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STUDIES ON EFFECT OF CEFOTAXIME AND *TERMINALIA CHEBULA* ON *ESCHERICHIA COLI*

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ABSTRACT

Infections caused by drug resistant Gram-negative bacteria are increasing day-by-day. An alternative mode of treatment needs to be considered to overcome the problem. With this aim in mind, the effect of Cefotaxime and aqueous extract of *Terminalia chebula* was studied on *Escherichia coli* by using Disc Diffusion Method and β-galactosidase assay. Disc Diffusion Method showed synergistic interaction between Cefotaxime and the aqueous extract of *Terminalia chebula* against *Escherichia coli*. The mode of action of the combination on *Escherichia coli* was evaluated using β-galactosidase assay. An increase in the level of β-galactosidase enzyme was observed in the cell suspension treated with the combination as compared to the controls. These results suggest that the active components present in the aqueous extract of *Terminalia chebula* should be an area of further *in-vivo* research so as to find leads of compounds which can act in combination with antibiotics.

KEY WORDS: **Cefotaxime, *Terminalia chebula*, Disc Diffusion Method, β-galactosidase assay, synergistic interaction.**

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INTRODUCTION

Throughout history, there has been a continual battle between humans and the multitude of micro-organisms that cause infection and disease. However, the euphoria over the potential conquest of infectious diseases was short lived. Almost as soon as antibacterial drugs were deployed, bacteria responded by manifesting various forms of resistance. As antimicrobial usage increased, so did the level and complexity of the resistance mechanisms exhibited by bacterial pathogens[1]. Both the amount of antibiotics used and how they are used contribute to the development of resistance. The use of broad-spectrum antibiotics rather than narrow-spectrum drugs is known to favour the emergence of resistance by broadly eliminating competing susceptible flora[2]. The World Health Organization (WHO) has identified antimicrobial resistance as one of the three most important problems for human health[3]. Today a number of organisms can be listed in both hospitals and the community that thwart treatment because they are resistant to not one, but to many different antibiotics. Among these opportunistic pathogens are the enterococci, the coagulase-negative staphylococci, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*[4]. One strategy employed to overcome the resistance mechanism is the use of combination of drugs. Inhibitors of β-lactamase have been long known and they are administered with antibiotics as co-drugs[5]. However, combination therapy is also proving to be ineffective these days.

Plant based antimicrobial compounds have gre at therapeutic potential as they have lesser side effects as compared with synthetic drugs and also little chance of development of resista nce.

There is also a possibility that herbs act as inhi bitors of enzymes. Moreover, the plant extractc an have synergistic effect with an antibiotic[6]. Few studies have found that the efficacy of antimicrobial agents can be improved by combining them with crude plant extracts against different pathogens[7]. Thus, the present study was undertaken to study the effect of Cefotaxime with *Terminalia chebula* on drug resistant *Escherichia coli*.

MATERIALS AND METHODS

(i) *Culture*

Isolate of *Escherichia coli* was collected from Brahmakumari hospital in Mumbai, India. The isolate was brought to the research laboratory in transport medium and checked for purity. The culture was maintained on Nutrient Agar slant containing 50µg/ml of Cefotaxime. Inocula were prepared by diluting overnight culture in saline. Fresh subcultures were used for each experiment.

(ii) *Antimicrobial Susceptibility Testing (AST)*

The isolate was subjected to antimicrobial susceptibility testing as per the Kirby Bauer disc diffusion method[8]. The medium used was Mueller-Hinton agar obtained from Hi-Media, Mumbai. The density of the culture was adjusted to 0.5 McFarland standard. The various antibiotics were used to determine the resistance of the test cultures. The antimicrobial agents tested were Cefotaxime (CTX 30), Ceftazidime (CAZ 30), Cefaclor (Cj 30), and Ciprofloxacin (CF 10). The culture was swabbed on Sterile Mueller-Hinton agar plates using sterile cotton swab. Using a sterile forcep, the discs of various antibiotics were placed aseptically on the plate. The plate was incubated overnight at 37°C for 24 hours. The zone sizes were interpreted as per the standard chart and the organisms were classified as sensitive, intermediate and resistant to the various antibiotics.

(iii) *Collection and Authentication of Terminalia chebula*

The dried plant part under study namely *Terminalia chebula* was provided by Konark Herbals and Healthcare, Mumbai. For the present study, the dried herb was examined for its authenticity by the Botany Department of Mithibai College, Mumbai.

(iv) *Preparation of Hot Aqueous Extract of Terminalia chebula*

Hot aqueous extract was prepared by boiling 10 g of *Terminalia chebula* fruit powder in 100 ml of distilled water for 30 mins and kept in a

conical flask for 24 hours undisturbed[9]. The aqueous extract of *Terminalia chebula* thus prepared was then kept in the refrigerator for further use.

(v) *Antimicrobial Activity of Cefotaxime and Terminalia chebula by Disc Diffusion Method*

For the Disc Diffusion method, the medium used was Mueller Hinton agar. The density of the culture was adjusted to 0.5 McFarland standard. With the help of sterile cotton swab, *Escherichia coli* was inoculated on the medium so as to obtain a lawn culture. Discs of Cefotaxime (30µg), discs containing 100µg/10µl of *Terminalia chebula* plant extract and discs of Cefotaxime (30µg) containing 100µg/10µl of *Terminalia chebula* plant extract were used. As the MIC of *Terminalia chebula* against *Escherichia coli* was 0.6, a low concentration of 100µg of hot water extract of *Terminalia chebula* per disc was used to check for synergistic interaction with Cefotaxime. Using a sterile forcep, these discs were placed on the surface of inoculated Mueller Hinton agar by pressing slightly. The plate was incubated at 37°C for 24 hours. At the end of the incubation period, the zone of inhibition formed was measured in mm.

(vi) *Antimicrobial Activity of Cefotaxime and Terminalia chebula by β-Galactosidase Assay*

The cells used for the assay were 24 hours old *E. coli* cells grown on Nutrient agar slant containing lactose. The cell growth was washed at the end of 24 hours and resuspended in 0.05M phosphate buffer. The culture suspension was adjusted to a cell density of 0.1 at 530nm. To study the mode of action of phytochemical on the cell wall, 0.5 ml of the culture suspension of *E. coli* was treated with 0.1 ml of 0.1% of phytochemical from *T. chebula* for 30 minutes at 37°C and centrifuged to remove cells. Washed cells of *E. coli* (negative control) as well as washed cells treated with toluene to rupture the cell wall (positive control) were used as controls. After incubation, 1ml of 0.005M ONPG in phosphate buffer (pH- 7.0) was added and the tubes incubated at 37°C for 15 minutes. At the end of the incubation period, 0.5ml of 0.5M Na_2CO_3 was added in all the tubes. The tubes were then centrifuged at 3000rpm for 5 minutes and O.D. was taken at 420nm.

RESULTS AND DISCUSSION

Isolate of *Escherichia coli* obtained from Brahmakumari hospital was subjected to AST to get the antibiogram. The antibiotics tested were Cefotaxime (CTX 30), Ceftazidime (CAZ 30), Cefaclor (Cj 30), and Ciprofloxacin (CF 10). *Escherichia coli* did not give any zone of inhibition to Cefotaxime, Ceftazidime and Cefaclor whereas 10mm zone of inhibition was observed against Ciprofloxacin. Since *Escherichia coli* did not give the zones of inhibition at all to Cefotaxime, they were considered ESBL[10]. The most prevalent Gram-negative pathogens, such as *Escherichia coli*, *Salmonella enterica*, and *Klebsiella pneumoniae*, cause a variety of diseases in humans and animals, and a strong correlation between antibiotic use in the treatment of these diseases and antibiotic resistance development has been observed over the past half-century. This is especially apparent with the β-lactam class of antibiotics and their related inactivating enzymes, the β-lactamases[11]. Resistance to norfloxacin increased from 1.3% in 1989 to 5.8% in 1998 in *Escherichia coli*. The yearly increase of resistance to fluoroquinolones in *E. coli* from UTI may stem from increased prescription of fluoroquinolones for UTI. Resistance of *E. coli* to these agents is likely to increase further as fluoroquinolone use increases in future[11]. Increasing prevalence of multidrug-resistant Gram-negative organisms has led to a rise in clinically significant infections with these organisms and an increasing therapeutic dilemma[12]. In Disc Diffusion Method, Cefotaxime did not give any zone of inhibition against *E. coli*. Aqueous extract of *Terminalia chebula* inhibited *E. coli* giving the zone of inhibition of 12.3mm. However Cefotaxime and the aqueous extract of *Terminalia chebula* inhibited *Escherichia coli* giving the zone of inhibition of 17.3mm. Thus synergistic interaction was observed between Cefotaxime and the aqueous extract of *Terminalia chebula* on *Escherichia coli* by Disc Diffusion Method. β-Galactosidase Assay was

performed to study the effect of the phytochemical of *Terminalia chebula* on the cell wall of bacteria. The supernatant of untreated washed cells of *E. coli* produced 20 µmoles of ONP (orthonitrophenol) indicating extremely low level of β-Galactosidase enzyme present in the supernatant. The supernatant of toluene treated cells produced 585 µmoles of ONP indicating rupture of cells by toluene leading to release of enzyme β-Galactosidase. The supernatant obtained from cells treated with the phytochemical and centrifuged immediately so that no effect of phytochemical occurs on the cells provided 22 µmoles of ONP on reaction with ONPG. This result indicates that when the cells are not exposed to the phytochemical for longer time, there is no effect on them and also, the phytochemical by itself is not adding to the false result. The supernatant of *Terminalia chebula* treated (30 minutes) cells provided 245 µmoles of ONP on reacting with ONPG. This indicates that the phytochemical ruptured the cells in 30 minutes.

Table 1
β - Galactosidase Assay for E. Coli

Cells	*E.coli* Lac cells	Toluene treated cells	*Terminalia chebula* treated cells
ONP (µmoles)	20	585	245

CONCLUSION

The resistance pattern of *Escherichia coli* was studied and was found to be ESBL producer. Disc Diffusion Method showed synergistic interaction between Cefotaxime and the aqueous extract of *Terminalia chebula* against *Escherichia coli*. β-Galactosidase assay proved that the phytochemicals act on the cell wall of bacteria leading to increased leakage of the enzyme.Thus,it can be seen that the combination of medicinal plant extracts and known antibiotics offers significant potential for the development of novel antimicrobial therapies and the treatment of various diseases caused by micro-organisms. Further research should be carried out to explore new medicinal plants which can exhibit synergistic behaviour with antibiotics. The mechanism involved in synergistic behaviour should be understood along with the safety aspects of the same. *In-vivo* studies should also be carried out so as to predict what would happen if such combinations are given to humans.situation.

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REFERENCES

1. Tenover FC, Mechanisms of Antimicrobial Resistance in Bacteria, The American Journal of Medicine, 119(6A): S3-S10, (2006).
2. Barbosa TM, Levy SB, The impact of antibiotic use on resistance development and persistence, Drug Resistance Updates, 3: 303-311, (2000).
3. Sekhri, K, Antimicrobial Resistance: Understanding Solutions and Future Developments, International Journal of Pharma and Biosciences, 4(2): 338-343.
4. Levy SB, Factors impacting on the problem of antibiotic resistance, J Antimicrob Chemother., 49: 25-30, (2002).
5. Hemaiswarya S, Kruthiventi AK, Doble M, Synergism between natural products and antibiotics against infectious diseases, Phytomedicine, 15: 639-652, (2008).
6. Deepak S, Kamat SD and Kamat DV, Effect of aqueous extract of *Terminalia*

chebula on Metallobetalactamase, International Journal of Pharmacy and Pharmaceutical Sciences, 2(4): 172-175, (2010).
7. Adwan G and Mhanna M, Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from Clinical specimens, Middle-East Journal of Scientific Research, 3(3): 134-139, (2008).
8. Bauer AW, Kirby WM, Sherris JC and Turck M, Antibiotic susceptibility testing by a standardized single disk method, Am J Clin Pathol, 36: 493-496, (1996).
9. Bag A, Bhattacharyya SK, Bharati P, Pal NK and Chattopadhyay RR, Evaluation of antibacterial properties of Chebulic myrobalan (fruit of *Terminalia chebula* Retz.) extracts against methicillin resistant *Staphylococcus aureus* and trimethoprim-sulphamethoxazole resistant uropathogenic *Escherichia coli*, African Journal of Plant Science, 3(2): 025-029, (2009).
10. Chaudhary U and Aggarwal R, Extended spectrum β lactamases (ESBL) – An emerging threat to clinical therapeutics, Ind J Med Microbiol, 22: 75-80, (2004).
11. Goettscha W, van Pelta W, Nagelkerkea N, Hendrixb MGR, Buitingc AGM, Petitd PL, Sabbee LJM, Van Griethuysenf AJA and de Neelinga AJ, Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the Netherlands, J. Antimicrob. Chemother., 46 (2): 223-228, (2000).
12. Segal-Maurer S, Mariano N, Qavi A, Urban C and Rahal JJ, Successful treatment of ceftazidime-resistant *Klebsiella pneumoniae* ventriculitis with intravenous meropenem and intraventricular polymyxin B: case report and review, Clin Infect Dis., 28(5): 1134-1138, (1999).