Effect of cow urine on wound healing property in Wister Albino Rats

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Published online 30-04-2011

Abstract

The wound healing activity of cow urine was studied in Wistar albino rats. The efficacy of wound healing property was evaluated by excision wound model. The parameter studied was the rate of wound contraction. The studies on excision wound healing revealed that all the groups showed decreased wound area from day to day. On day 4th, the external application of urine showed significant increase in wound healing in male and female rats compared to all other groups. However, on 14th post wounding day, Group I and VIII animals showed 0 % and 0.40.4 % of healing was left, which may be due to normal immunity of the animals. Where as nitrofurazone treated animals showed 0 % and 0.50.3 % healing .The study revealed that the cow urine on external application to the wound, hastened the wound healing process.

Key words: Cow urine, Wound healing, Nitrofurazone, Rats.

Introduction

Cows were regarded as wealth and were the backbone of the economy of ancient Indians. Cattle were one of the most frequently used animals described in Vedas. Cows were regarded as mother ("*Gau-mata*") and referred to as *Aghnya*. Cattle husbandry was well developed during the Rigvedic period (1500–1000 BC) and the cow (*Kamadhenu*) was adored and considered the 'best wealth' of mankind. Urine was also considered as an antidote to poisons (Sushrut Samhita).

In Sushrut Samhita, several medicinal properties of cow's urine have been mentioned and cow urine was known to cause weight loss and to cure leprosy, cardiac and kidney problems, indigestion, stomach ache, edema, *etc*. (Kaviratna and Sharma., 1996). This kind of alternative treatment, termed as 'panchgavya therapy' or 'cowpathy', (Dhama, K, *et al.,* 2008) has been reported to be beneficial even for dreaded diseases like cancer, AIDS and diabetes.

"Kamadugha Yojane" has been drawn up to protect Indian cows in appreciation of the multifarious uses of "Panchagavya," which comprises cow dung, urine, ghee, curd and milk, which is found to be effective in treating major diseases such as cancer and diabetes. The fact that cow urine costs more than milk speaks of its limitless medicinal use (Raghaveshwara Bharati Swamiji, 2006). United States Patent and Trade mark Office had granted Patents No 6410059 and No. 6896907 to an "Indian innovation which has proved that cow urine can make antibiotics, anti-fungal agents and also anti-cancer drugs more effective". Besides, cow urine is said to be a very effective insect repellent when mixed with certain herbs (Nair, 2002).

There are numerous uses of cow urine for various human ailments like, cancer, osteoarthritis, allergies, kidney failures, skin diseases, healing of wounds, *etc*. Need of hour is another revolution; through extensive research in cowpathy for its scientific validation and a wider popularityVisualizing the potential use of cow urine in several ailments including even cancer, the use of *Gomutra* (cow urine) and its scientific therapeutical validation is required for its worldwide acceptance and popularity.

The redistillate of cow's urine was found to possess total antioxidant status of around 2.6 m mol, contributed mainly by volatile fatty acids (1500 mg/L) as revealed by the GC-MS studies. These fatty acids and other antioxidants might cause the observed protective effects (Krishnamurthi *et al*., 2004). Olusi and Ojewole (1978) revealed that oral administration of cow's urine concoction in rats causes the activation of the third complement component in the serum. The product of this activation has some histamine-releasing effects and causes a characteristic acute fall in neutrophil and monocyte counts in the peripheral blood which is reversed within four hours.

Table. Experimental design

Group	Male	Dose	Groups	Female	Dose
I	6	Control	VIII	6	Control
II	6	Nitrofurazone ointment	IX	6	Nitrofurazoneointment
III	6	0.05ml	X	6	0.05ml
IV	6	0.1ml	XI	6	0.1ml
V	6	0.2ml	XII	6	0.2ml
VI	6	0.3ml	XIII	6	0.3ml
VII	6	External application	XIV	6	External application

The detailed studies on pharmacological activities and safety evaluation of cow urine employing controlled studies in experimental animals are scanty.

Hence, the present study was undertaken on cow urine in male and female Wistar albino rats with the objective to study the effect of cow urine on wound healing.

Materials and Methods

Selection of experimental animals : Wistar albino rats procured from the Laboratory Animal Facility of Veterinary College, Hebbal, Bangalore, were used in the present study. They were aged between six to seven weeks and weighed 150-200g. They were acclimatized to the experimental conditions for one week. After acclimatization, rats were grouped and housed in standard polypropylene cages during the experiment. They were maintained under standard laboratory hygienic conditions, providing standard laboratory animal feed and water *ad libitum*. The approval of the Institutional Animal Ethics Committee was obtained prior to start of the experiment.

Selection of urine source : Holstein-Friesian (HF) cross- bred, milking cows aged about 2-3 years were selected for urine collection. Animals were hygienically maintained in Karnataka Veterinary, Animal and Fisheries Sciences University regional campus, Dept. of Livestock Production and Management, Veterinary College, Hebbal, Bangalore. All the cows were fed with the concentrate prepared at the dairy farm and were provided clean drinking water *ad libitum* and daily animals were also taken outside for grazing in the university grazing land. All the animals were monitored carefully throughout the experimental period.

Collection of urine : Natural voiding mid- stream urine was collected in a sterile glass container. Collected urine was stored in refrigerator at 4ºC and utilized for the experiment within two hours of collection. Precaution was taken to avoid any external contamination. Urine was collected from the same cows and used in each experiment. Immediately after collection physical, chemical and microscopic examinations were done. Urine specimens were collected and stored as part of standard protocols in cohort studies designed to assess exposure to non persistent environmental contaminants (Gunter, 1997; Landi and Caporaso, 1997).

Selection of doses for pharmacological assays: The doses corresponding to the dose of 15 ml, 30 ml, 60 ml and 75 ml per day in a human being weighing around 60 kg. The doses administered were 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml/100 gm body weight in rats (The higher range of rat's body weight was considered for dosage calculation).

Administration of doses: The fresh urine at 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml/100 gm was selected and was made up to 2 ml by adding distilled water and was gavaged using a stomach tube daily for a period of 14 days.

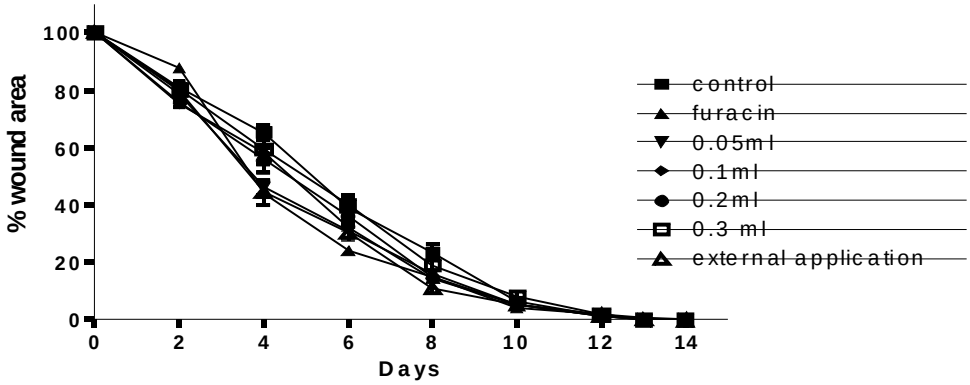
Six groups were made (n=6). The group I and VIII was considered as the control in male and female rats, the group II and IX served as reference standard and treated with 1% (w/w) Furacin® ointment, the group III – VI and X- XII animals were treated with

Table-1. Effect of cow urine on wound healing activity in Male rats

Group	Wound (%)							
	0 Day	2 Day	4 Day	6 Day	8 Day	10 Day	12 Day	14 DAY
Group I (Control)	100	80.9±2.4	65.2±2.3	39.0±4.5	23.4±2.8	6.5±0.9	1.4±0.3	0.5±0.0
Nitrofurazone ointment Groupt-II	100	87.8±1.2	43.9±4.0***	24.1±1.4***	14.7±1.6*	4.1±0.8	0.4±0.1	0.0±0.0
Group III (0.05 ml)	100	78.7±2.0	46.0±2.5	30.8±3.3	16.2±1.7	5.4±0.6	2.0±0.1	0.3±0.2
Group IV (0.1 ml)	100	75.0±1.2	58.3±4.2**	32.0±4.1	14.3±1.4*	5.4±0.8	1.5±0.1	0.1±0.1
Group V (0.2 ml)	100	75.9±1.9	55.7±4.4	36.1±5.1	14.9±1.1*	5.4±0.8	1.2±0.2	0.1±0.1
Group VI (0.3 ml)	100	80.2±1.7	59.4±1.2***	39.8±2.2*	19.0±0.8	7.9±0.8	1.8±0.2	0.1±0.1
External application-VII	100	80.0±2.8	44.3±4.2***	30.4±3.0*	10.9±1.1***	5.2±1.3	1.6±0.2	0.0±0.0

Values are mean SE, *P<0.05, **P<0.01, ***P<0.001, n = 6.

Fig 1. Effect of cow urine on wound healing in male rats



different doses of cow urine in male and female rats, respectively.

The urine gavaged once in a day, till the healing was complete. The parameter studied was wound closure time. Per cent wound closure was calculated.

Procedure: The rats were inflicted with excision wounds as described by Morton and Malon (1972). Excision wound model were used to evaluate the wound healing activity. Circular wounds of approximately 10 mm diameter were inflicted on the dorsal area skin under mild ether anaesthesia (Jalalpure *et al*., 2003). The entire wound left open (Patil and Kulkarni, 1984). The areas of the wound were measured (sq. mm) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it. This was taken as the initial wound area reading. Group-I and VIII served as negative control. Group-II and IX served as positive control to which nitrofurazone (0.2 % w/w in simple ointment I.P.) was applied topically. Group III to VI and IX to XII were treated with urine. Group VII and XIV were treated by externally applying cow urine. All the test samples were applied once daily. The wound area of each animal was measured on 2nd, 4th, 6th, 8th, 10th, 12th and 14th post wounding day. The percentage of wound contraction was calculated from the days of measurements of wound area.

Statistical analysis: The data were analyzed by One-way ANOVA with Dunnett's post test, using Graph Pad Prism statistical software, version 4.01, San Diego, California, USA (2004).

Results and Discussion

Male rats : The percentage of wound in group I, II, III, IV, V, VI and VII on 2nd day was 80.92.4, 87.81.2, 78.72.0, 75.01.2, 75.91.9, 80.21.7 and 80.02.8, respectively.

The percentage of wound in group I, II, III, IV, V, VI and VII on 10th day was 6.50.9, 4.10.8, 5.40.6, 5.40.8, 5.40.8, 7.90.8 and 5.21.3, respectively.

The percentage of wound in group I, II, III, IV, V, VI and VII on 12th day was 1.40.3, 0.40.1, 2.00.1, 1.50.1, 1.20.2, 1.80.2, and 1.60.2, respectively.

The percentage of wound in group I, II, III, IV, V, VI and VII on 14th day was 0.5 0.0, 0.00.0, 0.30.2, 0.10.1, 0.10.1, 0.10.1, and 0.00.0 respectively (Table. 1 and Fig. 1).

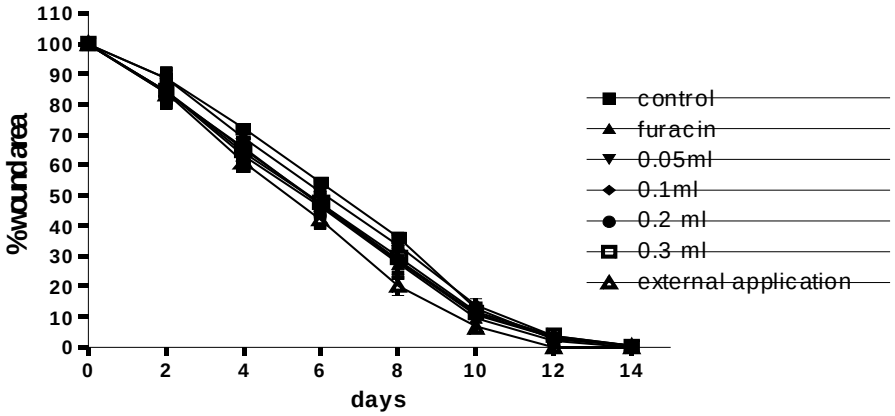
Female rats: The percentage of wound area in group

Table-2. Effect of cow urine on wound healing activity in Female rats

Group	Wound (%)							
	0 Day	2 Day	4 Day	6 Day	8 Day	10 Day	12 Day	14 DAY
Group VIII Control	100	88.5? 2.2	72.1? 1.7	54.3? 1.6	36.1? 1.2	12.9? 1.5	2.6? 0.8	0.4? 0.4
Nitrofurazone ointment IX	100	83.9? 3.9	65.6? 3.2	47? 3.5	28.4? 4.4	10.8? 1.6	2.9? 0.6	0.5? 0.3
Group X (0.05 ml)	100	88.4? 3.1	68.7? 3.2	51.3? 3.4	33.6? 3.8	14.1? 1.7	3.8? 0.6	0.6? 0.2
Group XI (0.1 ml)	100	84.4? 3.9	64.2? 3.1	47.6? 3.8	28.5? 4.5	11.2? 1.6	3.3? 0.7	0.5? 0.3
Group XII (0.2 ml)	100	84.5? 4.0	65.1? 3.6	47.7? 4.4	29.8? 5.1	11.7? 2.2	3.8? 1.0	0.5? 0.3
Group XIII (0.3 m)	100	84.3? 3.8	63.1? 2.9	46.4? 3.8	27.5? 4.5	9.5? 1.8	2.3? 0.8	0.0? 0.0
External application- XIV	100	83.6? 4.4	60.7? 2.7*	42.1? 2.8**	20.1? 3.0***	6.6? 1.1	0.0? 0.0	0.0? 0.0

Values are mean SE, *P<0.05, **P<0.01, ***P<0.001, n = 6.

Fig 2. Effect of cow urine on wound healing activity in female rats



VIII, IX, X, XI, XII, XIII and XIV on 2nd day was 88.52.2, 83.93.9, 88.43.1, 84.43.9, 84.54.0, 84.33.8 and 83.64.4 in female rats.

The percentage of wound area in group VIII, IX, X, XI, XII, XIII and XIV on 4th day was 72.11.7, 65.63.2, 68.73.2, 64.23.1, 65.13.6, 63.12.9 and 60.72.7 in female rats. There was significant increase in the percentage of wound healing in external applied urine treated group ($P<0.05$).

The percentage of wound area in group VIII, IX, X, XI, XII, XIII and XIV on 12th day was 2.60.8, 2.90.6, 3.80.6, 3.30.7, 3.81.0, 2.30.8 and 0.00.0 in female rats. There was significant increase in percentage of wound healing in externally applied urine treated group at $P<0.01$.

The percentage of wound area in group VIII, IX, X, XI, XII, XIII and XIV on 14th day was 0.40.4, 0.50.3, 0.60.2, 0.50.3, 0.50.3, 0.00.0 and 0.00.0 in female rats. (Table 2 and Fig. 2).

Wound healing property of cow urine: The wound healing efficacy of urine was evaluated by excision wound model. The parameter studied include rate of wound contraction.

The study on excision wound healing revealed that all the groups showed decreased wound area from day to day. On day 4th the external application of urine showed significant increase in wound healing in male and female rats compared to all other groups. However, on 14th post wounding day, Group-I and VIII rats showed 0.5 0.0 % and 0.40.4 % of healing was left, which may be due to normal immunity of the animals. Where as nitrofurazone treated rats showed 0 % and 0.50.3 % healing was left. The significant increase in per cent wound healing in external applied urine treatment could be correlated with the U S patent No. 6410059 for cow urine distillate, which claimed a novel pharmaceutical composition (Khanuja, 2002) and was effective as an antimicrobial and antifungal.

Conclusion

In the pharmacological studies, cow urine samples from HF cross bred cows was screened for wound healing activity. In the present study during assessing wound healing activity, the cow urine which has been applied externally to the wound, hastened the wound healing process. Further research needed to isolate and identify the active molecule/molecules responsible for wound healing activity.

Acknowledgement

Authors are thankful to Dept of pharmacology and Toxicology, and Then Dean, Veterinary College, Hebbal, Bangalore for providing necessary facilities.

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