

# An Investigation into Multifaceted Mechanisms of Action of Allantoin in Wound Healing

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Conflict of Interest:

AP: Scioderm consultant, honoraria, investigator, grants.

RN: Consultant to Amicus. HD, AR, CV, JC, JB: Amicus employee

## Background

- Allantoin is a heterocyclic organic compound produced by animals, bacteria, and plants
- While different allantoin-containing preparations have been used clinically to study its therapeutic effects in wound healing, its mechanisms of action are not known with certainty

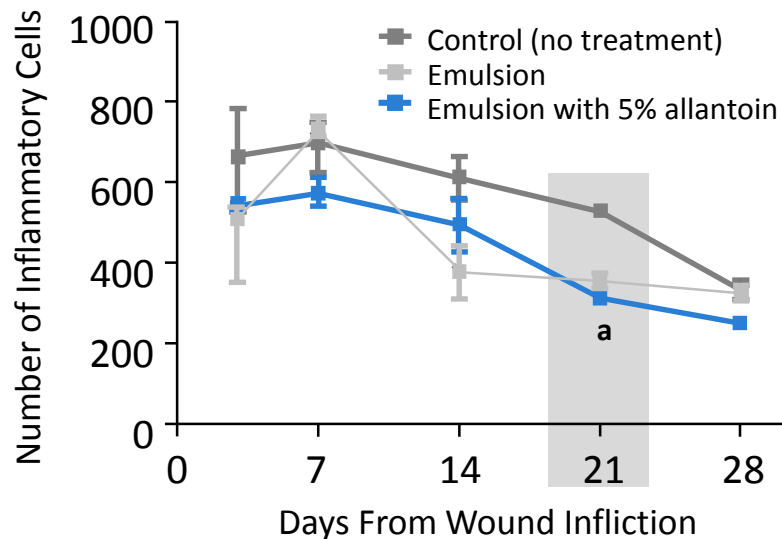
## Methods

- Systematic literature search for preclinical and clinical studies to further understand the mechanism of action (MOA) of allantoin in the context of wound healing
- The following search terms were used: wound, burn, scar, pruritus, anti-inflammatory, antioxidant, fibroblast, collagen, antifungal, antimicrobial, necrotic, and keratolytic
- ~100 preclinical studies (in vitro and animal models) and ~30 clinical studies were found focusing on MOA impacting the wound healing process

## Anti-Inflammatory Effects

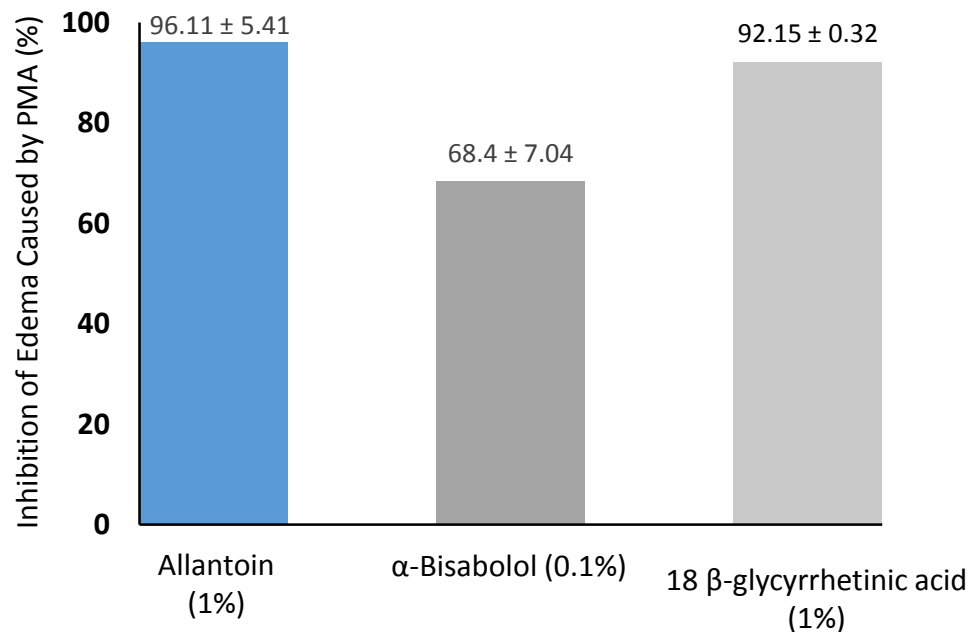
- Allantoin 5% emulsion resulted in fewer inflammatory cells in wounds compared with no treatment at all time points (Wistar rat model).
- Allantoin 1% treatment resulted in nearly complete inhibition of edema caused by chemical burn with phorbol myristate acetate (PMA) applied to mouse ear.

**Number of Inflammatory Cells in a Rat Wound Model by Treatment**



<sup>a</sup> $P < 0.05$  for emulsion containing 5% allantoin compared with control group.

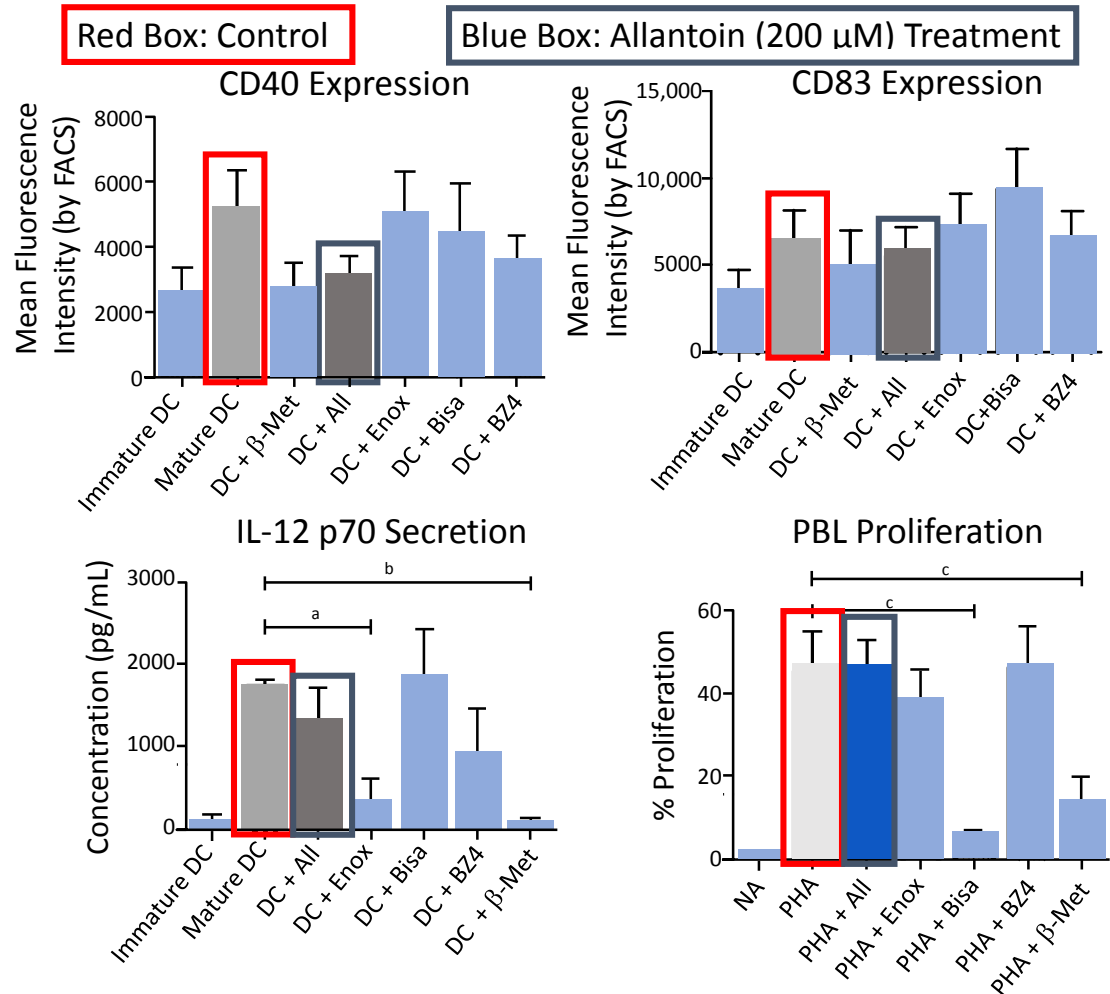
**% Reduction of Edema Caused by PMA**



# Anti-Inflammatory Effects

- In cell cultures, allantoin 200  $\mu$ M led to downregulation of maturation surface molecule CD40 and dendritic cell maturation marker CD83, and a slight increase in the secretion of tumor growth factor (TGF)- $\beta$

## Allantoin Reduced Expression of Pro-inflammatory Markers and Cytokines in Cell Culture Assays



<sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ .

TGF- $\beta$ , tumor growth factor beta; FACS, fluorescence-activated cell sorting; DC, dendritic cells; DC + B-met, DCs incubated with  $\beta$ -methasone (50 ng/mL); DC + All, DCs incubated with allantoin (200  $\mu$ M); DC + Enox, DCs incubated with enoxolon (50  $\mu$ m); DC + BISA, DCs incubated with bisabolol (0.01%); DC + BZ4, DCs incubated with benzophenone-4 (0.01%); PHA, phytohemagglutinin; ELISA, enzyme-lined immunosorbent assay.

Frikeche J et al. *Arch Dermatol Res.* 2015;307:211-218.

# Keratolytic Activity

- In patients with psoriasis, 14-days open-label treatment with allantoin decreased disease manifestations including a reduction in hyperkeratosis

## Effects of Allantoin (2% w/w) in Psoriasis Example Study Results: Effect of Allantoin Preparation With Lanacolic Vehicle on Hyperkeratosis<sup>1</sup>

Degree <sup>a</sup> of hyperkeratosis	+++	++	+	0
Patients prior to therapy, n	9	6	0	0
7 days post-treatment	3	7	5	0
14 days post-treatment	0	6	2	7

<sup>a</sup>Degree expressed as: + + + (very expressed), + + (moderately expressed degree), + (poorly expressed), 0 (not expressed).

# Anti-microbial Effects

- Allantoin exhibited antibacterial activity against many bacterial strains<sup>1</sup> and exhibited antifungal and antiviral activity against several strains of microorganisms in vitro<sup>2</sup>
- Allantoin in a solid lipid nanoparticle containing copaiba oil resulted in potent antifungal activity<sup>3</sup>

## Antibacterial Activity Tested Against Standard and Isolated Strains of Microorganisms<sup>1</sup>

Compound	<i>Escherichia coli</i> MIC (µg/mL)		<i>Pseudomonas aeruginosa</i> MIC (µg/mL)		<i>Proteus mirabilis</i> MIC (µg/mL)		<i>Klebsiella pneumoniae</i> MIC (µg/mL)	
	ATCC 35218	Isolated strain ESβL+	ATCC 10145	Isolated strain	ATCC 7002	Isolated strain ESβL+	RSKK 574	Isolated strain ESβL+
<b>Allantoin</b>	<b>8</b>	<b>128</b>	<b>4</b>	<b>32</b>	<b>8</b>	<b>128</b>	<b>8</b>	<b>128</b>
Ampicillin (reference)	2	>128	-	-	2	>128	2	>128
Ofloxacin (reference)	0.12	0.5	1	64	<0.12	1	<0.12	0.5

Compound	<i>Acinetobacter baumannii</i> MIC (µg/mL)		<i>Staphylococcus aureus</i> MIC (µg/mL)		<i>Enterococcus faecalis</i> MIC (µg/mL)		<i>Bacillus subtilis</i> MIC (µg/mL)	
	RSKK 02026	Isolated strain	ATCC 25923	Isolated strain MRSA	ATCC 29212	Isolated strain	ATCC 6633	Isolated strain
<b>Allantoin</b>	<b>2</b>	<b>64</b>	<b>16</b>	<b>&gt;128</b>	<b>8</b>	<b>128</b>	<b>8</b>	<b>16</b>
Ampicillin (reference)	2	>128	<0.12	>128	0.5	>128	0.12	0.5
Ofloxacin (reference)	0.12	64	0.25	64	1	32	-	-

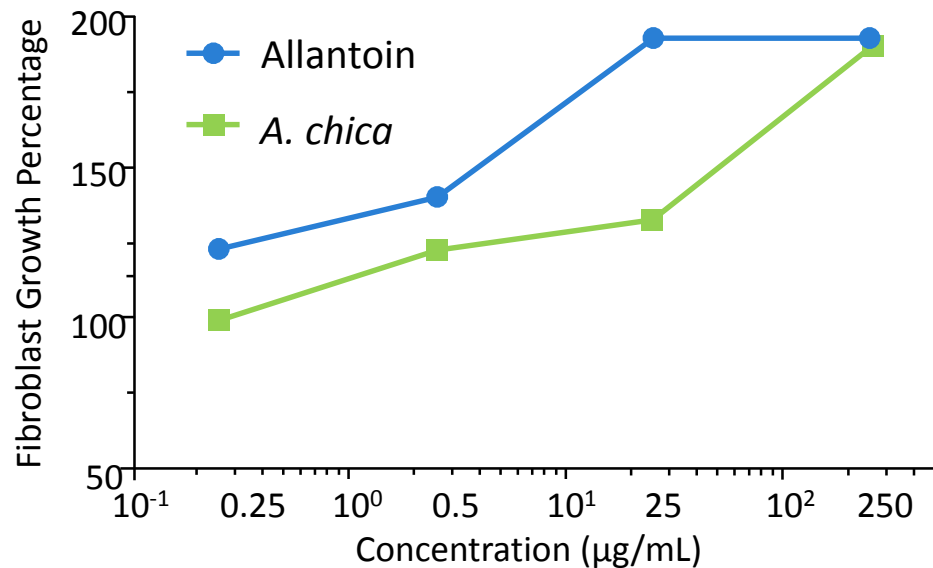
MIC, minimum inhibitory concentration.

1. Özçelik B et al. *Pharm Biol.* 2011;49:396-402. 2. Özçelik B et al. *Pharm Biol.* 2011;49:396-402. 3. Svetlichny G et al. *Pharmazie.* 2015;70:155-164.

# Fibroblasts

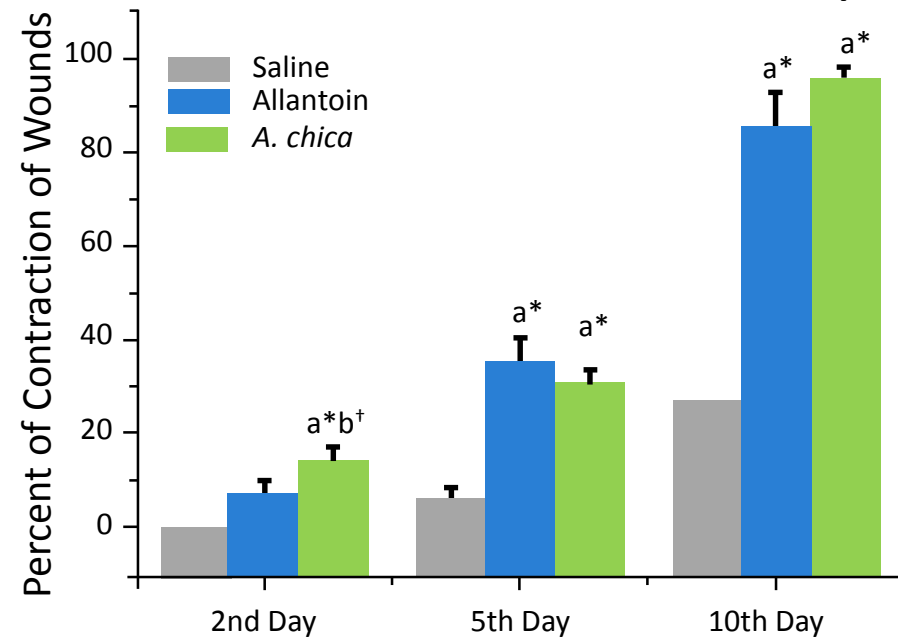
- Allantoin and crude extracts of allantoin-containing *Arrabidaea chica* added to human fibroblast cell cultures demonstrated a concentration-dependent increase in fibroblast proliferation
- Allantoin and *A. chica* extract significantly improved surgically induced wound healing in Wistar rats by days 5 and 10 compared with saline control

**Concentration-Dependent Fibroblast Growth Percentage Induced by Allantoin and *A. chica* Extract**



Jorge MP et al. *J Ethnopharmacol.* 2008;118:361-366.

**Wound Healing Observed as Percent of Contraction for Each Treatment Group**



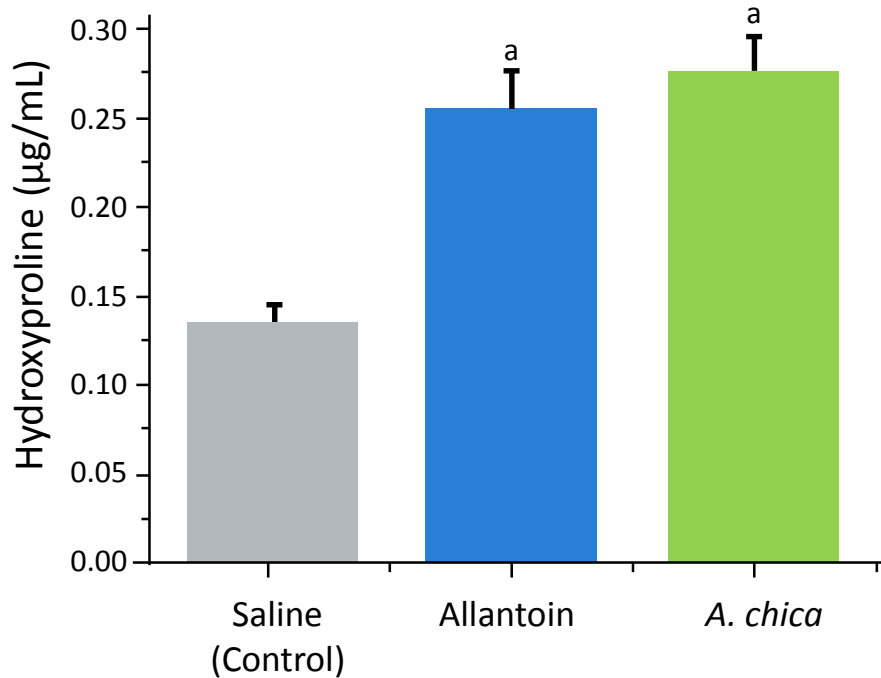
<sup>a</sup>Different from saline; <sup>b</sup>different from allantoin (100 mg/mL). \**P*<0.001 †*P*<0.01.

Jorge MP et al. *J Ethnopharmacol.* 2008;118:361-366.

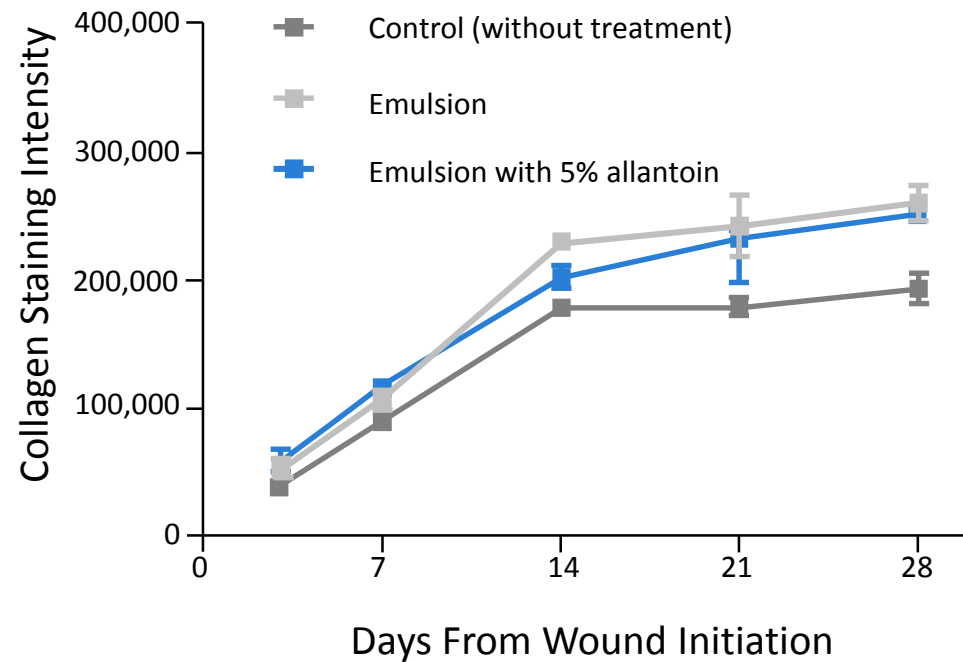
# Collagen Synthesis

- Allantoin and *A. chica* extract improved collagen synthesis in the wounds of Wistar rats analyzed 11 days after injury
- Allantoin 5% emulsion and the emulsion alone increased collagen stimulation compared with control (without treatment) at all times

**Effects of Allantoin and *A. chica* Extract on Collagen Synthesis in Wistar Rat Wounds**



**Effects of Allantoin on Collagen Synthesis in Wistar Rat Wounds**



<sup>a</sup> $P < 0.001$  compared with fibroblast control group.  
Jorge MP et al. *J Ethnopharmacol*. 2008;118:361-366.

Araújo LU et al. *Acta Cir Bras*. 2010;25:460-466.



# Summary

Anti-inflammatory Effects	Keratolytic	Antimicrobial	Fibroblast Proliferation	Collagen Synthesis
<ul style="list-style-type: none"><li>▪ Allantoin reduced chemotaxis of inflammatory cells into rat wounds</li><li>▪ Allantoin application prevented the edema associated with chemical burns in mice</li><li>▪ In cell culture, allantoin decreased immune activity and inhibited cytokine secretion</li></ul>	<ul style="list-style-type: none"><li>▪ In patients with psoriasis, allantoin decreased disease manifestations</li><li>▪ <i>In vitro</i>, allantoin dispersed psoriatic scales into solution</li></ul>	<ul style="list-style-type: none"><li>▪ Allantoin showed <i>in vitro</i> antiviral, antibacterial, and antifungal activity based on 2 separate studies</li></ul>	<ul style="list-style-type: none"><li>▪ Allantoin demonstrated increased fibroblast proliferation in cell culture assays</li><li>▪ Allantoin increased tissue regeneration and epithelialization in rat wound models</li></ul>	<ul style="list-style-type: none"><li>▪ Allantoin demonstrated increased collagen synthesis in cultured fibroblast</li><li>▪ Histology of rat wound models demonstrated increased collagen synthesis</li></ul>

- Studies used different formulations of allantoin and there was a lack of information on the stability or dermal penetration of allantoin
- Additional research is warranted to further characterize clinically relevant mechanisms of action in human skin and to determine the relative role of these various mechanisms in wound healing