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Wound healing activity of human urine in rats

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ABSTRACT

Urine is used traditionally in India for the treatment of burns and wounds. It is believed that applying urine over the wound increases healing. The present study was carried out to evaluate the effect of human urine, urea (2.5%), and urea (5%) on experimentally induced wounds in rats and compare the effects observed with an antiseptic agent, povidine iodine solution. The models selected were excision wound, incision wound, burn wound and dead space wound. In the excision wound and burn wound models, a significant decrease in period of epithelization and wound contraction-50% was observed in all the treatment groups when compared to control except low dose of urea (2.5%), which showed reduction in only period of epithelization. In the incision wound model, a significant increase in the breaking strength was observed. Human urine treatment orally produced a significant increase in the breaking strength, dry tissue weight and hydroxyproline content in dead space wound model. It was concluded that human urine applied topically or administered orally (10 ml/kg, p.o) possesses wound healing activity.

Keywords: Human urine, incision wound, excision wound, dead space wound, burn wound.

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INTRODUCTION

The use of urine for the treatment of diseases is an ancient practice described in many Hindu and Chinese literature. It is commonly called as auto-urotherapy, urotherapy or urine-therapy. It is believed that urotherapy was also used by indigenous Americans and ancient Egyptians [1]. Urine therapy may have been referenced in the Bible: "Drink waters from thy own cistern, flowing water from thy own well" [Proverbs 5:15]. There are very few scientific reports on the effect of urine. However, the uses of urine have been mentioned in ancient literatures in different countries including India. In India, it is known as Amaroli, Shivambu or Autourine therapy. This practice is derived from yoga wherein urine is treated as food, medicine and an immune booster. Urotherapy is believed to be beneficial for the treatment of wide variety of disorders from sores to cancer. Urine is said to be effective against the flu, the common cold, fever, broken bones, toothaches, dry skin, psoriasis and all other skin problems. It is said to deter aging and may be helpful against AIDS, cancer, allergies, animal bites, asthma, heart disease, hypertension, burns, fatigue, infertility, baldness, insomnia, gangrene, chicken pox, tuberculosis, and a countless number of other diseases and disorders [2]. Urotherapy is reported to be beneficial for the treatment of cancer [3]. The American Cancer Society recommends urine therapy for the treatment of cancer [4]. It is also known to treat problems related to skin and hair like acne, hair loss, warts, wrinkles and infections [5].

One of the important uses of human urine is for treatment of burns and wounds. It is believed traditionally in India that applying urine over the wound increases healing. Saharan Bedouins also used urine to cleanse burns and wounds. Urotherapy for treatment of wounds is also mentioned in the Ebers Papyrus of 1500 B.C, one of the oldest surviving documents of Egyptian history [1]. The wound healing activity of urine is believed to be due to the presence of urea, which is an effective antibacterial and antifungal agent [6].

Although, human urine has been recommended for the treatment of wounds from time immemorial, there are no scientific reports to confirm the effect of urine on the wounds. The present study was undertaken to evaluate the effect of effect of human urine on experimentally induced wounds in rats.

MATERIALS AND METHODS

Experimental animals

Albino Wistar rats of either sex weighing 200-225 gm and albino rabbits weighing between 1.5 to 2.0 kg were used. Animals were maintained under hygienic conditions and they were provided with commercial food pellets and tap water *ad libitum*. Cleaning and sanitation work were done on alternate days. Paddy husk was provided as bedding material, which was changed everyday. The cages were maintained clean and all experiments were conducted between 9 am to 5 pm.

Chemicals

Ketamine injection was procured from Prem Pharmaceuticals Pvt. Ltd. [Indore, India] and xylazine was from Indian Immunological Ltd. [Guntur, India], hydroxyproline and paradimethylamino benzaldehyde were procured from SD Fine Chemicals Pvt. Ltd. [Mumbai, India], sodium hydroxide, hydrogen peroxide and copper sulphate were purchased from Nice Chemicals Pvt. Ltd. [Mumbai, India], hydrochloric acid was obtained from Ranbaxy Fine Chemicals Pvt. Ltd. [Mumbai, India].

Selection of dose and treatment period

First void urine in the morning without any dilution was used for topical application in excision, incision and burn wound models. Urine was applied to cover the entire wounded area. In dead space wound model, urine [10 ml/kg] was administered orally. The treatment period was 10 days for incision and dead space wound models and in case of excision and burn wound models, the treatment was continued till the day of scar falling.

Urine analysis

Qualitative urine analysis was carried out to detect the presence of normal and abnormal constituents [7].Quantitative urea analysis was carried out every alternate day during treatment by diacetyl monoxime method [8].

Excision wound [9, 10]

The animals were anesthetized by using ketamine [100 mg/kg, im] and xylazine [16 mg/kg, im] [11]. An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved one day prior to the experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The animals were then grouped and treated as follows: Group I: saline [0.9% w/v]. Group II: povidine iodine [5% v/v]. Group III: low dose of urea in saline [2.5% w/v].Group IV: high dose of urea in saline [5%] Group V: human urine. Wound area was measured by tracing the wound on a millimeter scale graph paper on predetermined days i.e., 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 days post-wounding for determination of wound contraction-50%. Falling of scar leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization.

Incision wound [12, 14]

Para vertebral straight incision of 6 cm length was made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete

homeostasis, the wound was closed by means of interrupted sutures placed at equidistance points about 1 cm apart. Animals were treated daily with drugs, as mentioned above under excision wound model from 0^{th} day to 9^{th} post-wounding day. The wound breaking strength was determined on 10^{th} day by continuous, constant water flow technique.

Burn wound [15, 16]

Partial thickness burn wounds were inflicted on overnight-starved animals under ketamine [100 mg/kg, im] and xylazine [16 mg/kg, im] anesthesia by pouring hot molten wax at 80 ^{0}C. The wax was poured on the shaven back of the animal through a cylinder of 300 mm^2 circular opening. The wax was allowed to remain on the skin till it gets solidified. Immediately after the injury and on subsequent days, the drugs or vehicle was applied topically as mentioned above.

Dead space wound model [17]

This type of wound was created by implanting subcutaneously a 2.5x0.5 cm polypropylene tube in the lumber region of dorsal side in anesthetized rats. Animals received one of the following treatments from 0^{th} day to 9^{th} post wounding day.

Group I: control group: animal of this group received saline [1 ml/kg, p.o].
Group II: low dose of urea [250 mg/kg, p.o].
Group III: high dose of urea [500 mg/kg, p.o].
Group IV: human urine [10 ml/kg, p.o].

On the 10^{th} post wounding day, granulation tissue harvested on the implanted tube was carefully dissected out along with the tube. The tubular granulation tissue was cut along its length to obtain a sheet of granulation tissue. The breaking strength was measured as described under incision wound model. The pieces of granulation tissue were collected, dried at 60 ºC for 24 h to get a constant weight and weighed. The tissue was then used for the determination of hydroxyproline content [18].

Skin irritation study [19]

This study was carried out on rabbits. The skin of the animal was shaved at four different positions on the dorsal side, each about 6 cm in length [approx]. The first area was kept as control, to which vehicle was applied. To the second area, human urine was applied. The third area was treated with low dose of urea in saline [2.5%] and the fourth area was treated with high dose of urea in saline [5%]. After 4 h, the skin was observed for signs of inflammation and scored as follows: No erythema, no oedema-0; very slight erythema, very slight oedema -1; well defined erythema, slight oedema -2; moderate to severe erythema, moderate oedema -3; severe erythema, severe oedema -4. The study was carried on two different animals and average of the two scores was taken as an index of skin irritation.

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STATISTICAL ANALYSIS

Results are expressed as mean ± SEM. The difference between experimental groups was analyzed using one-way Analysis of Variance [ANOVA] followed by Bonferroni test and were considered statistically significant when $P<0.05$.

RESULTS

Urine analysis

Qualitative analysis of the human urine revealed the presence of following normal constituents; chloride, ammonia, urea, creatinine, and uric acid. The abnormal constituents of urine such as bile salts, ketone bodies and reducing sugar were absent. The urea content in the urine samples during treatment was in the range of 3.9 g/dl to 6.9 g/dl.

Effect on excision and incision wound

A significant decrease in period of epithelization was observed in all the treatment groups when compared to control. Comparative analysis revealed that human urine, urea [5%] and povidine iodine had almost equal wound healing activity. There was a significant reduction in wound contraction-50% in all the treatment groups except low dose of urea [2.5%] [Fig.1]. In incision wound model, the breaking strength of 10 day old wound was significantly increased by all treatments when compared to control [Fig. 2].

Effect on burn wound

Like excision wound, application of human urine, urea [5%] and povidone iodine topically shortened the period of epithelization and wound contraction– 50% significantly when compared to control while the low dose of urea [2.5%] was effective only in reducing the period of epithelization [Fig. 3].

Effect on dead space wound

The breaking strength of 10 days old granulation tissue was significantly promoted by human urine [10 ml/kg, p.o], high dose of urea [500 mg/kg, p.o] and low dose of urea [250 mg/kg, p.o]. The dry tissue weight and hydroxyproline content of granulation tissue was also increased significantly by all the treatments except low dose of urea [2.5%] [Table I].

Skin irritation study

There was no evidence of any noticeable inflammation when human urine and urea was applied over rabbit's skin indicating that it does not possess any irritant effect.

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Table I: Effect on breaking strength, dry tissue weight and hydroxyproline content in dead space wound model.

Treatment	Breaking strength (ml)	Dry tissue weight (mg)	Concentration of Hydroxyproline (mg/g)
Saline (1ml/kg, po)	227.50 ± 3.594	76.60 ± 4.539	0.2566 ± 0.0133
Urea (250 mg/kg, po)	278.33 ± 10.138*	92.16 ± 6.26	0.2683 ± 0.0164
Urea (500 mg/kg, po)	431.66± 12.758***	133.75 ± 3.83***	0.3400 ± 0.0158*
Human urine (10 ml/kg, po)	455.00 ± 12.111***	136.50 ± 10.90***	0.3466 ± 0.0152**

All values are mean ± SEM, n=4 - 6, *P<0.05, **P<0.01 ***P<0.001 Vs control.

DISCUSSION

The result of the present study show that human urine possesses good wound healing activity and it substantiates the traditional belief that human urine promotes healing of wounds. The work was carried out using different models of experimental wounds to evaluate the effect on breaking strength, epithelization and collagenation of wounds. Urea, which is the main constituent of urine was used at two different doses because the reported normal value of urea is human urine is around 2.5 g/dl [8] while the urea content of the urine used in present study was around 5 g/dl.

Collagenation, wound contraction and epithelization are crucial phases of wound healing. An intervention into any one of these phases by drugs leads to either promotion or depression of the collagenation phase of healing. Growth hormone is known to promote the healing process by enhancing epithelial cell proliferation and collagen formation. The collagen synthesis is stimulated by various growth factors [20]. Growth hormone is also known to promote the proliferation of fibroblasts [21] and fibroblast proliferation form the granulation tissue. In the dead space wound model, oral treatment with human urine increased the breaking strength of granulation tissue. The human urine contains many growth factors [22] and the effect on collagen synthesis may be due to the presence of these growth factors. Furthermore, urea is also reported to enhance collagen deposition in wounds [23]. Hence, the effect of urine on collagenation may be due to both presence of growth factors and urea in urine.

Lipid peroxidation is an important process of several types of injuries like burn wound, inflicted wound and skin ulcers. Agents that inhibit lipid peroxidation are believed to increase the viability of collagen fibrils. Several antioxidants such as vitamin C, metronidazole and vitamin E are reported to increase the wound healing [24]. Urine has good antioxidant effect [25]. Hence, it can be suggested that the wound healing activity of urine may also be due to its antioxidant activity.

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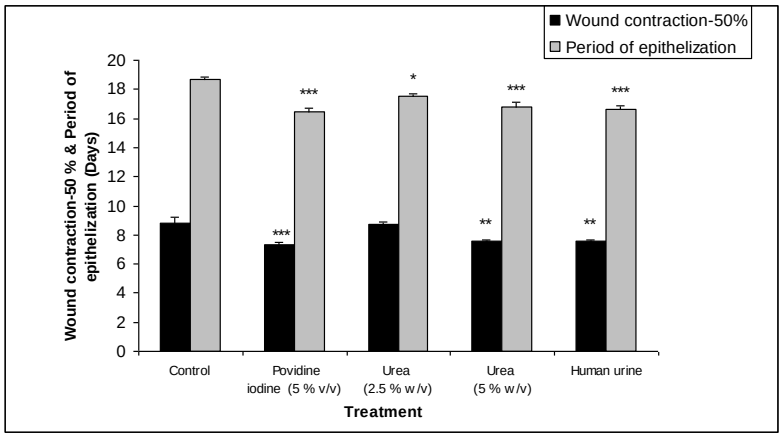
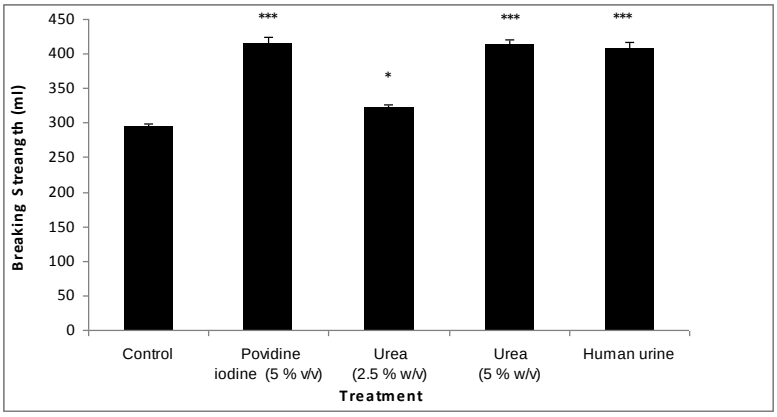
Figure 1: Effect on period of epithelization and wound contraction in excision wound model



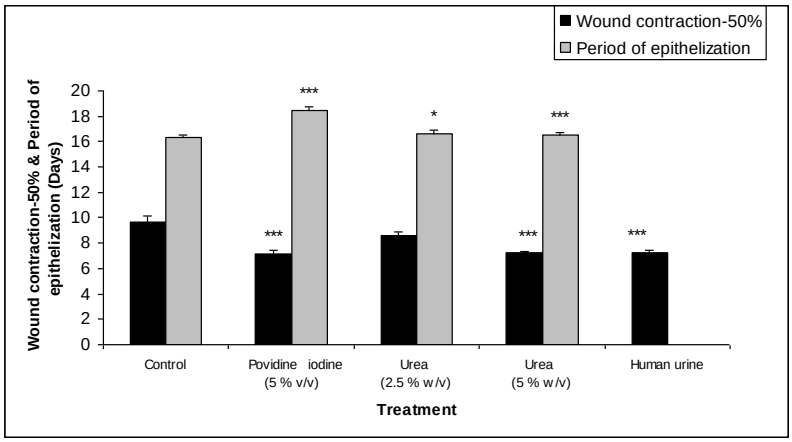
All values are mean ± SEM, n=6, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ Vs control.

Figure 2: Effect on breaking strength in incision wound model.



All values are mean ± SEM, n=6, $^{*}P<0.05$, $^{***}P<0.001$ Vs control.

Figure 3: Effect on period of epithelization and wound contraction in burn wound model.



All values are mean ± SEM, n=6, $^{*}P<0.05$, $^{***}P<0.001$ Vs control.

Earlier studies carried out on human urine suggest that human urine has good antibacterial activity [26]. The antibacterial effect is reported to be due to osmolality, urea concentration and ammonium concentration. Out of these, urea concentration was more important determinant of antibacterial activity than osmolality or ammonium concentration. In the present study, the effect of urea [5%] and human urine were almost similar. So, it is speculated that the wound healing activity of human urine may be due to combination of its antibacterial, antioxidant and growth promoting effects.

The human urine did not produce any skin irritation study indicating that is safe for local application. To conclude, human urine possess good wound healing activity when applied locally or administered orally. The effect observed is similar to that produced by urea.

CONCLUSION

The results are the present study show the human urine affects wound healing in experimental animals. It showed a significant decrease in period of epitheliazation and wound contraction in excision and burn wound model, an increase in breaking strength of 10 day wound in incision wound model and 10 day old granulation tissue, dry tissue weight and hydroxyproline content were significantly increase in dead space wound model. From the above results it was concluded that human urine has significant wound healing activity in excision, incision, burn and dead space wound model.

REFERENCES

[1] http://www.heartlandhealing.com/pages/archive/urine_therapy/index.html Retrieved on 26.09.2006, 11.49 AM IST.
[2] http://curezone.com/forums/fm.asp?i=124372. Retrieved on 26.09.2006, 11.55 AM IST.
[3] Eldor J. Urotherapy for patients with cancer. Med Hypotheses. 1997; 48(4):309-15.
[4] http://www.cancer.org/docroot/ETO/content/ETO5_3X_Urotherapy.asp?siteareа=ETO. Retrieved on 23.09.2006, 10.55 AM IST.
[5] http://www.dailynewarticles.com/article/201/20327/Urotherapy_A_Treatment_With_The_Help_Of_Urine.html. Retrieved on 22.09.2006, 11.55 AM IST.
[6] Gloor M, Reichling J, Wasik B, Holzgang HE. Res Complement Class Nat Med 2002; 9: 153-159.
[7] Rajagopal G, Thoora BD. Practical biochemistry. 1st ed. New Delhi: Ahuja book company Pvt Ltd; 2001; 39-42.
[8] Rajagopal G, Thoora BD. Practical biochemistry.1st ed. New Delhi: Ahuja book company Pvt Ltd; 2001;89-90.
[9] Kamath JV, Rana AC, Chowdhury AR. Phytother Res 2003; 17: 970-972.
[10] Morton JJ, Malone MH. Arch Int Pharmacodyn Ther 1972; 196: 117-126.

[11] Vogel HG. Drug discovery and evaluation, 2nd ed., Springer-Verlag Berlin Heidelberg, Berlin, 2002.pp: 1739.
[12] Lee KH. J Pharmacol Sci 1968; 57:1238-1240.
[13] Ehrich HP, Hunk TK. Ann Surg 1969; 170: 203-206.
[14] Somayaji SN, Jacob AP, Bairy KL. Indian J Exp Biol 1995; 33(3):201-204.
[15] Holla RK, Sequeria RP, Kulkarni DR. Indian J Exp Biol 1998; 26: 869-873.
[16] Rao CM, George KM, Bairy KL, Somayaji SN. Indian J Pharmacol 2000; 32: 282-287.
[17] Padmaja PN, Bairy KL, Kulkarni DR. Fitoterapia 1994; LXV: 298-303.
[18] Neuman RE, Logan MA. J Biochem 1950; 186: 549-552.
[19] OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Acute Dermal Irritation/Corrosion. Available from URL: http://ecb.jrc.it/documents/Testing-Methods/ANNEXV/B04web2004.pdf.
[20] Corton SR, Kumar V, Collins T. Robbins Pathologic Basis of Disease. 6th Ed. New Delhi: Harcourt Limited; 2003: 96-111.
[21] Williams TC, Frohman LA. Pharmacotherapy 1986; 6**:** 311-318.
[22] Starkey RH, Cohen S, Orth DN. Science 1975; 189: 800 – 802.
[23] Telgenhoff D, Lam K, Ramsay S, Vasquez V, Villareal K, Slusarewicz P, Attar P, Shroot B. Wound Repair Regen 2007 Sep-Oct; 15(5): 727-35.
[24] Rao CM, Ghosh AIndian J Pharmacol 1997; 29: 29.
[25] Kirschbaum B. Clin Chim Acta 2001; 305:167-173.
[26] Donald Kaye J. Clin Invest 1968; 47(10): 2374–2390.