTION

Diseases caused by *Staphylococcus aureus* are protean: from minor skin infections such as pimples and impetigo; conjunctivitis in neonates, and carbuncles, to life-threatening situations such as pneumonia and toxic shock syndrome. This organism invades skin, soft tissues, respiratory tract, bone joints, endovascular and wound infections (Giordano *et al.,* 2007). Ciprofloxacin a second generation quinolone has been found to appear safe and effective for a wide variety of clinical infections both *in vitro* and in animal studies. Like other fluoroquinolones, it is a class of highly potent, orally active broad spectrum antibacterial (Galante *et al.,* 1986),) effective against both Gram positive and Gram negative bacteria. It has been found useful in the management of skin and soft tissue infections. Like other fluoroquinolones, it acts on DNA, preventing super coiling by acting on topoisomerases II and IV. They both resolve topological constraints resulting from DNA replication and function (Drlica, 1997). Like other fluoroquinolones, it forms a tertiary complex with DNA gyrase and DNA resulting in the

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inhibition of the DNA activities in bacteria (Drlica, 1999).

The non-steroidal anti-inflammatory drugs (NSAIDs) are among the oldest and most widely used drug in human history. The ancient Greeks chewed the bark of the willow tree to alleviate pain and fever. Salicin which is metabolized to salicylate was isolated from the willow bark (Gupta and Dubois, 2001). Acetylsalicylic acid was synthesized at the end of the 19th century and marketed for its anti-pyretic, anti-inflammatory and analgesic properties. Other compounds with similar properties as the salicylates were discovered and were collectively named non-steroidal anti-inflammatory drugs, (NSAIDS) distinguishing them from the steroids. The most prominent NSAIDS include: Acetylsalicylic acid, (ASA, Aspirin) Piroxicam, Indomethacin, Ibuprofen, mafenamic acid, celecoxib, Diflunsal and Paracetamol.

They are generally indicated for the symptomatic relief of rheumatoid arthritis, osteoarthritis, inflammatory osteoarthropathy, ankylosing spondylitis, psoriatic arthritis, acute gout, bone pains, migraine and post-operative pains and headache (Gotzsche, 1989). Indomethacin is given to neonates whose *ductus arteriosus* do not close within 24 hours of birth (Sekar and Corff, 2008). Aspirin is used in the management of arterial thrombosis and in the prevention of cardiovascular effects of NSATDS. Most NSAIDS act as non-selective inhibitors of Cyclooxygenases which catalyse the formation of prostaglandins and thromboxane from arachidonic acid.

Quinolones and NSAIDS are known to interact with other medications. Concurrent use of an NSAID and quinolone including ciprofloxacin for instance, may increase the risk of quinolone' adverse central nervous system effects including seizures (Aronoff and Nelson, 2008; Koeberle and Werz, 2009; Akberpour *et al.,* 1985) in established *Staphylococcus aureus* infections. There is paucity of information on the effects of concomitant administration of the quinolone and NSAIDS in the management of *Staphylococcus aureus* infections. The aim of this study is to evaluate the suitability of co-administration of ciprofloxacin and these NSAIDS (ASA, indomethacin, piroxicam and paracetamol) *in vitro*.

EXPERIMENTAL

The stages involved in this *in vitro* study were as follows:

Authentication of organism. The *Staphylococcus aureus* used in this study was a wound isolate from the swab bench of the Department of Medical Microbiology of University of Benin Teaching Hospital, Benin City. The organism was grown on Mannitol Salt agar, and later subjected to Gram staining, physiological and biochemical test procedures as recommended by Cheesbrough (2004).

Preparation of organism. The organism was inoculated into Muller Hinton(MH) broth and incubated over-night at 37^{o} C. The over-night broth culture was doubly diluted with ¼ strength Ringer's solution and matched with McFarland's 0.5 turbidity standard.

Laboratory animals. Swiss albino mice were obtained from the Animal House unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were fed on standard feeds (Bendel Feeds Plc, Ewu, Edo State. Nigeria) and drank University of Benin tap water. The animals were acclimatised for at least two weeks prior to any experiment.

Ethical approval. The experimental protocol and procedures used in this study were approved by the Animal Ethics Committee of the Faculty of Pharmacy, University of Benin.

Preparation of drugs for the experiments. All the concentrations of NSAIDs used in the

in vitro experiments were derived from the growth curve experiments of the *Staphylococcus aureus* in the presence of the different NSAIDs using Spectrophotometer 'T 70' at a wave length of 300 nm. The stock solution of 1mg/ ml of acetyl salicylic acid was prepared by dissolving l0 mg of acetyl salicylic acid powder in 0.2 ml of methanol and making it up to l0 ml with 9.8 ml of sterile distilled water and sterilised by filtration through 0.22 µm Millipore membrane (MM) filter. A stock solution of 500 µg/ml of piroxicam was prepared by dissolving l0 mg of piroxicam powder in 2mls of methanol, diluting with l8mls of sterile distilled water and sterilising using 022 µm MM filter.

A stock solution of 1 mg/ml of indomethacin was prepared by dissolving 10 mg of indomethacin powder in 1 ml of 0.1N $NaHCO_3$ solution and diluting up to 10 ml with 9 ml of sterile distilled water and sterilizing with 0.22 µm MM filter. A stock solution of 5mg/ml of Paracetamol was prepared by dissolving 50 mg paracetamol powder in 2 ml dimethyl sulphoxide and made up to l0 ml with sterile distilled water and was sterilised using a 0.22 µm MM filter. A stock solution of 1 mg/ml of ciprofloxacin was prepared by dissolving 10mg of ciprofloxacin hydrochloride powder in 0.5 ml sterile distilled water, then making up to 10ml with 9.5 ml of sterile distilled water and was sterilised using a 0.22 µm MM filter.

Minimum inhibitory concentration (MIC) of Ciprofloxacin on *Staphylococcus aureus.* The agar dilution method as recommended by the British Society for antimicrobial chemotherapy (BSAC) Working party on antibiotic sensitivity testing (1991) and Andrews (2001) was used. The MIC was evaluated using final concentrations of 0.25 µg/ml, 0.5 µg/ml, 1 µg/ml, 2 µg/ml 4 µg/ml, and 8 µg/ml, of Ciprofloxacin solution respectively in 20mls Mannitol Salt agar (MSA). Each concentration was in triplicate plates of 20 ml of MSA. When the agar medium was set, was set, the plates were dried at 45^{o}C for ten minutes and inoculated by spotting with 0.02 m1 of 10^6 cfu/ml of overnight culture of *Staphylococcus aureus*. The plates were left on the bench for 30 minutes to allow the water content in the inoculum to be absorbed into the agar and were incubated at 37°C and observed for growth of *Staphylococcus aureus* after 24 hours.

***In vitro* growth of *Staphylococcus aureus* (only) in MH broth (CONTROL).** 20 ml sterile MH broth was inoculated with 0.1 ml of 10^8 cfu/ml of overnight broth culture of *Staphylococcus aureus*.in a universal bottle The bottle was set in shaker-water-bath incubator at 37°C for 24 h during which aliquots of 0.l ml were removed at thirty minutes interval for the first two hours and subsequently at two hours intervals, serially diluted in sterile ¼ strength Ringer's solution to obtain $l0^6$cfu/ml from which 0.l ml was taken and plated out in triplicates by spread plate method on MSA plates, incubated at 37°C and observed for growth of *Staphylococcus aureus* after 24 hours and the number of colony-forming units recorded.

***In vitro* effects of the NSAIDs on the activity of ciprofloxacin.**

In vitro effect of acetylsalicylic acid (ASA), indomethacin, piroxicam and paracetamol on antibacterial activity of ciprofloxacin against Staphylococcus aureus. The MIC (0.5 µg/ml) of ciprofloxacin was incorporated with 0.75 µg/ml of acetyl salicylic acid in a final volume of 20 ml of MH broth. The mixture was inoculated with 0. 1 ml of 10^8 cfu/ml of overnight culture of *Staphylococcus aureus*. The bottle was set in shaker-water-bath incubator at 37°C for 24h during which aliquots of 0.1 ml were removed at thirty minutes interval for the first two hours and subsequently at two hours interval, serially diluted in sterile ¼ strength Ringer's solution

to obtain 10^6 cfu/ml from which 0.1 ml was taken and plated out in triplicates by spread plate method on MSA plates, incubated at 37°C and observed for growth of *Staphylococcus aureus* after 24 hours and the number of colony-forming units recorded. This procedure was followed very strictly in similar interaction studies of ciprofloxacin (0.5 µg/ml) and indomethacin (150 µg/ml), piroxicam (150 µg/ml) and paracetamol (5 mg/ml) respectively.

RESULTS

Authentication of *Staphylococcus aureus*. The Gram staining procedure yielded Gram-positive cocci in clusters. When grown on Mannitol Salt agar, it produced golden yellow colonies which released gas when mixed with 3% hydrogen peroxide. When it was macerated with a loop-full of diluted human plasma on the slide, it agglutinated. In a tube coagulase test formed a large clump (Table 1). The MIC of ciprofloxacin against *Staphylococcus aureus* is 0.5 µg/ml (Table 2).

***In vitro* effect of ciprofloxacin on *Staphylococcus aureus* compared with growth of *Staphylococcus aureus* in MH broth.** The growth of Staphylococcus aureus in broth with and without ciprofloxacin is shown in Fig. 1 The effect of ciprofloxacin in restricting the growth of the bacterium is observed until the 12th hour when there was gradual and steady rise in the number of CFU. On the other hand, the tube without ciprofloxacin showed optimum growth of the *Staphylococcus aureus.*

The *in vitro* effect of acetylsalicylic acid on the activity of ciprofloxacin on the growth of *Staphylococcus aureus.* The *Staphylococcus aureus* was exposed to the minimum inhibitory concentration of ciprofloxacin (0.5 µg/ml), in the presence of 75µg/ml. Acetyl salicylic acid. There was no significant growth in the tube containing 0.5 µg/ml ciprofloxacin and the *Staphylococcus aureus* until the 12th hour when the number of cfu increased rapidly. (Fig. 2). At the same time, it was observed that there was an increase in the number of cfu in the tube containing *Staphylococcus aureus*, ciprofloxacin (0.5 µg/ml) and acetyl salicylic acid (75µg/ml).

The *in vitro* effect of indomethacin on the action of ciprofloxacin against growth of *Staphylococcus aureus* is shown in Fig. 3. There was an initial decline in the number of colony-forming units up to the 30th minute of the experiment in the tubes containing organism alone, organism and indomethacin, while in the tube containing combination of organism, indomethacin and ciprofloxacin there was increased and significant growth was observed at the hour. At the same time, growth of the organism in the tube containing ciprofloxacin, organism and indomethacin remained low (low number of colony-forming units) until the 12th hour when there was a rapid increase in the number of colony-forming units as shown in Fig. 3.

The *in vitro* effect of piroxicam on the antibacterial action of ciprofloxacin against *Staphylococcus aureus* is represented in Fig. 4. There was a low count in the colony-forming units within thirty minutes of the experiment. The tube containing organism alone showed a steady increase in the colony-forming unit. The combination of piroxicam organism showed an initial low number of colony-forming units up to the second hour. The tube containing only ciprofloxacin and organism remained low in the number of colony-forming units up to the 12th hour when there was steady increase in the number of colony-forming units to the 24th hour.

The *in vitro* effect of paracetamol on the anti-bacterial action of ciprofloxacin on the growth of *Staphylococcus aureus* is represented in Fig. 5. The number of colony-forming units resulting from the combination of ciprofloxacin and paracetamol remained low until after the 12th hour. When compared

with organism alone, the paracetamol-ciprofloxacin number of colony-forming units was much lower.

DISCUSSION

The results of the *in vitro* effects of the NSAIDs on the action of ciprofloxacin against *Staphylococcus aureus* showed that all the NSAIDs impaired the effect of ciprofloxacin by reducing its antibacterial action against *Staphylococcus aureus.* The in *vitro* study revealed that acetyl salicylic acid had the greatest effect on the antibacterial action of ciprofloxacin, impairing its antibiotic activity against *Staphylococcus aureus* giving rise to the highest number of colony-forming units of *Staph, aureus*. This was followed by piroxicam, indomethacin and paracetamol respectively in descending order of activity.

The in vitro studies of inhibitory effect of salicylic acid and acetylsalicylic acid to the growth and sporulation of some plant pathogenic bacteria were evaluated by Nehal S. El-Mongy (2002). He found them to show more inhibitory effect against bacteria than even streptomycin sulphate and suggested they could be used as alternative antimicrobial substances which can be manipulated to improve antifungal and antibacterial substances.

Table 1. Authentication of *Staphylococcus aureus*

Procedure	Result
Gram stain	+
Mannitol (fermentation)	+
Catalase	+
Coagulase (Slide)	+
Coagulate (Tube)	+
Sucrose (fermentation)	+
Glucose (fermentation)	+

+ = Positive

Table 2. The Minimum inhibitory concentration of ciprofloxacin against *Staphylococcus aureus* was determined as 0.5µg/ml.

Organism	Concentrations of ciprofloxacin (µg/ml)					
	0.25	0.5	1.0	2.0	4.0	8.0
Staphylococcus aureus	+	-	-	-	-	-

+ = Growth. - = No Growth

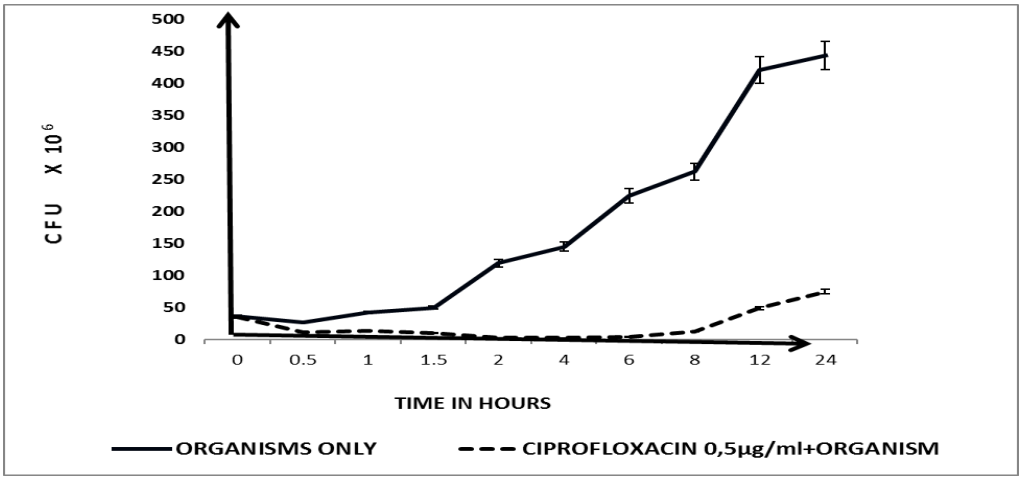

Fig. 1. *In vitro* interaction of ciprofloxacin with *staphylococcus aureus* (Value are given as Mean ±SEM)

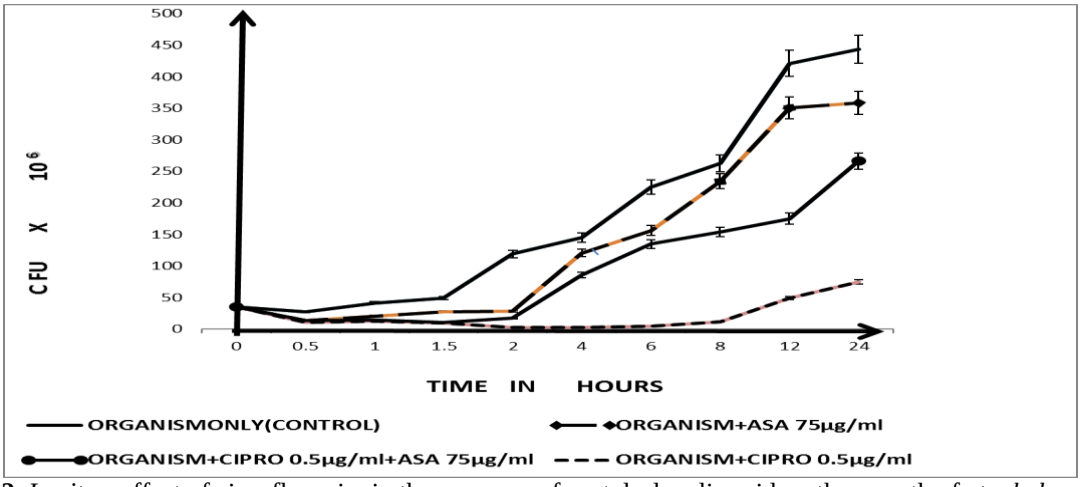

Fig. 2. *In vitro* effect of ciprofloxacin in the presence of acetylsalycylic acid on the growth of *staphylococcus aureus* (Value are given as Mean ±SEM)

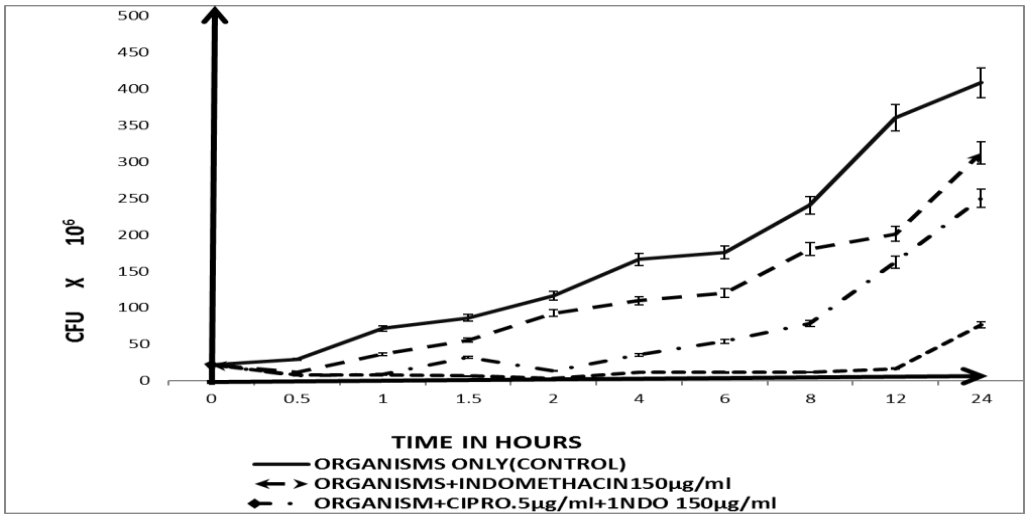

Fig. 3: *In vitro* effect of ciprofloxacin in the presence of Indomethacin on the growth of *Staphylococcus aureus* (value are given as Mean ± SEM)

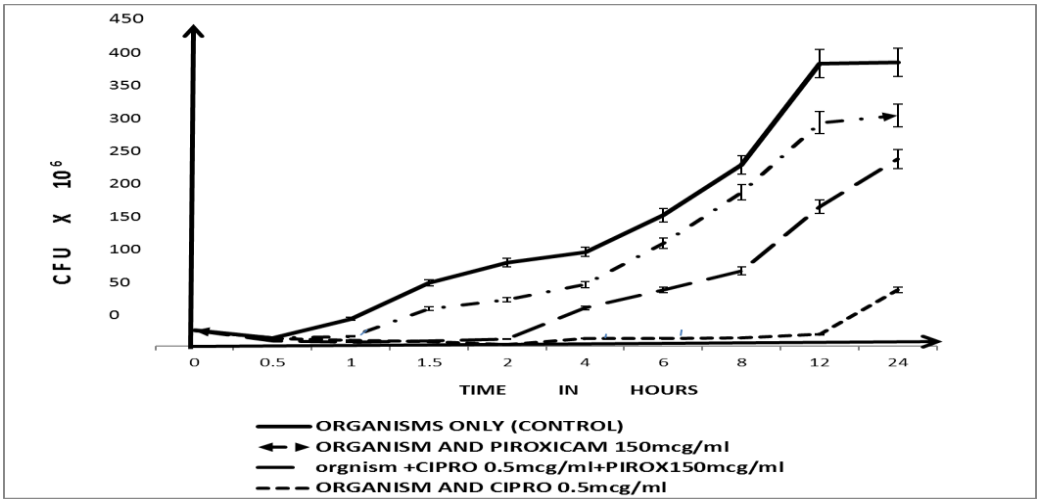

Fig. 4. *In vitro* effect of ciprofloxacin in the presence of piroxicam on the growth of *staphylococcus aureus* (Values are given as Mean ±SEM)

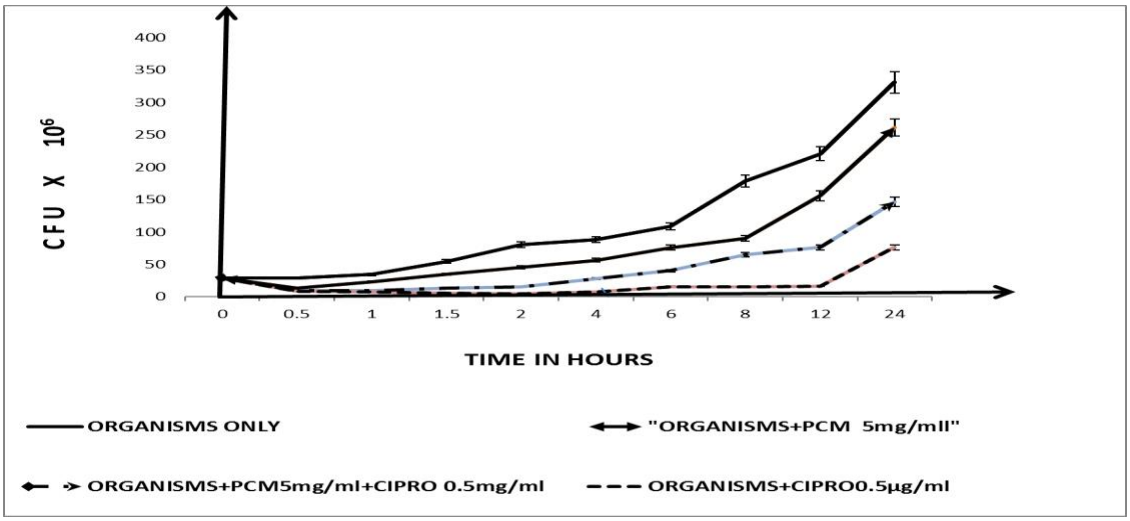

Fig. 5. *In vitro* effect of ciprofloxacin in the presence of paracetamol on the growth of *staphylococcus aureus* (Value are given as Mean ±SEM)

Kupferwasser *et al.* (2002), in their work demonstrated that acetylsalicylic acid reduces vegetation bacterial density, hematogenous bacterial dissemination, and frequency of embolic events in experimental *Staphylococcus aureus* endocarditis through antiplatelet and antibacterial effects. A mechanism, probably similar to that in acetyl salicylic acid, might be in operation in the other NSAIDs used in this study.

The effect of acetyl salicylic acid (75µg/ml) on the MIC of ciprofloxacin (0.5µg/ml) on *Staphylococcus aureus* in which there was a marked increase in the number of colony-forming units inferred reduced activity of ciprofloxacin against the *Staphylococcus aureus*. This finding appears to agree with an earlier work by Gustafson *et al.,* (1999). They demonstrated in their in *vitro* work that growth of *Staphylococcus aureus* in the presence of salicylate induced resistance to strains susceptible to fluoroquinolone and increased resistance to fluoroquinolone in resistant strains. In their work however, salicylate was added to already grown cultures of *Staphylococcus aureus* resulting in what was described as many fold increase or decrease in growth with probable transformation of the organisms. In this study acetyl salicylic acid (or the other NSAID) was added to ciprofloxacin before inoculation with *Staphylococcus aureus* in the *in vitro* experiments. This allowed for a uniform interaction of the NSAID with both the ciprofloxacin and the organism. Moreover, the incubation here was for 24 hours only.

Fluoroquinolones form a ternary complex with DNA gyrase and DNA activities in bacteria (Drlica, 1999). Some fluoroquinolones have higher affinity for topoisomerase iv than DNA gyrase This dual inhibition pathway is responsible for the lethality of fluoroquinolones to different bacteria. For example, the primary target of fluoroquinolones in *Staphylococcus aureus* and *Streptococcus pneumonia* is topoisomerase iv (Cooke *et al.,* 1996, and Claridge *et al.,* 1997). The 4-keto-3carboxylic acid moiety of the fluoroquinolones constitutes the active site of the quinolones. Ionization of the fluoroquinolones by the loss of H^+ makes the molecule capable of interacting with any in-coming entity. Thus the NSAIDs may bind to this active site of the fluoroquinolone rendering it inactive and hence it cannot inactivate DNA gyrase and topoisomerases IV in the bacterium. At the

same time the NSAIDs are known to have low pKa (3-5) which may affect the physico-chemical nature of the fluoroquinolones (like the ionization state) which will affect its solubility and hence its bioavailability. Thus lethal concentration levels may not be attained.

There is a suggestion that the studied NSAIDs and perhaps NSAIDs generally should not be co-administered with ciprofloxacin at the same time.

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REFERENCES

Akbarpour, F., Afrasiabi A., and Vaziri N. (1985). "Severe Hyperkalaemia caused by indomethacin and Potassium supplementation". *South Med Journal* **78** (6) 756-7.

Andrews, MJ. (2001) Determination of Minimum inhibitory concentrations. *Journal of antimicrobial Chemotherapy.* **48** supp **S1** 5-16.

Aronoff D M, Neilson E G (2001) "Antipyretics: mechanisms of action and clinical use in fever suppression". *Am. J. Med* **111** (4):304-15.

Cheesbrough, M. (2004). "District Laboratory Practice in Tropical Countries". Part 2 *LP* Edition. *Circulation.; **105:746**-75.*

Claridge, J E and Wessfield AS (1987) Enterobacteriacae infection, In: Wentworth BB, Baselski VS, Doen GV, Eds. Diagnostic procedures for bacterial infections 7th Ed. *American public Health Association Washington D C pp233*-296.

Cooke, C. Sklar, G Nappin, J. (1990). Possible pharmacokinetic interactions with quinidine. Ciprofloxacin or metronidazole? Annals Pharmacother. **30**:364-366.

Drlica, K (1999). Mechanisms of Fluoroquinolone action. Cua. Opin. Microbiol. 2: 377-392.

Drlica, K, Zhao X.K. (1997). "DNA gyrase/topoisomerase IV, and the 4 - quinolones". *Microbial. Mol. Biol. Review.* **61**(3): 377-92.

Galante D, Esposito S, Barba D, Giusti G. (1986), Ciprofloxacin in the treatment of urinary and respiratory tract infections in patients with chronic liver disease. Chemioterapia. **5**(5):322-6.

Giordano, P, Weber K., Gesing G., and Kubert J. (2007). Skin and Skin Structure Infections: "Treatment with newer generation Fluoroquinolones". *Therapeutics and clinical Risk management* **3**(2) 309-3 17.

Gotzsche, Pc (1989). "Methodology and overt and hidden bias in reports of 196 double blind trials of no steroidal anti-inflammatory drugs in rheumatoid arthritis". *Controlled clinical trials* **10**(1): 3 1-56.

Gupta, RA and Dubois RN (2001). Colorectal cancer prevention and treatment by inhibition of cyclooxygenase 2, Nature *reviews cancer*, **1** 11-21.89.

Gustafson, J E, Candelaria P V, Fisher SA, Goodridge P J, Lichocck M T, MacWlliams M EU, Price T C, O'Brirn G F, and Grubb B W. (1999). Growth in the presence of Salicylate increases fluoroquinolone resistance in *Staphylococcus aureus*: *Antimicrobial agents and chemotherapy* vol. **43** No 4 p 990-992.

Koeberle, A, Werz 0 (2009). "Inhibitors of the microsomal prostaglandin E (2) synthase-1 as alternative to non-steroidal anti-inflammatory drugs (NSAIDs)-a critical review". *Curr Med. Chem.* **16** (32):4274-96.

Kupferwasser L I, Yeaman M R, Shapiro S M, Nast C, Arnold S. Bayer A S. (2002)

Nehal S. El- Mongy (2002). Egypt J. Phytopathol, **vol 30, 2**, .41-45**.**

Sekar, K C, Corff IE (2008). "Treatment of Patent ductus arteriosus". *Journal of Perinatology.* **28**: 560-562.