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Wound Healing Activity of Methanolic Extract of Indigofera Trita Linn in Rats

Authors

Dr. V. Ramamurthy¹, M. Sathiyadevi²

^{1,2}P.G. & Research Department of Biochemistry Marudupanidyar College, Vallam Post, Thanjavur – 613 403 Tamilnadu, India.

Email: cibisathya@gmail.com

ABSTRACT

The study was carried out to evaluate the wound healing activity of whole plant methanolic extract of Indigofera Trita Linn (MIT) in rats. Group 1 (Positive Control) was provided mupirocin ointment, Group 2 (test dose) with 5% w/w Indigofera Trita ointment & group 3 (test dose II) with 10% w/w Indigoferatrita Ointment. The total exposure of the study was 16 days. The group were compared for the percentage of wound healing. It was observed the group treated with mupirocin ointments showed an increaseing the rate & percentage of wound contraction & period of epithelization compare to the treated group. The methanolic extract of Indigofera trita (5% & 10%) ointment increased rate of wound contraction. The study evaluated the wound healing activity of the methanolic extract of Indigofera Trita linn and identified it to possess significant wound healing activity.

Key words: *Indigofera Trita Linn, Wound healing, epithelization.*

INTRODUCTION

inescapable Wounds are event of life. Management of under healing of wounds is complicated and expensive program & research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different type of wounds. (1)

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (2) The most important of these bioactive constituents of plants are alkaloids, tanins flavonoids, and phenolic compounds (3) The use of herbal medicine for the treatment of diseases and infection is as old as mankind. The world Health organization supports the use traditional medicine provided they are proven to be effic acious & safe in developing countries, a huge number of people

lives in extreme poverty and some are suffering and dying for want of safe water and medicine. They have no alternative for primary heath care (4) many medicinal plants are claimed to be useful for wound (5) wound healing is the process of repair that follows injury to the skin and other tissues. Following injruy, an inflammatory response occurs and the cells below the dermis beings to increase protein (collagen) production, later, the epithelias tissue is regenerated ⁽⁶⁾.

Indigofera trita linn, a family of fabacea in an under shrup widely distriuted in India, Ceylon, South Africa and North Australia the plant was known as Kattuavuri and punal murugai in Tamil. The entire plant is traditionally used for various ailments including liver disorders and tumors. (7) It is found to be active againt transplantable tumors (8) the plant also possesses strong anti – oxidant and anti inflammatory and analgesic activity ⁽⁹⁾.

Hence the study was designed to investigate wound healing effect of methanolic extract of Indigofera trita (MIT) on various experimental model.

MATERIALS AND METHODS

I. Collection and Identification

Indigofera trita linn was collected from Ammapettai in Thanjavur District. The plant was authenticated by Dr. Jayaraman Director plant anatomy & research centre W. Thambaram, Chennai (PARC/2015/3042). The specimen were stored in our lab.

II. Preparation of plant extract

The entire plant were shade dried and pulverized. The Coarse powder of 500gm packed in a sox let apparatus ot continuous hot percolation, for 8 hours using 1.5 litres of methanol as a solvent. The extract was concentrated under vaccum & dried in a dessicator yield 23.25g (6.7% w/w)

III. Animals

The study was approved by Instutional Animal Ethical committee, Department of Pharmacology, periyar College of Pharmaceutical Sciences, Trichy – 1 The Registration number is CPCSEA / 265. Healthy male albino rats weighing (150 – 200g) were maintained on the standard rodent fed and water ad libitum, The excision & dead space wound models were used to evaluate wound healing activity of *Indigotera trita* extract. The Animals were randomly distributed into five

groups of 6 each in excision and four groups of 6 each in dead space wound models.

Excision wound model.

Animals were anaesthetized with diethyl ether by open mask method and shaved on both sider of the back with an electric clipper. The area of wound to be created was outlined on the back of the animals with methylene blue using a stainless steel stencil. The entire wound was left open. Animals were closely observed for any infection were separated, excluded from the study and replaced.

Animals were divided into five to group of 6 each. The normals controls (group -1) were applied with ointment base two times a day, experimental control (group 2) experimental controls (group 3) were applied 5% & 10% w/w ointment in saft paraffin base extract of I trita two times a day, (Group -1) positive controls received an application of mupirocin ointment two times a day. The treatment was done topically in all the cases. Wound area were measured on day 1,5 and 10 & 16 all the groups using a transparency sheet and a permanent marker. Recording of the wound areas were measured on graph paper. (10,11)

% Wound Enclosure

Wound area in day O – wound area onday (n)

Wound area on day O

were n = numbers of days 2th 4^{th} 6^{th} 8^{th} 10^{th} 12^{th} 14^{th} 16^{th}

Table – 1	Percentage	of wound	l contraction
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Group	Day 0 %	Day 2 (%)	Day 4 (%)	Day 6 %	Day 8 %	Day 10 %	Day 12 %	Day 14 %	Day 15 %
Positive	0	25.08	41.167	55.362	70.183 <u>+</u>	80.543 <u>+</u>	84.123 <u>+</u>	91.182 <u>+</u>	95.012 <u>+</u>
Group		<u>+</u>	<u>+</u>	<u>+</u>	1.528	1.093	1.014	0.625	0.233
		2.009	3.417	2.514					
Solvent	0	37.800 <u>+</u>	43.438 <u>+</u>	56/883 <u>+</u>	79.215 <u>+</u>	84.750 <u>+</u>	91.233 <u>+</u>	91.233 <u>+</u>	95.361 <u>+</u>
group		1.135	0.748	3.811	1.189	1.896	0.562	0.562	0.162
Test	0	24.312 <u>+</u>	39.343 <u>+</u>	53.417 <u>+</u>	80.760 <u>+</u>	84.150 <u>+</u>	89.983 <u>+</u>	94.117 <u>+</u>	98.812 <u>+</u>
dose I		0.581	1.135	1.417	0.850	1.005	1.060	0.946	0.583
Test	0	14.683 <u>+</u>	29.433 <u>+</u>	43.172 <u>+</u>	61.412 <u>+</u>	68.550 <u>+</u>	77.500 <u>+</u>	87.305 <u>+</u>	93.834 <u>+</u>
dose		1.360	3.380	1.417	2.707	2.497	2.278	1.220	0.785

Values are given as mean + SEM of six rats statistically analysis was done by one way analysis of variance ANOVA

Dead space wound model:

Dead space wounds were influcted by implanting sterile cotton pellets (10mg each), one on either side in the groin and axilla on the ventral surface of the each rat. The animals were divided into groups of 6 each. The normal controls (Group - I) were provided plain water & group II extract

given the orall in a dose 200 mg / kg for 10 days; on the 10^{th} post – wounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anesthesia.. After noting the wet weight of the granulation tissue, the tissue was dried at 60C for 12 hr and weights was recorded $^{(12)}$

Table – 2 Wound healing activity of the *Indigafera Trita in* rats (dead space wound model)

Parameter	Group - 1 Normal	Group - 2 MIT Treated
Wet granulation weight mg/100g rat	99.56+8.168	145.8 + 17.54**
Dry Granulation Weight (mg/100g rat)	38.16 + 3.151	34.82 + 6.13

The values are showed as mean + SE from 6 animals in each group.

RESULT AND DISCUSSION

The excision wound models was carried out to study the topically applied *Indigofera trita* extract on wound healing and contraction. Increase in the wound healing activity was observed in extract treated rats. On 10th day animal group 3 showed greater percentage of wound contraction when compared with the animals of group. The same pattern observed on 16th day also. The wound contraction results of extract treated animals were comparable with positive controls shown in Table – 1

The dead space wound model was used to study difference in matrix synthesis between drug treated and control groups. Oral administration of the plant extract appears to increase the mass of granuloma in both groups. However the dry granuloma mass is increased by extract treatments. The normal treated group 2 had greater wet / day ration (Table 2)

From these animal studies it can be concluded that significant increase in the wound healing activity was observed in MIT extract treated rats. In excision wound model, animals of groups 2 showed a decrease in the epithelialization period and increased percentage of wound contraction when compared to group – I (Table – I) on day 16, the extract treated animals (group2) & groups 3) showed wound contraction by 93% compared

with 40% of wounds of the control groups. The wound contraction results of extract treated animals were comparable with positive controls. In the dead space wound model (Table -2) the extract treated animals in group 2 showed significant increase in the dry weight of the granulation tissue in the animals treated with the extract was observed. Overall the weights of the animals did not differ for any of the study groups.

The methanolic exract of Indigofera Trial inn showed significant wound healing activity when topically administered on rats. These results offer pharma cological evidences on the traditional use of Indigofera trita linn for healing wounds. Further studies are heeded to better assess the potential value of Indigofera Trita extracts as wound healing agents.

^{**} Shows significant as compared to normal control (P < 0.01)

Photograph showing wound healing effect of Indigofera trita linn



0 Day Control Wound

Photograph showing wound healing effect of Indigofera trita linn



10th Day

Photograph showing wound healing effect of Indigofera trita linn



16th Day Extract Treated

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