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Research Article

Screening of Diclofenac for Antibacterial activity against Pathogenic Microorganisms

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ABSTRACT

Diclofenac 2- (2,6 dichloranilino) phenylacetic acid is a widely prescribed non-steroidal anti inflammatory drug. It is used to reduce pain and treat inflammation in some disorders like rheumatoid arthritis, osteoarthritis, ankylosing spondilytis, gout, dysmenorrhea, spondylarthritis etc., It acts by inhibiting the action of cycloxygenase enzymes COX-1 and COX-2 which are responsible for the synthesis of prostaglandins. Apart from anti-inflammatory property, Diclofenac also has powerful antibacterial potential. This study aims to assess the antibacterial effect of diclofenac against five pathogenic microorganisms *Bacillus subtilis, Staphylococcus aureus, Eschereschia coli, Pseudomonas aeruginosa, Salmonella typhi.* The Agar well diffusion method was adopted to assess the antibacterial activity. The inhibition of bacterial growth was studied taking test drug in three different concentrations 25µg/ml, 50µg/ml and 100µg/ml. The findings of the study indicated the inhibition of growth of bacteria.

Key words: Diclofenac, antibacterial activity, human pathogens, Agar well diffusion method.

INTRODUCTION

Bacteria constitute a large group of prokaryotic microorganisms which are either beneficial or harmful. The harmful bacteria interact negatively with human and other animals. There are about 50 known human bacterial infections that are caused by several pathogens. Antibiotics are used to treat the bacterial infections[1]. In recent times, many bacteria are acquiring resistance to conventional antibiotics[2]. A search for other drugs with antibacterial properties is going on for the treatment of bacterial infections[3]. Non steroidal anti-inflammatory drugs are a class of compounds which also possess antibacterial potential[4].

Diclofenac is a widely prescribed anti inflammatory drug, and was found to possess antibacterial properties[5]. It has exhibited antibacterial action against both Gram positive and Gram negative bacteria[6]. It is a non-steroidal anti-inflammatory drug with analgesic, antipyretic and anti inflammatory properties[7]. It is effective in reducing pain, and treating inflammation in some disorders like rheumatoid arthritis, osteoarthritis, ankylosing spondilytis, gout, dysmenorrhea, spondylarthritis etc,[8]. It acts by inhibiting the cycloxygenase enzymes that are essential in the biosynthesis of prostaglandins[9]. By blocking the effect of COX enzymes it helps in the less production of prostaglandins reducing pain and inflammation.[10]

Diclofenac is 2-(2,6 dichloranilino phenylacetic acid) with the molecular formula $C_{14}H_{11}Cl_2NO_2$ and molecular weight of 318.13. It is almost white crystalline powder which is hygroscopic. It is sparingly soluble in water and freely solube in organic solvents as methanol and butanol. It is a stable compound with the melting point 288-290^{o} C.

MATERIALS AND METHODS

Diclofenac sodium was obtained from Sara Exports Limited, Ghaziabad, U.P., India. The bacterial strains were obtained from IMTEC, Pune. Five different strains of bacteria were selected for the antibacterial activity. Two of them were Gram positive bacteria - *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96) and three were Gram negative

bacteria *Escherischia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) and *Salmonella typhi* (MTCC 98). All the bacterial strains were maintained in the nutrient agar medium and sub cultured once in every two week. Later the bacterial strain was inoculated into the tubes containing sterilized medium. They were incubated at 37°C for 24h and stock culture was maintained. The bacterial inoculums were prepared by transferring a loop full of stock culture to nutrient medium. The tubes were incubated at 37°C for 48hrs before the activity.

The antibacterial activity was conducted by using Agar well diffusion method[11,12]. All the procedures were carried out in total aseptic conditions. The cleaned petriplates and flasks were sterilized in an autoclave for 121°C for 30 min. The sterilized agar medium was prepared and poured into the petriplates. They were allowed to solidify at room temperature. The bacteria were seeded gently on the petriplates using a sterile cotton swab to ensure even distribution of bacteria on the petriplate. Five wells of 4mm diameter and 5mm depth were punched in the agar on each plate using a sterile borer. All the plates were labelled perfectly according to the respective test organism and the concentrations. The test solution was prepared by dissolving the test drug in 10 ml of methanol. Streptomycin was selected as the standard drug for the activity and its stock solution was prepared by weighing accurately. Streptomycin was taken as positive control and Methanol as negative control. Into each petriplate, 30µl of 25µg/ml, 50µg/ml and 100µg/ml concentrations of test solution was added. The positive and negative control solutions were also added in 30µl to the respective wells. The plates were kept undisturbed for 1 hour to allow the diffusion of the solution in the agar medium. Then petriplates were incubated at room temperature in a laminar flow for 24 hours at 37°C. The zone of inhibition around each well was measured with the help of a antibiotic zone reader[13]. All the tests were performed six times to minimize the test error.

RESULTS

All the pathogenic microorganisms tested have shown inhibition of bacterial growth at almost all concentrations of the test drug. *Staphylococcus aureus* has not shown any inhibitory effect at the 25µg/ml concentration but has shown 16mm inhibition at 50µg/l, and 21mm of inhibition at 100µg/l and 15mm of inhibition with the standard drug. *Bacillus subtilis* has shown 18mm of inhibition at 25µg/l, 16mm of inhibition at 50µg/ml, 19mm of inhibition at 100µg/ml and 25mm of inhibition with the standard drug. *Pseudomonas aeruginosa* has shown inhibition of 24mm at 25µg/ml, 25mm at 50µg/ml, 26mm at 100µg/ml and 21mm with the standard drug. *Salmonella typhi* has shown inhibition of 22mm at 25µg/ml, 23 mm at 50µg/ml, 22mm at 100µg/ml and 21mm with the standard drug. *Escherischia coli* has shown 24mm zone of inhibition at 25µg/ml, 25mm at 50µg/ml , 25mm at 100µg/ml and 21mm with the standard drug. Methanol was taken as negative control for all the test pathogens and no zone of inhibition was observed around that well. The mean zone of inhibition is given in the Table1.

DISCUSSION

The antibacterial effect of Diclofenac was reported in various earlier studies. About 397 bacteria were evaluated for in vitro antibacterial activity and the results have shown that most of them were inhibited at 50-100µg/ml concentrations of Diclofenac[5]. Diclofenac has shown significant antibacterial effect in synergism with aminoglyosides both in invitro and invivo studies[14]. Diclofenac has exhibited invitro inhibitory action against 45 different strains of bacteria at 10- 25µg/ml concentration[15]. A study on clinical strains of *Escherischia coli* in hospitals have indicated that diclofenac has shown antibacterial activity against many strains of bacteria from 5-50µg/ml and was effective in treating urinary tract infections[16]. The non –antibiotic drug, Diclofenac was found to protect mice from *Salmonella* infection more effectively when combined with Sterptomycin than used alone[17]. It was bactericidal at 40µg/ml for *Mycobacterium tuberculosis* and along with streptomycin it exerts profound effect in mice[18]. Diclofenac was found to protect from murine listeriosis, tuberculosis, salmonellosis within the maximum dose given to human[6]. Diclofenac administered to female mice against *Listeria monocytogenes* at 2.5µg/ml has reduced bacterial counts in liver and spleen[19]. It has shown inhibitory action at 50µg/ml and bactericidal effect at 100g/ml in *Listeria monocytogenes*[20]. It was found to exhibit noteworthy inhibition of bacterial growth against four strains tested at lower concentrations.[21] A study on *Enterococcus faecalis* was done to evaluate antibacterial effect of diclofenac in comparison with ibuprofen, calcium hydroxide and amoxicillin. The results have depicted significant antibacterial activity of Diclofenac and Ibuprofen at 50µg/ml and above concentrations[22]. A comparative study was conducted on the efficacy of antibacterial action of sodium diclofenac, sodium diclofenac and streptomycin, tri antibiotic and calcium hydroxide against *Enterococcus faecalis* biofilm. The study has inferred that Diclofenac has shown more antibacterial effect than calcium hydroxide suggesting diclofenac sodium as an alternative drug to calcium hydroxide in

root canal therapy[23]. A study on antibacterial activity and anti-biofilm activity of some non-steroidal anti-inflammatory drugs and N-acetyl cysteine was conducted against some uropathogens. The results have depicted that Diclofenac sodium has shown powerful antibacterial action in tested pathogens and highest effect was against *Klebesiella pneumoniae*. It has also shown inhibitory effect on the adherence of *Staphylococcus aureus*, *Klebesiella pneumoniae*, and *Proteus mirabilis* to the plastic surfaces of catheters. Diclofenac has also shown maximum disruptive effect on the mature biofilms formed by test organisms[24]. The inhibition of microorgasnisms was found to be mainly due to the inhibition of DNA synthesis[25] or the disruption of membrane activity[17]. Recent study on some non-steroidal anti-inflammatory drugs has reported that they inhibit *Escherischia coli* sliding clamp that is essential for the DNA replication and repair[26]. Agar well diffusion is the simplest and cost effective standardized test for antibacterial activity[27]. It is easier to perform and the results are obtained quickly. Some of the earlier studies have shown that diclofenac caused inhibition of bacterial growth at 50-100µg/l concentration. In this study, diclofenac has shown zone of inhibition of bacterial growth even at 25µg/l concentration. It is a drug with multiple utilities and can be used for the treatment of various bacterial infections. It was also found to show antibacterial action effectively when used in synergism with other drugs.

CONCLUSION

The emergence of antibiotic resistant bacterial strains with the use of regular antibiotics have revived the search for antibacterial property from drugs of different pharmacological classes. This study reveals the antibacterial potential of Diclofenac sodium which is a non-steroidal anti inflammatory drug. It has shown zone of inhibition of bacterial growth even at less (25µg/ml) cconcentration of the drug in four bacterial strains (*Bacillus subtilis*, *Escherischia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi)*. This study supports the usage of alternative drugs to the antibiotics for the treatment of various bacterial infections. Diclofenac can be employed as a potent drug to treat bacterial infections either alone or in combination with other drugs. Further research is needed in this area to know the efficacy of diclofenac and other non-steroidal anti inflammatory drugs in treating various clinical infections caused by pathogenic bacteria.

Table 1
The mean zone of inhibition of bacteria against human pathogens.

Test Drug	*Escherischia coli*	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*
Diclofenac 25µg/ml	24mm	22mm	24mm	18mm	No activity
Diclofenac 50µg/ml	25mm	23mm	25mm	16mm	16mm
Diclofenac 100µg/ml	25mm	22mm	26mm	19mm	21mm
Streptomycin Standard drug	21mm	21mm	21mm	25mm	15mm

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