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Original Article

Determination of Wound Healing Potential of Pharmaceutical Formulations (Gel and Paste) Prepared by Using Sea Water Snail

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ABSTRACT

Background: Marine species have long been valued as both nutritious foods for human diets and as a good source of medicinal chemicals. **Purpose:** The present work aimed to investigate wound healing potential of prepared gel and paste formulation. **Materials and Methods:** The work involved the preparation of extracts, preparation of formulation using extract and shells of *Tibia curta* sea water snail and determination of action against wound healing. Sea water snail extract was utilised to make Gels with varying compositions (G1-G9), and snail shells were used to make Paste (P1-P6). The gel and paste were evaluated based on different parameters. **Results:** The formulations with a neutral pH and excellent spreadability (G7 and P1) were chosen for further wound healing studies (since neutral pH does not cause irritancy and improved spreadability results in increased medication effectiveness). The extract, as well as both formulations, were used to treat excise wounds. **Conclusion:** The reduction in skin thickness reveals that formulations formulated using snail extract and shell were more efficient than simple snail extract.

Keywords: Shell, Mollusca, Gel, Paste, Wound healing.

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INTRODUCTION

The marine environment is extremely competitive, producing essential chemicals with industrial and medicinal uses.[1,2] Molluscs are the second biggest phylum in the marine environment in terms of species diversity.[3,4] Numerous scientists are drawn to their morphological and physiological characteristics.[5,4] Molluscs have a significant economic influence on India. Among molluscs, gastropods play an especially important role in the commercial shell craft sector. There are several species on land, in fresh water, and in the sea. Marine gastropods account for a relatively tiny portion of marine fisheries resources. Numerous species are exported for use in the manufacture of decorations, curios, and a variety of other commercially valuable artefacts. Women and children forage for gastropods and bivalves in shallow estuaries. Gastropods have been exported from the Kerala coast, particularly from Neendakara and Fortkochi, to a number of states for use in the production of manure, fish feeds, and chicken feeds. Shells and gastropod shell crafts are the primary source of income for the native peoples around the Kanyakumari and Mahabalipuram (Tamil Nadu) coasts. Additionally, marine bivalves and gastropods contain a plethora of physiologically active chemicals.[6,7] Numerous species are historically fished for food and shell due to its medicinal and commercial characteristics.[8]

Wound healing is a dynamic and active process in which the injured environment varies according to the individual's changing health status. The information on the physiology of the typical wound healing process as it progresses through the phases of hemostasis, inflammation, granulation, and maturation establishes a framework for considering the fundamental principles of wound healing. Through this consideration, health care professionals may enhance their abilities to care for injured patients, and the body can aid in the intricate task of tissue healing. A persistent wound should encourage the health care provider to look for unresolved underlying reasons. Chronic wound healing involves patient-centered, holistic, multidisciplinary, cost-effective, and evidence-based therapy.

A wound is an injury to the body that results in an opening or a break in the skin. They have a major influence on public health and health care spending. Wound healing mechanisms are well-ordered biochemical and cellular activities that result in the expansion and renewal of injured tissue in a unique manner.

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Acute wound healing in an animal model has been demonstrated to occur in four stages. Chronic wounds, it is believed, must also go through the same fundamental phases. According to some authors, the first two steps must be combined.[9]

The wound healing stages

Stage of inflammation.

Stage of debridement.

Stage of proliferation.

Stage of maturation or remoulding.

The various marine species has been reported for wound healing activity.[10-15] In the present work *Tibia curta* was selected and evaluated for wound healing action. *Tibia curta*, commonly termed as the Indian tibia, is a huge sea snail species belonging to the Strombidae family of marine gastropod mollusks. The present analysis aimed to develop formulations using the extract and shell of a marine water snail and to assess their wound healing properties.

MATERIALS AND METHODS

Materials

Carbopol 940, guar gum, xanthan gum, glycerin were procured from the S.D. Fine Chemicals, Mumbai, India.

Animals

Wistar strains rats (12-week old healthy) weighing 150-200 g of both sex were Global bioresearch center, Shirwal (Regd. No.1899/PO/Bt/16/CPCSEA) selected for wound healing evaluation. They were kept in a controlled environment with 10-14 hr cycles of light and dark at a temperature of 23°C ±2, 50± 5 RH. Individual polypropylene cages were utilised to house the animals, who had unlimited access to food and water *ad libitum*.

Methods

Procedure for extraction of marine snail

The gastropods (*Tibia curta*) were collected (in a month of January) and rinsed with sterile seawater to remove associated debris. The 500 g of mass was separated from shells and was macerated in water (250 mL × 3) for 7 days each cycle below 20°C. After each cycle the extracted solvent was replaced by fresh volume. The extracted solvent was concentrated under vacuum by using Rota-evaporator.

Procedure and formula for preparation of gel using marine snail extract

Dispersed carbopol 940 used as a binder in quantity of water and mixed slowly for approximately 15 min to avoid agglomeration. Mentioned quantity of guar gum or xanthan gum was added in the glycerin. Mixed well for 15 min to produce thick and smooth dispersion. Combined both dispersion and mixed for 10 min until the clear and consistent gel base produced. Nine different gel formulations were prepared by using marine snail extract. Detailed composition of formulations were given in Table 1.

Procedure and formula for Preparation of paste using marine snail shell powder

The quantity of guar gum or xanthan gum was added in the glycerin. Mixed well for 15 min to produce thick and smooth dispersion. The amount of powder was added to the water to produce thick paste. Then added to the dispersion. Sufficient amount of water was added to it to produce the paste given in Table 2.

Evaluation of formulations

Determination of the pH

The pH of the gel was measured using a digital pH metre by completely submerging a glass electrode in the gel system. The measurement was performed three times, and the mean of the three measurements was recorded.

Spreadability

It is an unofficial test. In this two glass slides of dimensions 25cm × 7.5cm were taken. 0.1g of the formulation was weighed and placed on one slide and then the approximate diameter of the formulation applied was measured in mm and then the second slide was placed on top of that slide and then 100g of weight was placed on it. The weight was kept for 1 min and after 1 min again the diameter of the formulation was measured.

Table 1: Formula for preparation of gel.

Sl. No.	Ingredients	Quantity (%w/w)								
		G1	G2	G3	G4	G5	G6	G7	G8	G9
1	Marine snail extract	5	5	5	5	5	5	5	5	5
2	Carbopol 940	1	--	--	2	--	--	3	--	--
3	Guar gum	--	1	--	--	2	--	--	3	--
4	Xanthan gum	--	--	1	--	--	2	--	--	3
5	Glycerin	10	10	10	10	10	10	10	10	10
6	Water q.s.	84	84	84	83	83	83	82	82	82

Table 2: Formula for preparation of paste.

Sl. No.	Ingredients	Quantity (% w/w)					
		P1	P2	P3	P4	P5	P6
1	Marine snail shell powder	70	70	70	70	70	70
2	Guar gum	0.5	--	1	--	1.5	--
3	Xanthan gum	--	0.5	--	1	--	1.5
4	Glycerin	5	5	5	5	5	5
5	Water (q.s.)	24.5	24.5	23.5	23.5	23	23

The increase in diameter of the formulation tells about its spreadability.

Extrudability

The formulation was filled in the 5g tubes. The tubes were filled completely and crimped. The tube was placed on the table at a height. The weighing machine was placed on the floor holding a petri plate. The tube was pierced and the specific weight was placed on it and the amount of the formulation that falls in the petri plate in 1min was recorded by recording its weight.

Wound healing activity

The efficiency of the formulations were studied by inducing excision wound on the rats through topical application. The rats were divided into four groups. Group I was a control group, group II wound treated with sea water snail extract, group III wound treated with Gel formulation (G7), group IV wound treated with Paste (P1). Each group of animals containing six rats in each group will be anesthetized with anesthetic ether. The rats will be depilated on the back and a predetermined area of 500 mm^2 full thickness skins will be excised in the dorsal interscapular region. Rats will be left undressed to the open environment.

The formulations will be applied daily until for 14 days. In this model, wound contraction and epithelialization period will be monitored. Histological Examinations will be done after complete healing. Measurement of wound contraction was done after each 2 days interval.

Ethical statement

The experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPSCEA) and Animal house of Rajaram and Tarabai Bandekar college of Pharmacy, Ponda CPCSEA Reg. No. 1659/PO/S/12/CPCSEA.

RESULTS

Evaluation of formulation

Both the formulations gel (G1-G9) and paste (P1-P6) prepared using carbopol 940 were evaluated for pH, spreadability, and extrudability. Results of the study were recorded in Table 3.

Table 3: Evaluation of formulations.

Sl. No.	Formulation code	Parameters		
		pH	Spreadability	Extrudability
1	G1	5.01	Not easily spreadable	Good
2	G2	5.97	Not easily spreadable	Good
3	G3	6.05	Easily spreadable	Good
4	G4	5.3	Not easily spreadable	Good
5	G5	8.4	Easily spreadable	Excellent
6	G6	5.54	Not easily spreadable	Good
7	G7	7.60	Easily spreadable	Excellent
8	G8	Not stable	Not stable	Not stable
9	G9	Not stable	Not stable	Not stable
10	P1	7.59	Easily spreadable	Excellent
11	P2	5.54	Not easily spreadable	Good
12	P3	6.27	Not easily spreadable	Good
13	P4	6.68	Easily spreadable	Good
14	P5	Not stable	Not stable	Not stable
15	P6	Not stable	Not stable	Not stable

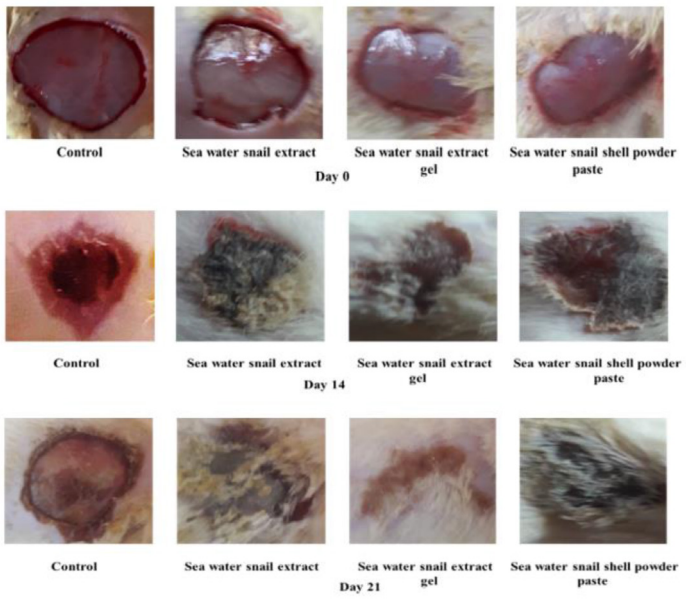

Figure 1: Effects of formulations on healing rate.

Wound healing activity

Effect of formulations on healing rate

Figure 1 illustrates the images of excision wound after 0, 14 and 21 days of administration of formulations (G7 and P1). The results concluded that the wound of each group healed with time. The excision wound treated with sea water snail extract, gel and paste begun to heal noticeably after 14 days. After 21 days, the wounds of excision treated with formulation (G7-Gel prepared by using sea water snail extract) was mostly healed.

Histopathological Evaluation of excision wound

Histopathological staining analysis was carried out to determine the effect of sea water snail extract, sea water snail extract gel and sea water snail powder paste on the wound given in Figure 2.

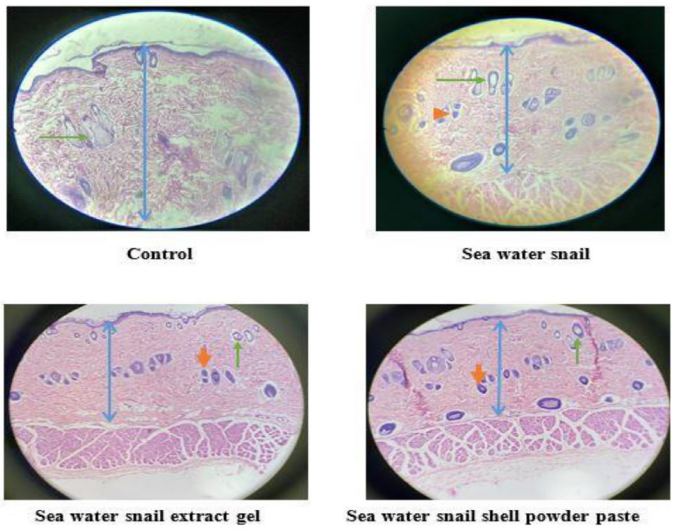

Figure 2: Histopathology study of skin.

Blue double arrowed line indicates the thickness of the skin in all three group.

Green arrowed line indicates the hair follicles.

Orange arrowed line indicates the blood vessels.

After 21 days of completion of treatment, the rats were sacrificed to obtain their skin samples. Observing these images revealed that reduction in the thickness of skin indicating the healing of skin. The extract of sea water snail was found to be less effective than gel and paste compared to control group. The hair follicles were observed near the surface in case of treated group when compared with untreated group. The blood vessels were also more observed in treated group than untreated group. The overall results concluded that the prepared formulations (G7 and P1) were more effective than extract.

DISCUSSION

Both the formulations gel (G1-G9) and paste (P1-P6) prepared and were evaluated for pH, spreadability, and extrudability. Prepared gels and pastes were found to be homogeneous and in good appearance and consistency. The pH values of all the formulations were ranges from (5.01-8.4). The pH formulation G7 and P1 was found to be 7.60 and 7.59 which is neutral and hence it caused no skin irritation. The G7 and P1 formulations were easily spreadable. Among all the gel and paste formulations G7 and P1 have excellent extrudability.

Based on the studied parameters, G7 and P1 were found to be excellent and used for further wound healing studies. After applying the extract and both formulations to excision wounds, each group's wound eventually healed. After 14 days, the excision wound started to heal noticeably after being treated with sea water snail extract, gel, and paste. After 21 days, the excision wound was mostly healed after being treated with the formulation (G7) gel made from sea water snail extract). The fact that the thickness of the skin was reduced during the histological evaluation suggests that formulations made with snail extract and shell were more efficient than those made with just snail extract.

CONCLUSION

Tibia curta, often known as the Indian tibia, belongs to family Strombidae. This study was done to develop formulations using a marine water snail's extract and shell and evaluate their wound-healing potential. Furthermore the formulations were evaluated for excise wound healing. The sea water snail extract was used to produce gels with different compositions (G1-G9) whereas shells of snails used to produce paste (P1-P6). The evaluation of gel and paste was carried out based on basic parameters such as pH, spreadability and excrudability. The formulations with neutral pH and excellent spreadability were selected for further wound healing studies (G7 and P1) as neutral pH do not produce irritancy and better spreadability provide more efficacy of drug. The extract and both the formulations were applied against excise wound, the wound of each group healed with time. The excision wound treated with sea water snail extract, gel and paste begun to heal noticeably after 14 days. The excision wound treated with formulation (G7 gel prepared by using sea water snail extract) was mostly healed after 21 days. In the histopathological evaluation, there was reduction in the thickness of skin observed indicates that formulations prepared by using extract and shell of snail were more effective than the plain extract of snail.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

pH: Potential of Hydrogen; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **g:** Gram, **cm:** Centimeter; **min:** Minute; **°C:** Degree Celsius; **mL:** Milliliter; **G:** Gel; **P:** Paste.

SUMMARY

Tibia curta, often known as the Indian tibia, is a huge Strombidae marine gastropod mollusc. This study developed marine water snail extract and shell wound-healing compositions. For excision wound healing, compounds were tested. Snail shells and sea water snail extract were utilised to make gels (G1–G9) and paste (P1-P6). Gel and paste were evaluated using pH, spreadability, and excrudability. The formulations with neutral pH and great spreadability were selected for future wound healing tests (G7 and P1) as neutral pH do not generate irritancy and superior spreadability provide more efficacy of medicine. The extract and both formulations cured excise wounds in each group. The excision wound treated with formulation G7 gel made by employing sea water snail extract was mostly healed after 21 days. In the histological assessment, there was reduction in the thickness of skin observed indicating that formulations formed by using extract and shell of snail were more effective than the basic extract of snail.

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