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WOUND HEALING ACTIVITY OF THE ETHANOLIC EXTRACT OF *MICROCOSMUS EXASPERATUS* HELLER, 1878

M.I. Delighta Mano Joyce*[1], V.K. Meenakshi*[2], M. Paripooranaselvi[2] and S. Gomathy[2]

[1]Department of Zoology, V.O. Chidambaram College, Tuticorin, Tamilnadu, India
[2]Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin, Tamilnadu, India

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Abstract

Wound is termed as any injury that disrupts epithelial integrity of the skin thereby altering the normal function of tissue. Healing of wound is a physiological process accompanied by different phases. The aim of the present study is to evaluate the wound healing potential of ethanolic extract of simple ascidian *Microcosmus exasperatus* using excision, incision and dead space models. Extract treated groups showed a significant dose dependent increase in the percentage closure of wound by enhancing epithelialization in both excision and incision models. In dead space, the tensile strength, wet, dry weight and hydroxyproline content recorded a highly significant increase in the extract administered groups compared to that of control and standard. Histopathological examination of granulation tissues exhibited well formed collagen fibres and fibroblasts without any inflammatory cells in the group treated with the highest dose than the control. The results obtained indicate that the extract of *Microcosmus exasperatus* has good potential in wound healing process.

Key words: *Microcosmus exasperatus*, framycetin, hydroxyproline.

*Corresponding authors: midelighta@gmail.com
vkmeenakshi.apcm@gmail.com

Introduction

Any injury resulting in the disruption of tissues or organs is termed as wound. There are various types of wounds such as abrasions, burns, incisions, ruptures, punctures, lacerations and bite, but the principles of wound healing behind them are the same. Wound healing is a complex and dynamic physiological process dependent on a number of inter-related factors. Review of literature has shown that many species of plants aid the wound healing process (Jalalpure *et al., 2002*). But from marine organisms only a very few has been reported (Gopalakrishnan *et al., 2012*, Subathra *et al., 2015*). *Microcosmus exasperatus* is a simple ascidian belonging to the family Pyuridae, found attached to the rocks, hull of ships and materials used for aquaculture. The animal has been reported to possess various activities such as antibacterial, acute, sub chronic oral toxicity, antidiabetic, hepatoprotective, CNS depressant, antitumour, antifertility, cardiotoxicity, antihyperlipidemic, anaesthetic, analgesic, antipyretic, nutritional value, and biochemical components (Senthamarai *et al., 2012*, Meenakshi *et al., 2012a, b, 2013a, b, c, 2014a, b, c*, Delighta *et al., 2015*, Karthikeyan *et al., 2010, 2011*). The present study was carried out to identify the wound healing activity of the extract of *Microcosmus exasperatus*, through excision, incision and dead space models.

MATERIALS AND METHODS:-

Collection of animal material

Fresh samples of *Microcosmus exasperatus* (Family: Pyuridae) were collected from Tuticorin harbour area, by SCUBA diving. They were identified and authenticated using key to identification of ascidians (Meenakshi, *1997*). A voucher specimen No: AS 2240 has been deposited in the museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin.

Preparation of extract

Collected samples were washed thoroughly with sea water to remove the adhering epibionts, dried under shade and homogenized. The moderate coarse powder obtained was stored in an airtight container. 100 g powder was extracted with ethanol in a soxhlet apparatus, cooled to room temperature, concentrated in a rotary evaporator until a sticky mass was obtained. For topical application, the extract ointment at three different formulations of 5, 10 and 15% (w/w) were prepared by incorporating 5, 10 and 15 g of extract in 100 g of simple ointment base. For acute oral toxicity studies, the extract was suspended in 1 % gum acacia, blended with vanillin and administered orally.

Experimental animal

Wistar albino rats weighing 180-200 g were selected. They were hoaxed in a ventilated cage and maintained under standard conditions of temperature at 26± 2° C, 12 hrs of dark, light schedule. All animals had access to clean water and standard pellet diet (Hindustan lever Ltd., India) "ad Libitum".

Acute toxicity studies

The minimum lethal dose of the ethanolic extract of *Microcosmus exasperatus* was determined as per OECD guidelines (OECD, *2002*). To the overnight fasted rats, a dose of 2000 mg/kg body weight of the extract was given orally using intra gastric catheter. The animals were observed for the first 3 hrs continuously for any gross behavioral changes and toxic manifestations like hypersensitivity, grooming, convulsions, sedation, hypothermia and mortality. After 24 hrs, the numbers of dead and surviving animals were recorded. With the same dose of the extract, the experiment was repeated for 7 more days. Thereafter the animals were continuously monitored at regular intervals for 14 days.

Excision wound model

The rats were anaesthetized with light ether and placed on the experimental table. On the dorsal thoracic region, an infliction was made 5 mm away from the ears using a round seal. A single circular wound of 500 mm^2 was made by excising the skin on the impressed area. The animals were grouped into five of six each. Group I was treated with simple ointment and group II

which served as standard with 2% framycetin sulphate cream (FSC). Ethanolic extract of *Microcosmus exasperatus* at a concentration of 5, 10 and 15% as ointment was applied to group III, IV and V. Using a fine brush, the ointment was applied topically once in a day till the wound was completely closed (Morton *et al*., *1972)*.

Determination of wound contraction

Wounds were traced planimetrically by using graph paper from the day of wounding and subsequently on days 2, 4, 8, 10, 12 and 14. Contraction of wound contributes to the reduction in size of wound area which is expressed in percentage. The days required for complete epithelialization was also recorded. Percentage of wound contraction was calculated by applying the following formula

$$\text{Percentage wound contraction} = \frac{\text{Initial wound size - specific day wound size}}{\text{Initial wound size}} \times 100$$

Incision wound model

On either side of vertebral column of rats, incisions of 6 cm were made through the entire thickness of the skin under light ether anaesthesia. Wounds were covered with interrupted sutures, using silk threads and round bodied needle. Then, the animals were divided into five groups of six in each. Group I was treated with simple ointment, group II with 2% framycetin sulphate (FSC) and group III, IV and V received 5, 10 and 15% of the ethanolic extract of *Microcosmus exasperatus.* Complete epithelialization period was also noted (Ehrlich *et al*., *1969*).

Dead space wound model

After induction with light ether anaesthesia, subcutaneous dead space wounds were imposed in the region of the axilla and groin into a pouch made by a small superficial cut in the skin. In order to harvest granulation tissue, cylindrical grass piths each measuring 2.5 cm in length and 0.3 cm in diameter was introduced into the pouch. Two grass piths were made in different locations in each animal. The wounds were sutured and cleansed with an alcoholic swab. Excision of the granuloma from the surrounding tissue was carried out on the 10th post wounding day. Granulation tissues around the grass piths thus excised were slit open. The breaking strength was determined on 10th post wounding day by a continuous constant water flow technique (Lee, *1968*).

Histopathology

For histopathological examination, tissues were collected and fixed in 10% formalin solution for 24 hrs. Using ethanol-xylene solution, the dehydration process was carried out sequentially. Sectioning of tissues with 10µ thickness was taken after embedding with paraffin at 40º-60º C. They were stained with haematoxylin - eosin and observed under light microscope.

Statistical analysis

The results obtained from the wound models are presented as mean ±SEM. p values were calculated using Student's t-test by comparing the treated groups with control and standard. Values less than 0.05 were considered as moderately significant, 0.01 significant and 0.001 highly significant.

RESULTS AND DISCUSSION

Observations recorded for the contraction of excision wound using the ethanolic extract of *Microcosmus exasperatus* are depicted in Figure 1. In excision wound healing, topical application of extract ointment showed a significant healing process. A high significant increase in the percentage closure of wounds by enhanced epithelialization was noted in the extract treated Group IV and V from 8th to 14th day when compared to control and standard drug.

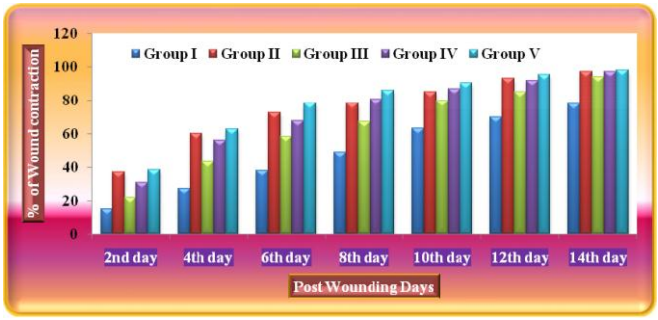

Figure 1: Effect of ethanolic extract of *Microcosmus exasperatus* on the contraction of excision wound

The effect of the extract on the contraction of incision wound model is given in Figure 2. A significant reduction in the size of the wound diameter was observed from 12th day onwards in the extract treated groups and on 16th day a significant dose dependent gradual increase in the percentage of wound closure was noted. The group which received the highest dose showed a decrease in wound diameter compared to that of standard drug framycetin. A gradual decrease in the epithelialization period was observed in the extract administered groups and in group V it was significant compared to control and standard

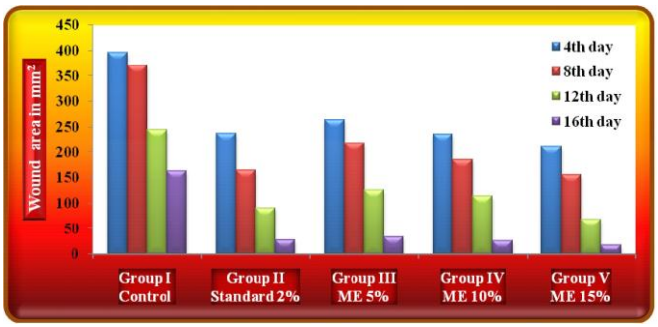

Figure – 2: Effect of ethanolic extract of *Microcosmus exasperatus* on the contraction of incision wound

The granuloma weight of wound was estimated for the evaluation of wound healing activity by dead space method in different groups. The results are given in Figure 3. A dose dependent highly significant increase in the tensile strength, wet, dry weight and hydroxyproline content were noted in the extract treated groups compared to that of control and standard. Group V exhibited highly significant increase than that of standard drug treated group in all the parameters studied. Histopathological examination of granulation tissues of control and extract treated are presented in plate 1. Group I represents control with indistinguishable collagen fibres. In group II, which received the standard drug, granuloma showed a high level of fibrosis and well formed collagen fibres. The granulation tissue of III and IV which was applied with the extract exhibited moderate collagenation and fibrosis. In group V treated with the highest dose of the extract, the granuloma tissue shows well formed collagen fibres and fibroblasts without inflammatory cells. The ethanolic extract of *Microcosmus exasperatus* at a dose of 15% (w/w) was more effective than the standard in promoting collagen formation indicating the process of wound healing.

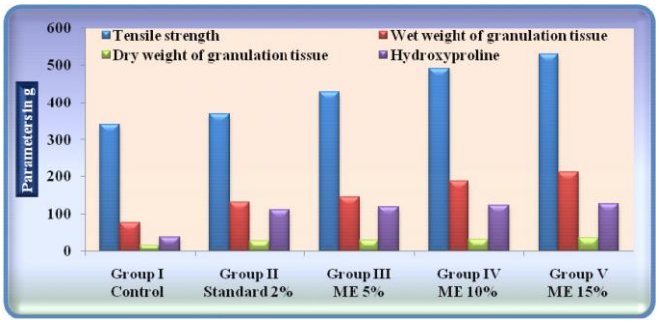

Figure – 3: Effect of ethanolic extract *of Microcosmus exasperatus* on dead space wound

Healing is a process of repairing the damaged tissue by replacement of connective tissue which then forms a scar. The phases of wound healing can be attributed to the types of wound. Each and every wound follows a common wound healing pathway consisting of four consecutive phases such as acute inflammatory, debridement, proliferation and remodeling or maturation (Flanagan, *2000*). Use of single model is inadequate to assess the effect of extract on wound healing process.

Group I shows increased number of fibroblasts and indistinguishable collagen fibres; Group III and IV ME extract- 5 and 10 %, moderate collagenation and fibrosis; Group II and V framycetin sulphate (2%) and ME extract15%, a high level of fibrosis as well as well-formed collagen fibres.

Hence, the present study has used three models to explain the mechanism of action. During the inflammatory phase the wound fills with blood and lymph followed by an immediate vasoconstriction mediated by serotonin, catecholamines, bradykinin, histamine, prostaglandins and minimizes the blood loss (Hosgood, *2006*). After an injury within 24-48 hrs local monocytes migrate into the wound and become macrophages which produce essential growth factors. The wound macrophages and fibroblasts mediate the healing process and they are more dominant from day 5 onwards. Fibroblasts invade the wound and form a new matrix in the form of collagen and from this, new blood capillaries grow and form connective tissue. This growth of new blood vessels, termed as angiogenesis is stimulated by a variety of substances produced by macrophages (Knighton *et al.*, *1981*). The fibroblasts gather around the wound margin, contract and pull the wound's edges together. This plays a significant role in the healing process (Harding and Cutting *1994*). When the wound starts contracting the surrounding skin becomes thin and it will be restored by the proliferation of epithelial cells and connective tissue (Pavletic, *2010*). The condition of granulation tissue indicates how far the wound is healed. In the maturation phase, an increase in the strength of scar occurs by remodeling of the tissue. Cells which are not needed further are removed by apoptosis (Brown, *1988*).

Increased wound contraction in extract treated groups in both excision and incision models might be due to the activity of fibroblasts in the regenerated wound tissue. In dead space wound model, a dose dependent increase in tensile strength was noted in extract treated groups and it may be due to repair process which involves remodeling of dermal tissues to produce greater tensile strength (Keast, *2004).*

Wet and dry weight of granulation tissue in extract treated groups showed a gradual increase in a dose dependent manner. This significant increase in the granulation tissue might be an indicative of increased protein synthesis and represents the proliferative and remodeling phase of wound healing process (Gopalakrishnan *et al., 2012*). A notable increase in the content of hydroxyproline in extract administered groups reflected the increased collagen levels thereby gain in granulation tissue. This was further supported by histopathological studies. Similar results with an increase in tensile strength, wet, dry and hydroxyproline content was observed with the methanolic extract of *Phallusia nigra* and ethanolic extract of *Phallusia arabica* (Gopalakrishnan *et al ., 2012*, Subathra *et al., 2015).*

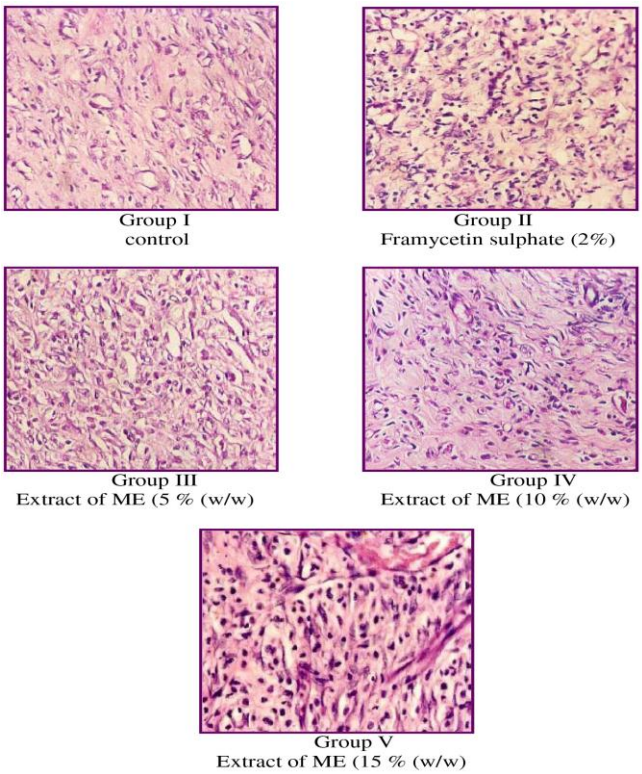

Plate - I: Photomicrograph showing histopathological changes in the granulation tissues

Various studies have reported that alkaloids, phenolic compounds and flavonoids possess potent wound healing properties (Inayathulla *et al., 2010*). Chemical investigation of *Microcosmus exasperatus* has revealed the presence of alkaloids, phenolic compounds and flavonoids (Meenakshi *et al., 2012c, 2014d*). In addition, GC-MS analysis of the ethanolic extract of *Microcosmus exasperatus* showed the presence of compounds like n-Hexadecanoic acid, Tetradecanoic acid, 26-Nor-5-cholesten-3á-ol-25one, (Z,Z,Z)- phenylmethyl ester of 6,9,12-octadecatrienoic acid, cholestan-3-01and N-[-4-bromo.n-butyl-]- 2-piperidinone having antimicrobial and antioxidant activities (Meenakshi *et al., 2012d*). Thus enhanced wound healing activity of ethanolic extract of *Microcosmus exasperatus* may be due to the presence of these biochemical constituents.

CONCLUSION

The present study suggests that the ethanolic extract of *Microcosmus exasperatus* may have a beneficial effect on the various phases of wound healing. Isolation, characterization, structure determination and identification of nature of the exact chemical constituent responsible for the wound healing process and their mechanism of action are to be studied further.

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References

Brown G.L. (1988). Acceleration of tensile strength of incisions treated with EGF and TGF. *Annals of Surgery*, 208: 788-794.

Delighta Mano Joyce M.I., Meenakshi V.K., Paripooranaselvi M. and S. Gomathy, (2015). Anaesthetic, Analgesic and Antipyretic activities of *Microcosmus exasperatus* Heller, 1878. *World Journal of Pharmaceutical Research*, 4(7): 1770-1779.

Ehrlich H.P. and T.K. Hunt, (1969). Effect of cortisone and anabolic steroids on tensile strength of healing wounds. *Annals of Surgery*, 170: 203-206.

Flanagan M. (2000). The physiology of wound healing. *Journal of wound care*, 9(6): 299-300.

Gopalakrishnan S., Shanmugapriya D. and V.K. Meenakshi, (2012). Wound healing activity of the methanolic extract of *Phallusia nigra* Sav. *International Journal of Chemical and Pharmaceutical Sciences*, 3(3): 45-51.

Harding K. and K.Cutting, (1994). Criteria for identifying wound infection. *Journal of Wound Care*, 3(4): 198-201.

Hosgood G. (2006). Stages of wound healing and their clinical relevance. *Veterinary clinics of North America: Small Animal Practice*, 36**:** 667–685.

Inayathulla I., Karigar Asif A., Shariff W.R. and S. Sikarwar Mukesh, (2010). Wound healing property of alcoholic extract of leaves of *Alangium salvifolium*. *Journal of Pharmacy Research*, 3(2): 267-269.

Jalalpure S.S., Patil M.B. and K.R. Alagawadi, (2002). Wound healing activity of the galls of *Quercus infectoria* Olivier. *Journal of Natural Remedies*, 2(1): 54-58.

Karthikeyan M.M., Ananthan G. and A. Jaffar Ali, (2010). Nutritional values of solitary ascidian *Microcosmus exasperatus* Heller, 1878. *Global Veterinaria*, 4(3): 255-259.

Karthikeyan M.M., Ananthan G. and T. Balasubramanian, (2011). Biochemical components of a solitary ascidian *Microcosmus exasperatus* Heller, 1878 (Ascidiacea: Pyuridae). *Journal of Marine Biological Association India*, 53(1): 139-141.

Keast D'Orsted H. (2004). The basic principles of wound healing. *Wound care Canada*, 9(2): 16-25.

Knighton D.R., Silver I.A. and T.K. Hunt, (1981). Regulation of wound healing angiogenesis: effect of oxygen gradients and inspired oxygen concentration. *Surgery*, 90**:** 262-270.

Lee K.H. (1968). Studies on mechanism of action of salicylates II. Retardation of wound. *Journal of Pharmacological Sciences*, 57: 1042-1043.

Meenakshi V.K. (1997). Biology of few chosen ascidians, Ph.D. thesis, Manonmaniam Sundaranar University, Tirunelveli, India.

Meenakshi V.K., Gomathy S. and K.P. Chamundeswari (2012 a). Acute and subchronic oral toxicity of *Microcosmus exasperatus* Heller, 1878. *Journal of Microbiology and Biotechnology Research*, 2(1): 94-98.

Meenakshi V.K., Gomathy S., Paripooranaselvi M. and K.P. Chamundeswari, (2012b). Antidiabetic activity of the ethanol extract of simple ascidian *Microcosmus exasperatus* Heller, 1878. *International Journal of Chemical and Pharmaceutical Sciences*, 3: 33-39.

Meenakshi V.K., Gomathy S., Senthamarai S., Paripooranaselvi M. and K.P. Chamundeswari, (2012c). Analysis of vitamins by HPLC and phenolic compounds, flavonoids by HPTLC in *Microcosmus exasperatus*. *European Journal of Zoological Research*, 1(4): 105-110.

Meenakshi V.K., Gomathy S. and K.P. Chamundeswari, (2012d). GC-MS analysis of the simple ascidian *Microcosmus exasperatus* Heller, 1878. *International Journal of ChemTech Research*, 4(1): 55-62.

Meenakshi V.K., Gomathy S., Senthamarai S., Paripooranaselvi M. and K.P. Chamundeswari, (2013a). Hepatoprotective activity of the ethanol extract of simple ascidian *Microcosmus exasperatus* Heller, 1878. *European Journal of Zoological Research*, 2(4): 32-38.

Meenakshi V.K., Delighta Mano Joyce M.I., Paripooranaselvi M. and S. Gomathy, (2013b). CNS depressant activity of simple ascidian *Microcosmus exasperatus* Heller, 1878. *International Journal of Current Microbiology and Applied Sciences*, 2(10): 16-25.

Meenakshi V.K., Senthamarai S., Paripooranaselvi M., Gomathy S., Sankaravadivu S. and K.P. Chamundeswari, (2013c). *In vitro* and *in vivo* antitumor and immunommodulatory studies of *Microcosmus exasperatus* against DLA bearing mice. *European Journal of Applied Engineering Scientific Research*, 2(3): 18-25.

Meenakshi V.K., Gomathy S., Paripooranaselvi M., Senthamarai S. and K.P. Chamundeswari, (2014a). Antifertility activity of simple ascidian *Microcosmus exasperatus* Heller, 1878. *International Journal of Pharmaceutical Sciences Review Research*, 24(1): 230-236.

Meenakshi V.K., Delighta Mano Joyce M.I., Paripooranaselvi M., Gomathy S. and K.P. Chamundeswari, (2014b). Protective effect of *Microcosmus exasperatus* against isoproterenol induced myocardial ischemia - A biochemical and histopathological approach. *International Journal of Pure and Applied Bioscience*, 2(1): 62-70.

Meenakshi V.K., Delighta Mano Joyce M.I., Paripooranaselvi M. and S. Gomathy, (2014c). Antihyperlipidemic activity of *Microcosmus exasperatus* Heller, 1878. *Journal of Chemical, Biological and Physical Sciences*, 4(3): 1379-1387.

Meenakshi V.K., Gomathy S., Paripooranaselvi M., Senthamarai S. and K.P. Chamundeswari, (2014d). Pharmacognostical evaluation and chemical screening of *Microcosmus exasperatus*. *International Journal of Phytopharmacy Research*, 5(2): 80-86.

Morton J.J.P. and M.H. Malone, (1972). Evaluation of vulnerary activity by an open wound procedure in rats. *Archives Internationales De Pharmacodynamie Et De Therapie*, 196: 117-126.

OECD (Organization for Economic Cooperation and Development). OECD Guidelines for the Testing of chemicals/ selection 4: Health Effects Test No 423: Acute Oral Toxicity – Acute Toxic Method. OECD, Paris, 2002.

Pavletic M.M. (2010). Atlas of small animal Wound Management and Reconstructive Surgery, 3rd edn. Wiley–Blackwell, Ames, pp.17-50.

Senthamarai S., Meenakshi V.K., Gomathy S., Paripooranaselvi M., Shanmugapriya D. and K. P. Chamundeswari, (2012). Antibacterial activity of ascidian *Microcosmus exasperatus* against human pathogens. *Proceedings of 8th all India Conference of KAAS* - Vol. III. Sciences, Zoo. 14-22.

Subathra Christy K.H., Jothibai Margret R. and V.K. Meenakshi, (2015). Evaluation of wound healing activity of *Phallusia arabica*. *World Journal of Pharmaceutical Research*, 4(7): 1202-1213.