

RESEARCH ARTICLE

Wound Healing Effect of Ointments Containing Cow Urine Concentrate on Rats

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ABSTRACT:

In the present study ointments containing cow urine concentrate were prepared and tested for their wound healing activity. The concentrate was obtained by distilling cow urine. Excision wound was produced in rats by removing the skin with the help of a sterilised blade under ether anaesthesia by making a mark with the help of coin. The ointments were applied daily for a period of 18 days. The diameter of the wound contraction was measured and compared with standard nitrofurazone ointment treated animals.

INTRODUCTION:

Cow-urine is considered to be the most effective animal origin substance or secretion having intrinsic property of general health improvement<sup>1</sup>. Ayurved has been using cow-urine for betterment of physical as well as mental health of mankind since thousands of years. Ayurvedic texts and literature recommend it for variety of conditions and ailments including injuries, wounds, skin disorders, etc<sup>2</sup>.

In its lifetime, skin gets damaged due to various causes. They may alternatively be traced to physical damage, irritant or allergic reaction to foreign substances, an underlying pathophysiological condition or microbial infections. Wound is a visible result of cell death or damage, and can be classified by site, size, depth, cause (surgery/ accident) or circulatory failure<sup>3</sup>. Wound healing is a process, which is fundamentally connective tissue's response. Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other extracellular macromolecules which are later remodelled to, form a scar. Several factors delay or reduce wound healing, including bacterial infections with blood supply, lymphatic blockage and diabetes mellitus. Generally, if the above condition could be altered by any agent, an increased healing rate could be achieved.

A large number of agents have been incorporated into the topical drug delivery system for their therapeutic effectiveness. The action may be local or systemic.

The agents are local anaesthetics, anti-inflammatory, corticosteroids, antibacterial, antifungal, antiseptics, scabicides, pediculicides, antileprotics, enzymes, enemas and sunscreen agents<sup>4,5</sup>. Taking clue from ancient Ayurvedic texts it was planned to investigate wound healing activity of cow urine.

MATERIAL AND METHODS:

1. Cow urine collection and distillation:

Go-Vigyan Anusandhan Kendra is a research centre established at Deolapar, in Nagpur district. It maintains a well equipped *Gaushala* which has almost all types of Indian breed cows. The Kendra is fully dedicated towards the research activity and also production of different formulations from cow's urine.

The samples of cow urine procured from *Gaushala* were first filtered through ordinary filter papers to remove all the visible extraneous matter. The filtered cow urine was subjected to distillation to obtain a viscous, semisolid residue. This mass was, then, treated with 5% Hydrochloric acid. This yielded a buff coloured solid crystalline matter. This crystalline solid was designated as cow urine concentrate (CUC).

2. Preparation of ointments:

Four different bases were used to prepare four formulations<sup>6</sup>. They were-

- Water soluble base containing PEG400, propylene glycol and water -F1;
- Oil soluble base containing white petrolatum, white bees wax and wool fat -F2;
- Modified hydrophilic base containing molten white petrolatum and steryl alcohol blended with hot solution of propylene glycol, sodium lauryl sulphate, methyl, paraben and sodium metabisulfite in water-F3; and

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Table I. Effect of formulated ointments and 0.2% nitrofurazone ointment on wound area (mm<sup>2</sup>) (Mean±SEM) and percentage wound contraction on excision wound model in rats.

Day	Wound area(mm <sup>2</sup> )		F1	F2	F3	F4
	Control	Standard				
0	308.70± 0.785	309.5± 1.570*(0)	300.20± 1.535*(0)	304.80± 3.095*(0)	306.40± 1.545*(0)	302.20± 1.532*(0)
6	203.60± 2.530 (34.0)	126.70± 1.990 (59.0)	143.80± 3.665 (52.0)	143.20± 2.120 (53.0)	141.0± 2.105 (53.3)	134.80± 2.055 (55.3)
12	107.9± 2.885 (65.0)	41.18± 0.6750 (86.6)	69.41± 1.480 (76.8)	73.15± 0.7550 (76.0)	60.19± 3.435 (80.3)	50.90± 0.6300 (83.1)
18	69.19± 3.205 (77.5)	10.25± 0.550 (96.6)	27.79± 0.4850 (90.7)	31.69± 1.495 (89.6)	21.41± 1.780 (93.0)	13.21± 0.6450 (95.6)

Standard = 0.2%w/w nitrofurazone ointment, F1 = 3% w/w Water soluble base ointment, F2 = 3% w/w Oil soluble base ointment, F3 = 3% w/w Modified Hydrophilic base ointment, F4 = 3% w/w Absorption base

iv) Absorption base containing homogenised mixture of cow ghee, cetyl alcohol and propylene glycol in water. -F4 In all these bases, 3% w/w CUC was incorporated at appropriate stages.

Nitrofurazone ointment was considered as standard and ointment bases was considered as control.

### 3. Excision wound healing studies:

For carrying out this study using albino rats, prior permission from CPCSPA was obtained. Young albino rats of either sex weighing between 150- 200g were used for the experimental work<sup>7</sup>. In the experiments, the rats were divided into six groups, each group consisting of two animals. Group 1, was considered as control (without any drug); group 2, treated with standard (0.2% w/w nitrofurazone) ointment; group 3, treated topically with 3% w/w CUC water soluble base ointment; group 4, treated topically with 3% w/w CUC Oil soluble base ointment; group 5, treated topically with 3% w/w CUC modified hydrophilic base ointment; and group 6, treated with 3% w/w CUC absorption base ointment. All the animals were housed in standard metal cages and provided with adequate food and water.

Each group was anaesthetized by open mask method with anaesthetic ether. The rats were depilated on the back and a predetermined area of 300 mm<sup>2</sup> full thickness skin was excised in the dorsal interscapular region. Rats were left undressed to open environment. The formulated ointments and standard ointment were applied daily until the complete healing was observed. The wound areas were measured on the 0<sup>th</sup>, 6<sup>th</sup>, 12<sup>th</sup>, 18<sup>th</sup> days, by which time healing was complete. A graph paper with a millimetre scale was used for the measurement of wound contraction. Wound contraction was expressed as percentage reduction of original wound.

### RESULTS AND DISCUSSIONS:

In the excision wound studies, there was significant healing in animals treated with formulated ointments F1 to F4 as compared to control group and among these formulations F4 exhibited more pronounced healing effect as compared to F1 to F3. Although, this effect was less than the standard nitrofurazone ointment. The results are summarized in Table I.

Values are mean ±SEM of 2 animals in each group. Number in parenthesis indicates percentage wound contraction. All values are significant at  $p > 0.05$  as compared to control and asterisk (\*) indicate as not significant. P values were calculated by one-way analysis (ANOVA).

### CONCLUSION:

The formulated ointments containing cow urine concentrate were notably effective as wound healing preparations. The effectiveness can be attributed to the antimicrobial activity of cow urine. The chemical moieties present in the concentrate which include urea, uric acid, tannins, etc may be responsible for this effect. Separate experimentation for investigating the chemical composition of the residue can throw light in this regard. However, as a wound healing preparation, the formulated ointments can be found useful.

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