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Combined wound healing activity of *Calendula officinalis* and Basil leaves

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Abstract

The present study is to screen wound healing activity of carbopol gels prepared from hydro alcoholic extracts of *Calendula officinalis* (CE) and *Ocimum basilicum* Linn. (OE) in excision wound model and burn wound models in albino mice. Formulations of the extracts was prepared in the gels of carbopol individually and also in combination in equal ratio. In excision and burn wound models, the so treated animals showed significant reduction in period of epithelization and wound contraction and in combined gel showed accelerated wound healing activity. This may be due to synergism. The enhanced wound healing activity of hydro alcoholic extracts may be due to free radical scavenging action and the phytoconstituents present in it which either due to their individual or additive effect fastens the process of wound healing. Presence of flavonoids in alcoholic extracts was confirmed by phytochemical investigation and TLC methods.

Keywords: *Calendula officinalis* and *Ocimum basilicum* Linn, wound healing activity and hydro alcoholic extract

1. Introduction

A wound is a disruption of tissue integrity that results in damage and is typically associated with loss of function. Healing of wound is a biological process that is initiated by trauma and often terminated by scar formation. The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collage nation and tissue remodeling. Healing of wounds usually takes place in a direction away from its normal course and under healing, over healing or no healing of wound is common [1]. Management of under healing of wounds is a complicated and expensive programme and research on drugs that increase wound healing is a developing area in modern biomedical science. Many ayurvedic medicinal plants have a very important role in process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way.

Calendula officinalis, or pot marigold, is a common garden plant belonging to the Compositae family. Native to Southern Europe, *Calendula* grows up to 60 cm in height and produces large yellow or orange flowers [2-4]. The flowers are the part of the herb used medicinally, [6] either in the form of infusions, tinctures, liquid extracts, creams or ointments, or in one of a number of skin and hair products available over-the-counter across the globe.

This extract is derived from the plant of *Ocimum sanctum* belonging to family Labiatae. It has been widely grown throughout the world and commonly cultivated in gardens. Traditionally *Ocimum sanctum* is used in malarial fevers, gastric disorders and in hepatic infections. *Ocimum sanctum* leaves are also used in bronchitis, ringworm and other cutaneous diseases and earache. The leaves are used as a nerve tonic and to sharpen memory. *Ocimum sanctum* leaves are abundant in tannins like gallic acid, chlorogenic acid etc. and also contain alkaloids, glycosides, and saponins along with the volatile oil. The major active constituent of Holy basil leaves include ursolic acid. It contains 70% eugenol, carvenol and eugenol-methyl-ether [2]. Both the plants especially leaves are having good wound healing activity individually. The present study has been undertaken to ascertain the combined wound healing effect of carbopol gels of GE and TE leaves on experimentally induced wounds in mice [3].

2. Materials and Methods

2.1 Plant material

Leaves of *Calendula officinalis* and *Ocimum sanctum* Linn were collected from local areas of Gujarat, shade dried and were authenticated from botany department, South Gujarat University, Surat.

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2.2 Preparation of extracts

100g of leaves of both the plants were powdered to coarse form. The powdered materials were loaded in Soxhlet extractor and defatted with petroleum ether (40–60 °C). The marc was dried and extracted with ethanol (50% v/v) in a same extractor up to three cycles. Finally the extracts were concentrated to semi solid mass using rotary evaporator under vacuum. The traces of solvent were removed by keeping the dried extract in to a desiccator. *Calendula officinalis* (CEE) extract was labeled as CES and *Ocimum sanctum* (OSE) Linn extract was labeled as OSE.

2.3 Phytochemical studies

The individual extracts were subjected to qualitative chemical investigation for the identification of the phytoconstituents: sterols, alkaloids, glycosides, saponins, carbohydrates, flavonoids and tannins^[4].

2.4 Thin layer chromatography (TLC)

TLC was performed for both the extracts by using suitable solvent system. Mobile phase for CEE is Chloroform-methanol-water (60:35:5) and for OSE is the toluene–ethyl acetate–formic acid (90: 10: 01). Pre coated silica gel is acted as stationary phase in both the experiments.

3. Experimental

3.1 Animals

Wistar albino rats of either sex weighing 120–200 g were maintained at 25±2 °C Temperature, 50±15 % relative humidity and normal photoperiod (12h dark/12h light) in plastic cages. The animals were fed standard pellet diet and water *ad libitum*. The animals were caged individually after wounding for treatment till completion of wound healing. In each group of different models six animals were used. All the animal experiments were carried out in accordance with the guidelines of CPCSEA and were approved by the Institutional Animal Ethical Committee.

3.2 Chemical

Metrozyl gel was procured from hetero pharmacy (Hyderabad) and carbopol, methyl paraben and propyl paraben were procured from SD fine chemicals Pvt. Ltd. (Mumbai).

3.3 Toxicity Studies^[5]

Toxicity studies of alcoholic extract were carried out in oral doses of 100 to 2000 mg/ kg- body weight using albino mice. After test extract administration, animals were observed 72 hr. period. The number of deaths was expressed as a percentile and the LD50 was determined by probate a test using the death percentage versus the log dose. Study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

3.4 Selection of gel base and formulations^[6]

A water soluble base like carbopol containing methyl paraben (0.01%) and propyl paraben (0.1%) was selected as base for

both the extracts individually and also in combination in equal ratio for local application as gel (carten sjet all).

Carbopol gel, 2.5%: Carbopol: 2.5g, methyl paraben: 0.01g, propyl paraben: 0.10gms, distilled water: 97.50ml.

CEE gel, 2.5%: CEE: 2.5g, carbopol: 2.5g, methyl paraben: 0.01g, propyl paraben: 0.10g, distilled water: 97.50ml.

OSE gel, 2.5%: OSE: 2.5g, carbopole: 2.5g, methyl paraben: 0.01g, propyl paraben: 0.10g, distilled water: 97.5ml.

Combined extract, CEgel, 2.5%: 1.25g, OSE: 1.25g, carbopole gel: 2.5g, methyl paraben: 0.01g, propyl paraben: 0.10g, distilled water: 97.50ml. Mixed vigorously to get gels of uniform consistency.

4. Evaluation of Wound Healing Activity^[8]

4.1 Excision model

Randomly collected mice of both sex, weighing between 25–40g. Divided them into five groups of six in each and are placed in different cages. Treatment groups: Group I: carbopol gel, Group II: CEE gel, Group III: OSE gel, Group IV: CE gel, Group V: Standard (metrozyl gel) for the excision wound study each group containing six animals was selected. A circular wound of about 10mm diameter was made on depilated dorsal thoracic region of mice under light ether anesthesia in aseptic condition and observed throughout the study. Animals were housed individually. Group-I animals are applied with 2.5% of carbopol gel. Group II & III are applied with 2.5% of CEE and OSE gels respectively. Group IV is applied with 2.5% of CE as thin layer twice daily. Group V animals are applied with metrozyl gel twice daily as thin layer. Wound area can be measured on 2,4,6,8,10,12,14,16, 18,20,22nd post wounding days. % of wound contraction was calculated from the day of measurement of wound area and epithelization period was also calculated.

4.2 Burn wound model^[9]

Wax is heated to a temperature above 100 °C and is poured as a drop on the mice skin to create a wound on the dorsal thoracic region 1cm away from the vertebral column and 5cm away from the ear. Area of the wound was measured in sqmm by placing a transparent polythene graph over the wound and then traced the area of the wound on it. This is taken as initial wound area reading. Group-I animals are applied with 2.5% of carbopol gel. Group II & III are applied with 2.5% of GSE and TEE gels respectively. Group IV is applied with 2.5% of CEE as thin layer twice daily. Group V animals are applied with metrozyl gel twice daily as thin layer. Wound area can be measured on 2,4,6,8,10,12,14,16,18,20,22nd post wounding day % of wound contraction was calculated from the day of measurement of wound area and epithelization period was also calculated.

5. Results and Discussion

CEE and OSE were subjected for the qualitative analysis to detect chemical constituents using standard procedures:

| Identification Test | Cee | Ose |
|--|-----|-----|
| Test for alkaloids □ Dragendroff's test □ Mayer's test □ Wagner's test | -Ve | -Ve |
| Test for saponins □ Foam Test | +Ve | +Ve |
| Test for Glycosides □ Killer-killiani test □ Libermann burchard's test | -Ve | +Ve |
| Test for anthraquinone glycoside □ Borntrager's test | -Ve | -Ve |
| Test for flavonoids □ Ferric chloride test □ Shinoda test □ Sodium hydroxide test □ Lead acetate test | +Ve | +Ve |
| Test for tannins Heavy metal test and FeCl ₂ | -Ve | +Ve |
| Test for Steroids | -Ve | -Ve |

5.1 TLC results: Sample was applied on TLC plates developed in Chloroform-methanol-water (60:35:5) as mobile phase and dried. Plates were then sprayed with 1% vanillin 5% sulphuric acid reagent and dried at 110 °C for few minutes. Spots appear as dark bluish to black spot [7]. *Ocimum sanctum*, the toluene-ethyl acetate-formic acid (90:10:1) is found to be more efficient. The Rf of eugenol in the

hydroalcoholic extract was recorded as 0.58.

Treated with CEE and OSE when compared with control. Even group treated with combined extract has shown much significant increase in % wound contraction when compared with control and it has shown synergistic effect when compared to groups.

Table 1: Excision wound model

| Groups | % wound contraction | | | | | Epithelialization Period |
|--------|---------------------|---------------------|---------------------|---------------------|----------------------|--------------------------|
| | 2 nd day | 4 th day | 6 th day | 8 th day | 10 th day | |
| I | 42.1+3.26 | 63.63+4.52 | 72.9+6.06 | 79.55+6.61 | 79.55+6.61 | 18.57 |
| II | 34+ 0 | 66.30+5.82 | 77.84+6.62 | 89.51+ 9.9 | 89.51 + 9.9 | 14.33 |
| III | 36.3 +2.43 | 70.3 +5.76 | 83.4+5.55* | 93.7 + 6.10 | 93.7 + 6.10 | 11.56 |
| IV | 41.83+2.27* | 72.59 +6.4 | 81.8 +6.39 | 91.63 +6.8 | 91.63 +6.8 | 11.15 |
| V | 43.26+3.6 | 52.34 +5.4 | 75.17+5.32 | 81.51+5.93 | 81.51+5.9* | 16.66 |

Significant at $P < 0.001$, P-value was calculated by comparing with control by ANOVA test, Values are expressed as mean \pm SEM (n=6).

Table 2: Burn wound model

| Groups | % wound contraction | | | | | Epithelialization Period |
|--------|---------------------|---------------------|---------------------|---------------------|----------------------|--------------------------|
| | 2 nd day | 4 th day | 6 th day | 8 th day | 10 th day | |
| I | 35.69+1.19 | 49.78+1.93 | 66.40+3.04* | 76.43+4.5 | 86+1.66 | 19.83 |
| II | 41.50+93 | 52.72+3.6 | 72.87+ 0.9 | 82.74+3.48 | 94.66+1.9* | 16.76 |
| III | 38.19 +5* | 60.86 4.5 | 74.85+2.12 | 90.18+3.97* | 96.12+3.45 | 15.47 |
| IV | 36.85+4.8 | 56.50+2.3 | 79.23+1.87 | 94.5+ 1.72 | 98.67+1.46 | 12.22 |
| V | 38.36+43 | 54.86+5.6 | 72.55+8.72 | 93.24+3.37 | 96.66+2.98 | 14.39 |

Significant at $P < 0.001$, P-value was calculated by comparing with control by ANOVA test, Values are expressed as mean \pm SEM (n=6)

The significant increase in the wound-healing activity was observed in the animals treated with the CEE, OSE and CE compared with those who received the control treatments. Table 2 showed the effects of the hydro alcoholic extracts of *Calendula officinalis* and *Ocimum sanctum* and combined extract of both the plants on wound healing activity in mice with excision wounds. Significant increase in % wound contraction is observed in groups treated with CEE and OSE when compared with control. Even group treated with combined extract has shown much significant increase in % wound contraction when compared with control and it has shown synergistic effect when compared to groups treated with CEE and OSE. The significant increase in the wound-healing activity was observed in the animals treated with the CEE, OSE and CE compared with those who received the control treatments. Table 3 showed the effects of the hydro alcoholic extracts of *Calendula officinalis*, *Ocimum sanctum*

and combined extract of both the plants on wound healing activity in mice with burn wounds. Significant increase in % wound contraction is observed in groups treated with CEE and OSE when compared with control. Even group treated with combined extract has shown much significant increase in % wound contraction when compared with control and it has shown significant synergistic effect when compared to groups treated with CEE and OSE.

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