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Original Research Article

Wound healing effect of *Parmelia perlata* (Huds.) Ach. in albino Wistar rats: an *in vivo* study

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ABSTRACT

Background: A wound is a disruption of soft tissue that heals with scar formation. Herbal medicines are widely preferred for wound management because of their cost-effectiveness and minimal side effects. In Unani medicine, Charela (*Parmelia perlata*), a lichen from Parmeliaceae family, found on rocks and tree trunks, is traditionally used for wound healing and other ailments. It contains several bioactive constituents, including Usnic acid, phenols, flavonoids, alkaloids, glycosides, steroid, tannins, vitamin A and C. Objective was to evaluate the wound healing activity of the ointments prepared from hydro alcoholic extract and powder of the Charela (*Parmelia perlata*) in albino Wistar rats.
Methods: Wound healing activity was evaluated using the excision wound model in albino Wistar rats divided into five groups (n=6). Group I served as untreated control, group II received ointment base (wax and Roghan Kunjad), group III received neomycin 0.5% ointment as standard treatment, group IV received ointment containing hydroalcoholic extract of Charela, and group V received ointment containing Charela powder. Treatments were administered until complete wound healing, which was evaluated based on the rate of wound contraction and period of epithelialization.
Results: Ointments formulated with the hydroalcoholic extract and powder of Charela (*Parmelia perlata*) demonstrated significant wound healing activity, evidenced by increased wound contraction and reduced period of epithelialization compared to the control group.
Conclusions: Ointments prepared from Hydro alcoholic extract and powder of Charela (*Parmelia perlata*) promote enhanced wound healing activity.

Keywords: Charela, Epithelialization, Parmelia perlata, USM, Wound contraction, Wound healing

INTRODUCTION

The WHO expressed that herbal remedies are favored by 80% of Asian and African people because of reachable, economical and with minimum undesirable side effects.[1] Study on wound repairing factors is one of the progressive areas in current biomedical studies. Various man-made medicines are presently in use for the repairment of wounds which in addition of cost, also cause unusual effects like side effects adverse drug reactions, hypersensitivity and multidrug resistance etc, and this problem has dragged scientists' attention to conventional drugs. However immense progress in the ground of artificial drugs in our time they start to have other complications while on other hand natural origin drugs have a peculiar place and have no ill effects.[2] Till date,

only few studies have been carried out on Charela (stone flower). Hence, the present study was carried out to evaluate wound healing effect of an ointment prepared with crude and hydro-alcoholic extract of the same drug in animal models.

Objectives of the study

To evaluate the wound healing effect of an ointment prepared with crude and hydro alcoholic extract of Charela (*Parmelia perlata*) in albino Wistar rats.

METHODS

Type of study

An In-vivo pre-clinical (animal trial) study done on experimental albino Wistar rats.

Planning

The study was conducted at the Regional Research Institute of Unani Medicine (RRIUM), University of Kashmir, Srinagar, from June to September 2021.

Collection of drug material

The whole part of *Parmelia perlata* was collected from Suraj Traders, Bohri Kadal, Srinagar which is an authorized drug dealer in Kashmir valley in July 2020.

Identification and authentication

The drug was identified and authenticated by Dr. Akhtar H. Malik, curator centre for biodiversity and taxonomy (CBT), department of botany, university of Kashmir under specimen voucher number 3729- KASH. A sample specimen of collected material was deposited in herbarium for the future references.

Preparation of extract

The four extracts of the crude drug were prepared by the hot extraction method using Soxhlet apparatus. The powder sample of Charela (*P. perlata*) was extracted in different solvents such as petroleum ether, ethylacetate, ethanol and hydro-alcohol. All the extracted samples were evaporated in vacuum under reduced pressure. The dried extracts were stored in glass bottles in a refrigerator at 4°C. The hydro-alcoholic extract used for making ointment was kept in flask and 100 gm powder of drug was placed in the thimble. The extraction was carried out at 70-80°C.[3]

Chemicals used

Injection phenobarbitone 50 mg/kg body weight i.p. (local anesthetic agent), bee wax (mom), Roghan Kunjad (sesame oil), neomycin 0.5% and sodium alginate.

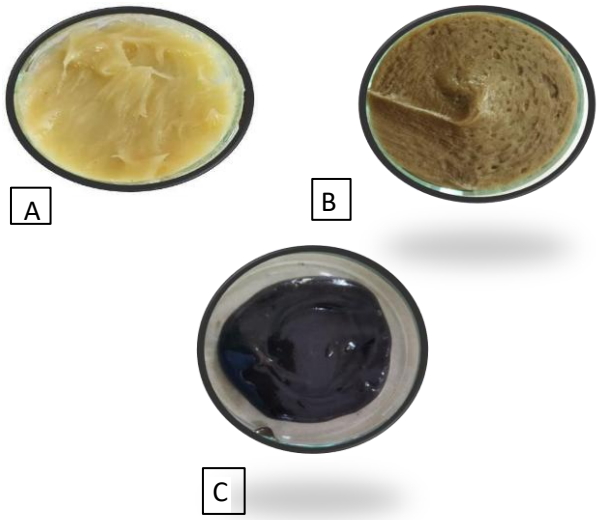

Figure 1: A) Base for ointment; B) ointment prepared from powder form; C) ointment prepared with hydroalcoholic extract.

Preparation of ointments

The ointment of hydro-alcoholic extract and powder of Charela (*Parmelia perlata*) was prepared separately in sodium alginate, Roghan Kunjad and mom (bee wax). The crude drug was first finely powdered and sieved through 100 no. mesh. Since Roghan Kunjad and mom are commonly used as base for the preparation of an ointments. Hence, the ointment was prepared with some modifications according to the formula mentioned in National Formulary of Unani medicine in which mom and Roghan Kunjad were taken in 1:6 proportions and according to the WHO guidelines for the preparation of herbal ointments. One more ointment composed of only mom and Roghan Kunjad was prepared for ruling out any wound healing effect of the base.[4]

Experimental animals

The study was carried out in albino Wistar rats of either sex weighing 150-200 gm (2-3 months old). All the animals were kept in the standard environmental conditions as per CPCSEA guidelines with free access to water and food pellets ad libitum. The animals were also acclimatized for a period of 7 days. The animals were randomly divided into 5 groups, 6 animals in each. Ethical clearance for experimental studies was obtained from Institutional ethical committee, Regional Research Institute of Unani Medicine (RRIUM), University of Kashmir, Srinagar under registration number 927/GO/RS/06/CPCSEA dated 16/09/2020.

Exclusion criteria

Rats outside the standard weight range (e.g., below 150 gm or above 200 gm) were abnormal weight:. Signs of illness/infection: rats exhibiting dullness, lethargy,

diarrhea, respiratory distress, or localized infections during the quarantine/acclimatization period (typically 7-14 days) were excluded. Lack of adequate acclimatization period. Physical anomalies included animals with visible injuries, ocular discharge, poor fur quality, or abnormal posture. Prior drug exposure: animals used in previous experiments or exposed to other medicinal agents.

Figure 2: Experimental albino Wistar rats.

Method of induction of wound

An excision wound model was used for the study of wound healing activity of the test drugs. The rats were fasted overnight and inflicted with an excision wound according to the method of Morton and Melon. A circular wound of about 500 mm^2 and 2 mm of depth was produced in dorsal thoracic region of rats under light anaesthesia using Inj. Phenobarbitone 50 mg/kg. i.p. Before excision of wound, the body hairs were shaved and ethanol (70%) was used as an antiseptic. The wound area was left undressed in the open environment.

Experimental design

Total 30 rats were randomly divided into 5 groups of 6 in each. The experimental design was as follows:

Group I (plain control 'A') group was left untreated and considered as plain control. Group II (plain control 'B') was treated with sufficient quantity of ointment prepared with mom and Roghan Kunjad. Group III (standard control) was treated with 0.5% neomycin cream. Group IV (treatment group 'A') was treated with sufficient quantity of ointment prepared with hydro-alcoholic extract of *Parmelia perlata*. Group V (treatment group 'B') was treated with sufficient quantity of ointment prepared with powder drug of *Parmelia perlata*.

The ointment and standard drug were applied daily once a day, starting from the day of excision of wound till complete epithelialization. The wound area was measured by tracing the wound on a millimetre scale graph paper on 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th}, 18^{th} and 21^{st} day and thereafter on alternate days. The percentage of wound healing was calculated. The period of epithelisation was calculated as number of days required for falling of the dead tissue remnants of the wound without any residual raw wound.

Measurement of contraction and epithelialization of wound

Contraction which mainly contributes for wound closure was studied by tracing the raw wound area on transparent paper every alternate day till wounds were completely covered with epithelium. These wound tracings were retraced on a millimetre scale graph paper to determine the wound area. Wound contraction was calculated as a percentage change in the initial wound size i.e. epithelialization period was monitored by noting the number of days required for Escher to fall away, leaving no raw wound area behind.[5]

$$WC\% \frac{\text{Initial wound size} - \text{Specific wound size x } 100}{\text{Initial wound size}}$$

RESULTS

Wound healing activity

Results of wound healing activity of Charela [*Parmelia perlata* (Huds.) Ach.].

Figure 3: Healing progress of the excised wound on (3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th}, 18^{th} and 21^{st} days) in the PCA, PCB, STD, TGA and TGB groups of albino Wistar rats.

Table 1: Effect of topical application of ointment prepared from hydro-alcoholic extract and powder of *Parmelia perlata* (Huds.) Ach. on excision wound model.

Days	Plain control A	Plain control B	Standard control	Treatment group A	Treatment group B
0th Day	500 mm^2	500 mm^2	500 mm^2	500 mm^2	500 mm^2
3rd	467±16	468.6±12	466.6±12.3 ns	338±12.28**	343.16±12.4**
6th	415.8±15	421.3±11.5	382.83±14.6**	318.16±14.5**	323.16±14.5**
9th	290.16±20.9	298±16.1	163.33±7.9**	205±30.9**	207.3±27.6**
12th	178.3±18.3	177±17.6	74.4±5.2**	49.5±4.4**	54.8±4.7**
15th	73±14.1	70.5±18.4	43.3±2.7**	35.9±2.9**	40.8±3.6**
18th	35.3±3.8	37.8±5.7	17.76±2.83**	11.45±2.56**	16±2.9**
21st	16.16±2.9	15.7±2.4	4.55±0.92**	5.6±0.69**	7.16±1.4**
Period of epithelialization	21.18±0.6	21.22±0.56	15.1±0.3**	16.6±0.8**	17.3±0.5**

Values are expressed as mean±SEM (n=6 animals in each group). *P<0.05, **p<0.01, ***p=0.05 were considered as significant, highly-significant, extremely significant and insignificant respectively compared with control. Data was statistically analysed by ANOVA followed by Dunnet's test.

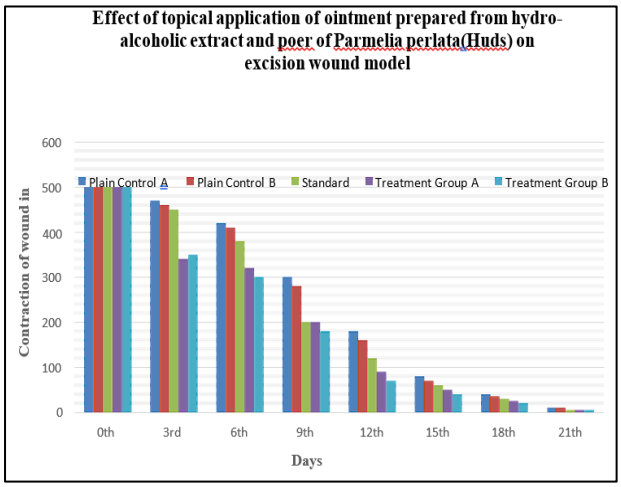

Figure 4: Graphical representation of topical application of ointment prepared from hydro alcoholic extract and powder of *Parmelia perlata* (Huds.) Ach. on excision wound model.

DISCUSSION

Jarahat (wound) is defined as discontinuation of living membrane of soft tissues without involvement of pus and healing by scar tissues (khatam). When a wound becomes putrefied then it is called Quruh (ulcer).[6] Ibn Hubal has defined the jarahat as breach in the continuity of soft tissues.[7] Furthermore, it is also defined as the dissolution in the continuity of skin and mucous membrane with or without involvement of underlying tissues. Mainly, two types of juruh (wound) have been described in classical Unani literature viz. Juruh Mufattiha (open wound), Juruh Qat'iya (incised wounds), Juruh Murfiyah/Razziya (lacerated wounds), and Juruh Wakhziya (punctured wound), Juruh Ghair Mufattiha (closed wound), Juruh-i-Razziya (contusion), blast injuries etc.[8] Wound healing is a dynamic complex process that leads to the re-establishment of tissue integrity and homeostasis and involves inflammation, re epithelialization granulated tissue formation, and neo-vascularization, wound contraction and remodelling of extracellular matrix.[9,10] Research on wound healing substances i.e. drugs is one of the developing or rising areas in modern, biomedical sciences and many traditional practices worldwide, particularly in countries like India and China, which have valuable information of many plants used for treating wounds and burns. This is one of the big reasons that traditional plant based drugs are back and find increasing demand for therapeutic agents.[11]

Under Unani Medicine, several drug substances of plants, animals, and mineral origin are described for their wound healing properties. *Parmelia perlata* is one of them which is mentioned in USM to have wound healing, cicatrizant, antiseptic etc properties. Due to its wound healing and cicatrizant properties, it is suggested to apply locally for the treatment of wounds and ulcers.[12-15] Till date, only few studies have been carried out on Charela (*Parmelia perlata*).

Wound healing is a primary response to tissue injury that results in the restoration of tissue integrity after connective tissue synthesis. Wound contraction, granulation tissue formation, and epithelialization are all part of the complex healing process. The contribution of these phenomena to healing is dependent on the type of wound. Wound contraction and epithelialization play an important role in excision wound healing. Charela (*Parmelia perlata*) in Unani literature has been described with actions e.g. styptic, wound healer, flesh grower and desiccant.[12,13,15] Excision wound models were used to investigate the drug. In this study, wound contraction was observed and in result it was found that contraction completed in 15 days in all the groups except plain control A and plain control B, whereas wound closure was completed after 21 days; similarly period of epithelialization in plain control A and B was more than 21 days where as it was 15 days in

standard control, 16 days in treatment group A and 17 days in treatment group B. The present study showed significant decrease in the epithelialization period. epithelialization was found to be enhanced significantly by the standard drug, thereafter by hydro-alcoholic extract treated group, followed by group treated with ointment prepared from powdered drug as evidenced by the shorter period required for eschar dropping as compared to the plain control A and B. The ointments prepared from both hydro-alcoholic extract and powder were also found significant in facilitating the wound contraction. The results showed that applying ointments prepared from hydro-alcoholic extract and powder of *Parmelia perlata* accelerated wound healing and repair, as evidenced by full thickness coverage of the wound area by an organised epidermis.

The test drug improved wound healing capacity could be explained by its astringent property attributed to the tannin present in it. The study on animal model revealed an increased rate of wound contraction and a significant improvement in healing.[16] In addition to this antimicrobial effect of *Parmelia perlata* was evaluated by Abdur et al against gram positive and gram negative bacteria and its anti-oxidant property screened by the estimation of vitamin C also favours the wound healing effect of *Parmelia perlata* by increasing immunity, bactericidal effect and wound healing property.[17]

The study is limited by sample size constraints and molecular-level challenges. The use of a limited number of animals often restricts sample size, which can reduce statistical power and affect the reliability and generalizability of results. At the molecular chemistry level, findings obtained in animal models may not accurately reflect human biological pathways due to species-specific differences in metabolism, receptor interactions, and biochemical responses. These factors can limit the direct extrapolation of results to clinical settings.

CONCLUSION

Charela (*Parmelia perlata*) has been described as astringent, anti-inflammatory, antimicrobial and wound healer. In the present study the wound healing activity of ointments prepared with Charela was evaluated on excision wound animal model, which showed significant decrease in the epithelialization period, enhanced epithelialization by the standard drug, thereafter by ointment prepared from hydro alcoholic extract treated animals and after that ointment prepared from powder drug as evidenced by the shorter period required for eschar dropping as compared to the PC group A and B. On the basis of the findings, it is suggested that, the hydro-alcoholic extract and powder of Charela should be tested in humans for wound healing activity of Charela using randomized controlled clinical trial.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee, Regional Research Institute of Unani Medicine (RRIUM), University of Kashmir, Srinagar under registration number 927/GO/RS/06/CPCSEA dated 16/09/2020

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