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Wound healing activity of hydro-alcoholic extract of *Ocimum basilicum* Linn. aerial parts in wistar rats

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Abstract

Objective: The main objective of this investigation was to develop a product, which may give a wound healing property and enhance wound healing process like increase the collagen synthesis, fibroblast proliferation, angiogenesis and epithelialization, because products which are available in market are either antiseptic or antimicrobial.

Methods: The hydro-alcoholic extract of aerial parts of *Ocimum basilicum* Linn. was used to evaluate wound healing activity using excision wound model. In excision wound model, the ointment of hydro-alcoholic extract of aerial parts was applied till complete epithelization occurs and evaluated for various parameters such as wound area measurement; percentage wound contraction and period of epithelization.

Results: There was a significant decrease ($P<0.01$) in the wound area (mm^2) in test groups as compared to the control which was measured on 0, 4^{th}, 8^{th}, 12^{th} and 16^{th} post wounding days. The rate of epithelization was a faster in animal of test groups as compared to the control group.

Conclusion: It may be concluded that the phytoconstituents present in the hydro-alcoholic extract of aerial parts of *Ocimum basilicum* Linn are responsible for wound contraction and increased rate of epithelialization.

Keywords: Wound healing, Excision wound model, Inflammation, *Ocimum basilicum* Linn

Wound can be defined as the disruption of anatomical or functional continuity of living tissues produced by physical, chemical, mechanical or microbial; insult to the tissue and wound healing refers to the restoration of continuity of living tissue. There are various types of wounds, including an incised wound, lacerated wound, abrasion, contusion, ulcer, and burn wound. Wound, one of the common clinical conditions has been targeted for exhaustive investigations so as to identify the ways and means of promoting the healing process. In India, there has been interest in the potential of medicinal plant for the development of drugs with wound healing properties as taught in a popular form of Indian medicine known as Ayurveda. Aerial parts of *Ocmium basilicum* Linn. belonging to family Labiatae is used as traditional drug. It is indigenous to lower hills of Punjab and cultivated throughout India. The plant has many medicinal properties such as flower is used as a carminative, diuretic, stimulant, and demulcent. The seed of the plant is used in gonorrhoea, antidysenteric and roots of drug are used in bowel complaints of children. Essential oil of the drug is used as a flavoring agent The plant has a local reputation as diuretic, expectorant, heart and brain diseases, chronic pain in joint, asthma, inflammation and ulcer [1]. It is also a source of aroma compound and essential oil containing biological active compound that possesses insecticidal [2], nematicidal [3], fungistatic [4] and antimicrobial activity [5]. A review of the literature revealed that the wound healing activity of this plant has been subjected to scientific evaluation.

Aerial parts of *Ocimum basilicum* were collected in the month of November to December 2015 around the Bhopal. Plants were identified and authenticated in the Department of Pharmacy, Barkatullah University, Bhopal (M.P.). The aerial parts were washed, dried in shade and plant material was powered moderately and passed through sieve no. 40. Specimen of the plant was submitted as herbarium to the department with herbarium number 4032 A/B.

Aerial parts of *Ocimum basilicum* were extracted by maceration process using hydro-alcoholic solvent [6]. The last traces of solvent were removed under vacuum and finally dried extract was stored in well closed container.

The ointment of the hydro-alcoholic extract of aerial parts of *Ocimum basilicum* was prepared in concentration of 5% (w/w) using the lipophilic base [7].

Excision wound model was employed to study the rate of percentage wound contraction and epithelialization. These parameters were selected because of easy availability of the albino rat and simplicity in handling them. After taking permission for animal studies from Institutional Animals Ethics Committee (IACE) in Department of Pharmacy, Barkatullah University, Bhopal, rats were procured and rats of either sex

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weighing 150-200 gm were selected, maintained at 24-28 ºC, housed individually with free access to food and water. The animals were left for 48 h to acclimatize to the animal room conditions.

In the excision wound model, rats were depilated by removing hairs at the dorsal thoracic region before wounding. Rats were anaesthetized by diethyl ether prior to excision. Circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats under aseptic conditions and were observed throughout the study. The rats were categorized into two groups (n=6). Group I was kept as control which received simple vehicle and Group II were considered as test group, which received extract of aerial part of *Ocimum basilicum* formulation. Topically ointment was applied once daily for 16 d, starting from the day of wounding. Observations of percentage wound closure were made on 4th, 8th, 12th and 16th, post wounding days. The areas of the wounds were measured (in mm^2) immediately by placing a transparent graph paper over the wound and then tracing the area of the wound on it (approx. area 500 mm^2). This was taken as the initial wound area reading. In excision wound model, percentage wound contraction and period of epithelization were evaluated [8].

The results were analyzed by one-way ANOVA and P-value less than 0.01 was considered significant [9].

Table 1: Percentage wound contraction and epithelization period in excision wound model

	Wound area measurement (mm^2) and epithelization period				
Groups	**4th day**	**8th day**	**12th day**	**16th day**	**Epithelization period (Days)**
I (CONTROL)	224.75±0.63 (55.04%)	160.16±0.63 (67.95%)	101.51±0.95 (79.69%)	59.13±0.86 (88.17%)	22
II (TEST-I)	163.93±0.38* (67.21%)	68.70±0.33* (86.25%)	19.95±0.79* (96.0%)	10.12±0.68* (97.97%)	18

Initial wound area approx. 500 sq mm; ≈ n = 6 animals in each group; ≠ Result expressed as mean ± S.E.M.; * P≤ 0.01 indicates significant when compared with control.

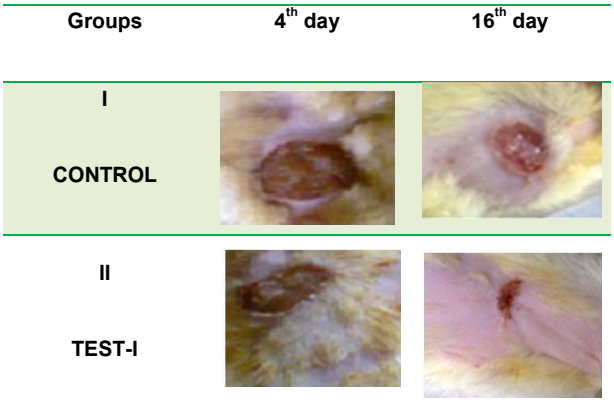

Figure 1: Macroscopic observation of excision wounds on day 4th &16th

The wound contraction was calculated as the percentage reduction in wound area with respect to the initial wound area while the time was noted as the number of days after required for wounding scar to fall off leaving no raw wound behind. In the excision wound model, table- 1 indicates the percentage wound contraction and epithelialization. There was a significant decrease (P<0.01) in the wound area (mm^2) in test groups as compared to the control which was measured on 0, 4th, 8th, 12th and 16th post wounding days (Figure 1). There was a faster rate of epithelization occurs in animals in test groups as compared to the control group.

Wound healing is a complex dynamic process. Wound environment changes with the changing health status of the individual. The knowledge of the physiology of the normal wound healing trajectory through the phases of homeostasis inflammation, granulation and maturation provides a framework for an understanding of the basic principles of wound healing [10]. Vasoconstriction in combination with clot formation and platelet aggregation occurs in response to the initial injury. Once aggregated, platelets degranulate and release mediators that help to form the fibrin clot together with growth factors and chemo attractants. During this initial hemostatic phase, platelets release several growth factors. Wound healing is a fundamental response to tissue injury that result in restoration not tissue integrity which is due to the synthesis of the connective tissue matrix. Collagen is the major protein of the extracellular matrix and is the components that ultimately contribute to wound strength. Breakdown of collagen librates free hydroxyproline could be used as an index for collagen turnover [11]. In previous investigation hydro-alcoholic extract of aerial parts of plant revealed the presence of steroids, flavonoids tannins and proteins. Flavonoids are known to reduce lipid per oxidation not only by preventing the onset of cell necrosis but also by improving vascularity. Any drug that inhibits lipid per oxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fiber, increasing the circulation, preventing cell damage and by promoting DNA

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synthesis. Tannins and flavonoids are also known to promote wound healing process mainly due to their astringent and anti microbial property [12]. The result shows that the hydro-alcoholic extract possess a definite pro healing action, this was demonstrated by a significant increase in the rate of wound contraction and by enhanced epithelisation. Hence, it may be concluded that the phytoconstituents present in the hydro-alcoholic extract of aerial parts of *Ocimum basilicum* Linn is responsible for wound contraction and increased rate of epithelialization.

Further studies are required for the extract of *Ocimum basilicum* Linn. to identify optimal treatment routes, dosages, and which constituent(s) may be conferring its wound-healing potential. Further the phytochemical fractionation and isolation of plant constituents which are responsible for wound healing property are needed to confirm its wound healing activity.

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