A COMPARATIVE STUDY OF WITHANIA SOMNIFERA WITH GENTAMICIN, CIPROFLOXACIN AND CEFOTAXIME AGAINST ESCHERICHIA COLI

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ABSTRACT

Background: Bacteria are the common cause of multiple infections and due to repeated use of certain antibiotics the growing resistance has become the major problem. Infections caused by *Escherichia coli* can be treated by using ciprofloxacin, ceftriaxone and gentamicin but excessive use of these antibiotics can lead to resistance against them. Thus new drugs that can effectively kill bacteria are constantly required to overcome this problem. *Withania somnifera* is a medicinal plant found in dry areas of India, Pakistan, China and Bangladesh. It has several medical uses. Research is needed to evaluate the ability of *Withania somnifera* extracts to kill bacteria and its role in minimizing development of resistance.

Objectives: To evaluate the antibacterial activity of *Withania somnifera* Root (WSR) extracts against *E. coli* and compare it with the antibacterial activity of gentamicin, ciprofloxacin and cefotaxime against *E. coli.*

Methods: Inoculums of the bacterial isolates were prepared. Nutrient agar plates were inoculated with the selected isolates @ 200 μl/ plate. Three standard drug discs Ciprofloxacin (CIP 5), Cefotaxime (CTX 30) and Gentamicin (CN 10), were placed against each *E. coli* isolate along with a filter paper disc, impregnated with WSR extract solution (100 μg/ 20 μl). After 18-24 hours of incubation at 37°C, diameters of the zone of inhibition were measured for each drug.

Results: The average zone values against *E. coli* isolates were found to be *Withania somnifera* (28.15 ± 2.39), followed by Ciprofloxacin (19.31 ± 9.30), Cefotaxime (16.46 ± 9.86), and Gentamicin (12.57 ± 2.19). Comparing resistant, sensitive, giving no response and re-growth cases, difference was significant as all the cases were with p-values <0.001. In all the cases of *E. coli* isolates, *Withania somnifera* was found to be effective and no resistance was found.

Conclusion: Withania somnifera root extract shows efficient antibacterial activity against Escherichia coli. If Withania somnifera extract is given along with other antibiotics, it will be having good synergistic activity.

K**ey Words:** Withania Somnifera, Gentamicin, Ciprofloxacin, Cefotaxime, Escherichia coli

INTRODUCTION

Escherichia coli (*E.coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium(1).Virulent strains of E. coli can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis and Chron's disease(2). The two important factors while choosing an effective antimicrobial drug are the type of the infecting pathogen and its sensitivity to available drugs. Ciprofloxacin (Flouroquinolone), cefotaxime (Cephalosporin) and gentamicin (Aminoglycoside) are commonly used to treat infections due to *E.coli* (3) but their widespread indiscriminate use causes development of resistance against these antibiotics (4). Enzymatic inactivation, alteration of target or binding site, and decreased drug accumulation are the main mechanisms due to which *E. coli* develops resistance against these antibiotics(5). The emergence of multi-drug resistance is a continuing problem.

Plants have been utilized for healthcare purposes in various regions of the world since old times and they have gained more interest as source of medicines in recent years (6). Research studies also show antimicrobial effect of compounds derived from different parts of plants (7). The global problem of resistance against antimicrobial drugs demands discovery of new drugs that can effectively kill microbes and minimize resistance (8).

Withania somnifera (Ashwaghanda, Indian ginseg)

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is a medicinal plant found in dry arid regions of Pakistan, India, China and Bangladesh (9). It is effective in the treatment of various conditions such as mental diseases, asthma, inflammation, arthritis, rheumatism, infections, fever, male sexual disorders and a variety of other diseases, including cancer(10, 11). Studies also show the antibacterial activity of *Withania somnifera* but a lot of work needs to be done on its antibacterial aspects. This research was conducted to evaluate the antibacterial activity of *Withania Somnifera* against *E. coli* and compare it with the antibacterial effect of gentamicin, ciprofloxacin and cefotaxime.

MATERIALS AND METHODS

The current was a descriptive study with cross-sectional time prospect. Roots of the *Withania somnifera* plants were collected from Shahpyaara Graveyard at Rawalpindi. These roots were washed, dried, and ground to powder. The extraction procedures were done using Chloroform in multiple phases (12, 13). The *Withania somnifera* extract thus obtained was subjected to qualitative test by Wagner's reagent and Thin Layer Chromatography(14). Forty pathogenic isolates of E. coli were procured from the microbiology laboratory of Fauji Foundation Hospital Rawalpindi and Pakistan Institute of Medical Sciences Islamabad.

Different concentrations of *Withania somnifera* extract were employed against pathogenic *E. coli* MQE 95 to get optimum inhibitory dose. 16, 20, and 27 mm zones we recorded for 40 μg/ 20μl, 80μg/ 20μl and 100μg/ 20 μl respectively of extract dissolved in Dimethyl sulphoxide solvent (Fig. 1A). The dose optimized was 100μg/ 20μl for *Withania somnifera* extract. To check the effect of solvents, discs of Dimethyl sulphoxide and phosphate buffer solution pH 7 were employed in a separate plate against *E*.coli. No clear inhibitory zones were recorded (Fig. 1B)

The antibacterial activity of the extract from roots of *Withania somnifera* plant was determined by the Kirby Bauer Technique(15). Inoculums of the bacterial isolates were prepared. Nutrient agar plates were inoculated with the selected isolates @ 200 μl/ plate. Three standard drug discs Ciprofloxacin (CIP 5), Cefotaxime (CTX 30) and Gentamicin (CN 10), were placed against each *E. coli* isolate along with a filter paper disc, impregnated with *Withania somnifera* root extract solution (100 μg/ 20 μl). After 18-24 hours of incubation at 37°C, diameters of the zone of inhibition were measured for each drug.

Comparison was made on the basis of statistical analysis by ANOVA and Chi-square. While comparing drugs against *E. coli* isolates results found to be significant with p-value <0.001. Data was entered and analyzed by using SPSS. Quantitative variables zone of inhibition were measured in mm and were reported with mean ± S.D for three medicines Gentamicin, Ciprofloxacin, Cefotaxime, *Withania somnifera* root extract for Gram-negative microorganisms and were compared by using ANOVA. Qualitatively sensitivity and resistance for each drug were reported by frequency and percentages. Comparisons for sensitivity and resistance among three drugs and plant extract were compared by using Chi-square in Gram Negative microorganisms.

RESULTS

The average zone values against *E. coli* isolates were found to be *Withania somnifera* (28.15 ± 2.39), followed by Ciprofloxacin (19.31 ± 9.30), Cefotaxime (16.46 ± 9.86), and Gentamicin (12.57 ± 2.19). *Withania somnifera* is giving significantly higher zone than all the

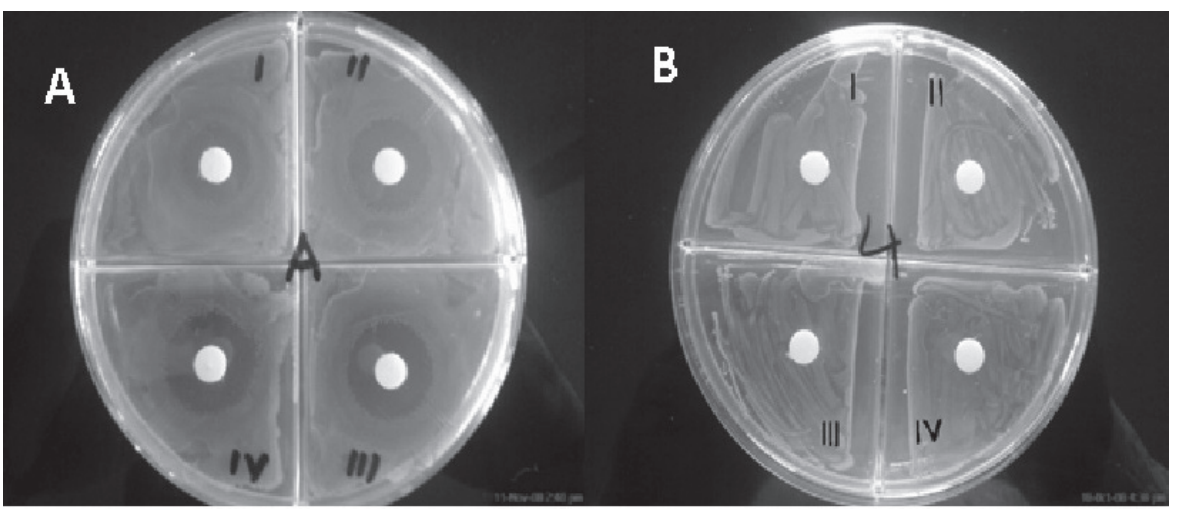

Figure 1: Escherichia coli media (A) Test samples treated with Withania somnifera extract, exhibiting different zones of inhibition at different concentrations; I. Withania somnifera 40 μg/ 20 μl, II. Withania somnifera 80μg/ 20μl, III. Withania somnifera 100μg/ 20 μl, IV. Withania somnifera 100μg/ 20 μl; (B) Dimethyl sulphoxide and Phosphate buffer containing negative controls; I. Dimethyl sulphoxide 20 μl, II. Dimethyl sulphoxide 20 μl, III. Phosphate Buffer pH 7, 20 μl, IV. Phosphate Buffer pH 7, 20 μl

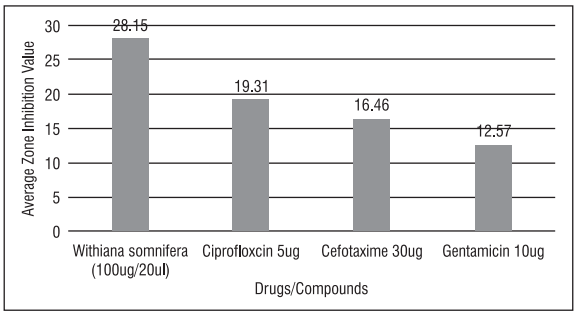

Figure 2: Average zone inhibition value of various drugs/compounds against Escherichia coli

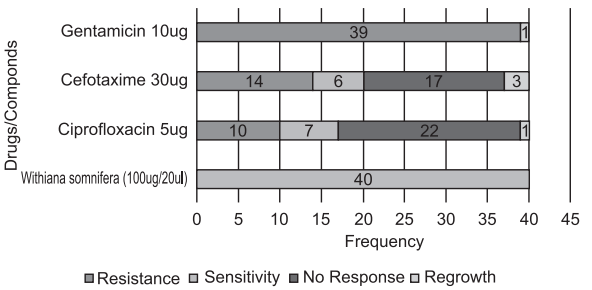

Figure 3: Resistance status of each drug/compound against Escherichia coli

three drugs (Fig.2). But Ciprofloxacin and Cefotaxime were having no significant difference. Comparing resistant, sensitive, giving no response and Re-growth cases, difference was significant as all the cases were with p-values <0.001. In all the cases of *E. coli* isolates, *Withania somnifera* was found to be effective and no resistance was found. While sensitivity of Ciprofloxacin, Cefotaxime, and Gentamicin, was 17.50 %, 15 %, and 2.50 % respectively. Ciprofloxacin and Cefotaxime showed no response in 55 and 42.5 % cases respectively, showing complete resistance (Fig.3).

DISSCUSSION

The results of our study show that WSR extract has antibacterial activity against E. coli and it is more effective in inhibiting the growth of E. coli as compared to ciprofloxacin, cefotaxime, and gentamicin. This study also shows that E. coli is most sensitive to antibacterial effect of WSR extract and most resistant to gentamicin. The studies conducted by other researchers also reveal the antibacterial activity of Withania somnifera. Kumar et al studied antibacterial activity of WSR extract against E. coli and concluded that WSR extract has significant antibacterial effect against E. coli(16). Similarly study carried out by Bish et al revealed that leaf extract of Withania somnifera has antibacterial activity against Gram-positive cocci(17).

CONCLUSION

Our study confirms the traditional usefulness of this plant against various infections. On the basis of our results and statistical analysis it can be concluded that *Withaniasomnifera* extract possesses specific compounds for antibacterial properties that can be used along with other agents for the therapy of infectious diseases caused by *E.coli*. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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