The safety profile of Bald's eyesalve for the treatment of bacterial infections

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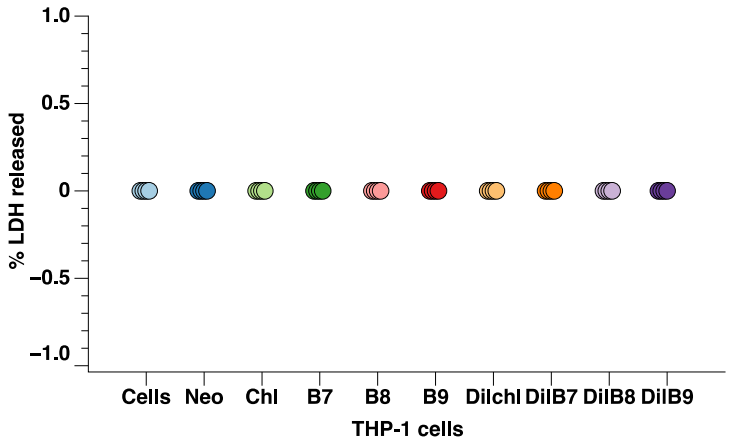

Figure S1: Lactate dehydrogenase assay of THP-1 cells treated with eyesalve. THP-1 cells were treated with three batches of eyesalve (B7, B8 and B9) in the undiluted and diluted (1/10) forms. The controls include cells only (untreated), Neosporin (Neo), a safe antibiotic for wound infections and Optrex^{TM} chloramphenicol (chl) treated cells (n = 4 replicates). The preface “dil” represents cells treated with a 1 in 10 dilution of either the chloramphenicol or the different eyesalve batches.

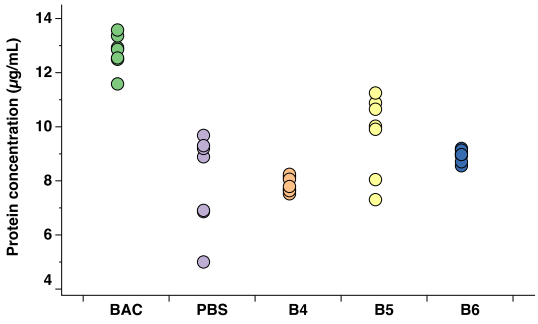

Figure S2: Protein concentration of mucus produced from slugs treated with eyesalve. Slugs were treated with three batches of eyesalve (B4, B5 and B6) and the protein concentration of the mucus measured using the NanoOrange kit. The positive control is benzalkonium chloride (BAC) and the negative control, phosphate buffered saline, PBS. ANOVA found significant higher protein concentration in the

positive control compared to the eyesalve treated slugs followed by Dunnett's test for multiple comparison, $F_{4,30} = 15.72$, $p < 0.002$, $n = 7$ replicates).

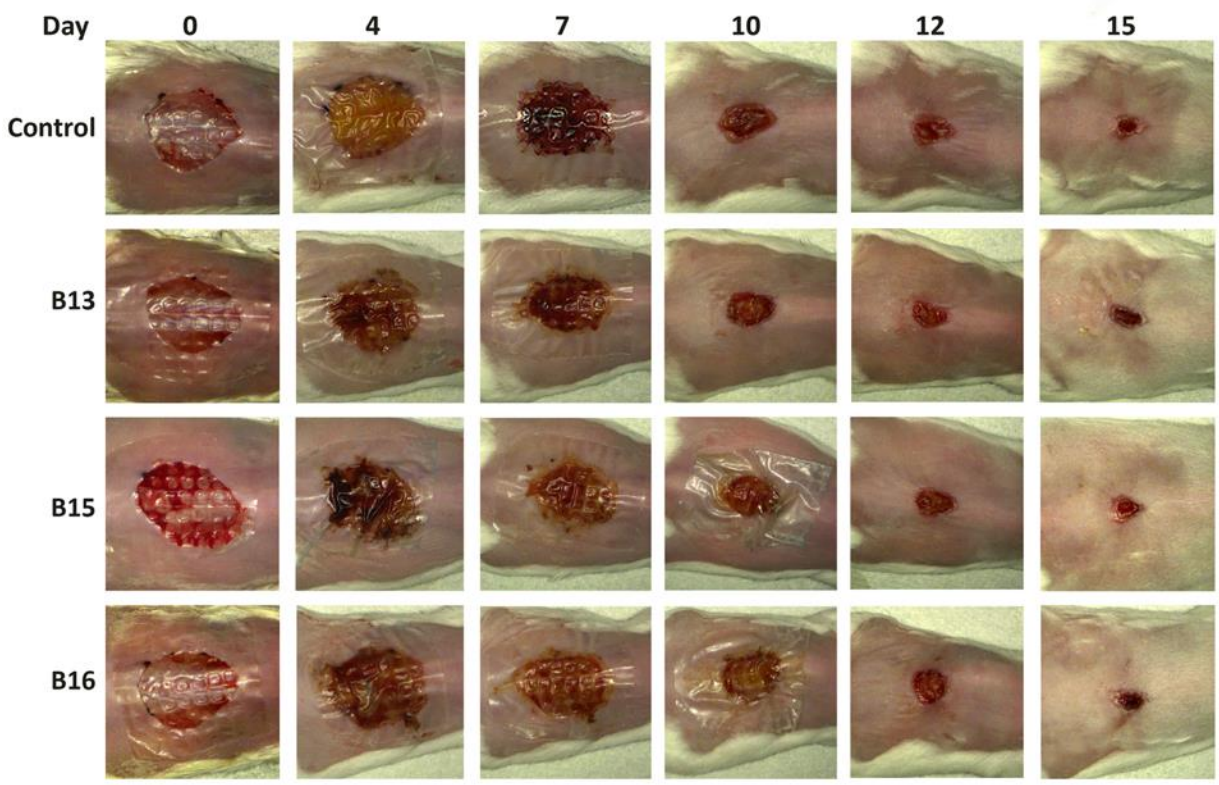

Figure S3: Images of the mouse wounds at different days of treatment with three batches of eyesalve showing closure of the wounds. The control is sterile water.

Supplementary Table 1: Bovine corneal opacity and permeability assay scoring matrix (modified from Van Erp & Weterings, 1990). Opacity is scored visually based on what is seen with the white light/unstained and epithelial integrity is scored following fluorescein staining visualised with a cobalt blue filtered light.

Opacity	Score	Epithelial integrity	Score	Cumulative score	Description
None	0	None	0	≤ 0.5	None
Slight	1	Diffuse and weak	0.5	0.6 - 1.9	Slight
Marked	2	Confluent and weak	1	2.0 - 4.0	Moderate
Severe	3	Confluent and intense	1.5	> 4	Severe