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ANTIBACTERIAL EFFECT OF *GLYCYRRHETINIC ACID* ON 55 HOSPITAL STRAINS OF *STAPHYLOCOCCUS AUREUS* AND 32 ACTINOBACILLUS *ACTINOMYCETEMCOMITANS*

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ABSTRACT

Glycyrrhetinic acid is a major component of a traditional plant called *Licorice*. This substance has been found to have some pharmacological properties including anti-inflammatory, anti-viral, anti-bacterial, anti-fungal, anti-allergic, anti-carcinogenic and anti-peptic-ulcer. *Glycyrrhetinic acid* also affects against some parasites such as *Trichomonas vaginalis*. In this study, 55 hospital strains of *Staphylococcus aureus* and 32 *Actinobacillus actinomycetemcomitans*, were isolated from patient's specimens by culture method. Antibacterial activities of *glycyrrhetinic acid* against those microorganisms were investigated by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. The MIC for *S. aureus* and *A. actinomycetemcomitans* were 64 and 8 μg/ml respectively. The MBC for *S. aureus* and *A. actinomycetemcomitans were* 64 and 16 μg/ml respectively. It is concluded that *Glycyrrhetinic acid* is effective against *Staphylococcus aureus* and *Actinobacillus actinomycetemcomitans* in appropriate concentrations.

Key words: Glycyrrhetinic acid, Antibacterial, *Staphylococcus aureus*, *Actinobacillus, Actinomycetemcomitans*

INTRODUCTION

Glycyrrhetinic acid is one of the major components of *Licorice* herbage, which is obtained in high purity extract from rhizomes of the plant. Glycyrrhetinic acid has some pharmacological properties including calmative, antiinflammatory, antiallergic, antitumor, antibacterial, and antifungal. Many studies have shown that glycyrrhetinic acid is able to carboxylate the DNA replication and inhibit production of microbial toxins and enzymes (1-8). *Staphylococcus aureus* is the most common pyogenic or pus-producing bacteria. It produces a large number of toxins and enzymes that act locally, chiefly to help them withstand phagocytosis by neutrophils. The organism can produce local abscesses in the body, from skin to bone marrow. Occasionally they cause specific diseases such as endocarditis (9) *Actinobacillus actinomycetemcomitans* is found in the mouth microflora, but clinically it has been isolated from blood, lung tissue, mouth abscess, and infected sinuses. The microflora associated with gingivitis and periodontitis may contain other bacteria like *Porphyromonas gingivalis, Prevotella intermedia, Eikenella corrodens* and *Capnocytophaga* spp. Based on this background we were interested to study antibacterial effects of glycyrrhetinic acid, against the above-mentioned bacteria that could provide primary information for formulation of different forms of the drug such as tooth paste, washing mouth and so on (10-13).

MATERIALS AND METHODS

In this study, 200 specimens were collected from patients between September 1998 and September 1999 in Tehran. The first 100 specimens were collected from patients, suffering from urinary infections, skin burn, sinuses or blood infection that admitted to Imam-Khomeini hospital. The specimens were cultured on blood agar (Difco) and incubated at 37 °C for 24 hours. Further investigations for identification of all isolated bacteria were made using biochemical tests (14). The second 100 specimens were collected from patients suffering from periodontal infections,

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referred to the periodontics clinic of faculty of Dentistry of Tehran university of medical sciences. The specimens were cultured on Trypticase Soy blood agar, that comprises Bacitracin and Vancomycin (TSBV), and incubated at 37 °C under 5% CO_2 for 24 hours. Direct observation was done to find the star shape colonies. Further investigations for identification of all isolated bacteria were made using biochemical tests (14,15). Investigation for antibacterial properties of glycyrrhetinic acid powder (Darupakhsh, Tehran) against *S. aureus* and *A. actinomycetemcomitans* was made as follow: Purified glycyrrhetinic acid powder was dissolved in sterile Mueller Hilton broth (Difco) and filtered using 0.5 μm pore size filter paper (Millipore). Different concentrations of glycyrrhetinic acid were used to prepare a series of diluted tubes. The MIC of tube series was performed by addition of 1*ml* bacterial suspension (containing 10^8) to each dilution tube aseptically. The blank tube contained the same amount of bacterial suspension, but without glycyrrhetinic acid. The tubes were incubated at 37 °C for 18-24 hours. The MIC was the first tube, which showed no bacteria growth. 0.1 ml of the MIC tube was inoculated on TSBV and blood agar medium and incubated at 37 °C for 24 hours. The MBC was the tube that contained the minimum amount of glycyrrhetinic acid with no growth of bacteria (14,16,17)

RESULTS

Of patients with various infectious diseases, 55 hospital strains of *Staphylococcus aureus*, and of patients with periodontal diseases, 32 *Actinobacillus actinomycetemcomitans* were isolated (Tables 1, 2 and Fig. 1).

The MIC for *S. aureus*, and *A. actinomycetemcomitans* were found to be 64 and 8 µg/ml respectively, while the MBC for *S. aureus* and *A. actinomycetemcomitans* was 64 and 16 µg/ml, respectively (Fig1).

DISCUSSION

Antibacterial resistance is an important issue that has created a number of problems in treatment of infectious diseases. The existence of resistance necessitates the investigation about natural antibacterials. The main aim of this study was to determine the MIC and MBC of glycyrrhetinic acid against *S. aureus* and *A. actinomycetemcomitans*. The MICs and MBCs for 55 *S. aureus* and 32 *A. actinomycetemcomitans*, isolated from patient's specimens were 64, 64, 8 and 16 µg/ml respectively. It has been shown that glycyrrhetinic acid inhibits DNA replication by carboxylating and inhibits production of microbial toxins and enzymes. It has a synergistic effect if used together with antimicrobial agents, mouth washing and gels for topical treatment of skin, oral and dental infections (5,18). Effectiveness of glycyrrhetinic acid in viral diseases like influenza and HIV has been reported in vitro (2,19,20) and remains to be studied in vivo. It is also considerable that this plant grows in many parts of Iran and glycyrrhetinic acid is produced with high quality and low price (4,21). Further studies to clarify the place of glycyrrhetinic acid in therapy of infectious diseases are suggested.

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Table 1. The frequency distribution of isolated *Staphylococcus aureus*

S. aureus	Urine (n=25)	Burn (n=25)	Skin (n=41)	Blood (n= 5)	Sinus (n=7)	Total (n=100)
Frequency	12	17	21	2	3	55
Percent	48	68	51.2	40	42.9	55

Table 2. The frequency distribution of isolated *Actinobacillus actinomycetemcomitans*

A. actinomy-cetemcomitans	LJ (Right) (n=25)	UJ (Right) (n=25)	LJ (Left) (n=25)	UJ (Left) (n=25)	Total (n=100)
Frequency	1	3	12	16	32
Percent	4	12	48	64	32

LJ (Lower Jaw), UJ (Upper Jaw)

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