Evaluation of antibacterial activity of photoactivated cow urine against human pathogenic strains

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Abstract

The bactericidal properties of cow urine have been known to humans from a long time. Cow urine is an effective antibacterial agent against a broad spectrum of Gram-negative and Gram-positive bacteria. In this work, we explored photoactivated cow urine as a potential antimicrobial agent. The antimicrobial activity of cow urine was tested by agar well method using Gram-positive bacteria *Bacillus cereus* (MTCC-1305), *Staphylococcus aureus* (MTCC-3160) and Gram-negative bacteria *Salmonella typhimurium* (MTCC-1253), *Aeromonas hydrophila* (MTCC-1739), *Enterobacter aerogenes* (MTCC-2823), *Micrococcus luteus* (MTCC-1809). Photoactivated urine showed highest antimicrobial activity against Gram-positive *Bacillus cereus* and Gram-negative bacteria *Aeromonas hydrophila*. Bactericidal effect showed against various bacteria depended on the concentration of photoactivated urine and the type of bacteria.

Key words: agar well method; antibiotics; bactericidal activity; cow urine.

Introduction

The discovery and development of antimicrobial drugs is among the most important medical advances of modern history, with antimicrobial agents playing a key role in control of infectious diseases. The increase in use of antimicrobial drugs has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill 1988; Ng 1994; Gonzalez et al. 1996; Dean, Burchard 1996). Drugs are not only expensive and inadequate for the treatment of diseases in developing countries, but also have side effects. Therefore, there is a need to develop new infection-fighting strategies to control microbial infections (Sieradzk et al. 1999).

Cow urine has great potential pharmacological importance as its medicinal utility has been mentioned already in Ayurveda. Panchagavya, a combination of five compounds obtained from the cow, namely urine, dung, milk, curd and ghee, is used as a medicine and sometimes as fertilizer for plants (Preethi et al. 1999). Cow urine has been found to be effective against reversal of certain cardiac and kidney diseases, indigestion, stomach ache, edema, skin disease, epilepsy, anemia, constipation, respiratory disease etc. (Ojewol et al. 1976; Chauhan et al. 2001). After photoactivation and purification, cow urine has been found to be effective against certain drug resistant bacterial strains (Biddle et al. 2007). Cow urine contains many essential components such as minerals (N, P, K, Ca, Cl), estrogen, pheromones and urinary protein (Lebeda et al. 1997; Bravo et al. 2003; Yen et al. 2007). Cow urine is also used by traditional homoeopaths in combination with herbs for treatment of fever, epilepsy and anemia. Cow urine exhibits both antioxidant and antimicrobial activities, which has been confirmed by recent studies (Edwin et al. 2008).

Therefore, in the present investigation we studied antimicrobial activity of photoactivated cow urine against various pathogenic bacterial strains: Gram-positive bacteria *Bacillus cereus, Staphylococcus aureus* and Gram-negative bacteria *Salmonella typhimurium, Aeromonas hydrophila, Enterobacter aerogenes, Micrococcus luteus*.

Materials and methods

Preparation of photoactivated cow urine

Healthy Hariana cows was selected from local cattle yards. Then fresh urine of cows was collected in sterile containers for antimicrobial studies. The cow urine used in the experiment was photoactivated by maintaining itin sun light for 48 h in a transparent glass bottle. Then the urine was filtered through Whatman No.1 filter paper (pore size 25 μm) to free it from debris and precipitated material. The photoactivated cow urine used in our study had pH of 7.8. The prepared samples were stored at 4 °C for further use.

Microbial cultures

All the test cultures were procured from Microbial Type Culture Collection Center (MTCC), Chandigarh, India. The photoactivated cow urine was tested against Gram-positive

bacteria *Bacillus cereus* (MTCC-1305), *Staphylococcus aureus* (MTCC-3160) and Gram-negative bacteria *Salmonella typhimurium* (MTCC-1253), *Aeromonas hydrophila* (MTCC-1739), *Enterobacter aerogenes* (MTCC-2823), and *Micrococcus luteus* (MTCC-1809). The cultures were maintained at 4 °C on nutrient agar (Hi-Media, India).

Well diffusion method

The antibacterial activity of cow urine against bacterial pathogens *Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium, Aeromonas hydrophila, Enterobacter aerogenes*, and *Micrococcus luteus* was performed by agar well diffusion method. Nutrient agar medium plates were prepared, sterilized and 0.5 mL of different bacterial cultures was inoculated in these plates. After solidification, wells were made and photoactivated cow urine was poured into each well on all plates using a micropipettes (5 µL, 10 µL, 25 µL and 30 µL). The plates were incubated at 37 °C for 24 h and the zone of inhibition was measured. Then the plates were incubated for 24 h at 37 °C. Tetracycline (30 µg mL) was used as a positive control and distilled water was used as a negative control. The results were evaluated by measuring diameter of the inhibition zone at the end of 48 h incubation. Zone of inhibition surrounding the discs was measured.

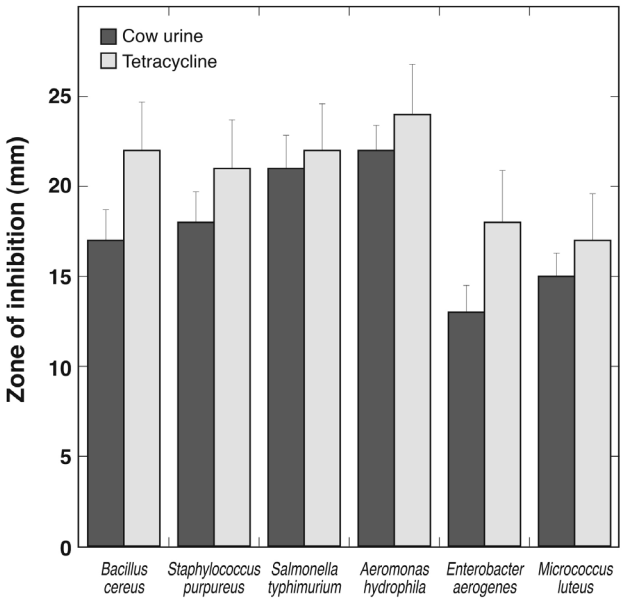

Fig. 1. Antimicrobial activity of photoactivated cow urine (30 µL per well) measured by agar disc diffusion method. The effectiveness is demonstrated by the size of the bacterial growth inhibition zone around the well, which is typically expressed as the diameter of the zone in mm. Data are means from three replicates ± SD.

Results

Antimicrobial activity of photoactivated cow urine against pathogenic bacterial strains estimated by agar well diffusion method showed different widths of zone of inhibition against various human pathogenic bacterial strains. Thus, photoactivated cow urine had significant antibacterial activity.

Antimicrobial activity was seen against bacterial pathogens *Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium, Aeromonas hydrophila, Enterobacter aerogenes*, and *Micrococcus luteus*. Inhibitory activity against growth of both Gram-positive and Gram-negative bacteria was evident, with the most pronounced effect at 30 µL dose (Fig. 1). The average width of inhibition zone at this dose was 15 mm. The highest antimicrobial activity, represented by a 22-mm inhibition zone was evident for *Aeromonas hydrophila* and the lowest activity (13 mm zone) against *Enterobacter aerogenes*.

Antibacterial activity of the 30 µL dose of photoactivated urine was comparable to that of standard tetracycline, which was used as a positive control (Fig. 1).

Discussion

Demand has raised the need in the scientific community to explore new antimicrobial agents (Murray et al. 2008; Shefer et al. 2008). Natural sources are among the best sources for discovering novel anti-bacterial substances. Several reports are available on a positive effect of cow urine in treatment of certain cardiac and kidney diseases, indigestion, stomach ache, edema, skin disease, epilepsy, anemia, constipation, respiratory disease etc. (Ojewol et al. 1976; Chauhan et al. 2001). Regarding a possible mode of action, cow urine can posses antimicrobial activity (Yadav et al. 2008). Therefore, the present study was designed to evaluate the antibacterial activity of photoactivated cow urine.

The present results clearly indicate that photoactivated cow urine exhibited high inhibitory potential against all human pathogenic bacterial strains tested. In this assay, photoactivated cow urine showed a great degree of antibacterial activity, comparable to that of antibiotics. The presence of certain volatile and nonvolatile components in urine is responsible for the antimicrobial activity (Hu et al. 2007; Shaw et al. 2007). Also, it has been reported that antibacterial activity of photoactivated urine may be due to its acidic pH (Sathasivam et al. 2010). However, that was not the case in the present study as pH of cow urine used was rather alkaline.

Beside this, the presence of amino acids and urinary peptides may enrich the bactericidal effect (Badadani et al. 2007) by increasing bacterial cell surface hydrophobicity. Photoactivated cow urine possesses higher antimicrobial activity than fresh cow urine due to formation of some inhibitory compounds, such as formaldehyde, sulfinol, ketones and some amines during photostimulation (Turi et al. 1997).

In conclusion, photoactivated cow urine possesses potential antibacterial activity and deserves attention for further studies on development of new drugs.

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