A CLINICAL EVALUATION OF ANTIMICROBIAL ACTIVITY OF GOMUTRA ARKA IN DUSTA VRANA

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ABSTRACT

Vrana chikitsha is very significant in field of Shalya tantra. Healing of Vrana is depends on many factors. Among them growth of microorganism play essential role to slow the process of healing. In Dusta Vrana to improve the healthy granulation and to reduce the colony count of microorganism specific Krimighna dravya is required for that in Shasthi upkrama under Krimighna karma Gomutra Arka is advised. Which has additional property of Lekhana and Ropana. Gomutra Arka is given importance in many oral classical formulation of Ayurveda to treat the Krimi, which is not evaluated for local application on Dusta Vrana yet.

Materials and Methods: A simple randomized controlled clinical study where 50 patients were equally divided as group A: Gomutra Arka (trial drug) and Group B: Povidone-iodine (standard drug) for 14 days. Observations and results: The study result revealed that Gomutra Arka proved to be better Antimicrobial agents than povidone-iodine with a highly significant p-value of 0.04 in comparison to 0.68. It was also established in the study that 10ml Gomutra Arka proved to be more effective on different microorganisms that are Staphylococcus Aureus, Pseudomonas Aeruginosa, Shigella, Salmonella, E. Coli, and Candida Albicans. Conclusion: The Gomutra Arka acts as an antimicrobial agent due to its property like Katu tikta kashya rasa which are Krimighna in nature by this inhibition of microorganism could happen also due to the Ushna tilkshna and Kshara guna gives unfavorable environment for bacterial growth. The presence of constituents like copper, aurum, urea, ammonia further helps in antimicrobial activity. Therefore, Gomutra Arka can be used in regular practice as its available at a low cost, affordable and the study proves its efficiency over the Povidone-iodine controlled group.

KEYWORDS: Antimicrobial Properties, Krimighna, Gomutra Arka, Povidone-Iodine, culture and sensitivity.

INTRODUCTION

Vrana is a phenomenon which consumes the tissue and after healing leaves behind a scar which remains for whole life on the individual[1]. An ulcer is a break in the continuity of the covering epithelium - skin or mucous membrane. It may either follow molecular death of the surface epithelium or its traumatic removal[2]. Even though healing of Vrana is a natural process of the body, its protection from Microorganism is important. Basic concept of wound cleansing, closure and splinting has been described in various medical systems. In Sashthiupkrama, Krimighana action is given prime role for healing of wound. Even diabetic or chronic wounds also show the presence of microorganism. In microorganism specifically bacterial presence are very common. Generally for Sudha Vrana, ropana agent or significant Dravya which promotes healing is mandatory. While in Dusta Vrana, first identification of microorganism to reduce the infection rate is necessary. Specifically, for cleaning and dressing of chronic wound, Povidone- Iodine is used in almost all wound clinics. The role of Povidone - iodine in wound care is primarily as an antimicrobial agent. Povidone iodine has been used and tested in wound healing for many decades. While Gomutra Arka is having Krimighnalekhn and Ropana properties which helps to promote healing as well as antimicrobial action[3]. Cow's urine has a unique place in Ayurveda and has been described Sushruta Sumhita and Ashtanga Sangraha as the most effective substance secretion of animal origin with innumerable therapeutic values. There are some studies on the use of cow urine, but the studies on Gomutra arka which is prepared by distillation of the cow urine and more palatable due to its lower ammonia content. Gomutra arka has also immunomodulatory and antioxidant effect[4]. The Gomutra Arka (cow urine distillate) has been patented as activity enhancer and availability
facilitator for bioactive molecules including anti-infective and anti-cancer agents (US Patent No 6410 059/2002). Cow urine patented (U.S.Patents No. 6896907 and 6,410,059) for its medicinal properties recently, particularly for its use along with antibiotics for the control of bacterial infection and fight against cancers.

**MATERIAL AND METHOD**

Research design and study population: it was a simple randomized controlled clinical study having two parallel groups (allocation ratio 1:1). The study population was composed of subjects diagnosed with Dusta Vrana which were selected form OPD, IPD of Sri Sri College of Ayurvedic Science and Research hospital and Government Community Health Centre Kaggalipura, Bengaluru. The ethical clearance from the institutional ethical committee was obtained before the initiation of research work.

**Eligibility criteria**

**Inclusion criteria**

- Patients were suffering from wound or ulcer with culture positive for micro-organism positive.

**Exclusion Criteria**

- Patients with ulcers due to Leprosy, Tuberculosis, HIV, HBSAg Positive patients were excluded.
- Patients with signs and symptoms of septicemia and wounds of a severe grade of infection.

**Diagnostic Criteria**

The diagnosis was made based on all type of Dustha Vrana with the following characteristic Symptoms:

- Deerghakaleena
- Pooi
- Pooya
- Ateeva Vedana
- Daha
- Kandu
- Shopha
- Shonitasrava

**Intervention**

- **Group A:** The Subject of this group were subjected to dressing with Gomutra Arka, once daily.
- **Group B:** The Subject of this group were subjected to dressing with Povidone-iodine solution, once daily.

**Assessment criteria**

The patients were assessed based on objective parameters before, during, and after the treatment.

**Objective parameter**

Swab Culture of ulcer discharge for growth of microorganisms and its colony count. In both groups, the swab of secretion/discharge was taken and sent for culture on 0, 7 and 14th day for analysis of the growth of bacteria and its colony count. The three assessment (0, 7 and 14th day) was done based on the presence of Microorganism during the trial period.

**Statistical Method:** The result was analyzed statistically, using the parametric test like paired t-test within the group, unpaired t-test between the groups with help of SPSS software version 20.

**OBSERVATION**

Among 50 patients who completed the study, a maximum patient 14 in number i.e. 28 % belong to age group 18-30 years, minimum patient 2 in number i.e. 4% belong to age group 81-90 years. In the age group, 41-50 years are 22%, 16% age group 61-70 and 31-40 age group 12%. Whereas 10% in 71-80 and 8% in the age group 51-60. (Table 1)

In the study, 23 male patients were contributing to 92% and 2 female patients 8% in group A whereas 21 male patients constituting 84 % and 4 female patients constituting 16% in Group B. (Table 2)

The observation on distribution of occupation revealed out of 50 patients, 19 patients were agriculture which constitutes 38%, 7 patients were in business constituting 14%, 6 patients were housewife constituting 12%, 6 patients were official job constituting 12%, 2 patients were student constituting 4% and 10 patients were other job constituting 20%. (Table 3)

The observation on distribution of site of the wound revealed Out of the total number of patients, a maximum number of patients, i.e., 10% had the site of the ulcer on Perianal region, 12% had on the heel, 14% had on upper limb, 30% had on the lower limb and 20% had an ulcer on other sites. (Table 4)

The observation on distribution of edge of the wound revealed Out of the total number of samples, the maximum number of patients i.e. edge of the ulcer was Slopping for 17 (34%), Raised for 6 (12%), Undermined for 1 (2%) and Punched out for 16 (32%) patients. (Table 5)

The observation on phase 1st trial revealed Bacteria/ fungus (Staphylococcus Aureus, Pseudomonas Aeruginosa, Shigella, Salmonella, E. Coli, Candida Albicans) + 1ml of Gomutra Arka was kept for 24 to 48 hour to observed that heavy growth of bacteria/fungus in colony count.

The observation with 5ml of Gomutra Arka was kept for 24 hours to observed that there was a slight growth of bacteria/fungus in colony count. Later after 48 hours, the sample was observed that with 5ml of Gomutra Arka there was no growth of bacteria/fungus in their colony.
The observation with 10ml of *Gomutra Arka* was kept for 24 hours and observed no growth of bacteria/fungus in colony count. Later after 48 hours, the sample was assured and it was observed that with 10ml of *Gomutra Arka* again there was no growth of bacteria/fungus in their colony.

The observation on phase 2nd trial revealed Out of the total number of patients, the maximum number of patients had *Staphylococcus aureus* (36%), *E. coli.* (28%) and *Klebsiella species* (16%), *Proteus mirabilis* (14%), *Acinetobacter* (2%), and *Pseudomonas* (4%) were observed over the ulcer.

To observe the Antimicrobial activity of *Gomutra arka* and povidone-iodine total 50 pt. was taken. In group A it was observed that out of 25 pt., 16 had no microorganism on the 7th day, 8 had on the 14th day and 1 pt. had a growth on the 14th day also. While in group B group out of 25 pt., 17 pt had growth till 14th day, 2 pt. had no microorganism on 7th day, and 6 pt. had no microorganism on the 14th day.

**Table 1: Showing the incidence of Disease with relation to Age**

| Age     | Group A | Group B | Total | Percentage |
|---------|---------|---------|-------|------------|
| 18-30   | 7       | 7       | 14    | 28%        |
| 31-40   | 4       | 2       | 6     | 12%        |
| 41-50   | 5       | 6       | 11    | 22%        |
| 51-60   | 2       | 2       | 4     | 8%         |
| 61-70   | 4       | 4       | 8     | 16%        |
| 71-80   | 3       | 2       | 5     | 10%        |
| 81-90   | 0       | 2       | 2     | 4%         |

**Table 2: Showing the incidence of Disease with relation to Gender**

| Gender | Group A | Group B | Total | Percentage |
|--------|---------|---------|-------|------------|
| Male   | 23      | 21      | 44    | 88%        |
| Female | 2       | 4       | 6     | 12%        |

**Table 3: Showing the incidence of Disease with relation to occupation**

| Occupation   | Group A | Group B | Total | Percentage |
|--------------|---------|---------|-------|------------|
| Student      | 1       | 1       | 2     | 4%         |
| Agriculture  | 11      | 8       | 19    | 38%        |
| Business     | 5       | 2       | 7     | 14%        |
| Housewife    | 3       | 3       | 6     | 12%        |
| Job          | 4       | 2       | 6     | 12%        |
| Others       | 4       | 1       | 10    | 20%        |

**Table 4: Showing the incidence of Disease on distribution of site of wound**

| Site of wound     | Group A | Group B | Total | Percentage |
|-------------------|---------|---------|-------|------------|
| Axilla            | 1       | 1       | 2     | 4%         |
| Elbow             | 1       | 2       | 3     | 6%         |
| Index finger      | 1       | 1       | 2     | 4%         |
| Lower abdomen ili | 1       | 1       | 2     | 4%         |
| Natal cleft       | 3       | 2       | 5     | 10%        |
| Knee joint        | 2       | 0       | 2     | 4%         |
| Perianal region   | 1       | 4       | 5     | 10%        |
| Medial malleolus  | 2       | 0       | 2     | 4%         |
| Dorsum part of foot | 1     | 4       | 5     | 10%        |
| Ankle joint       | 2       | 1       | 3     | 6%         |
| Heel              | 2       | 4       | 6     | 12%        |
| Great toe         | 2       | 1       | 3     | 6%         |
| other site        | 6       | 4       | 10    | 20%        |
Table 5: Showing the incidence of Disease on distribution of edge of wound

| Edge of wound | Group A | Group B | Total | Percentage |
|---------------|---------|---------|-------|------------|
| Slopping      | 14      | 13      | 17    | 34%        |
| Raised        | 1       | 5       | 6     | 12%        |
| Undermined    | 1       | 0       | 1     | 2%         |
| Punched out   | 9       | 7       | 16    | 32%        |

Table 6: Showing the Phase 1st trial (Effect of 1 ml Gomutra Arka on microorganism)

| Name of Species       | 1ml Gomutra in 24hr | 1ml Gomutra in 48hr |
|-----------------------|---------------------|---------------------|
| Staphalococcus aureus | 2                   | 2                   |
| Pseudomonas aeruginosa| 2                   | 2                   |
| Shigella              | 2                   | 2                   |
| Salmonella            | 2                   | 2                   |
| E. coli               | 2                   | 2                   |
| Candida albicans      | 2                   | 2                   |

Table 7: Showing the Phase 1st trial (effect of 5 ml Gomutra Arka on Microorganism)

| Name of Species       | 5ml Gomutra in 24hr | 5ml Gomutra in 48hr |
|-----------------------|--------------------|--------------------|
| Staphalococcus Aureus | 1                  | 0                  |
| Pseudomonas Aeruginosa| 1                  | 0                  |
| Shigella              | 1                  | 0                  |
| Salmonella            | 1                  | 0                  |
| E. Coli               | 1                  | 0                  |
| Candida Albicans      | 1                  | 0                  |

Table 8: Showing the Phase 1st trial (effect of 10 ml Gomutra Arka on Microorganism)

| Name of Species       | 10ml Gomutra in 24hr | 10ml Gomutra in 48hr |
|-----------------------|----------------------|----------------------|
| Staphalococcus Aureus | 0                    | 0                    |
| Pseudomonas Aeruginosa| 0                    | 0                    |
| Shigella              | 0                    | 0                    |
| Salmonella            | 0                    | 0                    |
| E. Coli               | 0                    | 0                    |
| Candida Albicans      | 0                    | 0                    |

Table 9: Showing the Phase 2nd trial (total number patients according to Microorganism)

| Name of Species         | Group A | Group B | Total Number | Percentage |
|-------------------------|---------|---------|--------------|------------|
| Acinobacter Baum        | 0       | 1       | 1            | 2%         |
| E.Coli                  | 8       | 6       | 14           | 28%        |
| Klebsiella Species      | 5       | 3       | 8            | 16%        |
| Proteus Mirabilis       | 3       | 4       | 7            | 14%        |
| Pseudomonas A.          | 1       | 1       | 2            | 4%         |
| Staphylococcus Aureus   | 8       | 10      | 18           | 36%        |

RESULTS: A total of 52 subjects participated in the study. There was 2 drop out who were lost to the follow-up. There were no patients in either group with any adverse effect.
The result for the first phase trial Analysis of Gomutra Arka on the microorganisms shows that Gomutra Arka is highly effective and curbs the growth of microorganisms. In a 10 ml quantity of Gomutra Arka, 1 loop culture of microorganism is mixed and found that there was no growth in 48 hours. Hence Gomutra Arka has antimicrobial activity on Bacteria such as Streptococcus aureus, Salmonella sps, E.coli, Pseudomonas, Shigella sps, and fungus Candida albicans.

The result of Second phase trial Analysis By considering the colony count on day 0 and day 14th, it was observed that mean of group A with P-value is 0.04 and that of group B is 0.68 showing the significance of group A that is Gomutra Arka over group B that is the povidone-iodine solution. Thus it can be stated that Gomutra Arka is proved to be having Antimicrobial activity.

**Table 10: Showing P values between the group**

| Parameters              | Group A | Group B | Mean+SE | P-value   | Interpretation |
|-------------------------|---------|---------|---------|-----------|----------------|
| Culture Colony count 14 days | 0.04    | 0.68    | 0.040   | <0.001    | Significant    |

**Discussion on Materials**

The present study is a clinical evaluation of the antimicrobial activity of Gomutra Arka in Dusta Vrana group A was treated with Gomutra arka, group B with povidone-iodine. **Gomutra Arka**: Cow urine patented for its medicinal properties recently, particularly for its use along with antibiotics for the control of bacterial infection. Gomutra arka has also immunomodulatory and antioxidant effects. Due to Katu, Tikta, Kashya it is...
strong antimicrobial agent. Same time it does the Ropana to the ulcer.

**Povidone-iodine**

The role of iodine in wound care is primarily as an antimicrobial agent. Povidone-iodine has been used and tested in wound healing for many decades. In povidone-iodine, iodine forms a complex with the synthetic carrier polymer povidone, which itself has no microbicidal activity. In an aqueous medium, free iodine is released into solution from the povidone-iodine complex and equilibrium is established, with more free iodine being released from the povidone-iodine reservoir as iodine-consuming germicidal activity proceeds.

**Drug storage:** The drugs Collected from the Sri Sri Tattva pharmacy were stored in the airtight container till the completion of the clinical trial.

**Discussion on Methods**

**Selection of the patients**

50 Cases of Dushta Vrana were selected from outpatient and inpatient departments of PG Studies in Shalyatantra, at Sri Sri college of Ayurvedic science and research, hospital and Community health center (C.H.C.) Kaggalipura, Bengaluru.

**Discussion on Intervention**

Vrana is cleaned by sterile clean swab then Gomutra Aarka was applied on Dustavrana once daily over it sterile gauze was placed and the dressing was done. If the bandage becomes wet completely before 12 hours re-bandaging was carried out.

**Discussion on Age**

Out of the total number of samples, the maximum number of patient's i.e., 28% belongs to the age group 18-30 yrs group. This may be due to complications of diabetic ulcer, exposure of ulcer to the unhygienic environment and less immunity.

**Discussion on Gender**

Out of 50 patients, 44 were male and 6 were female. The numbers of the female population enrolled in this study, as homemakers and hence were not exposed to outside work.

**Discussion on Occupation**

Out of the total number of samples, the maximum number of patients i.e., 18 (36%) belong to agriculture who would spend greater hours of the day toiling in the field and constantly in the presence of heavy utilities. The constant exposure to chemicals, unwarranted abrasions, and accidental trauma usually go unnoticed.

**Discussion on Religion**

Out of 50 samples recorded 47 were from the Hindu community, 1 from the Muslim community, and 2 from Christian communities.

**Discussion on Marital Status**

Out of the total number of patients, maximum patients were married i.e., 42, and 8 were unmarried.

**Discussion on Socio-Economic Status**

Out of the total number of patients, the maximum number of patients, i.e., 54% belonged to the lower-middle-class group, the remaining 32% middle class, and 14% belonged to the upper-middle-class group. The lower-income group might probably spend lesser income on personal care and hence they are more prone to diseases.

**On Wound**

A maximum ulcer was inflamed, tender, and slopping edge with regular margin. Shows the feature towards chronicity due to less care, poor hygiene. This might be also due to the systemic illness as well as the immunity of the patient or the indulgence in Apathya Ahara and Vihara.

Considering the type of wound/ulcer it was observed that for post incision and drainage cases the result was better than chronic leg ulcer. Due to Lekhana properties, it had a significant result on post fistulotomy wound.

**Discussion on Phase 1st Trial**

- Bacteria/fungus + 1ml of Gomutra arka was kept for 24 hours to observe any growth of bacteria/fungus increase in colony count.
- Later after 24 hours, the sample was assured and it was observed that with 1ml of Gomutra arka there was a growth of bacteria/fungus increase in their colony.
- Thus later bacteria/fungus + 5ml of Gomutra and after 24 hours to it was observed that growth of bacteria/fungus was not much evident but still the growth of bacteria/fungus observed.
- Later 1 loop bacteria/fungus +10 ml of Gomutra arka was kept 24hr -48hr. It was observed that no growth.
- It can be stated that a minimum amount of 10ml of Gomutra arka was required for 1 loop of culture media to kill microorganisms. From this, we can infer that a minimum of 10ml Gomutra arka is required for wound dressing.

**Discussion on Phase 2nd trial**

- This is a 2nd objective of my study.
CONCLUSION

The study highlights the principle of Ayurveda told by Acharya Sushruta in (Su.Chi.-1) Shasthi upakrama which still holds a strong stature of being Krimighna property (anti-microbial property) with Ropana action (healing) at a very affordable cost. The study reestablishes the potency of Gomutra arka with scientific evidence and investigations which prove it to be beneficial even to this date. Gomutra Arka proves to be effective on different microorganisms that are Staphylococcus Aureus, Pseudomonas Aeruginosa, Shigella, Salmonella, E. Coli, and Candida Albicans with minimum 10 ml quantity. Though both standard and trial drugs were beneficial to reduce colony count of Microorganism but Gomutra Arka (trial drug) proved to be having better antimicrobial activity than Povidone-iodine (standard drug). The Gomutra arka acts as an antimicrobial agent due to its property like Katu tikta kashya rasa which are Krimighna in nature. Inhibition of microorganism could happen also due to the Ushna tiksha and Kshara guna which gives an unfavorable environment for bacterial growth. The presence of constituents like copper, aurum, urea, ammonia further helps in antimicrobial activity. Therefore, Gomutra Arka can be used in regular practice as its available at a low cost, affordable and the study proves its efficiency over the Povidone-iodine controlled group.

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Pictures

Gomutra arka

Sample collection for culture by swab method

Cleaning and dressing of wound with Gomutra arka

Distillation Gomutra arka
Trial first *Gomutra* on bacteria and fungus

Second part of trial on *Dusta Vrana* (culture for swab)