

EVALUATION OF WOUND HEALING ACTIVITY OF *INDIGOFERA ASPHALATHOIDES* VAHL. EX DC. – A TRADITIONAL SIDDHA DRUG

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ABSTRACT

The present work aims at evaluating the wound-healing potential of a traditional Siddha plant “Sivanarvembu” botanically equated as *Indigofera aspalathoides* vahl. Ex DC. one of the controversial drug in Siddha system of medicine. This may contribute in developing a cost effective, safe and efficacious herbal wound healer. Traditionally this plant is used for ailments such as cancer, oedema, abscess and skin disorders. The chloroform extract of *Indigofera aspalathoides* vahl. Ex DC. was subjected to wound healing activity at two different dose levels employing excision wound model. The wound treated with plant drug showed higher rate of wound contraction, increased level of Hydroxy proline, Hexosamine, Superoxide Dismutase, Ascorbic acid and decreased levels of Lipid peroxides as well as histopathological studies also showed progressive collagenation and few macrophages compared to the control rats. Present work depicted that *Indigofera aspalathoides* Vahl. ex DC chloroform extract possess potent wound healing activity which may be due to the anti oxidant and antimicrobial properties of Flavonoids and triterpenoids present in the source taxon. Such Scientific evaluation studies are essential for the acceptance of Herbal based drugs as an integral part of modern drug therapy.

Keywords: Sivanarvembu, *Indigofera aspalathoides* Vahl. ex DC., Wound healing potential, Scientific evaluation, Herbal wound healer.

INTRODUCTION

Since time immemorial, Herbal medicines are contributing significantly towards the healthcare of human society.¹ Herbal medicines resulted out of therapeutic experiences of generations after generations through practices of physicians of indigenous systems of medicine over hundreds of years². In recent years, among the world population, there is an increasing trend towards the usage of herbal medicines which may be probably due to the side effects and enormous cost involved in modern medicines. At present people are turning towards the herbal wound healers so as to prevent allergy and other complications that are often encountered due to the application of synthetic wound healers. In the present work evaluation of the wound healing potential of a traditional drug source “Sivanarvembu” is carried out. This study will not only help in providing scientific evidences for the wound healing action of the herbal drug but also in assessing their safety and efficacy.

“Sivanarvembu” is botanically equated as *Indigofera aspalathoides* Vahl. ex DC. (IA) belonging to the family Fabaceae³. (Fig 1) In Sanskrit it is called as ‘*Sivanimba*’ and in tamil it is called as ‘*Sivanarvembu*’, ‘*Shiva malli*’.



Fig. 1: *Indigofera aspalathoides* Vahl. ex DC.

MATERIALS AND METHODS

Plant collection

Study materials (IA) were collected from Trichy region and authenticated (acknowledged) with the help of Flora of Presidency of Madras and authenticated with the specimens kept at RAPINAT Herbarium, St. Joseph’s College, Trichy, South India.

Preparation of plant extract

200g of coarsely powdered *Indigofera aspalathoides* Vahl. ex DC. was taken in an aspirator bottle and soaked in Chloroform for 48 hrs at room temperature. After 48 hrs the filtered solvent was distilled off and residue obtained was evaluated for wound healing activity.

Preliminary Phytochemical Studies

Preliminary phytochemical studies of drug powder as well as extracts were carried out as per the standard methods⁵

Experiment design

The animal experiments were conducted as per standard protocols after getting necessary approvals (790/03/ac/CPCSEA at Srimad Andavan College, Trichy).

Group I: Wounded- Controls

Group II: Wounded & treated with 1 g of chloroform extract of *Indigofera aspalathoides* Vahl. ex DC.

Group III: Wounded and treated with 2 g of chloroform extract of *Indigofera aspalathoides* Vahl. ex DC.

Group IV: Wounded and treated with 2 g of standard drug – Soframycin

Wound creation

Wounds created and experiments carried out as per standard procedures⁶ and also for marking areas where wound would be created. A full thickness excision wound was created in the dorsal region of the skin area measuring to 200mm² and 0.2 cm depth using a sharp surgical blade and pointed scissors.

Dressing of control rats were done using paraffin, while experimental rats were dressed with selected Herbal extracts and one group was also administered with standard drug. At regular intervals dressing were changed for 15 or 16 days and kept for observation.

Rate of contraction of wounds was monitored by measuring the wound area every 5 days until the wound healed. The contraction was measured by tracing the raw wounded area and recording diameter. The standard and mean deviations were given in sq.mm⁷.

Animals were sacrificed by cervical decapitation post-experimentation. Granulation tissue and blood samples collected⁸ were then assessed for hydroxyl proline⁹ and hexosamine content¹⁰ and the antioxidant status of experimental animals was evaluated by determining the content of Lipid peroxides¹¹, Super Dismutase¹² and Ascorbic acid¹³. The tissues were also subjected to histopathological studies¹⁴.

RESULTS AND DISCUSSION

Initial phytochemical studies revealed the presence of Flavonoids, Terpenes, Alkaloids, Coumarins, Lignins and Quinones in the drug powder and Flavonoids, Terpenes, Coumarins, Lignins and Quinones tests were positive in the chloroform extract.

Table 1: Shows the effect of chloroform extract of IA on the rate of wound contraction

Groups	Rate of Contraction in mm ²			
	0 th Day	5 th Day	10 th Day	15 th Day
Group I	2.1 ± 0.06	2.0 ± 0.12	1.5 ± 0.22	1.1 ± 0.23
Group II	2.4 ± 0.05a	1.8 ± 0.27a*	1.2 ± 0.18a*	0.8 ± 0.20a*
Group III	2.3 ± 0.05b	1.7 ± 0.31b*	1.0 ± 0.26b*	0.5 ± 0.24b*
Group IV	2.0 ± 0.03b	1.2 ± 0.09b*	0.4 ± 0.27b*	0 ± 0.03b*

Values in mean ± S.E (n = 6) Statistical Comparison

*P < 0.05 significant when compared with control a: Group I & II, b: Group I & III c: Group I & IV

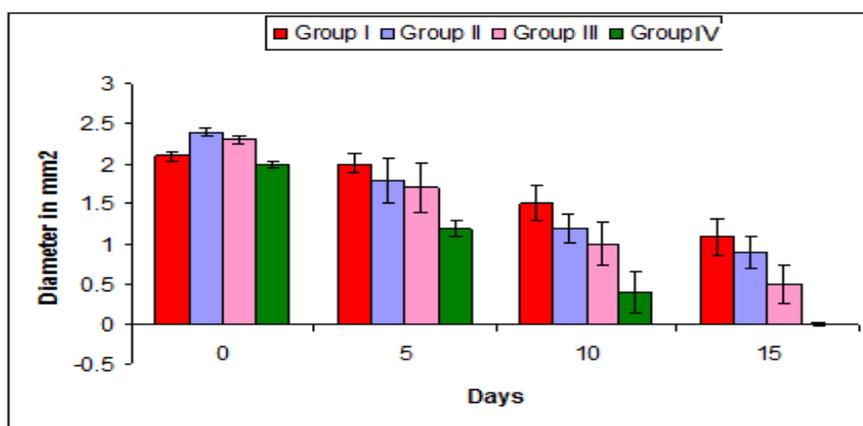


Fig. 2: Effect of chloroform extract of IA on the rate of wound contraction

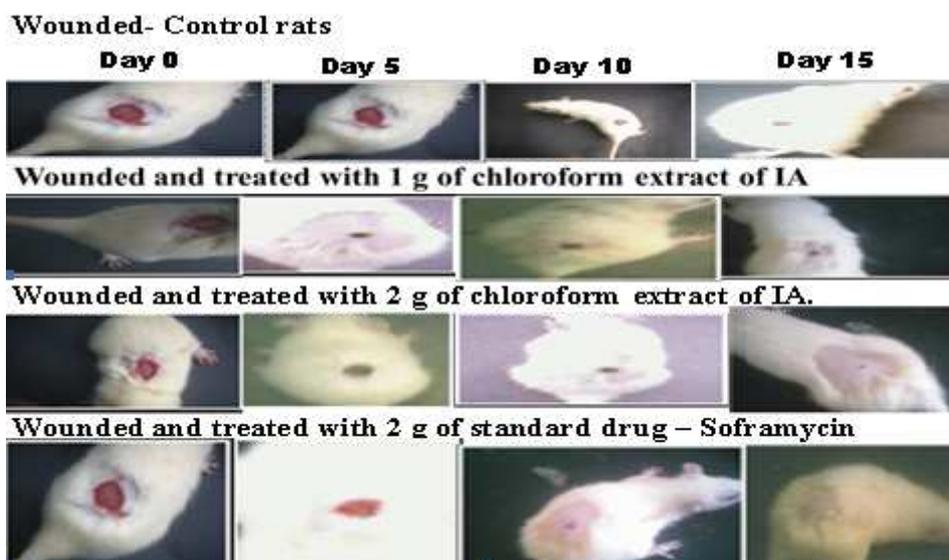


Fig. 3: Effect of chloroform extract of IA on the rate of wound contraction

The effect of chloroform extract of IA on wound contraction was measured and the values given in Fig 2&3. Wound healing potential is determined by the closure of wound area¹⁵. The rate of wound contraction increased in Chloroform extract of IA treated animals 1g/kg b.w. & 2g/kg b.w. from 0th day to 15th day. Group III (2g/kg b.w.) and Group II (1g/kg b.w.) showed significant decrease in mean scar area as compared to control. Group III animals showed

profound effect than Group II animals. The significant increase in wound contraction indicates rapid granulation, collagenation and collagen maturation properties of the test drug^{16,17}. Increased rate of wound contraction indicated the rapid granulation, collagenation and collagen maturation potentials of the extracts.

Biochemical Markers

The effect of Chloroform extract of IA on the hydroxy proline content was presented in Fig 4. The group III animals which received the dose of 2g/kg bw showed the increased hydroxy proline content (22.3 ± 3.93) as compared to Group II animals which received 1g/kg bw which also showed increased hydroxy proline content (18.89 ± 4.32). Both the doses of drug showed significant increase of hydroxy

proline content compared to the wounded control rats. Significant increase of hydroxyproline content is an correlated with increased collagen levels indicating improved cross linking of collagen fibres¹⁸. Collagen turnover was evidenced by elevated level of hydroxyl proline in plant treated experimental animals.

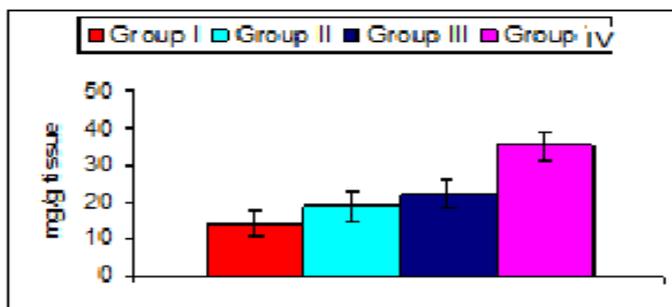


Fig. 4: Effect of test drug (IA) on Hydroxy proline content.

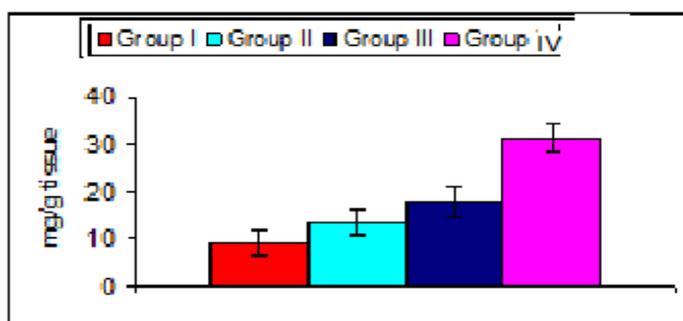


Fig. 5: Effect of Chloroform extract of IA on Hexosamine

Fig 5. shows the effect of test drug (IA) on Hexosamine content in the treated and untreated rats. Group I wounded control rats showed profound decrease in the Hexosamine level (9.0 ± 2.56) compared to Group II (13.3 ± 2.79) and Group III (17.56 ± 3.19). Hexosamine level indicate that the fibroblasts are actively synthesized. This is the

ground substance (mucopolysaccharides) on which the collagen can be laid on^{19, 8}. This pattern of hexosamine increase is another biochemical marker for determining wound healing potential. Fibroblasts formation was evidenced by elevated level of Hexosamine content among the IA extract treated animals.

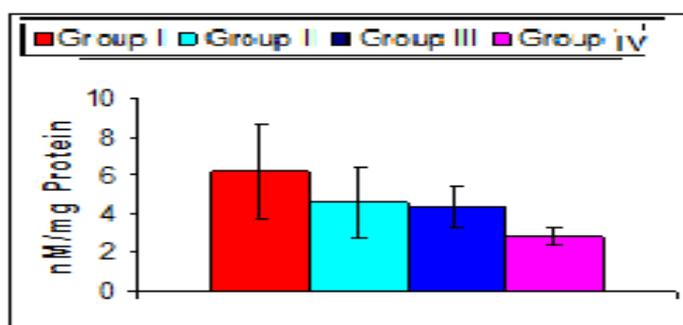


Fig. 6: Effect of test drug (IA) on LPO levels

The levels of LPO in Wounded control, drug treated and standard drug treated animals were given in Fig 6. The LPO level was elevated in the wounded control rats (6.2 ± 1.45) but on treatment with IA extract, group II and group III animals showed significant decreased levels of LPO ($4.56 \pm 0.85a$ & $4.34 \pm 1.1b$). The effect of plant extract was found to be dose dependent. After an injury the cytokine cascade will stimulate phagocytic cells leading to lipid peroxidation²⁰. The results obtained revealed the Free radical scavenging potentials of the plant.

Fig 7 shows the effect of test drug (IA) on SOD levels. Topical application of the IA extract at the dose level of 2g/kg bw & 1g/kg bw were found to have profound increase (3.0 ± 0.19 & 2.5 ± 0.32) in the SOD levels as observed in group I and group II animals whereas disease control (Group I) which were untreated had very low level (2.1 ± 0.75) of SOD. The observed increased level of Superoxide dismutase in the drug treated animals will retard the damage of cells caused by free radicals in line with reported activity of Superoxide dismutase²¹.

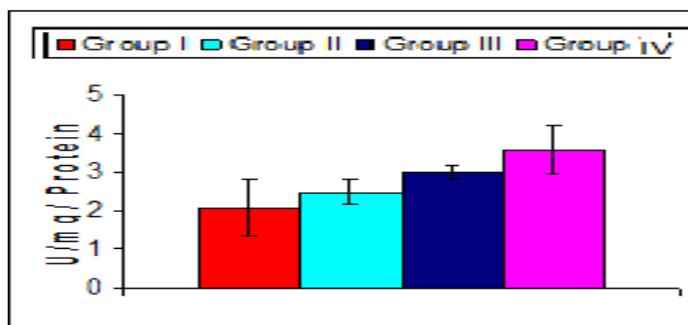


Fig. 7: Effect of test drug (IA) on SOD levels

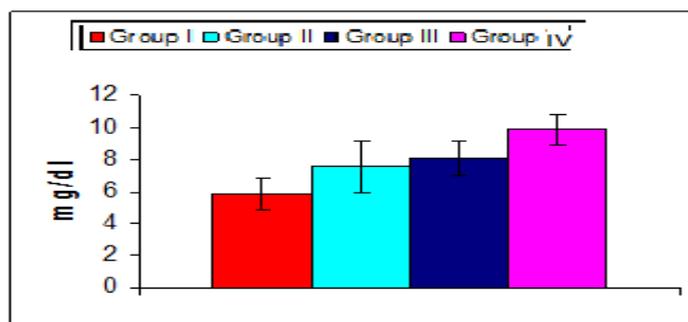


Fig. 8: Effect of test drug (IA) on Ascorbic acid content.

Fig 8. depicts the effects of IA extract on Ascorbic acid content. Wounded control animals showed decreased level of ascorbic acid compared to drug treated animals. Group III (8.08 ± 1.09) and Group II (7.58 ± 1.58) animals were found to have increased ascorbic acid content. Animals which received 2g/kg bw showed remarkable increase in the Ascorbic acid levels compared to other dose. The effect of plant extract was found to be dose dependent. Since ascorbic acid is known to have free radical scavenging activity and inhibition of lipid peroxidation²², elevated level of Ascorbic acid in the drug treated animals might have contributed to the wound healing.

Histopathological Studies

Histopathological observations recorded were given in Fig 9. Histopathological observations of the tissue obtained from IA treated animals showed progressive collagenation and few macrophages compared to the normal, control and standard drug treated experimental animals²³. Histopathological observations also

provided additional evidences in assessing the wound healing activity of the test drug.

The wound treated with plant drug showed higher rate of wound contraction, increased level of Hydroxy proline, Hexosamine, Superoxide Dismutase, Ascorbic acid and decreased levels of Lipid peroxides. Closure of wound indicate the rapid granulation, collagenation and collagen maturation potentials of the extracts. Collagen turnover and fibroblasts formation were evidenced by elevated level of hydroxy proline and Hexosamine content. Increased level of Super Dismutase and Ascorbic acid and decreased levels of Lipid peroxides in the drug treated animals depicted the anti oxidant potential of the test drug. Histopathological observations also provided additional evidences in assessing the wound healing potentials of the test drug. Hastening of the wound healing might be due to the anti oxidant and antimicrobial properties of flavonoids²⁴ and triterpenoids²⁵ present in this drug. Flavonoids and triterpenoids present in the drug might have contributed in hastening the wound healing process.

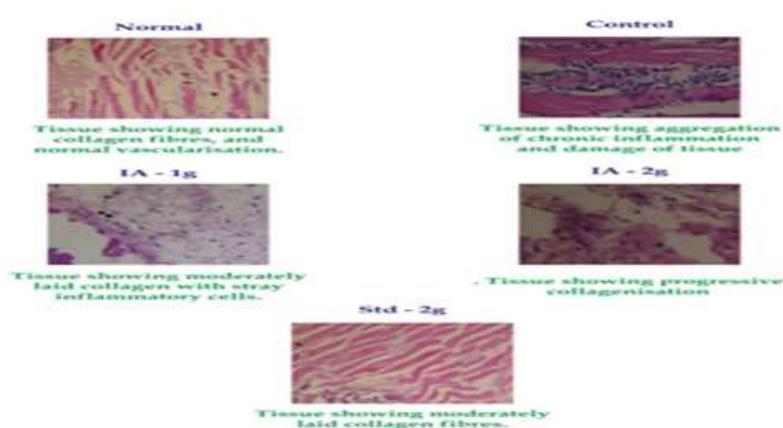


Fig. 9: Histopathological observations

CONCLUSION

Data obtained in the present work suggested that the chloroform extract from *Indigofera aspalathoides* Vahl. ex DC. possess potent wound healing activity especially in promoting collagen growth and hastening the healing process. Such Scientific evaluation studies are essential for the global acceptance of Herbal based drugs as an integral part of modern drug therapy.

REFERENCES

1. Yogayata SP, Vijay DW Herbal Medicines and Nutritional supplements used in the treatment of Glaucoma: A Review. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012; 3(1): 331-339.
2. Sanjoy KP, Yogeshwer S Herbal Medicine: Current Status and the Future. Asian Pacific Journal of Cancer Prevention 2003; 4: 281-288.
3. The Siddha formulary of India. Ministry of Health and Family Welfare. Part I. Government of India, New Delhi; 1992.
4. Krishnamoorthy JR, Ranjith MS, Gokulshankar S, Mohanty BK, Sumithra R, Ranganathan S et al., An Overview of Management of URTI and a Novel Approach Towards RSV Infection. Respiratory Diseases 2012; 179-194.
5. Harborne JB. Phytochemical methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, New York: 1984; 1-89.
6. Morton JJP and Malone MH Evaluation of vulnerary activity by an open wound procedure in rats. Arch int Pharmacodyn 1972; 196: 117-126.
7. Nayak BS and Lexley MPP *Catharanthus roseus* flower extract has wound -healing activity in Sprague Dawley rats. BMC Complement Altern Med 2006; 6: 41-49.
8. Brindha P, Radhika J, Saritha B and Sivaganesh M Evaluation of wound healing potentials of an Herbal formulation. Indian Drugs 2008; 45(1): 63-66.
9. Woessner JR The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch Biochem Biophys. 1961; 93: 440-447.
10. Wagner LA More sensitive assay discriminating galactosamine and glucosamine in mixture. Anal Biochem 1979; 94: 394-396.
11. Yagi K Assay for blood plasma or serum. Methods in Enzymology 1984; 105: 328-331.
12. Misra HP, Fridovich I The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for SOD. J Biochem 1972; 247: 3170-75.
13. Omayr. Selected methods for the determination of ascorbic acid in animal cell tissues and fluids. Methods in Enzymology 1979; 62: 3-8.
14. Sujai S, Histopathological Techniques in Handbook of CMAI Medical Laboratory Technology. Christian Medical Association of India: 1993. p. 508-541.
15. Esra KA, Ufuk K, Ipek P, Demet Y Evaluation of the wound healing potential of *Achillea biebersteinii* Afan. (Asteraceae) by in vivo Excision and Incision Models. Evidence-Based Complementary and Alternative Medicine 2009; 7.
16. Umesh KS, Abhishek S, Umashanker S, Maneesh K, Dhananjay R, Pawan A. Wound healing activity of *Kigelia pinnata* bark extract. Asian Journal of Pharmaceutical and Clinical research 2010; 3(4): 73-75.
17. Umachigia SP, Jayaveerab KN, Ashok Kumarc CK, Kumard GS, Vrushabendra swamy BM, Kishore Kumarf DV. Studies on Wound Healing Properties of *QuercusInfectoria*. Tropical Journal of Pharmaceutical Research 2008; 7 (1): 913-919.
18. Kumar R, Katoch SS, Sharma S β -Adrenoceptor agonist treatment reverses denervation atrophy with augmentation of collagen proliferation in denervated mice gastrocnemius muscle. Indian J Exp Biol 2006; 44: 371-376.
19. Mahesh SP, Patil MB, Ravi K, Sachin RP. Evaluation of aqueous extract of leaves of *Ocimum kilimandscharicum* on wound healing activity in albino wistar rats. International Journal of PharmTech Research 2009; 3: 544-550.
20. Victor MV, Kenneth J McCreath, Milagros R. Recent Progress in Pharmacological Research of Antioxidants in Pathological Conditions: Cardiovascular Health. Recent Patents on Anti-Infective Drug Discovery. 2006; 1: 17-31.
21. Somashekar S, Saraswati U and Laxminarayana U Evaluation of Antioxidant and Wound Healing Effects of Alcoholic and Aqueous Extract of *Ocimum sanctum* Linn in Rats eCAM. 2008; 5(1): 95-101.
22. Vandana P, Madhav S, Swati P Wound healing activity of *Ipomoea batatas* tubers (sweet potato). Functional Foods in Health and Disease 2011; 10: 403-415.
23. Sureendra BR, Shankrappa J, Shivakumar HG Formulation and evaluation of polyherbal wound treatments. Asian Journal of Pharmaceutical sciences 2007; 2(1): 11-17.
24. Ipek PS, Ufuk K, Esra KA, Demet Y, Murat A Assessment of Wound Healing Activity of the Aqueous Extracts of *Colutea cilicica* Boiss. & Bal. Fruits and Leaves. Evidence-Based Complementary and Alternative Medicine 2011; 2011: art. no. 758191.
25. Nayak BS, Pinto PLM *Catharanthus roseus* flower extract has wound healing activity in Sprague Dawley rats. BMC Complementary and Alternative Medicine 2006; 6: art. no. 41