ORIGINAL ARTICLE

The Role of Aloe Vera Extract on Bacterial Count in the Urine of Albino Rats Infected with *Proteus Mirabilis* and its Comparison with Ciprofloxacin

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ABSTRACT

Proteus mirabilis, a Gram-negative pathogen, is most frequently associated with urinary tract infections. When attached to urinary tract, this pathogen infects kidney more commonly than other pathogens. It may cause fever, bacteremia, acute pyelonephritis, and renal stones. If there is one-hundred thousand colony forming units per milliliter in the urine, this is indicative of a urinary tract infection. Aloevera has been documented to have an important antimicrobial activity as it efficiently kills or significantly decrease or remove the growth of Klebsiella pneumoniae, Staphylococcus aureus and Escherichia coli etc. The purpose of this study was to understand the roleof Aloe verain *Protues mirabilis* induced urinary tract infection and its comparison with Ciprofloxacin, considering bacterial count as a marker of infection in order to determine antimicrobial activity of Aloe vera. Five groups of 50 female albino rats, containing 10 in each group were used. Through artificial means, UTI was introduced in all animals. First group was kept as experimental control. The positive control group was given Ciprofloxacin. Three different doses of aqueous extract of Aloe vera whole leaf were used in group rest of three groups. Bacterial count in urine was calculated on day 0, 1, 5, 10 and 15, in advance and subsequent to the administration of Ciprofloxacin and Aloe vera extract (in three different doses). Assessment for bacterial count in urine among three groups showed that, at low doses Aloevera had insignificant effect, but had modest antibacterial outcome at intermediate dose (p-value 0.037),while at a high dose the result was nearly equivalent with Ciprofloxacin (p-value 0.970).

Conclusion: The reduction in urinary bacterial count 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: Aloevera, Urinary Tract Infections (UTI), Urinebacterial count, Ciprofloxacin

INTRODUCTION

UTIs due to *Proteus mirabilis* are well known and they significantly damage kidneys(1).Drugs available for *Proteus* infection include co-trimoxazole (TMP/SMZ), third generation cephalosporin, fluoroquinolones and gentamicin[2]. Ciprofloxacin (fluoroquinolones) is a known antibacterial drug for its action against gram-negative pathogen responsible for Urinary Tract Infections. Fluoroquinolones has shown a high incidence of resistance against urinary pathogens. This trend towards developing resistance against pathogen demands to re-assess the treatment for UTI[3].

Common name for Aloe verain Pakistanis *Quargandal*[4]. Phytochemical analysis of Aloevera showed approximately 75 nutrients and 200 active compounds containing sugar, vitamins, amino acids, minerals, enzymes, saponins, anthraquinoes and salicylic acid[5]. Aloe veragel efficiently kills or reduces the growth of *Streptococcus pyogenes, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli*[6]. Anthraquinones and saponin present in whole leaf of Aloevera have expressed direct antibacterial activity. The gel of Aloevera contains polysaccharides, which are responsible for indirect antibacterial activity through stimulation of phagocytic leucocytes[7]. In one of the studies, Aloevera inhibited the growth of 8 out of 14 bacterias, and this activity as determined from the zone of inhibition, was most prominent against Alcalgenesfaecalis and Protuesmirabilis[8].

The probable anti-microbial mechanism of Aloevera is because of the synergistic effects of various bioactive constituents, working through a number of modes[9]. Among these constituents, the phenol compounds i.e., Cinnamic acid; Pyrocatechol, Ascorbic acid and *p*-coumaric acid have shown maximum antibacterial activity by several mechanisms. For example, they act by disturbing cell membranes, destroying proteins of pathogens or by

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inhibiting glucose uptake and ATP assembly[10]. They may also act by prolonging the lag phase of the micro-organisms or by restricting the enzymatic activity of the bacteria, in addition the immune system may also be tempered. Acemannan, another constituent of Aloevera, may interfere the bacterial binding with epithelial cell[6].

Antibiotic resistance, worldwide is increasing to an alarming level and the trials and researches are being made to find out different antibacterial treatments. Aloevera and its components can be used as a substitute or adjunctive therapy[6]. This study was designed to assess the efficacy of Aloevera as a substitute treatment for infections with *Proteus mirabilis* by analyzing the fall in bacterial count in the urine of albino rats and comparing it with ciprofloxacin.

MATERIAL AND METHODS

In this experimental study various strengths of whole leave Aloe vera aqueous extract and Ciprofloxacin were given to 5 groups of rats for fifteen days. The change in the urinary bacterial count was observed at different intervals to assess and compare the antimicrobial activity of these strengths. Randomized control experimental study that was conducted in Pharmacology and Pathology Department of PGMI, Lahore, Pakistan. Female albino rats, 50 in number, weighing within range of 150 and 300 gm, were obtained and acclimatized before starting the research work. *Proteus mirabilis* was arranged from AFIP. Artificial UTI was introduced in all 50 rats. Infection was confirmed by culture and after confirmation through simple random balloting these rats was divided into 5 groups of 10 animals.

Group 1 (Control group): no treatment was given

Group2: 30 mg/kg of ciprofloxacin was given B.D for 15 days[11].

Group 3, 4, 5: rats in these groups were given increasing doses of Aloe vera25, 50 and 100 ppm respectively B.D for 15 days**[12].**

Whole leave extract of fresh Aloe leaves (250gm)was prepared by blending Aloevera leave and filtering them through sieve. Working dilution of 25,000ppm (stock solution) was prepared**[(12)]** and from this stock solution, three required dilutions were made i.e., 25 ppm, 50ppm & 100 ppm.

Proteus mirabilis, in dose of $5x10^7$bacteria/ml **(**in phosphate buffer saline solution**)** was adjusted with the help of optical density method according to McFarland scale[13].

Rats were anesthetized and infused slowly with 0.5cc of bacterial suspension of *Protues mirabilis*, through urethra by means of sterile polyethylene catheter **[(14)].** Morning urinary samples of all rats were collected 2 days after inoculation of infection that was considered day 1 and later on days 5, 10 and15**[(15)].** After 2 days of inoculation confirmation of urinary infection with *Proteus mirabilis* was done **[(16)],** and according to dosing schedule (mentioned earlier) treatment was started.

RESULTS AND DISCUSSION

Statistical Package for Social Sciences (SPSS) version 18.0 was used. ANOVA and Tukey's test for post hoc analysis were used. The difference was considered statistically significant if the 'p' value was < 0.05.

The data collected during this work indicated that Aloevera possess antibacterial activity which depends on both factors i.e. dose of the extract and duration of treatment .In this research the bacterial count was studied among the groups at different stages (days). Observation was made that the bacterial count constantly increased through research time in the group 1which was not given any treatment. This was comparable to group 3 in which low dose of aloevera was administered where the rise in bacterial count was observed in the same pattern (as with group 1) till day 15.In group 4 (given 50 ppm), a noteworthy drop in bacterial count was observed when compared with group 1 on day 15 with p value 0.001. In group 5 (given 100 ppm), the bacterial count dropped till last day, with p-values of 0.001. This observation proposed that by increasing the dose of Aloevera, there was a fall in bacterial count and in highest dose i.e. 100ppm this plant showed a better antibacterial action against Proteus mirabilis. In another research it was stated that Aloevera showed a significant in vitro antibacterial activity and it was particularly active against Proteus mirabilis[8]. This is close to this in vivo work, as Aloevera, in high dose, reduced the total number of urinary pathogen.

With Ciprofloxacin (group 2), however the bacterial counts started decreasing from day 1 and decrease levels of 2102±3338 were achieved on last day i.e. day 15. A significantly low number of bacteria were found in group 2 when compared with1 (at different days) with p-values shown in the table. This is in recognition with the statistics that Flourquinolones e.g., ciprofloxacin have known action against urinary pathogens including Proteus mirabilis[17].

On comparing different groups with group 2, it was found that at day 5, highly significant results were shown by group 2 as compared to 3 and 4 with p-values 0.001 and 0.002, but at day 5 on comparing 2 with 5 the difference was not significant with p-value 0.089. The statistical difference on day 10

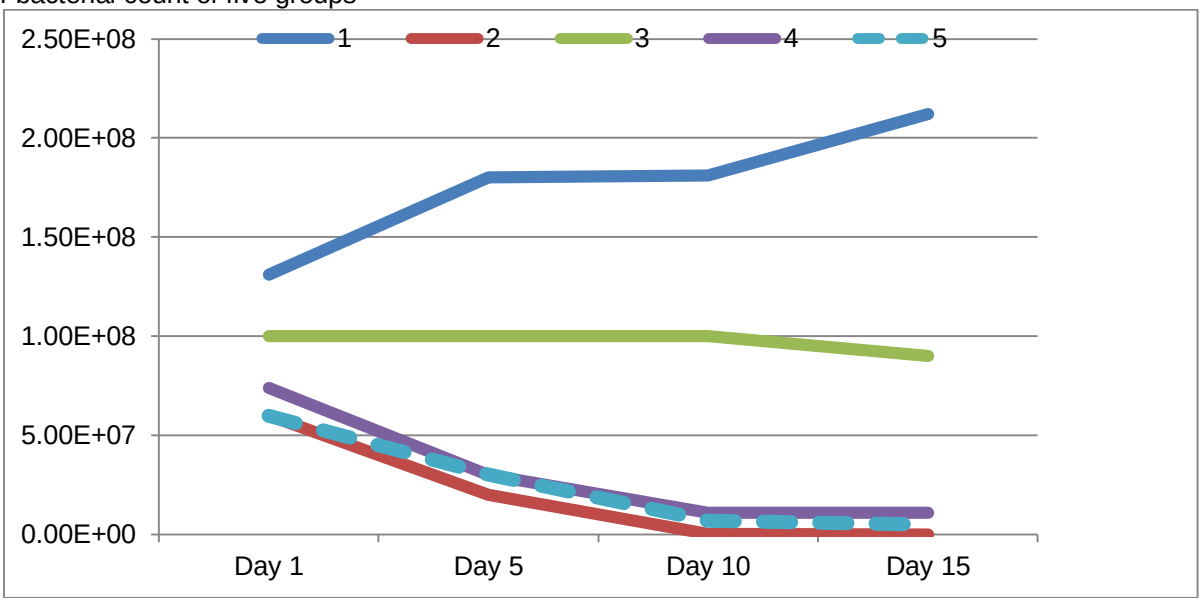
between 2 and 3was 0.001 and between 3 and 4was 0.034 which was significant. On the other hand comparison between 2 and 5was not significant, p-values 0.226. The results were significant again on day 15 i.e. between group 2 and 3p-values was 0.001 and 2 and 4it was 0.037, and insignificant p-value 0.970 amongst group 2 and 5.These results propose that Aloevera, in 25 ppm, showed no note worthy impact on bacterial count, but showed a reasonable antibacterial activity at intermediate dose. The results at highest dose i.e.100ppm with p-value of 0.970 on last day exhibited that the effect was practically equivalent when compared with Ciprofloxacin (p-value 0.970).

P value comparison between the bacterial count of groups

Days	1 & 2	1 & 3	1 &4	1&5	2&3	2&4	2&5	3&4	3&5	4&5
1	-	-	-	-	-	-	-	-	-	-
5	0.001 ***	0.596	0.130	0.002 ***	0.001 ***	0.002 ***	0.089	0.472	0.006 ***	0.010
10	<0.001 ***	0.089	0.001 ***	0.001 ***	0.002 ***	0.034*	0.226	0.151	0.013 **	0.496
15	<0.001 ***	0.049	0.001 ***	0.001 ***	0.001 ***	0.037 *	0.970	0.130	0.006 ***	0.151

*Significant ** More significant *** Highly significant

Graph of bacterial count of five groups



CONCLUSION

In this study it was observed that, Aloevera extract in different doses for continuous 15 days showed mild, moderate and high antibacterial action that finally lead to the lowering of the bacterial count in urine and this effect was most marked on day 15thday of research with 100ppm. This may be suggestive that for the treatment of UTI caused by Proteus mirabilis, extract of Aloevera in high dose may offer an attractive and suitable alternative, although it takes extended time to express its effect. The results of this research undoubtedly show that Aloevera has encouraging properties especially against UTI and make this plant a promising agent to be explored further.

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