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WOUND-HEALING ACTIVITY OF CENTELLA ASIATICA OINTMENT IN AN EXCISION WOUND MODEL

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Abstract

Ointments containing Centella asiatica (gotu kola) are widely used on wounds, burns and scars, and the excision wound in rats is the model most often used to test them. This review describes what the published literature reports on the plant's constituents, the way the excision model works, the choice of ointment base, and the available safety and clinical data. It reports no new experimental data. Animal studies are consistent in showing faster closure, more collagen and higher tissue antioxidant status, with triterpenoids such as asiaticoside implicated. Weaknesses include poorly standardised extracts, unjustified doses, limited reporting and very little clinical confirmation.

1. INTRODUCTION

Slow-healing wounds are an everyday problem for clinicians and pharmacists, and a quietly expensive one for health systems. Healing is not a single event but an overlapping sequence of haemostasis, inflammation, proliferation and remodelling, and a topical product can in principle act at several of these points [1, 2, 3]. Plant preparations have been put on wounds for centuries. *Centella asiatica* (L.) Urban, known as gotu kola, Indian pennywort or mandukaparni, is among the best known: a creeping perennial of the Apiaceae family used in Ayurvedic, Chinese and Malagasy practice for burns, ulcers and scars [4, 5]. This review brings together what has been published on the wound-healing activity of *C. asiatica* applied as an ointment to excision

wounds. It reports no new experimental data, and its aim is to explain what the excision model can and cannot tell us, and how far the findings carry over to people [6, 7].

The plant's reputation rests on a small group of pentacyclic triterpenoids: asiaticoside, madecassoside and their aglycones, asiatic acid and madecassic acid. Commercial extracts are often standardised to these compounds, and a titrated extract of the plant (TECA) has been used in European dermatology for decades [5, 6, 7]. Animal work published since the 1990s, mostly in rats, suggests that topical or oral exposure speeds wound closure and raises the tensile strength of healed skin [8, 9, 10]. Because the reports differ in extract type, vehicle, dose and wound design, a single headline figure for efficacy would mislead. The sections below are therefore organised by question: how the model works, which constituents seem responsible, how the ointment base shapes delivery, and what is known about safety and use in patients [4, 11].

Herbal wound products sit in an awkward regulatory position. Many are sold as traditional remedies on the strength of long use, while the evidence that would support a licensed product, such as controlled trials with defined endpoints, is thin. For research this means that preclinical studies carry much of the burden of proof. Authenticated botanical identity, a defined marker content and a stated extraction ratio are the minimum needed for results to be compared between laboratories [4, 5]. Without them, two papers on a so-called Centella ointment may be testing quite different materials, and differences in healing speed may owe more to the extract than to the biology.

2. THE EXCISION WOUND MODEL

The excision model is popular because it is simple and cheap. A full-thickness disc of skin, typically a few hundred square millimetres in a rat, is removed from the shaved dorsum of an anaesthetised animal, leaving a wound that closes mainly by contraction and re-epithelialisation [12, 13, 14]. The wound is traced or photographed at intervals, and the percentage contraction and the days to complete epithelialisation, taken as the day the scab falls off leaving no raw area, are the classic endpoints. The open-wound procedure described by Morton and Malone remains the template for many vulnerary screens [15]. The model has recognised drawbacks. Rodent skin is loose and contracts strongly, so healing in rats depends far more on contraction than it does in humans, and splinting is sometimes used in mice to reduce this [16, 17].

Healing biology gives the endpoints their meaning. After injury, platelets and fibrin plug the defect and release growth factors; neutrophils and then macrophages clear debris and microbes; fibroblasts and endothelial cells fill the space with granulation tissue; and keratinocytes migrate across it from the wound edge and from hair follicles [1, 2, 18]. Later, type III collagen is gradually replaced by stronger type I collagen, and the matrix is cross-linked and remodelled over weeks to months [3, 19]. Each phase can be disturbed independently. A compound that shortens closure time may therefore be acting on inflammation, on cell proliferation, on matrix synthesis, or simply on the bacterial load, and contraction rate alone cannot say which [19, 20].

Choice of species adds another layer of uncertainty. Rats are the usual subject in excision work because they are inexpensive, robust and easy to handle, and a large literature allows results to be set against earlier studies [13, 14]. Mice are favoured when genetic tools or splinting are needed [16, 17]. Pigs have skin structurally closer to human skin, with comparable epidermal thickness and healing mainly by re-epithelialisation, but they are costly and appear rarely in studies of herbal products [12, 14]. A finding that holds in one species can therefore shrink or vanish in another. For a plant product tested only in rats, the honest reading is that activity has been shown in a model that over-weights contraction, not that human wounds will behave in the same way [2, 16].

Measurement itself introduces variation. Wound area is traced onto transparent film, photographed with a scale bar and analysed with image software, or measured with callipers, and these approaches do not give

identical numbers [12, 13]. The percentage of contraction is calculated against the original area, so wounds that start at slightly different sizes can produce spurious differences, especially when group sizes are small. Observers who know which group an animal belongs to tend, quite unconsciously, to trace margins more generously for the treated side. Photographs taken at a fixed distance and angle, scored by someone unaware of treatment allocation, remove much of this bias at almost no cost [17, 21]. It is surprising how many published ointment studies do not say how area was measured at all.

Biochemical and histological endpoints are added to the wound area for that reason. Hydroxyproline, measured after acid hydrolysis of granulation tissue, is the standard surrogate for collagen content, using the colorimetric method of Woessner or its simplified variants [22, 23]. Hexosamine and uronic acid serve as indicators of ground substance. Tensile strength is measured on incision rather than excision wounds, which is why studies reporting both usually use two separate wound types [11, 13]. Histology with haematoxylin and eosin (H&E) and trichrome stains shows re-epithelialisation, new vessels, inflammatory cell density and collagen organisation. Lipid peroxidation, estimated as thiobarbituric acid reactive substances (TBARS) [24], and antioxidant enzymes are measured in tissue to gauge oxidative stress. Blinded scoring and predefined endpoints are not universal in the older papers [21].

Reporting quality is a recurring problem. Group sizes, randomisation, blinding, the strain, age and sex of the animals, housing, and the anaesthetic and analgesic used are often missing, although all can change healing speed. Analyses of risk of bias in animal research prompted fuller reporting requirements, and the ARRIVE guidelines were written with the same aim [21]. For wound work a few extra details matter: wound size and site, whether animals were caged singly to stop them chewing each other's wounds, the use of a positive control, and whether a vehicle-only group received the same ointment base [12, 13]. A treated group is informative only when it is compared with the base alone as well as with untreated skin.

3. CONSTITUENTS AND MECHANISMS

Among single compounds, asiaticoside has the longest record. In guinea pig punch wounds, topical asiaticoside was reported to raise hydroxyproline, tensile strength, collagen content and epithelialisation, and the same group found the compound active in burn-type wounds [9]. Kimura and colleagues later reported that asiaticoside promoted burn wound repair in mice at low doses, whereas higher doses lost the effect, which hints at a bell-shaped dose response [25]. That matters for ointment design, since more is not necessarily better, and few formulation studies test more than a single strength. Madecassoside and the aglycones have been studied less, although they are present in most standardised extracts and probably contribute to the overall activity [5, 6, 7].

Cell studies point to collagen synthesis as the central action. Extracts and isolated triterpenes stimulated type I collagen production in human fibroblasts [26], and asiaticoside promoted cell-cycle progression, proliferation and collagen synthesis in dermal fibroblast cultures [27]. Earlier work found that the triterpenoid fraction altered connective matrix macromolecules in cultured skin fibroblasts [28]. In rat wounds, triterpenes from the plant stimulated extracellular matrix (ECM) accumulation [10], and an aqueous extract was reported to raise hydroxyproline, tensile strength and cellular proliferation in dermal wounds [8]. The same chemistry can also restrain scarring: asiatic acid suppressed collagen expression induced by transforming growth factor-β1 (TGF-β1) in keloid fibroblasts [29]. The direction of effect appears to depend on cell type and context, much as fibrosis itself does [30].

Antioxidant activity is the second mechanism proposed again and again. Treatment of healing rat wounds with asiaticoside was accompanied by higher levels of enzymatic and non-enzymatic antioxidants, among them superoxide dismutase (SOD), catalase, glutathione peroxidase, vitamin E and ascorbic acid, and by lower lipid peroxidation

[31]. Excess reactive oxygen species (ROS) damage cells and matrix at the wound edge, so this could explain faster granulation. Angiogenic, antimicrobial and anti-inflammatory effects have also been suggested [4, 6, 7]. These claims rest on fewer experiments, and some come from in vitro systems in which the concentrations used may exceed anything reachable in skin [5, 20].

Anti-inflammatory and antimicrobial effects have also been put forward, though the evidence is thinner than for collagen. Inflammation is necessary early in repair but damaging when prolonged, and compounds that trim the neutrophil and macrophage response without blocking it could in theory shorten the lag before proliferation starts [1, 2, 18]. Reviews of the plant describe in vitro activity against skin pathogens and reductions in inflammatory mediators, but extract concentrations in these assays are high and the organisms vary [4, 5, 7]. Whether such effects occur at the wound surface after ointment application has hardly been tested. Wounds in clean laboratory conditions rarely become infected, so antimicrobial benefit in animals would in any case be hard to demonstrate [19, 20].

Quality of repair is a separate matter from speed. Faster closure is welcome, but a wound that closes quickly with disorganised, excessive collagen can leave a hypertrophic scar or keloid, which is a clinical problem in itself [3, 19]. The ratio of type I to type III collagen, fibre orientation and the timing of myofibroblast clearance all determine how the scar ends up [2, 30]. It is notable that the plant's triterpenes show both pro-collagen effects in fibroblasts from normal skin and anti-fibrotic effects in keloid cells [26, 29]. Studies of excision wounds seldom follow animals beyond the first three weeks, so late remodelling and scar appearance are largely unreported for ointments of this plant [8, 18].

Exposure of the wound to the active compounds is a further open question. The skin barrier slows entry of large, polar glycosides such as asiaticoside, but in an excision wound the stratum corneum is simply missing, so the ointment contacts the wound bed directly and absorption is likely to be much greater than through intact skin [9, 19]. Some studies compare topical and oral routes and report activity by both, which suggests that systemic exposure may add to local effects [8, 9, 25]. Pharmacokinetic work on wound-site concentrations has not been published to any meaningful extent, so the relationship between applied strength and tissue exposure remains inferred rather than measured [4, 5].

Whole extracts do not always behave like their marker compounds. Somboonwong and colleagues compared extracts prepared with different solvents in incision and burn models in rats and found that effectiveness differed between them [11]. Polarity, extraction method and the ratio of madecassoside to asiaticoside all shape activity, and plant chemotypes vary with geography and harvest time [4, 5]. For topical products the practical advice is simple: report the marker content of every batch, and keep the same batch through an experiment.

4. OINTMENT FORMULATION AND PERFORMANCE

Reported findings from excision and related wound studies on *C. asiatica* are summarised in table 1, and the distribution of the entries by type of evidence is shown in fig. 1. Most entries are rodent studies of extract or marker-compound preparations, with a smaller group of cell studies explaining mechanism and a handful of safety and clinical reports [4, 6, 7]. Ointment is the dosage form most often chosen, and for good reason. An occlusive hydrocarbon or absorption base keeps the wound moist, which in itself favours epithelial migration, and it holds the active against the wound bed instead of letting it run off [1, 19].

Base selection matters more than it is often given credit for. Simple ointments are made from white soft paraffin, wool fat or beeswax, while absorption and emulsifying bases accept aqueous extract. The triterpene glycosides are fairly polar and the aglycones lipophilic, so release from a hydrocarbon base may be slow, whereas an emulsion base can release more freely but dries out [4, 5]. Studies commonly use extract at a few per cent w/w, and the strength is rarely justified

on pharmaceutical grounds. A vehicle-only control is essential because occlusion alone speeds healing in rodents [13, 15]. Spreadability, pH, viscosity, content uniformity and stability are only occasionally reported for these preparations.

Table 1: Published findings on Centella asiatica and constituents relevant to wound healing

Preparation or model	Reported finding	Ref.
Asiaticoside guinea pig punch wound	Raised hydroxyproline, tensile strength and epithelialisation in healing wounds.	[9]
Asiaticoside mouse burn wound	Repair promoted at low doses, with loss of effect at higher doses.	[25]
Asiaticoside antioxidants	Higher antioxidant enzymes and vitamins and lower lipid peroxidation in rat wounds.	[31]
Triterpenes rat wounds	Stimulated extracellular matrix accumulation in experimental wounds.	[10]
Aqueous extract rat dermal wound	Higher collagen, tensile strength and cellular proliferation.	[8]
Solvent extracts incision and burn	Effectiveness differed between extracts prepared with different solvents.	[11]
Human fibroblasts	Asiaticoside and related triterpenes increased type I collagen synthesis and proliferation.	[26, 27]
Keloid fibroblasts	Asiatic acid reduced TGF-β1-induced collagen expression.	[29]
Patch testing	Weak sensitiser, with contact allergy possible in susceptible users.	[32]
Diabetic wound patients	Oral extract reported to aid healing, with few side effects.	[33]

Across the rodent literature the direction of effect is consistent. Treated wounds typically close earlier, contain more hydroxyproline and better organised collagen, and show higher tissue antioxidant levels than vehicle controls [8, 9, 11, 31]. Effect sizes vary widely, and comparators range from untreated animals to povidone-iodine and framycetin products. Dose-response data are scarce, apart from the low-dose signal noted for asiaticoside [25]. Where histology is presented it usually describes a thicker epithelium with more fibroblasts and new vessels, but scoring is subjective and seldom blinded [12, 18].

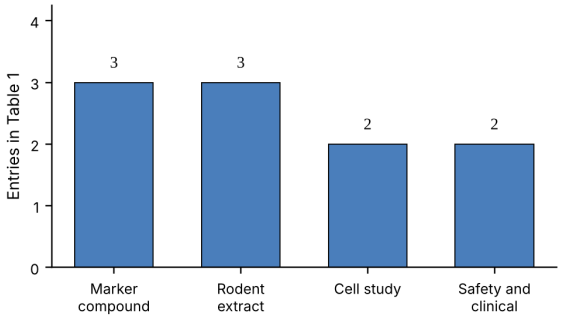


Fig. 1: Number of entries in table 1 by type of evidence

Dosing schedules vary almost as much as the products. In rodent studies the ointment is typically applied once or twice daily, starting on the day of wounding and continuing until the wound has closed, with wound area recorded at fixed intervals during the first two to three weeks [12, 13, 15]. The animals are usually kept singly so that they do not groom each other's wounds. Treatment start time is itself a design choice, since an agent that works on the proliferative phase may be missed if applied only after inflammation has resolved. Studies rarely state the quantity of ointment applied per wound, which makes cross-study comparison of exposure impossible [4, 11]. Without such details, even the best-run experiment is hard to reproduce.

Table 1 has to be read with these caveats in mind. Rows are grouped by the kind of evidence, not by weight, and a single well-run rat experiment is placed beside an in vitro assay as if they counted equally [8, 27, 29]. The entries on the marker compound come from a small number of research groups, so independent replication is thinner than the number of papers suggests [9, 25, 31]. Even so, the pattern across groups is coherent, with collagen, antioxidant status and matrix accumulation recurring in different models and laboratories [8, 10, 31]. The gap between the cell and rodent entries and the two safety and clinical entries is the most informative feature of the table [32, 33].

5. SAFETY AND CLINICAL EVIDENCE

Safety data for topical use are reassuring but limited. Contact allergy is the main concern.

Patch-test work in patients with dermatitis showed that the plant and some of its constituents could sensitise susceptible users, although the author called it a weak sensitiser given how widely it is used [32]. Reviews list occasional contact dermatitis, burning and itching with commercial preparations [6, 7]. Data in pregnancy and lactation are lacking, so use in those settings is not supported by evidence [4, 5]. Most animal studies report no obvious local toxicity at the strengths tested, but they were rarely designed to look for it.

Clinical evidence is much weaker than animal evidence. A review of the dermatological literature describes uses in venous insufficiency, scars, keloids, striae and chronic ulcers, but the trials are small and heterogeneous [6, 7]. A study of oral extract in patients with diabetic wounds reported benefit and few side effects [33], and the triterpenes have been explored for hypertrophic scars and keloids [29]. Nothing in the excision model can predict the outcome in an ischaemic or infected chronic ulcer, where healing is stalled by factors that a young, healthy rat does not have [3, 20].

6. LIMITATIONS AND FUTURE DIRECTIONS

The strongest conclusion that the evidence allows is a modest one: in healthy rodents, *C. asiatica* preparations accelerate closure and improve collagen deposition in acute excision wounds. Several weaknesses limit anything stronger. Extracts are poorly characterised, doses are chosen without pharmacokinetic justification, vehicles are not always controlled, and the endpoints are mostly short-term [5, 12, 21]. Healthy young animals with clean wounds are a poor stand-in for the diabetic, ischaemic or infected wounds that most need treatment [3, 16]. Future work would be more useful if it used standardised extracts, several strengths, blinded scoring and models with impaired healing, and if promising products moved on to properly controlled human trials [11, 20, 33].

Regulatory and quality considerations deserve more attention than they receive. Botanical drugs differ from synthetic ones in that the active principle is a mixture, and consistency has to be demonstrated by chemical fingerprinting and marker assays instead of by assay of a single molecule [4, 5]. Raw material of *C. asiatica* varies with origin, cultivar and harvest, and adulteration with other species sold as pennywort has been discussed in reviews [5, 6]. A wound product also has to be tested for microbial quality, since a non-sterile topical applied to broken skin carries obvious risks [1, 19]. Any development programme would need to settle these points before efficacy claims could be taken seriously [7, 20].

Several research questions follow naturally. The first is whether low-dose and high-dose effects of asiaticoside are reproducible, and whether the bell-shaped response seen in burns also appears in excision wounds [25]. The second is which constituent combinations work best, because whole extracts may behave differently from isolated compounds [11]. The third is how the plant performs in diabetic, aged or infected wound models, which would speak directly to clinical need [3, 16, 33]. Finally, comparison with an established dressing or topical agent would show whether any benefit is clinically meaningful and not merely statistically detectable [2, 3, 20].

7. CONCLUSION

Centella asiatica ointments have a coherent preclinical story. In acute excision wounds in rodents, the plant's triterpenoids are associated with faster contraction, more collagen, better organised granulation tissue and a healthier antioxidant balance, and cell studies offer plausible mechanisms through fibroblast activity and matrix synthesis [8, 9, 27, 31]. The direction of effect is consistent between laboratories, which counts for something given how varied the methods are [10, 11]. Cell studies add a mechanistic thread, in that fibroblast proliferation and type I collagen production recur across independent reports [26, 27, 28]. At face value, that is a respectable body of work for a traditional remedy.

What the literature does not yet provide is proof that the effect matters to patients. Extracts are inconsistently standardised, the ointment base and strength are seldom justified, the model overstates the role of

contraction, and clinical data are sparse and mostly oral [12, 16, 33]. Contact allergy is the only well-documented safety concern [32]. Standardised preparations, models with impaired healing and controlled trials are the logical next steps [3, 20]. Until such data exist, ointments of this plant are best regarded as a promising traditional product with supportive laboratory evidence, and not as a proven treatment for wounds in patients [4, 6, 7]. A pharmacist can reasonably describe the product as safe for short-term use on minor clean wounds, with a note about allergy, while being candid that proof of benefit in people is lacking [20, 32].

8. ABBREVIATIONS

ARRIVE-Animal Research: Reporting of In Vivo Experiments, ECM-Extracellular matrix, H&E-Haematoxylin and eosin, ROS-Reactive oxygen species, SOD-Superoxide dismutase, TBARS-Thiobarbituric acid reactive substances, TECA-Titrated extract of Centella asiatica, TGF-β1-Transforming growth factor-β1, w/w-Weight in weight

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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