SUPPLEMENTARY DATA

SUPPLEMENTAL METHOD SECTION

SM1: Method for Cytokine estimation

Briefly, RAW464.7 mouse macrophages (2.6 × 105 cells/well) were added into a 96-well microtiter plate and stimulated with lipopolysaccharide (LPS, 100 ng/mL). Stimulated cells were treated with different concentrations (0.45 µm filtered) of AE-VG111 (10, 20 and 40% [v/v]) in designated wells for predetermined time intervals (3, 6, 12, 24 and 48 h) while, the unstimulated cells served as control. The levels of various cytokines, including IL-6, TNF-α, IL-4 and IL-10, in cell supernatant were measured using the commercially available ELISA kit Standard curve of each cytokine was generated with serially diluted recombinant standard protein, and concentration in the cell supernatant was estimated in reference to a standard curve.

SM2: Method for Quantification of Matrix metalloproteinases (MMPs)

RAW464.7 mouse macrophages were plated in flat bottom 96-well plates (5×104 cells/well) for 18 h before treatment. After washing with PBS (pH 7.4), macrophages were challenged with 100 ng/ml purified LPS to induce production of MMPs. Culture supernatants were collected and stored at 80°C. Macrophages were treated with different concentrations of 0.2 micron filtered AE-VG111 (10, 20 and 40% [v/v]) in designated wells for predetermined time intervals (3, 6, 12, 24 and 48 h). The level of MMP-2 and MMP-9 in the medium was measured using ELISA kit (Krishgen Biosystems, Mumbai, India) according to the instructions of the manufacturer.

SM3: Method of HPLC fingerprint

VG111 (65 ml) was extracted with 100 ml of distilled water under reflux for 2 h. The water layer was separated and evaporated. The residue was weighed, dissolved in methanol, filtered and volume made to 5 ml. The final solution of 0.4 mg/ml was made by suitably diluting the solution with the mobile phase. The solution was filtered through a 0.25micron filter and a volume of 5 µm was injected into Waters Acquity H Class HPLC system fitted with PDA eλ detector and controlled through Empower 3 Software under following conditions.

Column: C18, 25 cm x 4.6 mm, 5 µm; Inertsil, G.L. Sciences, Japan

Mobile phase:

Time (min) % Water % Acetonitrile
0 100 -
30 70 30
35 50 50
40 50 50
45 100 -

Flow rate: 1 ml/min; Run time: 45 min; Temperature: 25°C; Detection wavelength: 280 nm

SUPPLEMENTAL RESULT SECTION

SR1: Case series in human and canine application

Human cases

Human wound applications[23]

Case 1: Diabetic foot ulcer on the 'fifth toe'

A 65-years old male with 7 years ago post-renal transplant and known diabetic for 20 years, presented with an ulcer on little toe of left foot for the past one month, preceded by trauma. On examination, the ulcer was noted to be oval, 1 x 1.5 cm in size with presence of dirty slough at the base and hyperpigmentation with erythema of the surrounding skin. Despite using povidone-iodine along with amoxicillin-clavulanic acid for 5 days, the ulcer continued to increase in size, therefore, the dressing was changed to VG111 and the wound healed with topical application of VG111 alone within 3 weeks despite the patient being on systemic steroids [Figure 8(i)].

Case 2: Pressure ulcer/bedsore in a 95-year-old female

A 95-years old immunocompetent female with bad Parkinsonism and bedridden for the past 2 years, presented with decubitus ulcer over two months. The ulcer was noted to be 4 x 3.5 cm in size, with sinus tract interacting with deep tissues. In spite of local dressings and systemic antimicrobials in the past. A dramatic reduction in size of the ulcer was noted within 5 days of application of VG111 and the sinus tract started filling with granulation tissue. The ulcer shrank completely and healed over the next 40 days. This much time was taken because of the repeated trauma while shifting the elderly patient from the bed by the relatives at home [Figure 8(ii)].

Case 3: Post Below-knee amputation recovery in a diabetic male

A 48-years old male patient, who has been a known diabetic (type 2 diabetes mellitus) for the past 4 years, hypertensive for 10 years and had a history of coronary artery disease, presented to the outpatient department with a history of non-healing ulcer over right foot. Clinical diagnosis of right

diabetic foot was made and right below knee amputation was planned. Pre-operative tissue cultures revealed presence of methicillin-sensitive *Staphylococcus aureus*. Intra-operative fluid was sent for microbiological culture and Gram-stain, which showed presence of pus cells with isolation of *Bacteroides thetaiotaomicron* and *Escherichia coli*. Tissue and pus cultures on D9 showed growth of *E. coli*, however, no antimicrobials were prescribed to the patient. The patient presented on postoperative day 11 (marked as day 1 in the figure 8(iii-g) with complaints of pain and purulent discharge over the stump, but was afebrile. Debridement of the wound was done and the patient was discharged. The wound worsened and a subsequent revision of the stump was planned. However, before revision of the stump, on microbiological consultation, daily dressing with VG111 was initiated on day 13. Within one week of daily application, the wound was noted to be healthy and had started to heal with the presence of granulation tissue (Figure 8(iii)-j) The same treatment was continued for 2 months. CT Angiography was done to plan for graft placement. Patient declined to be operated upon as his wound was healing well. And, after 4 months, complete resolution of the wound was noted without the use of antibiotics. It may be appreciated that this wound healed without the need of even a graft!! [Figure 8(iii)-l].

Canine cases

Case I: Post-malignancy surgical incision wound

A German Shepherd bitch aged 13 years was presented to the GADVASU hospital and was operated upon for mammary tumors on 13 October 2021. It was recuperating well but on 16 October 2021, in the 12-inch incision partial wound dehiscence occurred and part of the wound had one-inch depth. Due to the aggressive behavior of the animal, it was decided to treat the wound as an open wound. The dressing with povidone iodine was started on the day of gaping and dressing was done on alternate days for 6 days but there was no improvement in the condition of the wound. The swab taken from the wound area revealed *Pseudomonas aeruginosa* and based on susceptibility, ceftazidime was given 1 g iv bd for 5 days. On day 7 (day 0 of VG111), use of VG111 was started and by day 5 of VG111, there was no exudation and the wound was clean, which completely healed within 30 days [Figure 9- Case I].

Case II: Lacerated wound on dorsal part of spine with MSSA infection

Second case was of a female German Shepherd dog aged 4 years. It had a lacerated wound (5.5 cm x 4 cm) on the dorsal part of the spine. The swab taken from deep inside the wound revealed methicillin-sensitive *Staphylococcus aureus* (MSSA). VG111 was applied locally and was given cephalexin 300 mg od orally for 5 days. Within the first five days, no exudation and clear granulation tissue was observed and the wound healed completely within 11 days [Figure 9- Case II)].

Case III: Dog bite wound

A case of a 3 months old male dog was presented with a dog bite wound on the right side of chest. The bacterial isolation revealed *P. aeruginosa*. VG111 was applied locally and on the seventh day of treatment, no exudation was seen, and rapid healing was seen with almost 70% of healing on day 7 and it got completely healed with the regeneration of original, brown-coloured hairs [Figure 9- Case III].