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Evaluation of Wound Healing Activity of Methanolic Extract of *Tonna dolium* (L.1758)

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Abstract: Marine invertebrates offer a rich source of potential drugs with excellent biological activities. The present study was aimed at investigating the wound healing effect of methanolic extract of *Tonna dolium* (L.1758) using excision, incision and dead space wound model. Wound healing activity was evaluated in different concentrations prepared as simple ointment base BP using four groups of six Wistar albino rats in each model with povidone iodine (5%) as the standard drug. The wound healing was assessed by a rate of wound contraction, tensile strength and histological changes. Topical application of the methanolic extract ointments of *T.dolium* showed a significant and dose dependent effect on the healing process.

Keywords: *Tonna dolium*, methanolic extract, wound healing activity, povidone iodine

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I. INTRODUCTION

Nature is a primary source of effective medicinal agents and folk medicine has been the basis for the development of a large number of drugs, plasters, ointments and wound dressings based on animal fat, for instance have been used for millennia [1,2]. Wound refers to the disruption or opening in the epithelial tissue of the skin, caused by physical, chemical, thermal and mechanical integrity and infection occurred by microbes present in the environment and delay the wound repair [3]. Wound healing is a complex biological process that involves inflammation, reepithelialization, angiogenesis and formation of granulation tissue and interstitial matrix, as well as processes performed by specific cells such as keratinocytes, fibroblasts and endothelial cells [4,5].

Natural derivatives play an important role to heal the wound when compared to synthetic drugs. Moreover, cost and production of synthetic drugs is also high and they cause adverse effect when compared to bioactive naturally derived drugs [6]. Hence, intense research is under progress towards search for natural remedies with potent biological activities from marine organisms. Marine invertebrates offer a rich source of potential drugs with excellent biological activities [7]. So in the present study an attempt was made to investigate the wound healing effect of methanolic extract of *Tonna dolium*.

II. MATERIALS AND METHODS

Specimens of *Tonna dolium* used in the present study were collected from Gulf of Mannar Coastal region. Debris and epibionts adhering to the shells of *Tonna dolium* were carefully removed, washed several times with sterile sea water, shells were broken, and the soft tissues were removed dried at 56^0C in a hot air oven and powdered. 100g of powder was exhaustively extracted with methanol in a Soxhlet apparatus, concentrated in a rotary vacuum evaporator when 15g of brown sticky mass was obtained. The methanolic extract was incorporated into a simple ointment base BP. Two formulations of the extract ointment 30% (W/W) and 50% (W/W) were prepared by incorporating 30g and 50g of extract in a 100g of simple ointment base BP respectively for topical administration. The extracts were suspended in 1% carboxy methyl cellulose (CMC) and used for oral administration.

2.1 Selection of experimental animals

Adult male Wistar albino rats weighing about 150 - 180g maintained at SB College of Pharmacy animal house, Sivakasi were used for the present study with prior approval of Institutional Animal Ethics Committee. The rats were housed in standard environmental conditions, fed with standard food and water *ad libitum* during the whole period of the experiment. Excision, incision and dead space wound models were used to evaluate the wound healing activity of the methanolic extract of *T. dolium*.

2.2 Excision wound model

The Wistar albino rats in these studies were inflicted with an excision wound as described by [8], under light ether anaesthesia superficially a single wound of 200 mm^2 was made on depilated ethanol sterilized dorsal surface of thoracic region of rats with the help of sterilized surgical scissors and forceps under semi-aseptic condition. The slain impressed area was excised to the full thickness to obtain 200mm^2 width and 2mm depth. Each animal was housed individually in separate polythene cases. The animals were there randomized into four groups, each group consisting of 6 rats. Group- I received a topical application of 50mg of the simple ointment served as control. Group II received application of standard drug ointment i.e. povidone iodine cream (5%), Group III and IV received the topical application of test sample 30 mg/kg and 50 mg/kg of methanolic extract ointment respectively.

2.3 Measurement of wound area

Wound contraction was monitored planimetrically by tracing the wound margin on a transparent paper on 3rd, 6th, 9th and 12th days and retracing the wound margins on a millimeter scale graph paper. Wound contraction which contributes for wound closure or reduction in the wound area was expressed as percentage reduction of the original wound area (200mm^2).

$$\text{Percentage wound contraction} = \frac{\text{Healed area}}{\text{Total wound area}}$$

(Healed area = original wound area – recent wound area)

To apply this equation, the wound margins were traced and measured to calculate the non healed area which was subtracted from the original wound area to obtain the healed area.

2.4 Incision wound model [9]

The rats were divided into 4 groups each group consisting of six rats. The animals were anaesthetized using light ether. Para vertebral incision of 1cm was made on either side of vertebral column of rat with the help of sharp scalpel and incision was made more closely lateral to the vertebral column with sufficient care. The incision was closed with interrupted sutures. These rats were housed individually in different cases for 10 days. Group I (Ointment control) received a topical application of 50 mg of the simple ointment BP ; Group II (standard drug) were treated with topical application of 50 mg of 2% povidone, Group III and IV were treated with 30% and 50% methanolic extract of *Tonna dolium*. The animals were treated with ointment from 1st day to 10th day on 11th day of post wounding, wound breaking strength was measured by adopting continuous constant water slow technique as described by [10] and breaking strength of water necessary to bring about the gapping of the area.

2.5 Dead space wound model

For dead space wound model the animals were divided into 4 groups containing six in each. Group I served as the control that received 1 ml/kg of water orally. Group II (standard drug) received a physical application of 50mg/kg of povidone. The remaining Group III and IV were administered with oral dose of 30 and 50 mg/kg of test substance respectively. The animals were anaesthetized by light ether and dead space wounds were created through a small transverse incision made in the lumbar region with sterilized cylindrical grass piths (2.5 X 0.5cm) one on each side beneath the dorsal para vertical lumbar skin [11]. The day of the wound creation was considered as day zero. Granulation tissue formed on the grass pith was harvested by careful dissection on day 10th of post wounding period and was subjected to breaking strength and histological studies.

2.6 Histological study

The healing tissues obtained on the 12th day from all the three groups of animals of the dead space wound model were processed for histological study to determine the pattern of lay-down for collagen 10% of neutral formation solution was used to fix the granulation tissues for 24 hours and dehydrated with a sequence of ethanol-xylene series solution. The inflicted material embedded with paraffin at 40-60°C were subjected for microtome sections (10µ thickness), stained with haematoxylin – eosin and observed under microscope for any histological changes.

III. RESULT

The effect of methanolic extract of *Tonna dolium* on the contraction of excision wound is presented in Table.1. Topical application of the methanolic extract ointments of *T. dolium* showed a significant and dose dependent effect on the healing process. It could be noted that the wound contracting ability of extract

treated groups viz., group III (30 mg/kg) and group IV (50 mg/kg) were found to be highly significant from 6th to 12th day when compared to that of standard. At 30 mg/kg of the test drug treatment the wound closure was 90.31% on 12th day of the treatment, at 50 mg/kg of the (methnolic extract) test drug treatment the wound closure was 100 percentage on 12th day of experimental exposure. Epithelialization was also found to be highly significant in group IV (17.88±0.06) when compared to that of standard (15.68±0.12) (Table.1).

The effect of methanolic extract of *T. dolium* on the contraction of incision wound model, the extract treated groups even at low dose produced significant increase in wound contraction when compared with standard group. Highly significant increase was noticed on 12th day group IV showed 99.89 percentage of wound contraction than that of standard (98.11) Epithelialization period was also found to be highly significant in IV (10.11± 0.08) which is closely resembling with the standard (12.34±0.06) (Table.2).

The significant wound breaking strength was observed in animals treated with standard povidone iodine 2360±78.00 but the effects seem to be more than the corresponding extract concentration and the results are shown in table.3.That is at group III (30mg/kg) the breaking strength was 2085.71±106.11 and at group IV (50mg/kg) it was 2221.22±112.56 (Table.3).

In the dead space model, significant increase in the wet and dry weight of the granulation tissue were observed in the animals treated with the methanolic extract of *T.dolium.* There is a significant increase with the granuloma wet and dry weights at group IV that is 190.38±4.20 and 23.87±1.08 gms respectively (Table.4).

Histology of the granulation tissues of the control and methanolic extract treated animals were studied. The control showed less collagenation, more macrophages and lymphocytes. The standard (povidone iodine) treated granuloma showing more amount of collagen formation, tissue infiltration in the macrophages and lymphocytes. 30 mg/kg extract treated granuloma tissue showing less collagen fibres and less infiltration of tissue. 50 mg/kg extract treated granuloma showing more collagen and fibroblasts, in the absence of inflammatory cells when compared to the standard and control. The methanolic extract of *T.dolium* (50 mg/kg) was more effective in promoting collagen formation.

Table 1: Effect of methanolic extract of *Tonna dolium* on the contraction of excision wound on albino rats

Days	Control	30mg / kg	50 mg/kg	Standard (Povidone Iodine 5%)
3	14.34%	25.68%	41.88%	42.98%
6	32.86%	52.95%	80.01%	75.01%
9	58.75%	79.47%	93.77%	83.28%
12	66.08%	90.31%	100%	92.07%
Epithelization period	16.72±0.13	14.00±0.78	17.88±0.06	15.68±0.12

Table 2: Effect of methanolic extract of *Tonna dolium* on incision wound (mm^2)

Groups	Epithelization period	Wound area (mm^2) and percentage of wound contraction				
		0	3	6	9	12
Group I Control	24.18±0.11	230.21±1.23 22.17%	195.66±2.66 26.91%	150.13±5.66 58.37%	98.65±2.77	82.21±2.16 76.31%
Group II Standard	12.34±0.06	210.33±0.4	209.22±1.05 58.08%	113.38±1.87 73.16%	56.01±2.02 89.96%	27.44±0.82 98.11%
Group III 30mg/kg	11.86±0.01	201.01±10.08	132.88±11.72 50.18%	49.33±12.15 65.31%	19.01±2.91 82.41%	0.00 92.65%
Group IV 50mg/kg	10.11±0.08	200.03±1.07	130.34±1.07 39.87%	39.21±1.25 70.88%	12.07±1.99 90.08%	0.00 99.89%

Table 3: Effect of methanolic extract of *Tonna dolium* on incision wound breaking strength (g)

Group / Parameters	**Incision wound breaking strength (g)**
Control	19.30 ± 71.25
30 mg / kg	2085.71 ± 106.11
50 mg / kg	2221.22 ± 112.56
Standard povidone iodine (5%)	2360 ± 78.00

Table 4: Effect of wound healing activity on dead space wound

Parameters	Control	Standard	Group III 30mg/kg	Group IV 50mg/kg
Wet weight of granulation tissue (mg)	87±1.50	130.2±3.20	175.66±2.11	190.38±4.20
Dry weight of granulation tissue (mg)	12.67±1.02	20.33±1.88	21.08±0.33	23.87±1.08

IV. DISCUSSION

Wound healing is a complex and dynamic process by which the damaged cellular structures and tissue layers restored as closely as possible to its normal and original state. The healing process depends upon the repairative ability of the tissue, the type and extent of damage and general state of health of the tissue. The healing process consists of different phases such as rapid hemostasis, appropriate inflammation, mesenchymal cell differentiation, proliferation and migration of the wound site, suitable angiogenesis, prompt epitheliaization and proper synthesis, cross linking and alignment of collagen to provide strength to the healing tissue [12,13,14]. All these process are concurrent but independent to each other.

In the present study three different models were used to evaluate the effect of methanolic extract of *Tonna dolium*. In the excision wound model topical application of the methanolic extract ointment of *Tonna dolium* improved wound contraction and closure and the effects were distinctly visible starting from the 3rd day of the post wounding period. The wound healing process ending with the production of scar indicating that the tissue regeneration was completed in the rats which was administered by the 30mg/kg and 50mg/kg of the methanolic extract ointment of *Tonna dolium* on the 12th day of post wounding period. While in control animals the rate of wound contraction was slow and complete epithelialization of the excision wound was delayed. In the present investigation significant increase was observed in the rats which were treated with the methanolic extract ointment of *Tonna dolium*. Similar findings have been reported by [15] on *Cypreaea moneta*. On the other hand [16] evidenced that lipid extract from different species of mollusc *Mytilus galloprovincialis and Rapana venosa* were found to be more efficient in healing induced skin burns in Wistar albino rats.

The tensile strength or breaking strength of a healing wound is measured by the amount of force required to disrupt it. The breaking strength increases rapidly as collagen deposition increases and cross linkage are formed between the collagen fibres, than in the beginning where a wound will be having little breaking strength because the clot alone will be holding the edges together [17]. In the present study significant increase in the breaking strength was noticed in the animals treated with the methanolic extract of *Tonna dolium* on the 10th day of the post wounding period.

In dead space wound model increase in the tensile strength of treated wounds may be due to an increase in collagen formation per unit area and stabilization of the fibres [18]. Collagen which strength and supports extra cellular tissue is composed of aminoacid hydroxyproline which has been used as a biochemical marker for tissue collagen [19]. In dead space wound model granulation tissue of the wound is primarily made up of edema, fibroblasts, collagen and new bood vessels. The mesenchymal cells of the wound area adjust themselves into fibroblasts there begin migrating into the wound gap together with fibrin strands [20]. Similar observations have been have been reported by [21] and [22] in the phenolic extract of *Hyptis suaveolens* and *Elephantopus scaber*. In the present study the wet and dry weight of the granulation tissue were significantly increased in the test groups. It exhibited that some bioactive compound present in the methanolic extract of *Tonna dolium* is the primary cause for wound healing activity.

V. CONCLUSION

In conclusion, the result of the present study emphasized that the wound healing activity of the methanolic extract of *Tonna* dolium has the capacity to increase the wound healing activity. This in turn imply that some bioactive component is present in the marine gastropod *Tonna dolium*. However it needs further investigation and clinical trials to evaluate the bioactive component from the marine gastropod *Tonna dolium,* it will pave a better way to discover new drug lead.

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