Journal of Stress Physiology & Biochemistry, Vol. 20, No. 3, 2024, pp. 5-11 ISSN 1997-0838

ORIGINAL ARTICLE

OPEN ACCESS

Wound Healing Efficacy of Herbal Ointment Containing *Peristrophe paniculata* Forssk. on Incision Wounded Animals

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Received March 04, 2024

Objective: The present study is aimed to evaluate the curative effect of herbal ointment containing *Peristrophe paniculata* Forssk. against incision wound.

Methods: Animals weighing 150 -200g were divided into five groups each comprising of six rats: Group I served normal control, Group II served as incision wounded control and Group III, IV served as incision wounded animals were treated with 10% and 20% of herbal ointment (HO) containing *Peristrophe paniculata* Forssk. applied topically for 14 days respectively and group V served as incision wounded animals treated with reference ointment soframycin. The healing of wound was assessed by breaking strength, level of hydroxyproline, hexosamine, tissue and serum protein, ascorbic acid, Deoxyribonucleic Acid (DNA), Ribonucleic Acid (RNA), Superoxide Dismutase (SOD), Lipid Peroxidation (LPO).

Results: The topical application of herbal ointment treated groups showed increase in hydroxyproline, hexosamine, protein, breaking strength, DNA, RNA and SOD, Ascorbic acid and significant decrease in the LPO.

Conclusion: The results concluded that the *Peristrophe paniculata* Forssk. has the efficacy in the management of wound healing. Further in-depth studies will be carried out by the isolation of active compounds for drug discovery.

Key words: Wound, Antioxidants, Herbal Ointments, Peristrophe paniculata Forssk

A wound encompasses damage to anatomic structures and functions of the skin; consequently, it results in loss of epithelial continuity with or without loss of surrounding connective tissue. A wound can be classified in a variety of ways, but it is usually categorized as acute or chronic based on how much time it requires to heal. the wound healing process may be divided into 4 phases, I.e. haemostasis, inflammation, proliferation, and remodelling. Many factors can interfere with the wound healing process, thus causing improper or impaired wound healing. The factors include infection, poor nutrition, diabetes, and other diseases, alcoholism, drugs, smoking, insufficient oxygenation, prolonged inflammation, depression, and others. Impaired wound healing cause severe morbidity, requiring patients to stay in the hospital for an extended period of time. The goal of wound treatment is to lessen the time it takes for a wound to heal and the risk of complications.

Wound is a major public health concern, in both developing countries as well as the developed world. Though it has a treatment strategy high cost, drug resistance, and other factors made the management of wounds inadequate. Wounds are the leading cause of disability and lost productivity worldwide. Aside from the incidence, chronic wounds are associated with causing a variety of health concerns.

The herb selected for this study is *Peristrophe paniculata* Forssk., belonging to the family *Acanthaceae*. Tamil name is naganantha. The root is bitter, astringent, cooling and is useful in intermittent fever, intrinsic haemorrhage, ulcer, wounds, skin diseases, pruritus, worms, dental caries, leucorrhoea and insomnia. Indian tribes have been using the plant in the treatment of liver disorders, rheumatism, gout, antidote for snakebite, anti-nematode and pesticide (Chopra *et al*., 1956; Kirtikar & Basu, 1975)**.** It is also useful in psychosomatic disorders and possesses anti-venom activity. The 50% hydro ethanolic extract of the plant showed good anti-inflammatory and analgesic activity (Rathi *et al*., 2003). The aqueous extract of the plant also showed anti-inflammatory, analgesic and wound healing activity and antimicrobial activity has also been reported. Hence, the present study was initiated to assess the efficacy of herbal ointment containing *Peristrophe paniculata* Forssk. on incision wound model.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

Root of the *Peristrophe paniculata* Forssk. were collected in and around trichy, identified with the help of Flora of Presidency of Madras (Gamble 1928) and authenticated with the specimen deposited at RAPINAT Herbarium, St. Joseph's College, Trichy (Voucher specimen number: PARC/2012/1290).

EXTRACTION OF PLANT

Root parts of the *Peristrophe paniculata* Forssk. were shade dried and powdered coarsely using electrical blender. 200g of the plant powder was mixed with six parts of water. Then it was boiled until it was reduced to one third and filtered. Then the filtrate was evaporated to dryness. Paste form of the extract obtained was used for the preparation of wound healing ointment.

HERBAL OINTMENT PREPARATION

At the concentration of 10 and 20% of the wound healing ointment was prepared by mixing aqueous extract of *Peristrophe paniculata* Forssk. and ointment base.

EXPERIMENTAL ANIMALS

Healthy adult Wistar strain of albino rats of either sex, weighing 150 – 200 g were used as experimental models. Animals were kept in ventilated cages and fed with standard rat chow pellet obtained from Sai Durga Food and Feeds, Bangalore, India, and water ad-libitum. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

ANIMAL GROUPING

The rats were divided into five groups each containing of six animals.

GROUP I was served as Normal control,

GROUP II was served as incision wounded animals without treatment,

GROUP III and IV was served as incision wounded

animals treated with herbal ointment (HO) at 10% and 20 % (0.5g applied topically for 14 days),

GROUP V was served as incision wounded animals with Standard Drug SOFRAMYCIN OINTMENT (SO) (0.5g applied topically for 14 days).

Creation of incision wound:

The rats were anaesthetized prior to and during creation of the wounds, with diethyl ether. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision of 6 cm long was made through the skin and cutaneous tissue on the back. After the incision, the parted skin was sutured 1 cm apart using a surgical thread and curved needle. The wounds were left undressed. The rats were given herbal ointment topically at 10 and 20%. The sutures were removed on 10th post wound day and the application of the extract was continued. The skin-breaking strength was measured on the 14th day evening after the last application of the extracts (Lee, 1968).

Tensile Strength: (Saha *et al*., 1995)

On the 10th day after creating the wound the animals were anaesthetized. Healing tissue along with normal skin at two ends was excised for tensile strength measurement using Tensile Testing Machine TKG-20. Strips of 1cm width and 5cm length were cut out from the excised tissue in treated and control animals and were loaded between the upper and lower holder of the machine in such a way that the effective load bearing size was 2.5 x 2.5 cm with the wound remaining in the centre. The total breaking load is measured in Newtons and the tensile strength was calculated by the following equation:

Tensile strength=Total breaking load/Cross-sectional area

Parameters studied

After the experimental period, the animals were sacrificed by cervical dislocation and the blood and tissue samples were collected for analysing biochemical parameters such as hydroxyproline (Woessener, 1961), hexosamine (Wagner, 1972), DNA (Giles & Myers, 1965), RNA (Yoichi Endo, 1970), tissue protein (Lowry *et al*., 1951), lipid peroxide (Ohkawa *et al*., 1979), superoxide dismutase (Misra *et al*., 1972) Ascorbic acid (Omayae *et al*., 1979)

STATISTICAL ANALYSIS:

All the data were expressed as Mean ± SEM. The data were statistically analysed by one – way analysis of variance (ANOVA) and P values <0.05 were considered significant.

RESULTS AND DISCUSSION

The results of the measurement of tensile strength in incision wound was represented in **Table 1.** The tensile strength of the wound tissue was significantly increased in the herbal ointment treated animals (Group III & IV) when compared with the untreated controls (Group II). Resistance to breach beneath tension is known as tensile strength. It describes how much the repaired tissue resists to breaking under tension and may indicates the quality of repaired tissue. In the present study, the increased tensile strength in herbal ointment treated groups indicates the increase in collagen concentration and stabilization of the fibers that strengthen the damaged tissue.

Table 2 depicted the levels of Hydroxyproline, hexosamine, DNA and RNA of the wound tissues. Those levels were significantly reduced in Group II animals. Upon treatment with herbal ointment, those levels were revered back to normal.

Collagen is a principal protein of the extracellular matrix and is the major component that eventually contributes to wound strength (Pattanayak & Sunita, 2008). The collagen is breakdown to liberates free hydroxyproline and its peptides. Measurement of the hydroxyproline could be used as an indicator for collagen turnover (Sasidharan *et al*., 2012). In the present study, incision wound model were used to assess the effect of the herbal ointment containing *Peristrophe paniculata* Forssk. applied topically. The effectiveness of herbal ointment was compared to the animals treated with a commercial brand of soframycin. The profound increase in the hydroxyproline thereby increasing the rapid collagen formation.

Hexosamine and hexuronic acids are amorphous gel like matrix molecules, which act as ground substratum for the synthesis of fibroblast and new extracellular matrix. They are known to stabilize the collagen fibres by enhancing electrostatic and ionic interactions with it and possibly control their ultimate alignment and characteristic size. Their ability to bind and alter protein-

protein interactions has identified them as important determinants of cellular responsiveness in development, homeostasis, and disease (Trownbridge & Gallo, 2002). In the present study, hexuronic acid and hexosamine concentrations which are the components of glycosaminoglycans were significantly increased with herbal ointment which indicates the stabilization of collagen fibres by enhancing electrostatic and ionic interactions.

DNA is a diffusible oligonucleotide appears to be a stimulus and essential for fibroplasias of cells. Decreased level of DNA in incision wound control indicates disturbances in the cellular proliferation and RNA indicates the low level of transcription. The increased level of DNA and RNA in herbal ointment treated groups indicates the rapid formation of granulation tissue in response to injury and prophylactic action on protein synthesis (Madhura *et al*., 2003).

Table 3 demonstrated that the levels of protein were significantly increased in the HO treated groups (groups III, IV), when compared to immunocompromised incision control (group II. Protein is essential for enhancing the wound healing process. It is a part of inflammatory process, in the immune response and also in the granulation tissue development. The low level of protein content in incision wounded rats indicates the prolonged inflammatory processes thereby inhibiting the fibroblasia, collagen synthesis and remodeling. Simultaneous increase in the total protein content in herbal ointment treated animals suggesting active synthesis and deposition of matrix proteins in the granulation tissues and enhance the wound healing process (Agnel arul john *et al*., 2018). The low level of protein content in wounded controls signifies the delayed wound healing. Concomitant increase in the total protein content in herbal ointment treated animals signifying active synthesis and deposition of matrix proteins in the granulation tissues.

The results of the level of lipid peroxide was showed in the **Table 4.** There was significant increase in the level of lipid peroxide were observed in the Group II animals. The topical application of HO at the dose level of 10 and 20% normalized the lipid peroxide level in Group III & IV animals.

Lipid peroxidation is oxidative deterioration of poly unsaturated fatty acids which leads to cellular injury and also generate the peroxide radicals. The cytokine cascade activated after a wound injury which stimulates phagocytic cells that results in the formation of oxygen free radicals and lipid peroxidation. In group II animals showed an elevation in LPO which indicates the scavenging capacity of the wounded tissues. Decreased level of lipid peroxide in the herbal ointment treated groups indicates the anti-lipid peroxidative effect of herbal ointment containing selected plant.

Effect of herbal Ointment on SOD and Ascorbic acid in incision wounded Rats were represented in the **Table 5.** The levels of SOD and Ascorbic acid were increased in the HO treated groups compared than that of untreated groups.

The superoxide dismutase (SOD) level were found to be increased in the herbal ointment treated groups (III, IV) when compared to incision wound control group (II) which indicates that the tissue damage was being repaired by the scavenging activity appear to be a reflex mechanism to guard against the extra cellular oxygen derived free radicals. Low level of SOD in untreated animals showed to increased tissue damage and inhibit the healing process in control group. Thus, SOD enhanced wound healing may be due to the free radical scavenging action of the plants as well as enhanced antioxidant enzyme level in the granulation tissue (Meenakshi *et al*., 2006).

Ascorbic acid is necessary for the hydroxylation of proline and lysine residues in pro collagen, which is necessary for its release and subsequent conversion to collagen. In addition to collagen production, ascorbic acid enhances neutrophil function, increases angiogenesis and also functions as an antioxidant. These combined effect of ascorbic acid promote rapid wound healing. Ascorbic acid deficiency causes abnormal collagen fibers and alterations of the intracellular matrix that manifests as cutaneous lesions, poor adhesion of endothelium cells, and decreased tensile strength of fibrous tissue. Increased level of vitamin C in HO treated group (III, IV) indicates the rapid wound contraction by enhanced tissue repair and scavenging free radicals.

Table 1: Effect of Herbal Ointment on tensile strength by incision wound

Groups	Tensile strength
I	-
II	9.50±0.95
III	11.45±1.40
IV	14.60±1.10
V	13.20±1.83

Values are expressed as Mean ± SEM n=6,
*P< 0.05, when compared herbal ointment treated (group III, IV) with incision wound control groups (group II).

Table 2: Effect of herbal Ointment on Hydroxyproline, Hexosamine, DNA and RNA in incision Wounded Rats

Groups	Hydroxyproline (mg/g Tissue)	Hexosamine (mg/g tissue)	DNA (mg/g tissue)	RNA (mg/g tissue)
I	69.20 ±2.06	54.50 ±1.89	16.23 ±0.48	18.7 ±0.2
II	35.10 ±1.74^{*a}	18.23 ±2.16^{*a}	5.46 ±0.26^{*a}	10.54 ±0.2^{*a}
III	42.10 ±1.59^{**b}	33.60 ±1.33^{**b}	9.79 ±0.46^{**b}	13.20 ±0.3 ^{**b}
IV	58.40 ±2.11^{**b}	44.50 ±1.53^{**b}	13.13 ±0.40^{**b}	17.35±0.3^{**b}
V	64.10 ±1.91^{*c}	56.10 ±2.11^{*c}	14.50 ±0.25^{*c}	18.0 ±0.9^{*c}

Values are expressed as mean ± SEM n=6.
a* P< 0.05 statistically significant when immunocompromised excision wound control (Group II) compared with normal group (Group I), b** P< 0.05 statistically significant when HO treated (Group III, IV) compared with incision wound control groups (group II), c* P< 0.05 statistically significant when HO treated (Group III, IV) compared with Reference ointment treated (Group V).

Table 3: Effect of herbal Ointment on Tissue protein in incision wounded Rats

Groups	Tissue protein (mg/g tissue)
I	1.65± 0.04
II	0.55± 0.02^{*a}
III	0.80± 0.08^{**b}
IV	1.61± 0.15^{**b}
V	1.72± 0.09^{*C}

Values are expressed as mean ± SEM n=6
a* P< 0.05 statistically significant when incision wound control (Group II) compared with normal group (Group I), b** P< 0.05 statistically significant when HO treated (Group III, IV) compared with incision wound control groups (Group II), c* P< 0.05 statistically significant when HO treated (Group III, IV) compared with Reference ointment treated (Group V).

Table 4: Effect of herbal Ointment on LPO in incision wounded Rats

Groups	LPO (milli moles of MDA produced/g tissue)
I	4.40 ±0.13
II	7.65 ±0.19^{*a}
III	6.40 ±0.10 ^{b**}
IV	5.53 ±0.12 ^{b**}
V	4.25 ±0.09^{*c}

Values are expressed as mean ± SEM n=6

a* P< 0.05 statistically significant when incision wound control (Group II) compared with normal group (Group I), b** P< 0.05 statistically significant when HO treated (Group III, IV) compared with incision wound control groups (Group II), c* P< 0.05 statistically significant when HO treated (Group III, IV) compared with Reference ointment treated (Group V).

Table 5: Effect of herbal Ointment on SOD and Ascorbic acid in incision wounded Rat

Groups	SOD (mg of epinephrine oxidized /g tissue)	Ascorbic acid (mg/g tissue)
I	35.11 ±1.46	3.69 ±0.1066
II	14.21 ±2.37^{*a}	1.35 ±0.0252 ^{a*}
III	24.98 ±2.33 ^{b**}	2.40 ±0.0391 ^{b**}
IV	26.39 ±4.08 ^{b**}	3.29 ±0.0518 ^{b**}
V	33.31 ±2.30^{*c}	3.58 ±0.0867^{*c}

Values are expressed as mean ± SEM n=6
a* P< 0.05 statistically significant when immunocompromised excision wound control (Group II) compared with normal group (Group I), b** P< 0.05 statistically significant when HO treated (Group III, IV) compared with incision wound control groups (Group II), c* P< 0.05 statistically significant when HO treated (Group III, IV) compared with Reference ointment treated(Group V).

CONCLUSION

From the results obtained in the study interpreted that the topical application of herbal ointment containing *Peristrophe paniculata* Forssk**.** provides the beneficial effects on incision wounded animals by stimulating the tensile strength, increases the levels of collagen, hesosamine, DNA, RNA, antioxidants and alters the lipid peroxide. Further in-depth studies must be carried out to isolate the active constituents present in *Peristrophe paniculata* Forssk. and explore the complete mechanism of wound healing activity.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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