ORIGINAL ARTICLE

The Effect of Aloe Vera Extract and Ciprofloxacin on the pH of Urine in *Proteus Mirabilis* Induced Urinary Tract Infection in Albino Rats

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ABSTRACT

Proteus mirabilis is one of the common Gram-negative pathogens that infect the upper urinary tract.Once attached to urinary tract, it infects the kidney and is difficult to eradicate. The frequency of kidney infection by Proteus mirabilisis more than Escherichia coli. Proteus mirabilis may cause severe renal damage such as Acute Pyelonephritis, fever, bacteremia and renal stones. Urease enzyme present in Proteus mirabilis catalyzes the formation of struvite and apatite stones in the kidney and urinary bladder and increases the urinary pH. A rise in urinary pH is indicative of a positive Urinary Tract Infection due to Proteus mirabilis.Aloe vera has a long history of medicinal usage. This study was conducted to observe the effect of Aloe vera extract and its comparison with Ciprofloxacin against Proteus mirabilis induced increase in pH as marker to evaluate its antimicrobial activity against the organism. 50 female albino rats, divided into 5 groups, 10 in each were used. They were artificially induced UTI with Proteus mirabilis. The first group was not treated while the second group was given Ciprofloxacin. The rest of the groups were given Aloe vera whole leaf aqueous extract in three different doses. Urine pH was determined on Day 0, 1, 5, 10 and 15, before and after the administration of Aloe vera extract (in three doses) and Ciprofloxacin. At low doses, Aloe vera had no significant effect on the pH of urine, but a decline in pH was observed in the groups treated with intermediate and high doses of Aloe vera and Ciprofloxacin respectively, over the study period with p-value 0.510 and 0.709 respectively.

Conclusion: The reduction in urinary pH with Aloe vera extract is comparable to ciprofloxacin indicating its activity against Proteus mirabilis. It is a potential therapeutic remedy in patients where Ciprofloxacin cannot be used, but it takes longer time to exert its effect.

Keyword: Aloe vera, Urinary Tract Infections, Urinep H,Ciprofloxacin, *Proteus mirabilis*

INTRODUCTION

Urinary Tract Infection (UTI) is the second most common infection in public practice. Universally, about 150 million people are diagnosed with UTIs each year, costing the international economy of 6 billion US dollars (Astal ZE, 2005). UTIs due to Proteus mirabilis are well known and they significantly damage kidneys. The rise in urinary pH in subjects infected with this pathogen is attributed to the urease enzyme present which causes hydrolysis of urea into ammonia causing local pH to rise and may lead to precipitation of magnesium and ammonium crystals, as suggested by Melanie P. et al in 2008. (Li X et al., 2004; Pearson MM et al., 2008).

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Treatment in vogue for *Proteus* infection includes fluoroquinolones, co-trimoxazole (TMP/SMZ), third generation cephalosporin, such asceftrioxane and gentamicin (Stuble K, 2011).Amongst the quinolones Ciprofloxacin has shown exceptional activity against gram-negative bacteria responsible for Urinary Tract Infections.An increased incidence of resistance has been reported against antimicrobials including Fluoroquinolones. This emergence of drug resistance in various communities has raised the need to re-assess the choices for treating UTI (Kahlmeter G, 2003).

Aloe vera is known as *Quargandal* in Pakistan.It has been used for medicinal purposes since a very long time (Khiljee S et al., 2011). It is a perennial, drought-resisting, succulent plant belonging to the Lily (Liliaceae) family containing 300 different species with its origin in Africa.It is now reported that Aloe vera contains as many as 75 nutrients and 200 active compounds including saponins, vitamins, sugar, enzymes, anthraquinones, minerals, lignin, salicylic acid and amino acids (Kaur R, 2010). Aloe vera extract, as an antibacterial agent,

has an extensive activity against Gram negative and Gram positive pathogens. The gel effectively kills or significantly reduces or eliminates the growth of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiellapneumoniae, Pseudomonas aeruginosa,Salmonella typhi*and *Helicobacter pylori*(Lawrence R. et al., 2009). Alemdar and Agaoglu (2009) mentioned in their study that there is wide range of research work based upon different species of Aloe vera, showing anti-microbial activity of the Aloe veraextract against Gram-negative and Gram-positive bacteria (Alemdar S and Agaoglu S, 2009).Whole leaf components of Aloe vera have shown direct antibacterial properties. These components are anthraquinones and saponin.The polysaccharides, present in the gel, cause stimulation of phagocytic leucocytes to destroy bacteria and exert indirect antibacterial activity (Shilpakala SR et al., 2009).

In one of the research works regarding isolation, purification and evaluation of antibacterial agents from Aloe vera, the fractions showing maximum antibacterial activity were identified as phenol compounds i.e. Cinnamic acid, Pyrocatechol, Ascorbicacid and *p*-coumaric acid. These phenolic compounds act by destroying proteins and disturbing cell membranes. Pyrocatechol is a hydroxylated phenol, known to be lethal to micro-organisms (Cowan MM, 1999).

In addition to direct inhibitory effects on bacteria, Aloe vera components may also function by selectively modulating the cells of the immune system. Moreover, Acemannan also inhibits bacteria adhering to epithelial cells and establish an infection. It is likely that the anti-bacterial activity of Aloe vera extracts in vivo is due to the synergistic effects of multiple bioactive components, functioning through several mechanisms (Cock IE, 2011).

Global antibiotic resistance is becoming an interesting public health concern and the efforts are being made to discover new antibacterial remedies. Aloe vera, along with its recognized compounds with promising antibacterial activity, could be used as a substitute or adjunctive herbal remedy. These compounds also have been reported to have other benefits on human health, with very little side effects in the overdoses (Lawrence R et al., 2009).

The present study was,therefore, designed to evaluate the usefulness of Aloe vera as an alternative treatment for commonly occurring infections with *Proteus mirabilis* by analyzing the fall in pH (asthe rise in pH is an indicator of UTI with *Proteus mirabilis*). A comparison was made with ciprofloxacin, a commonly used antimicrobial for the same infection (Katzung BG et al., 2009).

MATERIAL AND METHODS

This Randomized control experimental study involved groups of rats who received Aloe vera extract and Ciprofloxacin for fifteen days. The change in the urinary pH was observed at different intervals to analyze the fall in pH toassess and compare their antimicrobial activity. The study was conducted in the laboratories of Pharmacology & Therapeutics Department and Pathology Department PGMI (Post Graduate Medical Institute), Lahore, Pakistan. They were fed with rat chow and water *ad libitum*. 50 female albino rats, weighing between 150 and 300 gram, were procured from National Institute of Health, Islamabad and kept in the animal house at the Post Graduate Medical Institute, Lahore. They were acclimatized for 2 weeks before starting research work.

All 50 rats were introduced UTI by *Proteus mirabilis* obtained from Armed Force Institute of Pathology (AFIP), Rawalpindi. After confirmation by culture, they were dived into 5 groups of 10 each by simple random balloting. Each group of rats was kept in a separate cage.

Group 1A (Control group): This group was not given any treatment.

Group1B: Ciprofloxacin was given for 15 days. (Kadurugamuwa JL et al., 2005)

Group 2A: 25 ppm of Aloe vera extract was given, twice daily for 15 days (TaiwoVO et al., 2005).

Group 2B: 50 ppm of Aloe vera extract was given, twice daily for 15 days (TaiwoVO et al., 2005).

Group2C: 100 ppm of Aloe vera extract was given, twice daily for 15 days (TaiwoVO et al., 2005).

Each group was kept at controlled room temperature (22±2^{o}C) and humidity of 55±10% (Sato T et al., 2005). They were kept under natural light and dark cycle. They were fed on normal rat chow and water *ad libitum.*

Preparation of aqueous extract of Aloe vera

Collection of plant material: Aloe vera leaves (10 Kg), were collected from the research field of Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore in the month of July 2011. Fresh leaves were washed with tap water and sliced with sterilized knife. During cutting of leaves (from the mature plant) and peeling off, the yellow sap expressed was not removed from the mucilaginous gel. 250gm of the cut leaves were pulverized in an electric blender and soaked in 2 liters of water for 3 hours .They were filtered through 1mm mesh sieve. The filtrate was made up to 10 liters with water, making a working dilution of 25,000ppm of the water extract of Aloe vera(Taiwo et al., 2005). From the above stock solution, three

required dilutions 25 ppm, 50ppm & 100 ppm were prepared.

Urine pH: The pH of the urine is directly related to the Urinary Tract Infection where *Proteus Mirabilis* is the causative agent (Jacobsen SM et al., 2008); therefore, it was used as a marker to assess the antimicrobial effect of the Ciprofloxacin and Aloe vera. Urine pH was tested by test strip (Medi-Test Combi 10 SGL) by Marcherey-Nagel

Standardization of bacterial dose: *Proteus mirabilis* was obtained from Armed Force Institute of Pathology (AFIP) in Rawalpindi. It was kept in SIMS Microbiology department laboratory. Bacterial specimen was cultivated in nutrient broth in (MacKonkeys agar) at 37^{o}C overnight. Next day, dose of *Proteus mirabilis* was adjusted by means of optical density method using McFarland scale (RiveroNl et al.,2012). Bacterial dose was 5x10^{7}bacteria/ml in phosphate buffer saline solution (Cheesebrough M, 2003; Sutton S, 2006).

Introducing UTI: Rats were anesthetized by chloroform. They were placed on a board whereperiurethral place was cleaned by spirit swab. A sterile polyethylene catheter was taken and inserted into the bladder through the urethra of the rat (Johnson DE et al., 1998; Burall LS et al., 2004). Lignocaine gel was applied and the tube was gently introduced through the urethra of the rat. 1 cc of syringe was securely fixed with the catheter and 0.5cc of bacterial suspension, containing *Proteus mirabilis*, was slowly infused through urethra. Catheter was held in place, firmly between thumb and index finger. Tube was gently removed from urethra and after gaining consciousness rats were put back in their cages (Larsson P et al., 1980).

Introduction of Urinary Tract Infection through urethra

Collection of urine sample: The periurethral area of rats was cleaned by spirit swab and urine sample was collected in Eppendorf tubes by gentle compression of the bladder at the mentioned time, which was 2 days after inoculation of infection i.e. day 1 and later on days 5, 10 and15. Early morning samples were collected. (Hvidberg H et al., 2000)

Confirmation of Proteus Infection: Urine sample was collected in the morning, by holding the rat from back. Abdomen was pressed and urine sample was taken in the Eppendorf tube. The sample was inoculated on CLED and incubated for 48 hours at 37°C. Proteus infection was confirmed after 2 days of inoculation. Individual blue green colonies of non-lactose fermenting pathogens were observed. Swarming was not observed because CLED is electrolyte deficient. The colonies on CLED from all rats were reconfirmed by cultural characteristics and biochemical profile. All were gram negative rods, non-lactose fermenting on CLED,Urease and H2S positive.Consequently,*mirabilis* infection was confirmed on 3rd day of urethral inoculation (Brooks GF et al., 2007). The infection was established in all the animals. After the confirmation of infection i.e. the third day after introducing the infection, the treatment was initiated according to the dosing schedule mentioned earlier. It was considered as “day 1”of study.**(**Kadurugamuwa JL et al., 2005)

Urine pH: The pH of urine, collected on the specific day, was measured with test strip (Medi-Test Combi 10 SGL) by Marcherey-Nage

Urine Culture: Urine culture was done in all the groups, before and after introducing infection. A culture was done before infection to confirm the absence of *Proteus mirabilis* in urinary tract and another culture was done after introducing the infection to confirm the presence of *Proteus mirabilis* infection. CLED (cystine lactose electrolyte deficient) medium was used for culture (Cheesebrough M, 2003).

Statistical Analysis: The statistical analysis was carried out using computer software Statistical Package for Social Sciences (SPSS) version 18.0.ANOVA and Tukey’s test for post hoc analysis were applied. The difference was regarded statistically significant if the ‘p’ value was < 0.05.

RESULTS AND DISCUSSION

In the present study, urinary pH was analyzed to observe the effect of Aloe vera extract on UTI. Urine was obtained from female albino rats on day 0, 1, 5, 10 and 15 after introducing transurethral infection. The data was collected and a comparison was made between the untreated group and the groups treated with Ciprofloxacin and Aloe vera. The indicator analyzed was urinary pH since *Proteus mirabilis* causes rise in urinary pH,

Urinary pH: Urine was obtained from female albino rats on day 0, 1, 5, 10 and 15 after introducing transurethral infection.The data was collected and a comparison was made between untreated group and the groups treated with Ciprofloxacin and Aloe Vera A decline was observed in the pH in the groups treated with Aloe Vera (intermediate and high doses) and Ciprofloxacin, respectively.Comparisons of the pH of urine sample were made between untreated group and groups treated with different doses of Aloe Vera and Ciprofloxacin.

In group 1A,(untreated group) the pH level showed a rise from 6.3±0.48 on day 1 to 8.6±0.52 on day 15. In 2A (low dose Aloe Vera), the pH level also increased from 5.6±0.52 at day 1 to 7.9±0.32 at day 15. The pH level in group 2B and 2C (given intermediate and high dose of Aloe Vera respectively) also increased to 7.7±0.48 and 7.7±0.67 respectively at day 5 but then declined to 6.5±0.85 and 6.4±0.84 respectively at day 15. Similarly, in group 1B,which was treated with Ciprofloxacin, the pH level was 7.0±0.47 on first day of infection, but fellto 6.0±0.82 on 15th day of treatment. ThepH level of group 2A was similar to 1A with p-value 0.149, 0.043, 0.062 and 0.18, indicating that at a low dose Aloe Vera was not effective in treating UTI; hence the urinary pH remained alkaline.

When the comparison was made between 1A and 2B and 1A and 2C, low pH levels were observed in groups 2B and 2C with significant p-values on specific days. On day 15,the p-value was highly significant i.e., <0.001, reflecting that intermediate(2B) and high doses (2C) of Aloe Vera had antibacterial activity. It was showed by a decline in the pH of the urine.Similarly, groups 2B and 2C had highly significant p values i.e. <0.001 as compared to 2A, again indicating that low dose of Aloe Vera was not enough to effectively treat the infection; hence, the fall in pH of urine was not observed when compared with intermediate andhigh doses.

The comparison between 2B and 2C showed insignificant p-values throughout the experiment. It indicated that the effect on pH of intermediate and high dose of Aloe Vera was almost similar.Therefore, there was no remarkable difference between their antibacterial activities. When group 1B treated with Ciprofloxacin was compared with untreated group (1A) the comparison of pH between the two groups showed insignificant results at baseline with p-value 1.000 and the difference was found significant at day 1, day 5, day 10 and day 15 with p-values 0.016, 0.043, <0.001 and <0.001 respectively

The average pH level was high at day 1 and low at other follow up days in group 1B, showing that Ciprofloxacin effectively treated the infection indicated by lowering urinary pH. In comparison of 1B with 2A the difference among the pH was appreciable with significant p-values i.e. <0.001 at day 1 and 15, showing the effectiveness of Ciprofloxacin versus low dose of Aloe Vera.

When group 1B was compared with group 2B and 2C,the only significant p-value was observed on day 5(<0.001).Otherwise, there was no significant difference found between these groups with p-values 0.253 and 0.062 at day 10 and 0.510 and 0.709 at day 15 respectively.This showed that the effect of intermediate and high doses of Aloe vera is comparable with Ciprofloxacin in treating the infection and consequently, lowering the pH of urine. This has been documented in studies by Griffith (1975) and Mobley (2003), where induction of transurethral infection, when kept untreated, caused a rise in urinary pH. However, with ciprofloxacin and intermediate and high doses of Aloe vera, the progression of infection was interrupted, resulting in the decline in pH of urine.

Our data showed that Aloe Vera had antibacterial activity dependent upon both, the duration of treatment as well as on the dose of the extract. Drugs to treat microbial infections are among the most frequently used and misused of all drugs. Their unjustified and otherwise widespread use has been the reason for emergence of antibiotic-resistant pathogens, leading to an ever-increasing need for new drugs. This research is a stimulus for future researchers to study the mechanism of action of Aloe Vera in treating UTI and its probable role in preventing renal stones due to urease producing pathogens such as *Proteus mirabilis*. Its role as an adjuvant, along with other antimicrobials, may also be studied.

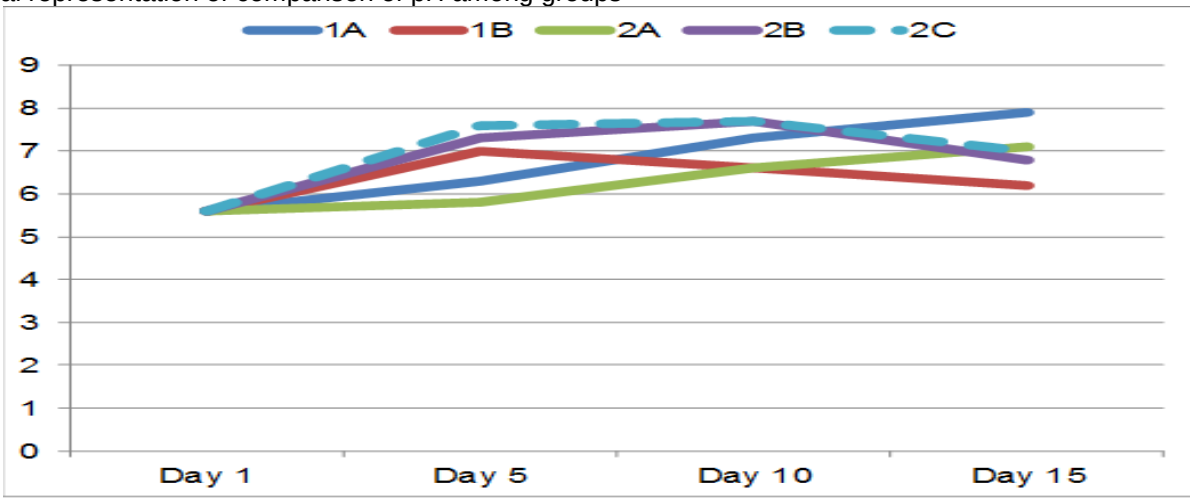
Urine pH value (Mean ±SD) of all groups

Time	**1 A**	**1 B**	**2 A**	**2 B**	**2 C**
Day 0	5.6±0.52	5.6±0.52	5.6±0.52	5.6±0.52	5.6±0.52
Day 1	6.3±0.48	7.0±0.47	5.8±0.42	7.3±0.48	7.6±0.52
Day 5	7.3±0.48	6.6±0.52	6.6±0.52	7.7±0.48	7.7±0.67
Day 10	7.9±0.32	6.2±0.92	7.1±0.32	6.8±0.79	7.0±0.67
Day 15	8.6±0.52	6.0±0.82	7.9±0.32	6.5±0.85	6.4±0.84

Comparison of p value among the pH of all groups

Time	1A Vs 1B	1A Vs 2A	1A Vs 2B	1A Vs 2C	1B Vs 2A	1B Vs 2B	1B Vs 2C	2A Vs 2B	2A Vs 2C	2B Vs 2C
Day 1	0.016**	0.149	<0.001 ***	<0.001 ***	<0.001 ***	0.625	0.053*	<0.001***	<0.001***	0.625
Day 5	0.043*	0.043*	0.470	0.470	1.000	<0.001 ***	<0.001 ***	<0.001***	<0.001***	1.000
Day10	<0.001***	0.062	0.004 ***	0.026*	0.026*	0.253	0.062	0.893	0.997	0.958
Day15	<0.001***	0.188	<0.001 ***	<0.001 ***	<0.001 ***	0.510	0.709	0.001***	<0.001***	0.998

Graphical representation of comparison of pH among groups



CONCLUSION

In our study Aloe Vera resulted in lowering of pH of urine which was finally observed on day 15th of experiment with 100 ppm which was comparable with ciprofloxacin. However, Ciprofloxacin effectively reduced the pH within 5 days of treatment. pH of urine can be used as marker for evaluating the antimicrobial activity against *Proteus mirabilis* induced urinary tract infection. Our data shows that Aloe vera extract (100 ppm) may be used as an alternative to the conventionally used drug such as Ciprofloxacin for treatment of UTI caused by *Proteus mirabilis*, although it takes longer time to show its effect.

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