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RESEARCH ARTICLE

Comparison of the Effects of Bitter Melon *(Momordica charantia)* and Gotu Kola *(Centella asiatica)* Extracts on Healing of Open Wounds in Rabbits

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Summary

This study investigated the effects of topically applied oily homogenized and powder forms of bitter melon (*Momordica charantia*) (MC) and ointment formulation of gotu kola (*Centella asiatica*) (CA) extract and compared the results with untreated control and pure olive oil groups on wound healing in rabbits. A total of 30 New Zealand rabbits were divided into five equal groups (oily homogenized form of MC, powder form of MC, ointment of CA, control, pure olive oil). Full-thickness 5x5 cm skin wounds were created on the right mid-dorsum area and experimental groups were treated daily with the above mentioned extracts. Wounds were observed daily. Planimetry was performed for the unhealed wound area and the percentage of total wound healing on days 0, 7, 14, 21 and 28. Median time for the first observable granulation tissue was shorter in all experimental groups than in the control group ($P<0.05$). Filling of the open wound to skin level with granulation tissue was faster in the oily homogenized form of MC and ointment of titrated extract of CA groups ($P<0.05$). The average time for healing was shorter in the oily homogenized form of MC and ointment of titrated extract of CA groups than in other groups ($P<0.05$). The results demonstrate that topical application of the oily form of MC and ointment form of CA results in significant improvements on wound healing in rabbits.

Keywords: *Wound healing, Rabbit, Momordica charantia, Centella asiatica*

Tavşanlarda Kudret Narı *(Momordica charantia)* ve Gotu Kola *(Centella asiatica)* Ekstraktlarının Açık Yara İyileşmesi Üzerine Etkilerinin Karşılaştırılması

Özet

Bu çalışmanın amacı; tavşanlarda açık yara iyileşmesinde topikal *Momordica charantia*'nın (MC) yağlı homojenize formu ve toz formları ile *Centella asiatica* (CA) ekstratının pomat formunun etkilerini araştırmak, saf zeytinyağı uygulanan ve sağaltım uygulanmayan kontrol grubu ile karşılaştırmaktı. Bu çalışmada kullanılan otuz adet Yeni Zelanda tavşanı 5 gruba ayrıldı: MC'nın yağlı homojenize formu, MC'nın toz formu, CA'nın titre edilmiş ekstratının pomat formu (Madécassol® pomat), kontrol ve saf zeytinyağı. Her bir tavşanda dorsal orta hattın sağ tarafında tam kalınlıkta deriyi kapsayan birer yara (5x5 cm) oluşturuldu ve yukarıda belirtilen ekstratlarla tedavi uygulandı. Yaralar günlük olarak izlendi ve 0, 7, 14, 21 ve 28. günlerde iyileşmemiş yara alanı ve total yara iyileşme yüzdesini ölçmek için planimetri uygulandı. İlk gözlenebilir granulasyon dokusu için ortalama zaman; tüm deney gruplarında kontrol grubundan daha kısa bulundu ($P<0.05$). Yara yatağının granulasyon dokusu ile deri düzeyine kadar dolması; MC yağlı homojenize formu ve CA titre edilmiş ekstratının pomat formu uygulanan gruplarda diğer gruplardan daha hızlı idi ($P<0.05$). Ortalama iyileşme zamanı, MC yağlı homojenize formu ve CA titre edilmiş ekstratının pomad formu uygulanan gruplarda, diğer gruplardan daha kısa idi ($P<0.05$). Bu çalışmada elde edilen sonuçlar; MC yağlı homojenize formu ve CA ekstratının pomad formunun topikal uygulamasının, tavşanlarda açık yaraların iyileşme prosesinde önemli gelişmelere yol açtığını göstermiştir.

Anahtar sözcükler: *Yara iyileşmesi, Tavşan, Momordica charantia, Centella asiatica*

INTRODUCTION

In the past decade, research has been focused on the scientific evaluation of traditional herbal drugs. Bitter melon *(Momordica charantia)* is one such plant that has been frequently used as medicine [1-3]. *Momordica charantia* Linn., family Cucurbitaceae, also known as bitter pear melon, bitter gourd, balsam pear or balsam apple is a tropical

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annual plant that grows freely around dwelling places in uncultivated open spaces [4,5]. *M. charantia* has been reported to possess antilipolytic [6], analgesic [7], abortifacient [8], antiviral [9], cytotoxic [10], hypoglycemic [11] and antimutagenic [12], and also antidiabetic, antileukemic, antibacterial, anthelmintic, antimycobacterial, antioxidant, antiulcer, antiinflammatory, hypotensive, immunostimulant, and insecticidal properties [13-17]. Recently, *M. charantia* is cultivated in the fields at Marmara region (western Anatolia) [17]. The effects of aqueous extracts of *M. charantia*, which are widely used for various purposes in Turkey had been previously investigated [18,19]. The other common preparation type of *M. charantia* is "oily extract". This extract is used externally for the rapid healing of wounds and internally for the treatment of peptic ulcers [1,14,20].

Gotu kola (*Centella asiatica*), has been used as a traditional herbal medicine in Asiatic countries for hundreds of years [21]. A dermal product containing ingredients of Centella is reportedly useful in wound healing [21-23], and is reported to have antiulcerogenic [24], antimicrobial [21], sedative, anti-depressant, analgesic, and anticonvulsive properties [25] as well in Europe. *C. asiatica* contains three principal triterpenoid ingredients: Asiaticoside, asiatic acid, and madecassic acid which were found to contribute to wound healing [26,27]. Asiaticoside is the main active ingredient of *C. asiatica* and exhibits significant wound-healing activity in normal and delayed-healing models [28]. The wound healing property of *C. asiatica* extract has led to its commercial introduction under the trade name, Madécassol® [29].

The present work was undertaken to study the effects of topically applied oily homogenized and powder forms of *M. charantia* and ointment formulation of *C. asiatica* extract on wound healing in rabbits and to compare the results with untreated control wounds.

MATERIAL and METHODS

Plant Material

Fresh fruits of *M. charantia* were purchased from a herbalist in Bursa, Turkey and was authenticated at the Department of Pharmacognosie, Division of Biology, Faculty of Arts and Sciences, Uludag University, Bursa, Turkey with accession number *BULU32549B*.

Preparation of the Plant Material

The mature, fresh fruits of *M. charantia* were cut into small pieces. Hundred grams of chopped plant was immersed in 500 ml of olive oil and put inside a jar of pure olive oil and left under sunshine until the fruit and the seeds dissolved for approximately 20 days. Then they were homogenized by pressing with a spoon and were put into a refrigerator. The undiluted oily homogenized form of *M. charantia* was used in the study. Another 100 g portion of bitter melon was chopped into small pieces and put into a jar. The jar was placed in shadow and was covered with a semi-porous sheet allowing the evaporation of the liquid content of the plant, but limiting the entrance of dust or other contaminants. The dried fruit was then grinded to produce powder form.

Madécassol® (1% ointment, Bayer Co, Istanbul, Turkey), a formulation based on the titrated extract of *C. asiatica*, was obtained from the local pharmacy and used in this study. Madecassol® contains hydrocotyle (*Centella asiatica*; reconstituted titrated dry extract containing 40% asiaticoside and 60% madecassic and asiatic acids) and the other ingredients are essential oils of lavender and geranium, and purified water.

Study Population

A total of 30, six-month-old, New Zealand female rabbits (average weight: 2-2.5 kg) supplied by the Experimental Animals Unit at Uludag University, were used in the study. The rabbits were kept in standard cages, one in each, with 12 h light-12 h dark cycles. The room temperature and humidity were maintained at 19±1°C and 55±10%, respectively. All rabbits were fed 160 g pelleted rabbit diet (Ankara Feed-stuff Industry, Ankara, Turkey) daily and water was available *ad libitum*. The study protocol was approved by Uludag University Animal Care and Use Committee (Approval Number: 01-04/2010).

Rabbits were divided into five groups of six animals each: Oily homogenized form of *M. charantia* (MC), powder form of MC, ointment of titrated extract of *C. asiatica* (CA) (Madécassol® ointment, Bayer Co.), control and pure olive oil. A complete blood count was performed for each rabbit on days 0, 7, 14, 21 and at the end of the study.

Anesthesia

After premedication with xylazine HCl (Rompun®, Bayer Co.) (3 mg/kg, IM), anesthesia was maintained with ketamine HCl (Alfamine®, Alfasan International BV, Woerden, The Netherlands) (50 mg/kg, IM). Bacterial prophylaxis was achieved using intramuscular cephazolin sodium (Cefozin®, Bilim, Istanbul, Turkey) (30 mg/kg). All animals received routine pain control with subcutaneous carprofen (Rimadyl®, Pfizer Inc., Zaventem, Belgium) (4 mg/kg) for two days after wounding.

Operative Procedure

Each rabbit was positioned in sternal recumbency and the dorsal hair was clipped with an electric razor. The skin surface was surgically prepared with povidone-iodine (Betadine®, Kansuk, Istanbul, Turkey), and then draped. Full-thickness skin wounds (5 × 5 cm) were made with #11 scalpel blade on the right mid-dorsum area of each rabbit by excising the skin and the underlying cutaneous trunci muscle *(Fig. 1)*. Hemostasis was achieved by compressing sterile surgical sponges.

Treatment Protocol

Wounds were treated topically each day with the oily homogenized form of MC in group I, powder form of MC in group II, ointment of titrated extract of CA in group III, and pure olive oil (group V) from the first day after wound creation until complete healing occurred. Control group rabbits were left untreated (group IV). After applications, the wound areas were bandaged with sterile non-adherent pads and porous adhesive tapes.

Evaluation of Wound Healing

- Planimetry

Planimetry was performed on days 0, 7, 14, 21 and 28 on anesthetized animals with similar protocol, although measurements were made daily until day 28. The wound area of each lesion on each evaluation day was obtained by tracing the perimeter of the wound onto a sterile piece of clear acetate film with a special marking pen. The outlined area was defined as 'total wound area'. Thereafter, the examiner traced the margin at the leading edge of the advancing epithelium. This area was defined as 'unhealed wound area'. Wound tracings were digitized using digital scanning software (Sigma Scan® Pro 5.0, Systat Software Inc., San Jose, CA, USA) and the percentage of total wound healing was calculated by using a previously described two-step formula [30]. The unhealed wound area and the percentage of total wound healing were recorded at each day of measurement and used for statistical analysis.

Step 1

$$\text{Open wound day}_n \text{ as \% of original} = \frac{\text{Open wound area day}_n \times 100}{\text{Original wound area (day}_0\text{)}}$$

Step 2

$$\text{\% total wound healing day}_n = 100 - \text{Open wound day}_n \text{ as \% of original}$$

- Observations During Daily Wound Care

The bandages were changed daily and medication was applied at each bandage change. Each wound was evaluated for the presence of exudate or other abnormalities and wound appearance during the bandage changes. Information regarding the day that the first granulation tissue was observed, the day that the wound was covered, and the day the wound was completely filled with granulation tissue and epithelialized were recorded. The observations were performed in a non-blinded manner.

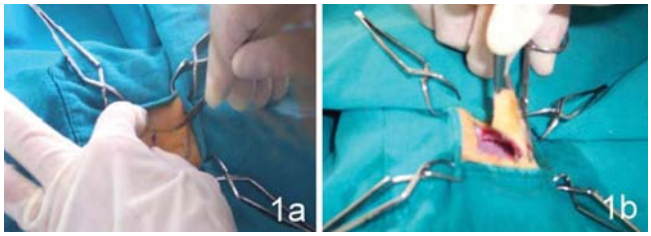

Fig 1. Excision of the skin and the underlying cutaneous trunci muscle with scalpel blade and scissors for creating identical full-thickness skin wounds (5 × 5 cm)

Şekil 1. İdentik ve tam kalınlıkta açık yara oluşturmak için deri ve altında bulunan *M. cutaneus trunci*'nin bistüri ve makasla eksizyonu (5 x 5 cm)

Statistical Analysis

All the data were calculated and the mean values were compared among the four groups using repeated measures model for analysis of variance (ANOVA). Where existed, the differences were determined by Duncan's multiple-range test. All analyses were performed using SPSS 13.0 (SPSS Science, Chicago, IL, USA). A *P*-value lower than 0.05 was considered significant.

RESULTS

Observations During Daily Wound Care

On the first day, all wounds appeared clean. In group II and IV, wounds had fresh view, while wounds in group I had initial signs of healing. All wounds were free of exudate throughout the study. On day 7, the wound size was reduced in all cases. In the group III, granulation tissue formation was easily noticeable at wound edges, especially it was more significant at the caudal region in three cases. In three cases of this group, black brown scab was noticed over the wounds. In the group I, granulation tissue formation was easily noticeable on the wound edges especially at caudal and ventral regions and black-grayish scabs were seen over some areas of the wound surface. In the group II, the powder accumulation was noticed as scab-like appearance over the wound surfaces with an increased amount of epithelial tissue at the wound edges. In the group IV and V, wounds exhibited thin granulation tissue formation at all wound edges on day 7 *(Fig. 2)*. On day 14, all wounds continued to reduce in size in parallel to wound healing. In the group III, a small elevation of granulation tissue in addition to gray-brownish scab was observed. In the group I, it had a crust-like appearance at wound rims. In the group II, when the hard crust over the wounds was removed, the underlying tissue was dark red and was considered as granulation tissue. Caudal and dorsal edges were still separate from the underlying tissues; adhesion was observed on cranial and ventral edges only. On day 21, coverage of the wound bottom and wound filling with granulation tissue were remarkable in the group I and III. In the group IV and V, wounds were usually flat with uncomplete epithelialization and no evidence of elevation with granulation tissue. On day 28, in the group I and III, all wounds had complete coverage of the wounds with granulation tissue and epithelialization, whereas wounds in the other three groups were not completely epithelialized *(Fig. 2)*.

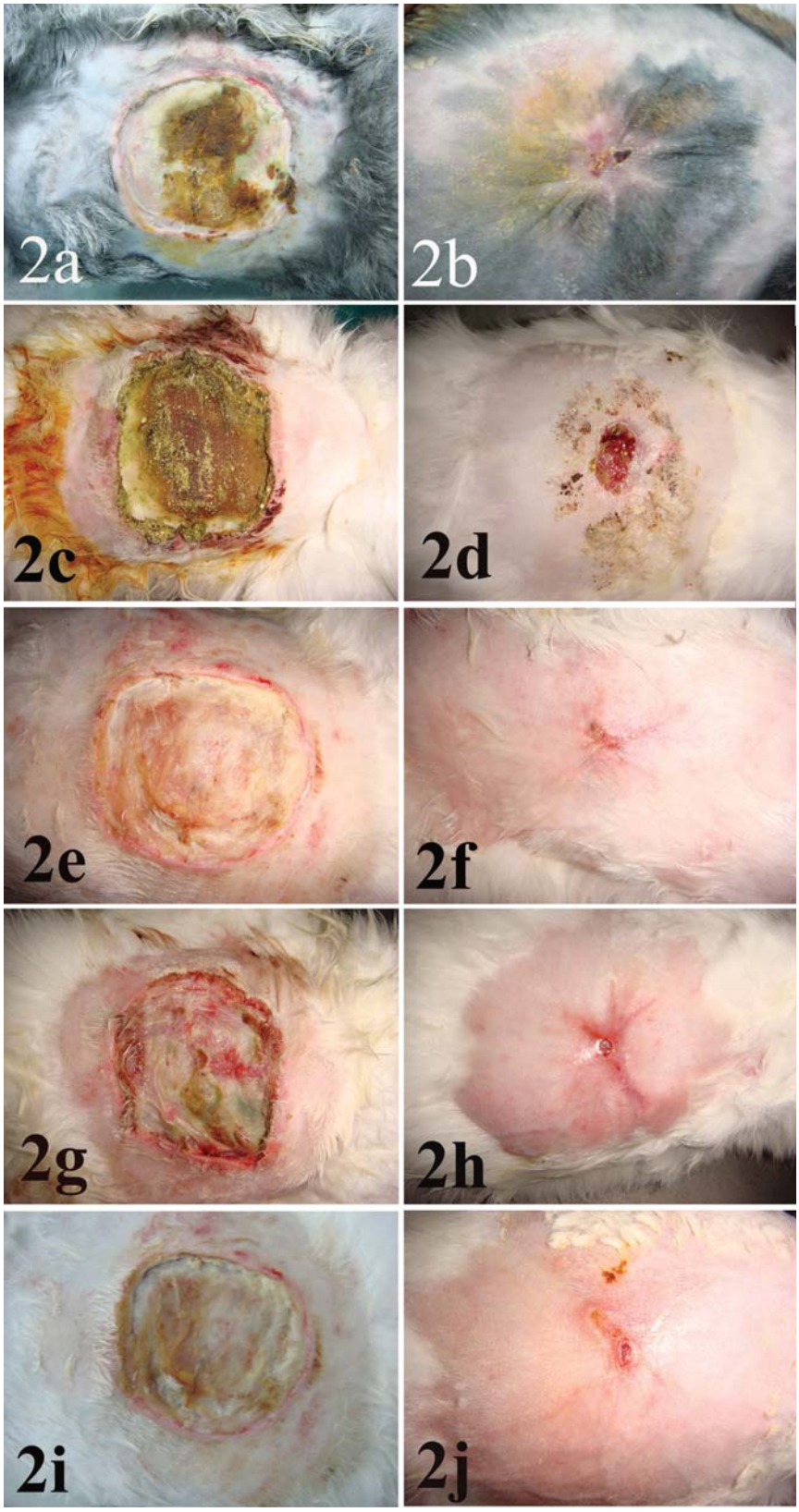

Fig 2. Progression of wound healing in the oily homogenized form-MC, powder form-MC, ointment form-CA, control and pure olive oil groups *(top to bottom, respectively)* on days 7 *(left column)* and 28 *(right column)*. Remarkable granulation tissue formation can be seen at the wound edges in the oily homogenized form-MC (a) and ointment form CA (e) treated animals on day 7. In the powder form of MC group, the wound size was reduced and scab-like powder accumulation was noticed over the wound surface (c). Thin granulation tissue formation is present at all wound edges in the control and pure olive oil group (g, i) on the same day. On day 28, all rabbits in the oily extract form of MC (b) and ointment of titrated extract of CA-treated animals (f) had complete coverage of the wounds with granulation tissue and epithelialization, whereas wounds in the other three groups were not completely epithelialized (d, h, j)

Şekil 2. Yukarıdan aşağıya sırasıyla MC- yağda hazırlanan homojenize formu, MC- toz formu, CA- pomadı ile tedavi edilen, kontrol ve saf zeytinyağı grubu tavşanlarda yara iyileşmesinin ilerlemesi (sol sütun 7. gün, sağ sütun 28. güne aittir). MC- yağda hazırlanan homojenize formu (a) ve CA- pomadı (e) uygulanan tavşanlarda 7. günde yara kenarlarında belirgin granulasyon dokusu gözükmekte. MC- toz formu grubunda, yara boyutu küçülmüş ve yara üzerinde kabuk benzeri toz birikimi gözlenmekte (c). Aynı günde kontrol ve saf zeytinyağı grubunda, tüm yara kenarlarında ince bir granulasyon dokusu gelişimi mevcut (g, i). MC- yağda hazırlanan homojenize formu (b) ve CA- pomadı (f) ile tedavi edilen tüm tavşanlarda 28. günde yaraların tümü granulasyon dokusu ve epitelizasyon ile kapanmışken, diğer üç gruptaki yaraların tam olarak epitelize olmadığı gözlenmekte (d, h, j)

The median time for the first observable granulation tissue was shorter in the group I, II and III than in the control and pure olive oil group (2, 2, 2 vs. 3.5, 3.3 days, respectively) (P<0.05), but was not different among these groups (2, 2 vs. 2 days) (P>0.05). Filling of the open wound to skin level with granulation tissue was faster in the group I and III than in the group II, IV and V (14, 16 vs. 23, 25, 24 days, respectively) (P<0.05), but was not significantly different between the group II, IV and V (23, 25 vs. 24 days, respectively) (P>0.05).

The average time for healing was shorter (P<0.05) in the group I and III than in the group II, IV and V (27.42, 27.66 vs. 30.66, 32.66, 31.58 days, respectively), but was not different between the group II, IV and V (30.66, 32.66 vs. 31.58 days, respectively) (P>0.05). Granulation tissue did not become excessive at any of the wounds either in the treated or control groups during this study. Complete blood count values were within normal limits on days 7, 14, 21 and 28 (data not shown).

Planimetry

A significant decrease in wound area was measured in group III when compared with the other four groups on day 7 (P<0.05, *Table 1*). The mean unhealed wound area in the group I and III was significantly smaller than in the other three groups on days 14 and 21 (P<0.05, *Table 1*). The mean percentage of total wound healing in the group III was significantly higher than in the other groups on day 7 (P<0.05). On days 14 and 21, the mean percentage of total wound healing in the group I and III was significantly higher than in the other groups (P<0.05), but no significant differences were observed between these two groups (P>0.05, *Table 1*). At the end of the study, all wounds in the group I and III had fully recovered, whereas wounds in the other three groups were not completely epithelialized.

DISCUSSION

Wound healing is a complex biological process, including inflammation, cell migration, angiogenesis, extracellular matrix synthesis, collagen deposition, and re-epithelialization [31]. Plant products are potential agents for wound healing and largely preferred because of their widespread availability, non-toxicity, ease of administration, absence of unwanted side effects and their effectiveness as crude preparations [32,33]. These findings prompted us to further investigate other tropical plants which had been reported to have medicinal values for *in vivo* wound healing.

M. charantia is a plant effectively used for the rapid healing of wounds in folk medicine [4]. Prasad *et al.*[34] researched wound-healing property of *M. charantia* and showed a statistically significant response (P<0.01) in terms of wound contracting ability, wound closure time, period of epithelialization when compared with the control group in an excision, incision and dead space wound model in rats. Sharma et al.[35] observed significant wound healing activity in animals treated with Momordica extract compared with other groups in rats. In our excision wound model, animals treated by oily homogenized form of *M. charantia* showed a significant reduction in wound area and time for epithelialization and these animals showed faster epithelialization of wounds than the powder form of *M. charantia,* the pure olive oil and control groups. Healing after *M. charantia* application was similar to that observed after *C. asiatica* application. We believe that the constituents present in the oily homogenized form of *M. charantia* may be responsible for promoting the wound healing activity. Our findings are in agreement with those demonstrated previously by Teoh *et al.*[36] and Ono *et al.*[37].

Madécassol®, a formulation based on the titrated extract of *C. asiatica* is a well-known commercial ointment for promoting dermal wound healing. This extract significantly shortens the wound-healing time, acting more specifically on the immediate process of healing [28]. Our findings are in agreement with those demonstrated previously by Shukla *et al.*[28], and Poizet and Dumez [29].

A dry, desiccated wound will not heal as good as a moist wound. Winter [38] proposed his classic hypothesis that the optimum environment for epithelialization is a moist environment. Topical ointments and gels provide such an environment and aid wound healing. In our study,

Table 1. *Comparison of the mean unhealed wound area and the percentage of total wound healing on days 7, 14, 21 and 28 among groups of rabbits treated with oily extract form-MC, powder form-MC, ointment form-CA, untreated controls and pure olive*

Tablo 1. *MC- yağda hazırlanan ekstratı, MC- toz formu, CA- pomadı ile tedavi edilen, kontrol grubu ve saf zeytinyağı grubu tavşanlarda ortalama iyileşmemiş yara alanı ve total yara iyileşme yüzdelerinin 7,14, 21 ve 28. günlerde karşılaştırılması*

Group	Day 0	Day 7		Day 14		Day 21		Day 28	
	Wound Area (cm^2)	Unhealed Wound Area (mm^2 ± SE)	Total Wound Healing (%)	Unhealed Wound Area (mm^2 ± SE)	Total Wound Healing (%)	Unhealed Wound Area (mm^2 ± SE)	Total Wound Healing (%)	Unhealed Wound Area (mm^2 ± SE)	Total Wound Healing (%)
Group I (oily homogenized form-MC)	25.0	19.64±4.81[a]	26.70±14.74[a]	4.85±2.17[b]	80.60±5.67[b]	0.74±0.25[b]	97.18±1.02[b]	0	100
Group II (powder form-MC)	25.0	19.95±4.52[a]	26.27±15.24[a]	11.39±3.12[a]	54.44±9.02[a]	5.69±3.30[a]	91.06±9.48[a]	1.73±0.52	99.23±1.87
Group III (ointment form-CA)	25.0	15.68±1.54[b]	30.17±10.69[b]	5.59±3.29[b]	77.64±28.67[b]	0.94±0.78[b]	95.13±4.41[b]	0	100
Group IV (Control)	25.0	19.59±4.17[a]	27.08±11.26[a]	12.59±4.82[a]	49.64±8.87[a]	6.92±2.03[a]	90.43±6.56[a]	1.78±1.12	98.23±1.59
Group V (Pure olive oil)	25.0	19.18±2.95[a]	27.26±11.80[a]	12.33±.3.00[a]	50.67±12.01[a]	6.37±2.26[a]	90.78±5.59[a]	1.74±1.63	98.96±2.24

[a,b] *Different superscripts within the same column indicate significant difference among groups (P<0.05)*

the rate of wound contraction in treated rabbits by the oily homogenized form of *M. charantia* and the ointment of titrated extract of *C. asiatica* was significantly higher. Furthermore, the period of epithelialization was shorter in treated wounds. These results further support the effectiveness of *M. charantia* and *C. asiatica* in wound healing.

We did not observe any abnormal findings in the complete blood count throughout the study and all rabbits were clinically healthy throughout the study which suggest that topical applications of *M. charantia* or *C. asiatica* do not result in systemic abnormalities.

The results obtained in the present study demonstrate that topical application of the oily homogenized form of *M. charantia* and ointment form of *C. asiatica* showed significant increase on the healing process of open wounds in rabbits and encourage us to carry out a wider and more profund study on these plants to obtain better knowledge about their therapeutic potentials.

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