

Dermatological evaluation of counter irritant potential of Human urine in rabbits

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Abstract

Objective: To confirm the effect of human urine in the treatment of wounds, burns and irritation.

Methods: The study was conducted at Bahauddin Zakariya University, Multan, in November 2013 and comprised 15 rabbits of either gender. Irritation was induced by clockwise frictional movement of fine sandpaper to the ear of the rabbits. The counter-irritant activity was determined by calculating the mean decrease in redness and erythema. A group of 3 rabbits each was used while experimenting with the main assay every time the concentration of irritants was increased. The ear treated with distilled water was used as a control. Sodium lauryl sulfate irritation model was also applied and the counter-irritant activity was evaluated. One-way analysis of variance followed by Bonferroni test were used for statistical analysis.

Results: The mean weight of the 15 rabbits was 1.5+/-SD kg. Urine (50 and 100µl) showed excellent counter-irritant activity when compared with control and standard dexamethasone. Both the doses showed counter-irritant activity, ranging between 76.84% and 95.68% in the sandpaper model, and 68.90% and 93.68% in the sodium lauryl sulfate model.

Conclusion: Human urine countered the effect of irritation in experimental animals and can be an alternative way of countering irritation.

Keywords: Counter-irritant, Dehydroepiandrosterone (DHEA), Human urine, Rabbit's skin, Dexamethasone. (JPMA 65: 9; 2015)

Introduction

Skin irritation is known as the production of reversible damage of the skin following the application of a test substance for up to 4 hours.¹ Pathological characteristics and manifestation of skin irritation such as, erythema and oedema are manifestations of dermal irritation. Irritation is initially manifested by redness (erythema), vesicles, serous exudates, serous scabs (escher) and various degrees of swelling (oedema). Over time, other reactions may also manifest, like small areas of scaling, hyperplasia, hyperkeratosis and alopecia. Histopathology is useful in evaluating responses. In most cases, inflammation is well developed within the first 72 hours of observation.²

Human urine is used for the bleeding wounds, rashes, inflammations and oedema in the subcontinent. Hakeems and vaydes advise patients to take a bath in the lakes that carry waste, including human urine. Hindu and Chinese literature advises the use of urine as a remedy for many diseases.

Urotherapy was also used by ancient Egyptians and indigenous Americans.³ Urine therapy may have been referenced in the Bible: "Drink waters from thy own cistern, flowing water from

thy own well" (Proverbs 5:15 Old Testament). In India, urine therapy is known as Amaroli, Shivambu or Autourine therapy. In yoga, urine is treated as food, medicine and an immunity booster. Urine is said to be effective against the common cold, burns, fever, the flu, broken bones, dry skin, psoriasis, toothaches and several other skin problems. It is said to deter aging and may be helpful against the acquired immunodeficiency syndrome (AIDS), animal bites, allergies, asthma, heart disease, hypertension, fatigue, insomnia, infertility, baldness, gangrene, chicken pox, tuberculosis, and a countless number of other diseases and disorders.⁴

Urotherapy is reported to be beneficial for the treatment of cancer.⁵ The American Cancer Society recommends urine therapy for the treatment of cancer.⁶ It is also known to treat problems related to skin and hair like acne, warts, wrinkles and infections.⁷ One of the important uses of human urine is for treatment of burns and wounds.⁸ It is believed traditionally in India that applying urine over the wound increases healing. Saharan Bedouins also used urine to cleanse burns and wounds. Urine therapy for treatment of wounds and inflammation also mentioned in the Ebers Papyrus of 1500BC which refers to the oldest surviving documents of Egyptian history.³

Urine is used for treatment of wounds, burns and irritation since time immemorial. However, there are no pharmacological reports to confirm the effect of urine in such disorders. Modern scientists are aware of the fact that urine is not a waste product, but valuable serum, which is a panacea for several major illnesses, including aging. Urine is flesh, blood and vital tissues

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in living solution. Apart from urokinase, several other injections like Profasi, Personal, Antineoplastens etc. are derived from urine. It is useful in aplastic anaemia, heart disease and aging.⁹ It is a sound treatment for inflammation, blood clots and artery vasodilation.¹⁰ It is also a source of antibodies of cholera, salmonella, diphtheria, tetanus and polio.¹¹

The current study was designed to evaluate the effect of human urine on experimentally-induced irritation in rabbits.

Material and Methods

The study was conducted at Bahauddin Zakariya University, Multan, in November 2013 and comprised 15 rabbits of either gender. The rabbits were purchased from the Hussain Agahi Animal Market, Multan. Dexamethasone sodium was purchased from Ethical Laboratories (Pvt.) Ltd. Pakistan, while sodium lauryl sulfate (SLS) was purchased from Sarco Chemicals, Multan.

All animals with a mean weight of 1.5 ± 0.3 kg were kept under one-hour observation initially for normal dermatological and allergic behaviour before evaluating the counter-irritant activity. Subsequently, the rabbits were kept under laboratory conditions at room temperature with 12h light and dark cycles. All animal experiments were carried out in accordance with the acts of the Animal Ethical Committee of Bahauddin Zakariya University, Multan.

Urine samples were collected from healthy volunteers in the morning after overnight fasting. The urine sample was authenticated and biochemically evaluated by an expert biochemist at the Department of Biochemistry, Multan Medical and Dental College, Multan.

Assay for the counter-irritant effect of human urine was performed with some specified modifications in assay described in literature.¹² Sandpaper with fine particles was used to irritate the inner surface of a rabbit's ear in a clockwise direction for 10 minutes. Irritation, redness and erythema were produced in an area of 2cm^2 in diameter. Then $100\mu\text{l}$ and $50\mu\text{l}$ human urine and dexamethasone (standard) was applied to the irritated area. The ear treated with distilled water was used as a control. Ears were examined for the

intensity of erythema. A group of 3 rabbits was used while performing the main assay for all the four dose variants. Including the controls. Rabbits were examined after every minute. The numbers of ears showing decreased irritation, redness and erythema were observed and recorded. The time, dosage and degree of counter-irritancy were noted.

SLS irritation model was used as described in literature.¹³ A well-known tensoactive agent was used as an irritant in the SLS group which also had 3 rabbits. It determines toxic dermatological effect. Concentration above 10% is harmful. Human urine was applied directly on the skin immediately after irritation was induced with SLS which is evident by the appearance of erythema. Decrease in redness, erythema and counter-irritancy time was observed and recorded.

Variables were first tested for normality through Kolmogorov-Smirnov test. The difference between experimental groups was analysed using one-way analysis of variance (ANOVA) followed by Bonferroni test and means were separated with Duncan's multiple range post-hoc test (DMR) at $p < 0.05$. Results were expressed as mean \pm standard error of mean (SEM).

Results

Qualitative analysis of the human urine revealed the presence of normal constituents like chloride, ammonia, urea, creatinine and uric acid. Abnormal constituents 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.

All doses exhibited moderate to strong counter-irritant response in dose-dependant manner on the rabbit skin in sandpaper irritation model ranging between 76.84% and 95.68% (Table). The maximum irritant healing response was demonstrated by the $100\mu\text{l}$ dose. Both the urine doses showed as quick a counter-irritancy effect as dexamethasone. The entire irritated area stood healed and light scars of the same size were observed compared to the dark scars of the controls (Figure).

In the SLS model, a similar phenomenon was observed, with counter-irritation ranging from 68% to 87%. However, it took more time compared to the sandpaper model in the $50\mu\text{l}$ dose.

Table: Counter-irritant activity.

Groups	Sandpaper irritation model			SLS irritation model		
	Mean degree of Erythema \pm S.E.M	Inhibition (%) of Erythema	Counter irritancy time	Mean degree of Erythema \pm S.E.M	Inhibition (%) of Erythema	Counter irritancy time
Control $100\mu\text{l}$	95 ± 1.66			90 ± 1.66		
Human urine $100\mu\text{l}$	6.3 ± 2.12	95.68%***	3min	11.3 ± 4.1	93.68%***	3min
Human urine $50\mu\text{l}$	22 ± 1.89	76.84%**	10min	27 ± 3.65	68.88%**	17min
Dexamethason $50\mu\text{l}$	1 ± 1.09	98.90%***	1min	1 ± 1.09	98.90%***	1min

#One-way ANOVA with DMR 0.05 (post-hoc test). SLS: Sodium lauryl sulfate. SEM: Standard Error of Mean

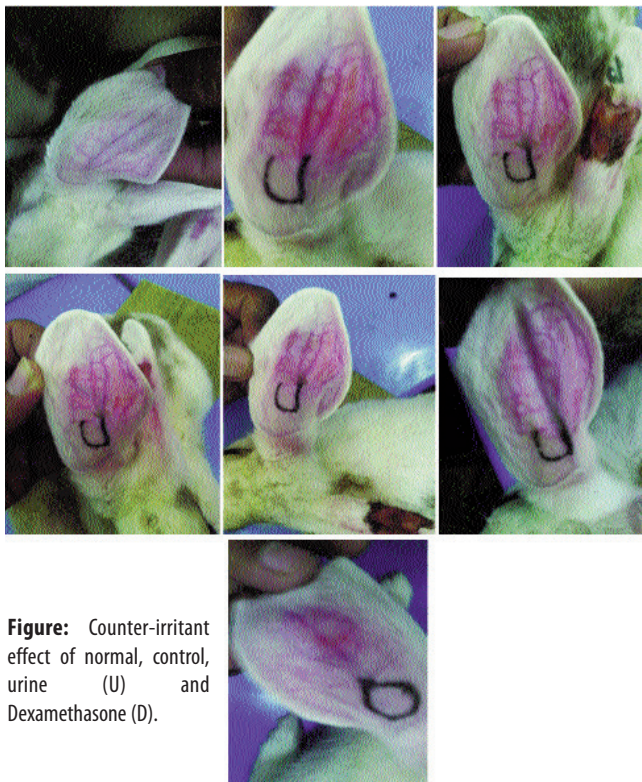


Figure: Counter-irritant effect of normal, control, urine (U) and Dexamethasone (D).

Discussion

Multiple pharmacological phenomena are responsible for counter-irritant activity. Dehydroepiandrosterone (DHEA) steroid from the human urine has been identified¹⁰ which is reported to be behind several activities. For instance, it prevents obesity, extends lifespan and is a possible cure for aplastic anaemia, diabetes and breast cancer in women. The steroid is among treatment options for allergy and irritation.¹⁴ It has advantages over the dexamethasone as it stimulates the production of bone marrow¹⁵ rather than suppression as does dexamethasone.¹⁶ It has allantoin-mediated wound-healing activity.¹⁶ It is useful against skin allergies and inflammations. The quick response in the current study shows that urine probably penetrated through the skin damage of the rabbit's ear with as much ease as dexamethasone. The presence of DHEA or allantoin was bound to react with the irritated, inflamed cell membrane and cellular content of both the superficial and deeper layers of the skin. As a result, the inflamed and the damaged superficial and deeper layers healed.

Conclusion

Human urine countered the effect of irritation in experimental animals, showing a significant effect in terms of dose and counter-irritancy time compared to the controls and standard dexamethasone.

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