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PHYTOCHEMICAL AND WOUND HEALING ACTIVITY OF *ALLIUM SATIVAM LINN.*, IN WISTAR ALBINO RATS

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ABSTRACT

Wound healing is an intricate process whereby the skin repairs itself after injury. The present study was evaluated that the wound healing activity of *Allium sativam Linn* on albino rats of Wistar strain using two different models viz, excision and dead space wound. There was a significant increase in wound closure rate, tensile strength, dry granuloma weight, wet granuloma weight and decrease in epithelization period in *Allium sativam Linn* treated group when compared to control and commercial drug treated groups. From the results, it may be concluded that, the ethanolic extract of *Allium sativam Linn* had greater wound healing activity than the soframycin ointment.

KEY WORDS: *Allium sativam Linn*, wound healing, tensile strength and granuloma.

INTRODUCTION

Wound healing, as a normal biological process in the human body, is achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling. For a wound to heal successfully, all four phases must occur in the proper sequence and time frame. Many factors can interfere with one or more phases of this process, thus causing improper or impaired wound healing [1]. The wound-healing process consists of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution [2]. Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns. *Allium sativum*, commonly known as garlic, is a species in the onion genus, *Allium*. Garlic cloves are used as a remedy for infections (especially chest problems),

digestive disorders, and fungal infections such as thrush [3,4] . Garlic can be used as a disinfectant because of its bacteriostatic and bactericidal properties [5]. The plant under study, namely *Allium sativam Linn* contains alkaloids, flavonoids, lignins, triterpenoids, fixed oils, fats, proteins and amino acids. A survey of literature revealed that not much work has been made to study wound healing activity of this plant; hence it was thought worthwhile to investigate the wound healing activity of *Allium sativam Linn* extract in efficient experimental models of wound in rats.

METHODS AND MATERIALS

Preparation of leaf extract

The Fresh leaves of *Allium sativam Linn* were collected and identified and confirmed with the voucher specimen kept in the Rapinat Herbarium, St.Joseph's College, Tiruchirappalli, Tamil Nadu, India.

The fresh leaves were shade dried at room temperature, pulverized by a mechanical grinder, sieved through 40- size sieve mesh. 1000g of fine leaf powder were suspended in 2000ml of ethanol for 24 hours at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No:1). The filtrate was placed in a water bath to dry at 40ºC and the final ethanol free clear residue was used for the study.

Qualitative phytochemical evaluation

The plant extract was subjected to qualitative tests by adopting standard procedure for the identification of the phytoconstituents present in it viz., alkaloids, carbohydrates, phytosterols, fixed oils, phenolic compounds, proteins, free amino acids, gums, mucilage, flavonoids, terpenoids, lignins and saponins [6].

Animal used for wound healing activity

Wistar albino rats (150-180g) were used and six rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment. They were provided with food and water *ad libitum.*

The work was carried out in CPCSEA registered (Reg. No: 265/ CPCSEA) Animal House of Periyar College of Pharmaceutical Sciences, Tiruchirapalli, during the year 2012-2013. It was approved by the IAEC of the above Institution.

Ointment Formulation

Two types of ointment formulations were prepared from the extract: 5% w/w, 10% (w/w), where 5g or 10g of the extract were incorporated in 100g of simple ointment base British Pharmacopoeia (B.P) respectively. Soframycin ointment (0.2% w/w,) was used as a standard drug for comparing the wound healing potential of the extract.

Excision Model [7]

Four groups with six animals in each group were anaesthetized with diethyl ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500mm^2 full thickness of skin from the depilated area, the wound was left undressed to open environment. Then the drugs, i.e., the reference standard, 0.2% w/w Soframycin ointment, simple ointment B.P., *Allium sativam Linn* extract ointment 5% w/w and 10% w/w were applied once daily till the wound was completely healed [8]. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as percentage reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day.

Dead Space Model [9]

Three groups of wistar albino rats (150-200g) were used. Dead space wounds were made by implanting, subcutaneously, a 2.5 x 2.5 cm polypropylene tube beneath the dorsal paravertebral lumber skin. Control animals received 2 ml of 1% carboxy methyl cellulose (CMC), orally, while the test groups received *Allium sativam Linn*(100 mg/kg & 200mg/kg) orally once daily for 10 days. On the 11th post-operative day, the dead space wound was excised. Wet weight was recorded and tensile strength determined [10].The granuloma was dried in an oven at 60ºC and the dry weight noted.

Measurement of Healing

Tensile strength, the force required to open a healing skin wound, was used to measure healing. The instrument used for this measurement is called tensiometer. It was designed on the same principle as the thread tester used in the textile industry. It consisted of a 6x12 inch board with one post of 4 inch long, fixed on each side of the longer ends. The board was placed at the end of a table. A pulley with a bearing was mounted on the top of one of the posts. An alligator clamp with 1 cm width, was tied on the tip of the post without pulley by a piece of fishing line (20-lb test monofilament) so that the clamp could reach the middle of the board. Another alligator clamp was tied on a piece of fishing line with a 1-L polyethylene

bottle tied on the other end. The excised granuloma tissue was then placed on a stack of paper towels that could be adjusted so that the polyethylene bottle was freely hanging in the air. Water added to the polyethylene bottle was weighed and considered as the tensile strength of the wound.

Statistical Analysis

Data are expressed as Means ± SEM and subjected to Analysis of Variance (ANOVA) test by comparing with different groups.

RESULT AND DISCUSSION

The ethanolic extracts of *Allium sativam Linn.,* have revealed the presence of alkaloids, Phytosterol, flavonoids, Phenolic Compounds & Tannins, saponins, Proteins & Amino Acids, lignins are shown in Table 1.Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

The measurements of the progress of the wound healing induced by the soframycin ointment (0.2% w/w), extract ointment 5% w/w and 10% w/w and the control group (i.e.) simple ointments in the excision wound model are shown in Table 2. It is observed that the wound contracting ability of the extract ointment was significantly greater than that of the control as well as reference standard, soframycin ointment. The extract ointment produced complete healing at 18^{th} day and 16^{th} day respectively when 5% w/w and 10% w/w extract ointments were used. The extracted treated wounds were found to epithelialize faster. The rate of wound contraction significantly increased in extract treated wounds compared with those in the control wounds ($P<0.001$). The extract *Allium sativam Linn* at (100 mg/kg & 200mg/kg) produced a significant increase in the wet granuloma tissue as well as in the dry weight. The tensile strength was also found to be increased ($P<0.001$) in the extract treated groups (Table 3).

Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. It has three phases: inflammatory, proliferative and maturational. It is dependent upon the type and extent of damage, the general state of the host's health and the ability of the tissue to respond. The inflammatory, phase is characterized by hemostasis and inflammation, followed by epithelization, angiogenesis and collagen deposition in the proliferative phase. In the

maturational phase, the final phase of wound healing undergoes contraction resulting in a smaller amount of apparent scar tissue. Granulation tissue formed in the final part of proliferative phase is primarily composed of fibroblast, collagen, edema and new small blood vessels.

Earlier studies showed the presence of triterpenoids which were responsible for the effective wound healing activity of *Cecropia peltata* [11] and *Pentas lanceolata* [12]. Earlier study demonstrated that an ethanol extract of *Leonotis nepetaefolia* R.Br., possess wound healing activity comparable to that of standard drug and control. Wound contraction and increased Hydroxyproline observed in the present work provide scientific evidence support the usage of the plant extract n the topical treatment and management of wounds [13]. Nithya and Anusha (2011) reported that ethanolic extract of *lawsonia ulba linn.,* leaves has properties that render it cabable of promoting accelerated wound healing activity compared with placebo control [14,15]. The previous study revealed that the wound healing activity of *Datura metel Linn.,* had greater wound healing activity than the Nitrofurazone ointment. There was a significant increase in wound closure rate, tensile strength, dry granuloma weight, wet granuloma weight and decrease in epithelization period in Datura metel Linn., treated group when compared to control and commercial drug treated groups [16].

A glucosidal mixture extract of *Allium sativam Linn* has been reported to be responsible for enhanced repair only in incised wounds and in stimulating collagen in human skin fibroblast cells. The wound healing property of the leaves extract of *Allium sativam Linn* appears to be due to the presence of its active principles, which accelerates the healing process and confers breaking strength to the healed wound. The present study has demonstrated that an ethanolic extract of *Allium sativam Linn* leaves has properties that render it capable of promoting accelerated wound healing activity compared with placebo control. Wound contraction, increased tensile strength activity support further evaluation of *Allium sativam Linn* in the topical treatment and management of wounds.

Table.1. Preliminary phytochemical Screening of the ethanolic extract of *Allium sativam Linn.*

Test / Reagents used	*Allium sativam Linn* Extract
Alkaloids	+
Reducing Sugar	-
Phytosterol	+
Fixed oil & fats	-
Phenolic Compounds & Tannins	+
Proteins & Amino Acids	+
Gums & Mucilage	-
Flavonoids	+
Lignins	+
Saponins	-
Triterpenoids	+

(+) Positive, (-) Negative

Table .2. Effect of *Allium sativam Linn* leaf extract and Soframycine on excision model

Post Wounding day	Wound Area (mm^2)			
	Control	Soframycin ointment (0.2% w/w)	Extract ointment (5% w/w each)	Extract ointment (10% w/w each)
0	511 ±12.1 (0.0)	509 ±18.7 (0.0)	502 ± 13.0 (0)	506 ± 29.8 (0)
2	478 ±16.7 (13.2)	425 ±11.6(17.8)	395 ± 18.1 (27)	435 ±14.8 (28)
4	423 ±19.4 (28.5)	329 ±28.3* (34)	336 ± 21.3* (35)	351 ± 17.9* (31)
6	371 ±18.6 (32.3)	248 ±21.4**(49.4)	284 ± 11.2* (46)	291 ±18.3** (43)
8	332 ±11.5(41.8)	174 ±16.6**(65.4)	168 ±16.5** (66)	141 ±8.8** (71)
10	294 ±11.6(44.9)	159 ±10.7**(74.0)	113 ±6.5** (73)	99 ±7.8** (78)
12	276 ±11.9(48.3)	103 ±9.8**(79.5)	95 ±8.4**(84)	85 ±8.1**(89)
14	247 ±11.3(51.2)	78 ±2.4**(83.2)	41 ±0.8**(87)	37 ±1.3** (92)
16	231 ±16.2(55.2)	49 ±10.8**(89.2)	21 ± 1.4* (92)	4 ± 0.5** (99)
18	209 ±15.3(60.1)	27.0**(94)	0.0** (100)	-

Values are means ±S.E of 6 animals in each group. A figure in parentheses indicates percentage of wound contraction. *Significant differences at P<0.01 when compared to control. **Significant differences at P<0.001 when compared to control.

Table . 3. Effect of *Allium sativam linn* on Dead Space Wound in Rats

Treatment	Wet Granuloma Weight (mg)	Dry Granuloma Weight (mg)	Tensile Strength (g)
Control	217.7 ± 8.2	43.2 ± 1.2	367 ± 11.5
***Allium sativam Linn* (5% w/w each)**	392.3 ± 10.5*	84.5 ±3.2*	481.4 ± 29.2*
***Allium sativam Linn* (10% w/w each)**	487.6 ± 27.2*	111.6 ± 7.6*	588.9 ± 27.5*

Significant differences at P<0.001 when compared to control. (Means ± S.E., n = 6)

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