

RESEARCH ARTICLE

WOUND-HEALING ACTIVITY OF DRIED SKIN EXTRACTS OF DUTTAPHRYNUS MELANOSTICTUS IN A RAT EXCISION WOUND MODEL

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ABSTRACT

Wound healing is important and complex process, and this includes events like haemostasis, proliferation of different cells and sequence of inflammation events that includes enhancing fibroblast activity and promoting collagen synthesis. There were many natural and synthetic compounds are there used since ancient times to produce better wound healing like turmeric (curcumin), quercetin and catechins (green tea) and thymol a very limited compounds have in this nature serve as wound healers. Animal origin wound healing compounds are very limited and still unexplored, our study aimed to explore the wound healing property from Indian toad dry skin extract (*Duttaphrynus melanostictus*) of acetate (EtOAc) and dichloromethane (DCM) with these formulated a typical ointment using normal petrolium jelly. The method of study includes induction of wounds (8mm diameter standard size) on the dorsal side and total study done for 16 days, penicillin was taken as standard drug. For every 4 days the wound size measured and noted the size reduction/contraction and EtOAc extract has shown marked enhancement of wound healing in terms of wound contraction compared to control. This study suggests that the Indian toad skin extract having significant and marked wound healing properties.

Keywords:

Duttaphrynus melanostictus, Wound healing, Amphibian skin extract, Excision wound model, Antimicrobial peptides, Wound contraction

INTRODUCTION

To restore tissue integrity after injury, wound healing is a basic physiological process. A disruption in the normal anatomical structure and function of skin or underlying tissues brought on by physical, chemical, thermal, or microbiological assaults is referred to as a wound [1]. Based on the severity of the injury, damage limited to the superficial epithelial layer or may extend into deeper subcutaneous structures such as muscles, tendons, blood vessels, and nerves, potentially leading to significant functional impairment [2]. Effective wound repair is therefore critical not only for structural restoration but also for the re-establishment of normal physiological function. Wound healing process is regulated and dynamic sequence of cellular and molecular events that occurs in four overlapping phases: haemostasis, inflammation, and remodelling [3]. Furthermore, studies have shown that amphibian skin peptides possess potent pharmacological properties, including broad-spectrum antimicrobial activity and immunomodulatory functions [4]. Notably, the antimicrobial peptide cathelicidin-DM, isolated from the skin of *Duttaphrynus melanostictus*, has been reported to exhibit strong antibacterial activity and to promote wound healing in experimental models, thereby supporting the therapeutic relevance of amphibian-derived peptides in tissue repair [5]. Additionally, bioactive compounds derived from toad skin extracts (commonly referred to as Chan Su in

traditional medicine) have demonstrated anti-inflammatory and regenerative properties that may further contribute to enhanced wound healing [6]. Given the documented antimicrobial and anti-inflammatory properties of compounds derived from toad skin, there is a plausible scientific basis to hypothesize that extracts of dried Indian toad skin may enhance wound healing through modulation of inflammatory responses, inhibition of microbial proliferation, and stimulation of tissue regeneration. Therefore, investigating the wound-healing potential of dried toad skin extracts may provide valuable insights into the development of novel bioactive agents for effective wound management.

MATERIALS AND METHODS

A crushing machine (Daiki Rika Kogyo Co., Ltd.) was used to grind the toad's dried skin. After that, 100 grams of the crushed material were extracted successively using dichloromethane (DCM) and ethyl acetate (EtOAc) while being continuously shaken on an orbital shaker for 48 hours. A rotary evaporator (Buchi Labortechnik AG) was used to filter and concentrate the extracts. Until they were needed for additional analysis, the concentrated extracts were kept in a refrigerator at 4 °C [7].

Chemical Screening

Preliminary chemical analysis of dichloromethane (DCM), ethyl acetate (EtOAc) extracts of the dried toad skin was

conducted [8], to qualitatively determine the presence of bioactive secondary metabolites.

Experimental Animals

25 female Wistar rats (180–250 g) were employed. The animals were housed under standard laboratory conditions in the animal facility and allowed to acclimatize for two weeks prior to the initiation of the experiment. Ethical approval (UCPSC/KU/12/2015) The animals were given with a pellet diet (Caps Feed) and provided water ad libitum throughout the experimental period [9].

Preparation of Ointment Formulations

Topical ointment formulations prepared separately from the DCM & EtOAc extracts of dried toad skin using petroleum jelly (Vaseline) as the vehicle. The petroleum jelly was liquefied in oven (Charles Hearson & Co.) at the temperature of 37 °C and each extract was incorporated individually under continuous stirring until a uniform and homogeneous pomade formulation was achieved [10].

Experimental Design

The animals randomly allocated into 5 groups (n = 5 per group) as follows:

Group A: Negative control (untreated)

Group B: Positive control (topical penicillin once a day)

Group C: DCM extract of dried toad skin (once a day)

Group D: EtOAc extract of dried toad skin (once a day)

Group E: Vehicle control (Vaseline, once a day)

Topical treatments were administered once daily for a duration of 16 days.

Excision Wound Model

The animals were weighed and given an intraperitoneal dose of ketamine (75 mg/kg body weight) to induce anaesthesia. An electric clipper was used to shave the dorsal fur after it had been neatly clipped using scissors. Before generating a wound, the exposed region was cleaned with 70% ethanol. On the dorsal surface, an 8 mm diameter excision wound was made with a small incision slit. The panniculus carnosus muscle was removed, and the base of the wound was formed by the fascia beneath. To produce analgesia, neurogenic ointment was applied right after the wound was created [11].

24 hours after wound induction, the respective topical treatments-DCM extract ointment, EtOAc extract ointment, Vaseline, or penicillin- applied to the wound site according to group allocation using sterile cotton applicators. Wound healing progression was evaluated by measuring wound area, percentage wound contraction, and epithelialization time [12]. Following the 16-day course of therapy,

the animals were put to sleep by inhaling chloroform, and the wound tissues were removed and preserved in 10% saline for later histological analysis.

Determination of wound Area

Wound area assessed at 4-day intervals using acetate tracing. The wound margins were carefully traced onto transparent acetate sheets. And the wound diameter was measured with a millimeter ruler. The wound area was calculated using the

formula:

$$\text{Wound Area} = \frac{\pi d^2}{4}$$

d represents the wound diameter $\pi = 3.14$.

Percentage Wound Contraction

Wound contraction quantified every four days until day 16 and expressed as a percentage reduction in wound size

$$\% \text{ Wound Contraction} = \frac{\text{Initial Wound Area} - \text{Wound Area on Specific Day}}{\text{Initial Wound Area}} \times 100$$

relative to the initial wound area [18], using the formula:

RESULTS

The comparative effects of the various treatments on wound area are presented in Table 1. There was no statistically significant difference ($P > 0.05$) at baseline (Day 0). in wound area among all groups, with mean values of approximately 50 mm². This indicates uniformity in wound creation prior to the commencement of treatment. A progressive reduction in wound area was observed in all experimental groups from day 4 to day 16. However, the magnitude of wound contraction differed significantly among the groups. By day 4, animals treated with the dichloromethane (DCM) extract (39.20 ± 0.10 mm²) and ethyl acetate (EtOAc) extract (37.10 ± 1.25 mm²) showed larger

wound area reductions than the negative control (46.02 ± 2.75 mm²). On day 8, further reductions were recorded, with the DCM and EtOAc-treated groups exhibiting smaller wound areas (18.30 ± 4.20 mm² and 19.40 ± 4.60 mm², respectively) relative to the untreated group (31.15 ± 3.42 mm²). By day 12, the DCM (5.20 ± 0.90 mm²), EtOAc (4.95 ± 0.90 mm²), and penicillin-treated (7.12 ± 1.58 mm²) groups showed significantly reduced wound areas compared to both the negative and vehicle controls ($P < 0.05$). At the end of the treatment period (day 16), the DCM extract group showed the lowest mean wound area (1.95 ± 0.60 mm²), by the EtOAc extract group (2.45 ± 0.55 mm²) and the penicillin group (3.80 ± 0.30 mm²). In contrast, the negative control (10.12 ± 1.85 mm²) and vehicle control (5.45 ± 3.50 mm²) groups presented larger residual wound areas. These findings indicate that

Table 1: Evaluation of the potential for wound healing of dried skin extracts of *Duttaphrynus melanostictus* in an excision wound model

Group	0 Day	4 Day	8 Day	12 Day	16 Day
Group A: Negative control (no therapy)	50.10 ± 0.00	46.02 ± 2.75	31.15 ± 3.42	19.02 ± 4.10	10.12 ± 1.85
Group B: Positive control (once daily penicillin ointment)	50.10 ± 0.00	43.90 ± 2.50	24.05 ± 3.05	7.12 ± 1.58	3.80 ± 0.30
Group C: Dichloromethane extract (once daily)	50.10 ± 0.00	39.20 ± 0.10	18.30 ± 4.20	5.20 ± 0.90	1.95 ± 0.60
Group D: One daily dose of ethyl acetate extract	50.10 ± 0.00	37.10 ± 1.25	19.40 ± 4.60	4.95 ± 0.90	2.45 ± 0.55
Group E: Control (Vaseline, once daily)	50.10 ± 0.00	50.10 ± 0.00 ^a	50.10 ± .00	50.10 ± 0.00	50.10 ± 0.00

the dried toad skin extracts significantly enhanced wound healing compared to

untreated ($P < 0.05$) (Fig. 1).

Values are expressed as Mean ± SEM.

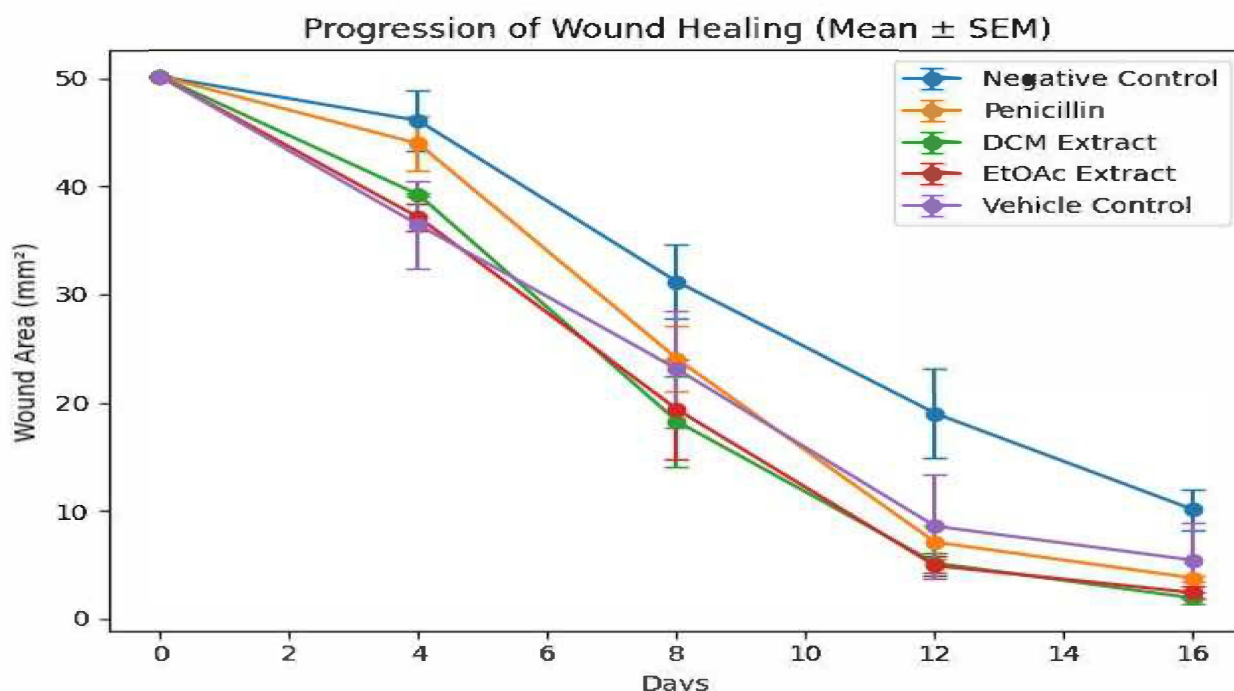


Fig. 1: Effect of Dried *Duttaphrynus melanostictus* skin extracts on wound contraction in the excision wound model (Mean $\pm$ SEM).

Means with different superscript letters within the same column are considered statistically significant at $P < 0.05$.

4. DISCUSSION

This study shows that in the excision wound model, topical application of dried toad skin extracts greatly increased wound contraction. The absence of significant differences in wound area at Day 0 confirms consistency in wound induction and validates the experimental design. Wound healing is a complex, dynamic process involving haemostasis, inflammation, proliferation, and remodelling phases [13-15]. Accelerated wound contraction observed in the extract-treated groups suggests that bioactive constituents of dried toad skin may positively influence one or more of these

healing phases. The more pronounced effect of the DCM extract indicates that moderately non-polar compounds extracted in dichloromethane may possess greater biological activity relevant to tissue repair [16-17]. Overall, the findings of this study suggest that dried toad skin extracts, particularly the DCM fraction, possess significant wound-healing potential. Further studies involving histopathological, biochemical, and molecular analyses are recommended [18].

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