International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-4, 2013 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Cow Urine: An Antimicrobial Activity against Pathogens and their Possible Uses

Pankaj K. Tyagi[1], Shruti Tyagi[1], Vikas Sarsar[2], Rochika Pannu[2]

[1]*Department of Biotechnology Meerut Institute of Engineering and Technology, Meerut-250005, Uttar Pradesh Technical University, Lucknow, U.P., India.*

[2]*Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal-131039, Haryana, India.*

Manuscript No: IJPRS/V2/I4/00242, Received On: 20/12/2013, Accepted On: 26/12/2013

ABSTRACT

Cow urine is an effective killing agent against a broad spectrum of gram-negative and gram- positive bacteria. The present investigation proved the antimicrobial action of cow urine against the microbes of gram positive bacteria, *Bacillus cereus, Staphylococcus aureus* and gram negative bacteria, *Salmonella typhimurium, Aeromonas hydrophila, Enterobacter aerogenes, Micrococcus luteus.* The activities of photoactivated urine were comparable with that of a standard tetracycline. It was observed that photoactivated cow urine was showing similar antimicrobial activity as tetracycline against all pathogenic bacteria. Highest antimicrobial activity against gram positive *Staphylococcus aureus* (zone of inhibition 18mm) and gram negative bacteria *Aeromonas hydrophila* (zone of inhibition 22mm) was observed in photoactivated urine. Cow urine kills the number of drug resistant bacteria and viruses. Medical uses of cow urine was reported in literature and indicated to be beneficial even for dreaded diseases like cancer, AIDS and diabetes. Recently the cow urine has been granted U.S. Patents (No. 6896907), for its medicinal properties, US Patent for India Patent (No. 6410059), Cow urine antimicrobial effect of antibiotic and Patent (No.7235262), Cow urine use of bioactive fraction as a bio-enhancer of anti-infective.

KEYWORDS

Cow Urine, Tetracycline, Gram Negative Bacteria, Gram Negative Bacteria, Antimicrobial Activity

INTRODUCTION

Cow, is a most valuable animal in all Veda and it is called as the Mother of all. The composition containing cow's excretions, urine, dung, milk, curd and ghee, five ingredients together known as "Panchagawya" is given to women after she delivers a baby. Panchagawya is the main ingredient of many of our ayurvedic preparations.[1]

***Address for Correspondence:**
Pankaj K. Tyagi
Department of Biotechnology,
Meerut Institute of Engineering and Technology,
Mahamaya Technical University,
Noida, U.P., India.
E-Mail Id: pktgenetics@gmail.com

Cow urine one of the ingredients in panchagawya is believed to have therapeutic value. In India cow urine is used by majority of rural population as folklore remedy in almost all the states. As per Ayurvedic literatures gomutra is useful in number of diseases particularly in gulma, kusta, ascitis, filaria, aburda (cancer), etc. Cow urine is also used along with herbs to treat various diseases like fever, epilepsy, anemia, abdominal pain constipation, etc by the traditional healers[2,3], Immunomodulatory[4], hypoglycemic[5] and cardio- respiratory effects[6] of cow urine were established scientifically. Bacterial infections are an emerging problem

worldwide, especially in developing countries, such as India[7]. Gram-positive bacteria, such as *Staphylococcus aureus*, are mainly responsible for postoperative wound infection, toxic shock syndrome, scaled skin syndrome, septicemia, endocarditis, osteomyelitis, and food poisoning[8]. *Streptococcus pneumoniae* causes lobar pneumonia, bronchopneumonia, bronchitis, endocarditic, sinusitis, and conjunctivitis[9]. Gram-negative bacteria such as *E. coli*, which resides in human intestine, causes lower urinary tract infection, cholecystitis or septicemia, and another strain, *Klebsiella pneumoniae*, causes chest infections, urinary infections and wound infections.[10,11] Various antibiotics are available on the market to treat the bacterial infectious diseases by working on various targets to inhibit pathogen growth. Gram positive and gram-negative bacteria can be inhibited by antibiotics viz. Chloramphenicol, Nalidixic acid, Rifampicin and Ampicillin, either by blocking protein, DNA, RNAs or peptidoglycan synthesis, respectively.[12] However, the development of bacterial resistance to presently available antibiotics enforces the search for new antibacterial agents.[13,14] WHO emphasized research for natural components from herbal medicines to find new antibacterial agents.[15] Therefore, to combat the problem of microbial infection and drug resistance new alternative of synthetic drugs have been explored, though antimicrobial activities of so many natural products have been explored. But very few report available on antimicrobial activity of cow urine. In spite of the fact that the cow urine has great pharmacological importance and has great aesthetic and medicinal value though it's utility has been mentioned in holy texts of Indian literature. Cow urine has certain volatile and nonvolatile components, which might have very high antimicrobial activity[16]. After photoactivation and purification cow urine was made free from microbes and it gains massive toxic potential to kill drug resistant bacterial strains. Cow urine consists few important components such as estrogen[17], Phosphorous[18], Nitrogen[19], chloride[20], Potassium[21] and Calcium urinary proteins[22] and pheromones[23]. In India, cows are very important animal resource and are highly useful in agriculture and dairy industry.[24] It has been observed that important forest dwelling cows secrete so many herbal compounds in urine, which are of high medicinal value. In such cows plant origin dietary organic and inorganic compounds effectively get absorbed in the rumen and digested by bacterial activity. But there are some compounds, which do not disturb by any microbial enzyme action and secreted in their natural form in cow urine. In the present investigation we have observed the antimicrobial activity of photo activated cow urine against seven pathogenic bacterial strains that is, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebisella pneumoniae*, *Lactobacillus acidophilus* and *Micrococcus luteus.*

METHODOLOGY

Collection and Purification of Cow Urine

Healthy pure Indigenous breed (Hariana breed) of cattle was selected for this study, due to their high disease resistance capacity to other breeds. Fresh cow urine was collected in contaminated free glass bottles. Before loading the experiments, fresh cow urine was photo activated for 72-144 h to direct sunlight in sealed glass bottles. It was purified by chromatographically by using silica gel G-25 column and passed through two separate columns simultaneously to get rid of all the precipitated material and debris. Purified cow urine was stored at 4°C for long-term use. Before evaluation of antimicrobial activity, cow urine was tested for presence of other pathogens microscopically as well as in broth culture.

Bacterial Culture

All the test cultures were procured from the Microbial Type Culture Collection Center (MTCC), Chandigarh, India. The photoactivated cow urine were tested against Gram positive bacteria, *Bacillus cereus* (MTCC-1305), *Staphylococcus aureus* (MTCC-3160) and Gram negative bacteria, *Salmonella typhimurium* (MTCC-1253), *Aeromona shydrophila* (MTCC-

1739), *Enterobacter aerogenes* (MTCC-2823), *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, 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 the plates with the help of micropipette (30 µl). The plates were incubated at 37°C for 24 h and zone of inhibition was measured. Then the plates were incubated for 24 h at 37°C for room temperature. Tetracycline (30 mcg/ml) was used as positive control. The results were recorded by measuring the diameter of inhibition zone at the end of 24-72 h. Zone of inhibition surrounding the discs was measured.

RESULTS AND DISCUSSION

Antimicrobial activity of photoactivated cow urine against pathogenic bacterial strains was performed by agar well diffusion method. The photoactivated cow urine and tetracycline (Positive control) showed different zone of inhibition in mm against six pathogen bacteria (Table 1). The result of the present study shows that photoactivated cow urine has effective antibacterial activity against various human pathogenic bacterial strains. Cow urine is an effective antibacterial agent against a broad spectrum of Gram-negative and Gram-positive bacteria.[25]

The presences of certain volatile and nonvolatile components in urine are responsible for the antimicrobial activity.[26] The antibacterial activity of photoactivated urine may be due to its acidic pH.[27] The Photoactivated urines showed highest antimicrobial activity against gram positive *Staphylococcus aureus* (MTCC-3160) and Gram negative bacteria *Aeromonas hydrophila* (MTCC-1739) (Fig.2).

Table 1: Antibacterial activity of Photoactivated cow urine and Tetracycline (Positive control) against six pathogenic bacteria measured by agar disc diffusion method, expressed as the diameter of the zone of inhibition in mm

Micro-organisms	**Photo activated urine (**zone of inhibition in mm **)**	**Tetracycline (**zone of inhibition in mm **)**
Bacillus cereus	17	22
Staphylococcus aureus	18	21
Salmonella typhimurium	21	22
Aeromonas hydrophila	22	24
Enterobacter aerogenes	13	18
Micrococcus luteus	15	17

The bactericidal activity of the present study is also constituent with the reports of other authors which also show potent antibacterial activity in fresh urine. The activities of photoactivated urine were comparable with that of standard or positive control tetracycline (Fig.1).

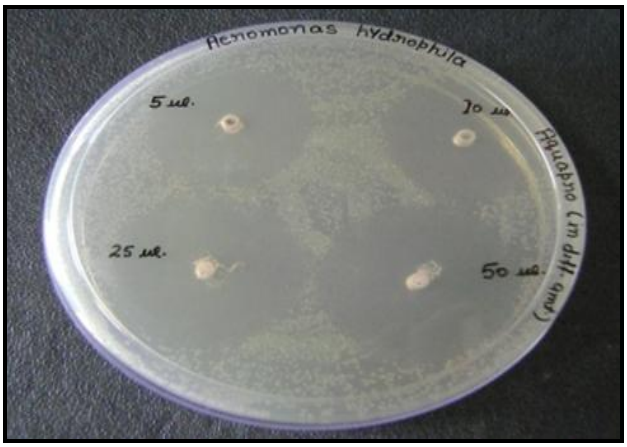

Figure 1: Anti-microbial activity of photoactivated cow urine against *Aeromonas hydrophila* measured by agar disc diffusion method, expressed as the diameter of the zone in mm

It was observed that photoactivated cow urine was sensitive for both gram positive and gram negative bacteria. The increasing prevalence of antibiotic resistance in infectious bacteria, ultimately increasing prevalence of infectious diseases in developed as well as developing countries, has raised the demand for the scientific community to search for new anti-bacterial components.[28,29]

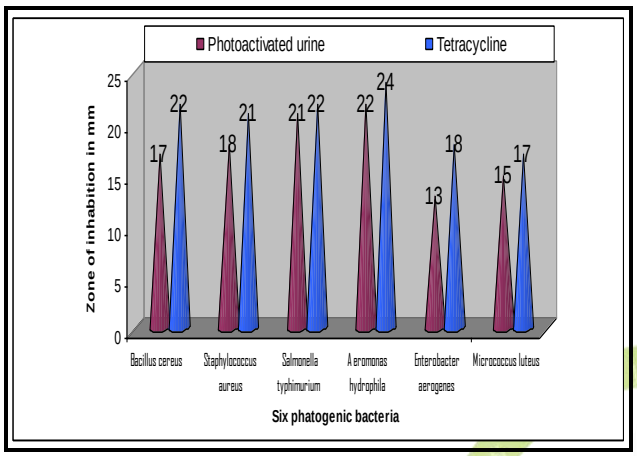

Figure 2: Results of antimicrobial activity (zone of inhabitation in mm) of photoactivated cow urine and tetracycline (Positive control) against six pathogenic bacteria

Natural sources are the best to find new and noble anti-bacterial substances that can help to resolve this problem to some extent.[30] In India, as well as various other parts of the world, large plants have been used as effective anti-microbial.[31] Therefore, the present study was designed to evaluate the anti-bacterial potential of cow-urine against six pathogenic bacteria. Surprisingly, we have observed that cow urine alone has the potential of an anti-bacterial property against both gram-positive and gram negative. Moreover, the results of the present study clearly indicate that Tetracycline had higher efficacy against all the selected strains from both gram positive and gram-negative groups, and similar efficacy is observed in the photoactivated cow urine, which indicates that this fraction had similar effects to Tetracycline by inhibiting bacterial growth. Furthermore, our preliminary results shown that cow urine has antibacterial properties due to the presence of various bioactive peptides that are under process to isolate characterize and study detailed mechanisms of action and cow urine also used for different possible use such as, medical, treatment, home remedies, antiseptic lotions.

POSSIBLE USES OF COW URINE

Medical uses of cow urine has been reported in a lot of literature and indicated to be beneficial even for dreaded diseases like cancer, AIDS and diabetes. Practitioners of Ayurvedic medicine from India routinely use cow urine as a remedy and the medicines made from it are used to cure several diseases. Improvements have been shown or reported with those suffering from flu, allergies, colds, rheumatoid arthritis, bacterial/viral infections, tuberculosis, chicken pox, hepatitis, leucorrhoea, leprosy, ulcer, heart disease, asthma, skin infections, aging, chemical intoxication etc. Cow urine can kill the number of drug resistant bacteria and viruses. Recently the cow urine has been granted U.S. Patents (No. 6,896,907) for its medicinal properties, particularly as a bioenhancer and as an antibiotic, antifungal and anticancer agent as an anti-cancer agent it increases the potency of "Taxol" (Paclitaxel) against MCF-7, a human breast cancer cell line, in in vitro assays (US Patent No. 6,410,059). Through extensive research studies a cow urine distillate fraction, popularly known as 'ark', has been identified as a bioenhancer of the activities of commonly used antibiotics, anti-fungal and anti-cancer drugs. Thus it can promote and augment the bioactivity or bioavailability or the uptake of drugs in combination therapy and reduce the dose and duration of treatment. These milestone achievements highlight the beneficial role of cow urine in treating bacterial infections and cancers and that cow urine enhances the efficacy and potency of therapeutic drugs. During the past few years cow urine therapy has provided promising and authentic results for the treatment of cancer, a deadly malady which is being faced by the mankind and the incidences of which are ever increasing in the current scenario of changed lifestyle and food habits along with exposure to predisposing factors of carcinogens such as tobacco chewing, smoking,

alcohol intake, environmental pollutants, occupational health hazards etc.

Anti-cancer potential of cow urine therapy has been reflected by several case reports, success stories and practical feedback of patients for the treatment of cancer. Cow urine enhances the immunocompetence and improves general health of an individual; prevent the free radicals formation and act as anti-aging factor; reduces apoptosis in lymphocytes and helps them to survive; and efficiently repairs the damaged DNA, thus is effective for the cancer therapy.

Cow Urine Products, India

According to International Research Center for Science, Nature, Religion (http://www.science-nature-religion.com/) report the cow urine products are used in India e.g. Cow urine medicines, Cow urine cool drinks, Cow urine fertilizers, Cow urine pesticides, Cow urine shampoos, Cow urine shave lotions, Cow urine hair oils, Cow urine phenyls.

Cow Urine - US Patent for India

Recently two patents are granted in uses of cow urine.

A) Patent 6410059: A pharmaceutical composition comprising an antibiotic and cow urine distillate in an amount effective to enhance antimicrobial effect of the antibiotic is disclosed. The antibiotic can be an antifungal agent. The antibiotic can be a quinolone or a fluoroquinolone. The antifungal agent can be azoles, clotrimazole, mystatin or amphotericin.

B) Patent 7235262: The invention relates to a novel pharmaceutical composition comprising an effective amount of bio-active fraction from cow urine distillate as a bioavailability facilitator and pharmaceutically acceptable additives selected from anticancer compounds, antibiotics, drugs, therapeutic and nutraceutic agents, ions and similar molecules which are targeted to the living systems.

ACKNOWLEDGEMENT

Authors are highly thankful to HOD of Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal, for provided necessary facility to conduct this work and also highly thankful to Director and HOD Department of Biotechnology, Meerut Institute of Engineering and Technology, Meerut for their continuous encouragement and problem solving assistance.

REFERENCES

1. Pathak ML, Kumar, A. Cow praising and importance of Panchyagavya as medicine, Sachitra Ayurveda, 2003, 5, 56-59.
2. Pathak ML, Kumar A, Gomutra a descriptive study, Sachitra Ayurveda, 2003, 7, 81-84.
3. Krishnamurthi K, Dutta, D, Devi SS, Chakrabarti T, Protective effect of diatillate and redistillate of cow's urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals. Biomedical and Environmental Science, 2004, 17, 57-66.
4. Chauhan RS, Singh BP, Singhal LK, Immunomodulation with kamdhenu Ark in mice. Journal of Immunology and Immunopathology, 2001, 71, 89-92.
5. Ojewole JA, Olusi SO, Effects of cow's urine concoction on plasma glucose concentration in fasted rats. Transactions of the Royal Society of Tropical Medicine and Hygiene, 1976, 71, 241-245.
6. Elegbe RA, Oyebola DDO, Cow's urine poisoning in Nigeria: the cardiotoxic effects of cow's urine in dogs. Transactions of the Royal Society of Tropical Medicine and Hygiene, 1976, 71, 127-132.
7. Sanders JW, Fuhrer GS, Johnson MD, Riddle MS, The epidemiological transition: the current status of infectious diseases in the developed world versus the developing world. Sci Prog, 2008, 91, 1-37.
8. Diederen BM, Kluytmans JA, The emergence of infections 1 with community associated methicillin resistant *Staphylococcus aureus*. Journal of Infection, 2006, 52, 157-168.

9. Riley C, Riley S, Influenza and pneumococcal disease in the community. Nursing Standard, 2003, 18, 45-51.

10. Pierce AK, Sanford JP, Aerobic gram-negative bacillary pneumonias. American Review of Respiratory Disease, 1974, 110, 647-658.

11. Beutin L, Emerging enterohaemorrhagic Escherichia coli, causes and effects of the rise of a human pathogen. Journal of Veterinary Medicine Infectious Diseases and Veterinary Public Health, 2006, 53, 299-305.

12. Williamson NR, Fineran PC, Leeper FJ, Salmond GP, The biosynthesis and regulation of bacterial prodiginines. Nature Reviews Microbiology, 2006, 4, 887-899.

13. Alanis AJ, Resistance to antibiotics: are we in the post-antibiotic era? Archives of Medical Research, 2005, 36, 697-705.

14. Okeke IN, Laxminarayan R, Bhutta ZA, Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infectious Diseases, 2005, 5, 481-493.

15. Mahady GB, Medicinal plants for the prevention and treatment of bacterial infections. Current Pharmaceutical, 2005, 11, 2405-2427.

16. Shaw SL, Mitloehner FM, Jackson W, Depeters EJ, Fadel JG, Robinson PH, Holzinger R, Goldstein AH, Volatile organic compound emissions from dairy cows and their wastes as measured by protontransfer–reaction mass spectrometry. Environmental Science and Technology, 2007, 14, 1310-1316.

17. Biddle S, Teale P, Robinson A, Bowman J, Houghton E, Gas chromatography-mass spectrometry/mass spectrometry analysis to determine natural and post administration levels of oestrogens in bovine serum and urine. Analytica Chimica Acta, 2007, 586, 115-121.

18. Bravo D, Sauvant D, Bogaert C, Meschv F, Quantitative aspects of phosphorous excretion in ruminants. Reproduction Nutrition Development, 2003, 43, 285-300.

19. Yan T, Frost JP, Keady TW, Agnew RE, Mayne CS, Prediction of nitrogen excretion in faces and urine of beef cattle offered diets containing grass silage. Journal of Animal Science, 2007, 85, 1982- 1989.

20. Coppock CE, Aguirre RA, Chase LE, Lake GB, Oltenacu EA, McDowell RE, Fettman MJ, Woods ME, Effect of a low chloride diet on lactating Holstein cows. Journal of Dairy Science, 1979, 62, 723-731

21. Lebeda M, Bus A, Effect of Potassium – hydrogen interaction in the excretory mechanism of the kidney on the acid –base and other biochemical values of the blood and urines in calves. Veterinary Medicine (Prabha), 1997, 22, 229-236.

22. Gabel M, Poppe S, Protein and amino acid metabolism in the intestinal tract of growing bulls. Arch. Tierernahr, 1986, 36, 709-729.

23. Tauck SA, Berardinelli JG, Putative urinary pheromones of bulls involved with breeding performance of primiparous beef cows in a progestin- based estrous synchronization protocol. Journal of Animal Science, 2007, 85, 1669-1674.

24. Jonker JS, Kohn RA, Using milk urea nitrogen to evaluate diet formulation and environmental impact on dairy farms. Scientific World Journal, 2001, 1, 852-859.

25. Sarsar V, Krishan KS, Manjeet KS, Rochika P, Tyagi PK, Evaluation of antibacterial activity of photoactivated cow urine against human pathogenic strains. Environmental and Experimental Biology, 2013, 11, 201–203.

26. HW W, Murphy MR, Constable PD, Block E, Dietary cation anion difference effects on performance and acid base status of dairy cows postpartum. Journal of Dairy Science, 2007, 90, 3367-3375.

27. Shafer RW, Rhee SY, Bennett DE, Consensus drug resistance mutations for epidemiological surveillance: basic principles and potential controversies. Antiviral Therapy, 2008, 2, 59-68.
28. Arun KS, Muthuselvam M, Rajasekran R, Antimicrobial activities of cow urine distillate against some clinical pathogens. Global journal of pharmacology, 2010, 4(1), 41-44.
29. Murray CK, Infectious disease complications of combat-related injuries. Critical Care Medicine, 2008, 36, S358-364.
30. Jain SK, Ethnobotany and research on medicinal plants in India. Ciba Foundation Symposium, 1994, 185, 153–164.
31. Craig WA, Pharmacokinetics/ pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clinical Infectious Diseases, 1998, 26, 1–12.

