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The Effect of *Withania somnifera* Extract on Drug Resistant Strains of *Escherichia coli*

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Background: Due to the increased resistance of pathogenic bacteria even towards new antibiotics, researchers have attempted to find herbal antimicrobial agents to replace chemical drugs.
Objectives: The purpose of this study was to evaluate the effect of *W. somnifera* extracts on drug resistant *E. coli* strains isolated from clinical samples.
Materials and Methods: We studied all drug resistant strains of *E. coli* collected from patients with urinary tract infections hospitalized at Zabol hospitals from January 2010 to January 2012. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts were determined by the dilution method with various concentrations of the bacteria. Sensitivity to multiple antibiotics was defined by the Kirby-Bauer disc diffusion test.
Results: The results showed that the isolated *E. coli* strains were sensitive to these antibiotics: erythromycin (52.94%), tetracycline (76.47%), ceftazidime (41.17%), cefixime (35.29%), penicillin (76.47%), ampicillin (58.82%) and nalidixic acid (41.17%). Examination of the herbal extracts showed that the highest maximum inhibitory concentration (MIC) against drug resistant *E. coli* was 200 ppm. The lowest MIC was 50 ppm, where three strains of *E. coli* were inhibited at this concentration.
Conclusions: Our results indicated that *Withania somnifera* extracts had antibacterial effects on drug resistant *E. coli* strains isolated from clinical samples. Further studies are required to evaluate the effective compounds of this plant on microbial agents.

Keywords:E. coli; Drug resistance; *Withania somnifera*

1. Background

Withania somnifera is an evergreen plant from the Solanaceae family. It grows widely in India, East Mediterranean regions and East Asia. In ancient times, roots and leaves of this plant were used for treatment of infectious diseases such as wound infections, painful abscesses, conjunctivitis, tuberculosis, and for sexually transmitted infections such as gonorrhea and syphilis (1-4). Previous studies have shown the antimicrobial activity of *Withania somnifera* extracts (1, 5). These extracts have also been used for dyspepsia and abdominal pain (6). Also, the entire plant, including its roots, leaves and fruits, due to containing polyphenols and antioxidant compounds is useful for human health (7). Previous studies have shown that in animal models, this extract increases memory and learning power (8). *Escherichia coli* belongs to the Enterobacteriaceae family that causes many infections such as urinary tract infection, sepsis, peritonitis, neonatal meningitis, gall bladder infections and pneumonia (9-12). Indiscriminate and careless use of antibiotics has led to the emergence of drug resistance to this bacterium.

2. Objectives

The aim of this study was to evaluate the effect of *Withania somnifera* extract on isolated *E. coli* strains from clinical samples.

3. Materials and Methods

3.1. Isolation of Escherichia coli

We evaluated all strains of *E. coli* isolated from urine cultures of hospitalized patients at Zabol hospitals (Southeastern Iran) with urinary tract infections from January 2011 to January 2012. The samples were examined microscopically by Gram staining. Gram-negative samples were inoculated on plates of nutrient agar, cysteine lactose electrolyte deficient (CLED) agar, MacConkey's and blood agar. Samples were then incubated at 37°C for 24 hours (13). The colonies that showed fermenting of lactose on MacConkey's agar and CLED agar media were purified and identified according to their morphology as circular, rose-pink to red colonies on MacConkey's agar

medium and yellow colonies on CLED agar. The isolates were identified by biochemical tests such as catalase enzyme test, potassium hydroxide test, indole and methyl red test, Voges Proskauer reaction, urease and citrate test, H_2S and oxidase test (14).

3.2. Agar Disc Diffusion Assay

Resistance to tetracycline, erythromycin, cefixime, ceftazidime, penicillin, ampicillin, and nalidixic acid was tested by the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocols. Antibiotic discs were obtained from the Patended -Iran. *Escherichia coli* isolates were evaluated based on their size of zones of inhibition and classified as susceptible (S), intermediate resistant (I) or resistant (R) according to the CLSI criteria (15).

3.3. Plant Material

The leaves of *W. somnifera* were collected from Zabol (located South-east of Iran) and approved by the Faculty of Agriculture of Zabol University. Specimens were then dried at room temperature. Samples were crushed and transferred to glass containers and maintained until the extraction procedure.

3.4. Preparation of Extracts

Twenty grams of fresh leaf materials were macerated with 60 mL of 95% ethanol, in a grinding machine for about 10 to 15 minutes to separate the extract phases during one day (shaking occasionally with a shaker). The supernatant was filtered through Whatman No. 1 filter paper. The extracts were preserved aseptically at 5°C for further use.

3.5. Minimum Inhibitory Concentration of Plant Extracts

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC). All tests were carried out in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Next, serial doubling dilutions of the extract were made in a 96-well microtiter plate ranging from 0.3 mg/mL to 10.00 mg/mL. To each well, 10 μL of indicator solution (prepared by dissolving a 10-mg extract in 2 mL of DMSO) and 10 μL of Mueller Hinton broth were added. Finally, 10 μL of bacterial suspension (106 CFU/mL) was added to each well to obtain a concentration of 10^4 CFU/mL. The plates were loosely held together with a cling film to warrant that the bacteria do not become dehydrated. Next plates were prepared in triplicates, and placed in an incubator at 37°C for 18-24 hours. Color change of the samples was then visually evaluated. The lowest concentration at which color change occurred was considered as the MIC value. The average of the three values was calculated providing the MIC values for the tested extracts. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate visible growth. Microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganisms were completely killed. The results are expressed as mean and or rank in order of importance as a percentage. The data were subjected to one-way analysis of variance (ANOVA), using the SPSS-17 software. $P < 0.05$ was considered significant.

4. Results

The results showed that 17 strains of isolated *E. coli* were resistant to antibiotics with different percentages; erythromycin (52.94%), tetracycline (76.47%), ceftazidime (41.17%), cefixime (35.29%), penicillin (76.47%), ampicillin (58.82%) and nalidixic acid (41.17%). Results from *W. somnifera* extract showed that the highest MIC (minimum inhibitory concentration) was 200 ppm. Four strains were inhibited at this concentration, and the lowest MIC was 50 ppm and three strains of *E. coli* were inhibited at this MIC (Table 1).

Table 1. Minimum Inhibitory Concentration/Minimum Bactericidal Concentration of *Withania somnifera* Extract on drug Resistant Bacterial Strains [a]

Bacteria	The least MIC/ MBC, PPM
1	No growth/No growth
2	100/200
3	100/100
4	200/200
5	No growth/No growth
6	No growth/No growth
7	200/200
8	100/100
9	50/100
10	100/200
11	100/200
12	100/200
13	200/400
14	50/100
15	200/200
16	100/100
17	50/50

[a] Abbreviations: MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration.

5. Discussion

Our results showed that the isolated *E. coli* strains were resistant to the following antibiotics: erythromycin, tetracycline, ceftazidime, cefixime, penicillin, ampicillin and nalidixic acid with different percentages and the

extract of *W. somnifera* had antibacterial effect on these strains. Due to the wide and indiscriminate use of antibiotics, we have faced drug resistant bacteria especially in during the recent years, even against new drugs such a quinolones, new penicillin groups and cephalosporins. Therefore, we need new substances to help us treat infectious diseases. Among these agents, *W. somnifera* has been shown to have antibacterial effects on some drug resistant bacteria. A study at Imam Ali hospital (in Chaharmahal Bakhtiari) showed that isolated *E. coli* were resistant to gentamicin (62.50%), co-trimoxazole (51.51%), nalidixic acid (21.21%), amikacin (66.67%), ciprofloxacin (41.86%) and ceftriaxone (57.14%) (16). In a report by Mohammadi Mehr et al., *E. coli* strains isolated from clinical samples were resistant to antibiotics including: gentamicin (27.77%), ampicillin (80.55%), sulfamethoxazole (47.22%), nitrofurantoin (13.88%), ceftazidime (63.89%) and amikacin (80.55%) (17). In Madani's study from Kermanshah, *E. coli* showed the greatest amount of resistance to ampicillin (91.4%), cotrimoxazole (61.1%), cefixime (46.8%), gentamicin (43.3%) and ceftazidime (38.8%) (18). The result of the herbal extraction experiment showed that the highest MIC was 200 ppm. Four strains were inhibited at this concentration. The lowest MIC was 50 ppm and two strains of *E. coli* were inhibited at this concentration. In Kaur's research, treatment of infected mice with a combination of *Asparagus racemosus* and *Withania somnifera* not only resulted in successful reduction of parasite level, but also, generated protective Th1 type of immune responses with normalization of biochemical and hematological tests suggesting their role as important anti-leishmanial agents (19). Bensod in his study showed that extracts of *Withania somnifera* with 100 μg concentration in each disk, made an inhibitory zones (15 ± 0.5 mm) against *Aspergillus fumigatus* (20). In Kuboyama's study, *Withania somnifera* and its constituents showed various activities against Alzheimer's disease and spinal cord injury. *Withania somnifera* extracts also showed ameliorative effects against other neurodegenerative disease models such as Parkinson's disease and Huntington's disease (21). Mir Ba et al. reported that *Withania somnifera* is a rich source of withaferin A and other bioactive withanolides, which are used for medical purpose (22). Results by Heidari Sour Shojani et al. in Iman Ali Hospital in Chaharmahal Bakhtiari showed that isolated *E. coli* were resistant to gentamicin (62.50%), co-trimoxazole (51.51%), nalidixic acid (21.21%), amikacin (66.67%), ciprofloxacin (41.86%) and ceftriaxone (57.14%) (16). In the report by Mohammadi Mehr et al., *E. coli* strains isolated from clinical samples were resistant to antibiotics including: gentamicin (27.77%), ampicillin (80.55%), sulfamethoxazole (47.22%), nitrofurantoin (13.88%), ceftazidime (63.89%) and amikacin (80.55%) (17).

In conclusion, our results and other previous results suggest that *Withania somnifera* extracts have anti-bacterial effect on even drug resistant bacteria such as *E. coli* and also, have a wide range of efficacy on neurodegenerative diseases such as Alzheimer's disease.

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