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Research Article

WOUND HEALING ACTIVITY OF *LEONOTIS NEPETAEFOLIA R.br.,* IN WISTAR ALBINO RATS

NITHYA V*, DR.BRINDA P[1] and ANAND K.V[2]

***Lecturer, Department of Biotechnology, Srimad Andavan Arts and Science College. [1]Dean, Life sciences, Srimad Andavan Arts and Science College, [2]Department of Biochemistry, Srimad Andavan Arts and Science College, Tiruchirappalli**
Email: nithya.gopinath@rediffmail.com

ABSTRACT

A Preclinical trial was conducted to study the effect of topical administration of ethanolic extract of the *Leonotis nepetaefolia R.Br.,* on the healing of burn wounds. The burn wound was created by using a rod of 2.5 cm diameter, which was heated to 80-85°C for 20 seconds. The control rats were undressed and the standard drug treated rats were dressed with soframycin, while experimental rats were dressed with plant extract ointment. The plant extract treated wound healed much faster as indicated by improved rate of contraction and a decreased period of epithelialization. Biochemical studies revealed a significantly increase in Hydroxy proline, Hexosamine, Super Oxide Dismutase and also reduced the Lipid Per Oxide in the granulation tissues of plant extract treated wounds when compared with control and standard drug. From the result, it has been concluded that, the ethanolic extract of *Leonotis nepetaefolia R.Br.,* has greater wound healing activity.

Keywords: *Leonotis nepetaefolia R.Br.,* Wound healing, Ethanolic extract, Hydroxy proline, Hexosamine.

INTRODUCTION

Wound healing is a process, which is fundamentally a connective tissue response; initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and other intracellular macromolecules that are later remodeled to form a scar[1].

Wound healing studies mainly aim to detect various means and factors influencing healing process, so that they could be either used or avoided in clinical practice to alter the healing process favorably. The process of wound healing occurs in four phases: (i) coagulation, which prevents blood loss, (ii) inflammation and debridement of wound, (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen deposition. Any agent that accelerates the above process is a promoter of wound healing. Many herbal plant extracts have been used for wound healing since several decades.

The plant under study, namely *Leonotis nepetaefolia R.Br.,* 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 potential of *Leonotis nepetaefolia R.Br.,* extract in experimental animal models by creating Excision wounds.

METHODS AND MATERIALS

Preparation of leaf extract

Fresh leaves of *Leonotis nepetaefolia R.Br.,* were collected in Tiruchirapalli during the month of January 2008. The collected plants were identified with the help of Dr.P.Brindha M.Sc., Ph.D., Dean life sciences Srimad Andavan Arts and Science College, Tiruchirapalli, Tamil Nadu, India and confirmed with the voucher specimen kept in the Rapinat Herbarium, St.Joseph's College, TamilNadu, India.

The fresh leaves were shade dried at room temperature, pulverized by a mechanical grinder, sieved through 40- size sieve mesh. 500g of fine leaf powder were suspended in 1500ml 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, glycosides, phytosterols, fixed oils, phenolic compounds, proteins, free amino acids, gums, mucilage, flavonoids, terpenoids, lignins and saponins[2]. The results are presented in Table 1.

Animal used for wound healing activity

Wistar albino rats (150-180g) were used as experimental models and five 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 approved (Reg. No: 790/03/ac/CPCSEA) Animal House of, Srimad Andavan Arts and Science College, Tiruchirapalli, Tamil Nadu, India.

Wound Creation

Wistar albino rats weighing 150-180g were used for investigation. Four groups of 5 rats each were taken for studies. The dorsal side of the rat was shaved with razor blade. Using a rod of 2.5cm diameter, which was heated to 80-85°C burn wounds were created by exposing dorsal side of rats for 20 seconds.

After 24hrs dead tissues were excised using surgical blade, and the wound site was sterilized by spirit Group-I rats were not treated with any drugs. The Group-II rats were treated with soframycin (2% w/w Aventis Pharma) and Groups III &IV were treated with 1g and 2g of ethanol extract of *Leonotis nepetaefolia R.Br.,* respectively**.**

All the rats were given regular feed and water *ad libitum. Rats* kept under observation for up to 24 days giving regular dressing with plant extracts. After the removal of the dead tissues the initial wound size was measured.

The animals surviving after treatment was sacrificed by cervical decapitation and blood was collected and serum was separated. This wound tissue was deserted out and washed in ice-cold saline and is used for various experiments.

Rate of Contraction

The rate of contraction wound site was measured regularly at every 5 days interval by tracing wound site with trace paper and measured graphically. The standard deviation and mean deviation were given in sq.cm^2

Collection of Granulation tissue

Granulation tissues from control, standard and test groups were collected and washed well in cold saline (0.9% NaCl) to remove the blood tissues and stored for various parameters. A part of granulation tissues was lyophilized for collagen and hexosamine analysis and a part was used freshly for (MMP) Matrix Metallo Protein expression studies.

Estimation of Hydroxy proline[3]

5mgs of lyophilized sample was hydrolysed with 5ml of 6N HCl at 110^{0}C for 18-20 hours in a sealed tube. After hydrolysis sample was evaporated to dryness in water bath. The residue was dissolved in known amount of water. Samples of varying concentrations were taken for analysis.

To this 1ml of chloramines T was added and incubated for 20 minutes and 1ml of 70% perchloric acid was added and incubated for 5 Minutes. Finally 1ml of PDAB (Para Dimethyl Amino Benzaldehyde) solution was added and the mixture was shaken well. The colour developed was read spectrophotometrically at 557nm.

Estimation of hexosamine[4]

5 mgs of lyophilized tissue sample was hydrolysed with 5ml of 2N HCl at 110^{0}C for 6 to 7 hours in sealed tubes and evaporated to dryness and the residue was dissolved in known amount of water. Samples of varying concentrations were taken for analysis. The solutions were treated with 1ml of freshly prepared 2% acetyl acetone in 0.5M sodium carbonate and boiled for 15 minutes. After cooling in tap water, 5ml of 95% ethanol and 1ml Ehrich's reagent were added and mixed thoroughly. The purple red color developed was read after 30 minutes at 530nm.

Estimation of Lipid PerOxide[5]

0.1 ml of sample, 0.9ml of 10% TCA and 2.0ml of 0.67% thiobarbituric acid regent were added and kept in boiling water bath for 20 minutes in the water bath. The tubes were cooled after centrifugation and the absorbance of the supernatant was read at 532nm.

Estimation of Ascorbic acid[6]

0.5ml of plasma, 0.5ml of ice cold 10% TCA was added and mixed thoroughly and centrifuged for 20 minutes. At 3500g, supernatant (0.5ml) was mixed with 0.1ml of DTC (dithiocarbamate) reagent mixed well and incubated at 37^{0}C for 3 hours. Then 0.75ml of ice cold 65% H_2SO_4 was allowed to stand at room temperature for 30 minutes. The yellow colour developed was read at 520nm. Ascorbic acid was used as standard.

Assay of Super Oxide Dismutase[7]

Superoxide dismutase was assayed following the method of Misra and Fridovich, (1972).

Required amount (100μl) of enzyme was added to tubes containing 0.5ml of carbonate buffer and 0.5ml of EDTA (Ethylene Diamine Tetra Acetic acid) solution. The final volume was made up to 2.5ml.

The reaction was initiated by the addition of 0.5ml epinephrine and the increase in absorbance at 480nm was measured in systronics 119 UV spectrometer. 100% auto oxidation of epinephrine to adrenochrome was performed in a control tube without the enzyme.

The enzyme unit of activity was defined as the enzyme required giving 50% inhibition of epinephrine auto oxidation.

RESULTS AND DISCUSSION

Wound healing is a process by which the damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of the area of the wound. The preliminary phytochemical analysis of the leafs extract showed the presence of tannins, triterpenoids, alkaloids, fixed oils, flavonoids amino acids and proteins (Table 1).

Plant products have been shown to possess good therapeutic potential as anti inflammatory agents and as a wound healing promoter, due to the presence of terpenes, and flavonoids. (Earlier[8] studies showed the presence of triterpenoids which were responsible for the effective wound healing activity of *Cecropia peltata*[9] and *Pentas lanceolata*[10].

The measurements of the progress of wound healing induced by the soframycin ointment (1g), plant extract ointment (1g) and (2 g) and the control rats are shown in Table 2 and corresponding to this value a graph is also plotted which is given in the Figure 1. It is observed that the wound contracting ability of the extract ointments were significantly greater than that of the control, and was comparable to that of the reference standard soframycin ointment. The extract ointment produced complete healing on 20th day.

In the burn wound, the *Leonotis nepetaefolia R.Br.,* extract significantly increased the Hydroxy proline, Hexosamine, Super Oxide Dismutase (SOD), Ascorbic acid, when compared to the control ,standard drug, whereas LPO also significantly decreased when compared to the control, standard reference as shown in Figure 2,3,4,5,&6 as respectively.

The granulation tissue of the wound is primarily composed of fibroblasts, collagen, edema and new small blood vessels. The collagen composed of the amino acid; L-Hydrophobic is the major component of extra cellular tissue, which gives strength and support. Break down of collagen liberates free hydroxy proline and its peptides. 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 epithelialization, 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.

Flavonoids, terpenoids are found to promote the wound healing process mainly due to their antimicrobial property. These phytoconstituents present in the plant drug under study may be responsible for wound contraction and increased rate of epithelialization observed in the present work.

Hexose amine content increases in the early stages of wound healing and indicated that the fibroblasts actively synthesized, ground substances (mucopolysaccharides) on which the collagen can be laid on [11].

The cytokine cascade activated after a burn injury with stimulation of phagocytic cells that result in the formation of oxygen free radicals and lipid per oxidation. The control group showed an elevation in the lipid per oxidation levels which indicates the decreased scavenging capacity of the wounded tissues. Lipid per oxidation is oxidative deterioration of PUFA (Poly Unsaturated Fatty Acids). Its leads to cell injury leading to generation of peroxides and lipid per oxidation.

Ascorbic acid is reported to have scavenging activities and inhibition of lipid per oxidation. In the present study it was found that the ascorbic acid level was higher in the test group when compared to the control group and hence a decline in the lipid per oxidation.

The Super Oxide Dismutase (SOD) levels were found to be increased in the treated animals. This indicates that the tissue damage was being repaired by the scavenging activity of SOD. The increased activity appears to be a reflux mechanism to guard against the extra cellular oxygen derived free radicals.

The present study thus 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.

Table 1: Preliminary phytochemical screening of the ethanolic extract of *leonotis nepetaefolia R.Br.,*

Test / Reagents used	Leonotis nepetaefolia R.Br.,*Extract*
Alkaloids	+
Reducing Sugar	-
Phytosterol	+
Terpenoids	+
Phenolic Compounds & Tannins	-
Proteins & Amino Acids	+
Coumarin	+
Flavonoids	+
Starch	+
Saponins	-
Quinone	+

(+) Positive, (-) Negative. It shows that preliminary phytochemical screening of the ethanolic extract of *leonotis nepetaefolia r.br.,*

Table 2: The rate of wound contraction on different days.

Days	Control	Standard (Soframycin)	Plant Extract(1g)	Plant Extract(2g)
0	4.98	4.98	4.98	4.98
5	4.20	4	3.91	3.9
10	3.91	3.62	2.85	2.75
15	2.80	2.46	1.52	1.32
20	1.72	1.25	0.2	0.1

It shows that rate of wound contraction on different days of healing (cm^2) days.

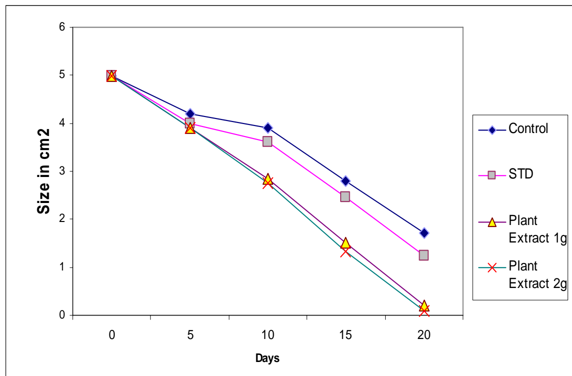

Fig. 1: The rate of wound contraction. It shows that the wounds increased with treatment and showed an appreciable decrease in wound size.

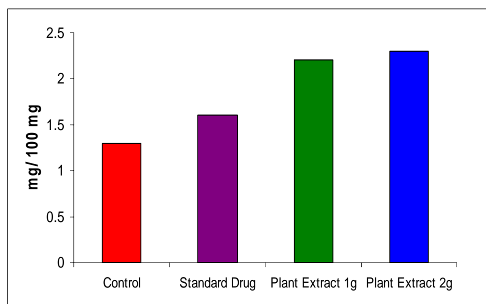

Fig. 2: Estimation of Collagen. It is evident from the graph that the amount of collagen has been increased in the test rats compared to control. It has been stated that collagen provides tensile strength to tissues especially of healing wounds.

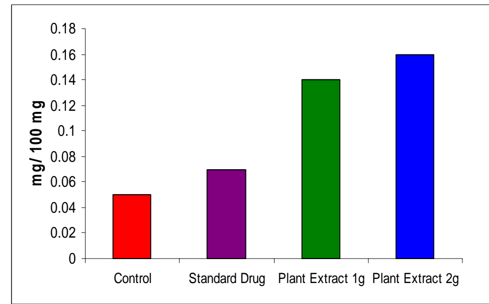

Fig. 3: Estimation of Hexosamine.The figure shows that the increase in hexosamine content is obvious in the test rats when compared to control. This indicates that the fibroblasts are actively synthesized. The ground substance (mucopolysacchoides) on which the collagen can be laid on.

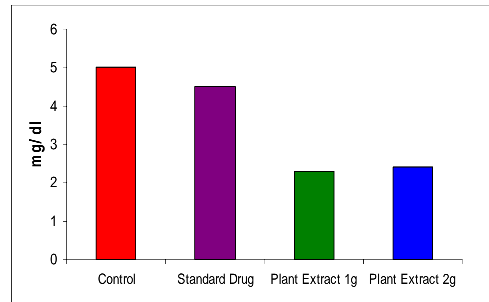

Fig. 4: Estimation of Lipid Per Oxide. It shows that the lipid peroxide levels were also found to be lowered in the treated rats. This also suggests that there is a disease in cellular destruction due to free radicals and can prevent per oxidation.

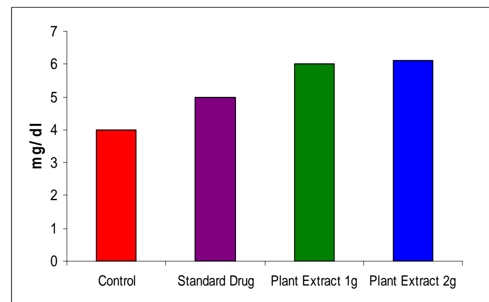

Fig. 5: Estimation of Ascorbic Acid. The ascorbic acid content was found to be increased in the rat treated with *leonotis nepetaefolia R.Br.,* the increase in ascorbic acid contributes to the decrease in lipid per oxidation. Ascorbic acid is associated with the scavenging activities that inhibits lipid per oxidation.

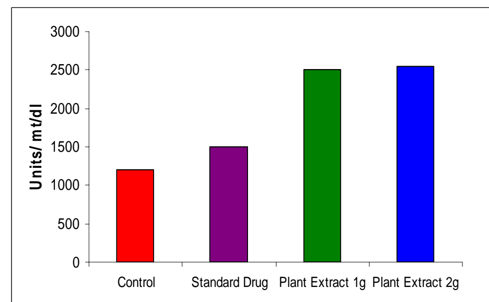

Fig. 6: Estimation of Super Oxide Dismutase. It shows that the Super Oxide Dismutase (SOD) levels were found to be increased in the treated animals. This indicates that the tissue damage

was being repaired by the scavenging activity of sod. The increased activity appears to be a reflux mechanism to guard against the extra cellular oxygen derived free radicals.

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