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Research Article

INVITRO EVALUATION OF WOUND HEALING ASSAY OF NEW HERBAL FORMULATION ON ATOPIC DERMATITIS

-A SPECIAL MEDICINE FOR CHILDREN

K.Vetrivel, Suvetha.C

1 Associate Professor, Dept of Paediatrics-National Institute of Siddha,Chennai.

2 Residential Medical Officer, Dept of Paediatrics-National Institute of Siddha,Chennai.

ABSTRACT

Siddha system of medicine is most ancient and spiritually enriched one.It is the tradition of tamil speaking world known for its proven ability in curing chronic diseases and their life threatening complications. In siddha literature view, karrapan(AD) it is characterised by erythema, papules, vesicles, and crushing with lichenification followed by scaling of healthy skin without scars. siddha system is very effective in chronic disorders especially in skin diseases. This drug is used as the external application to evaluate their efficacy in treating " Atopic dermatitis".

KEYWORDS

Atopic dermatitis, wound healing, scratch test, Karappan

INTRODUCTION

Atopic dermatitis (AD) is a very common skin disease. It affects between 8.7% to 18.1% of all infants and children. It is an itchy, red rash. It can appear all over the body. Many people have it on their elbows or behind their knees. Babies often have eczema on the face, especially the cheeks and chin. They can also have it on the scalp, trunk (chest and back), and outer arms and legs. Children and adults tend to have eczema on the neck, wrist and ankles and in the areas that bent, like the inner elbow and knee. People with eczema are usually diagnosed with it when they are babies or young children. Eczema symptoms often become less severe as children grow into adults. The rash of eczema is different for each person. It may even look different or affect different parts of your body from time to time. It can be mild, moderate or severe. Generally, people with eczema suffer from dry, sensitive skin. Eczema is also known for its intense itch. The itch may be so bad that you scratch your rash skin until it bleeds, which can make you rash even worse, leading to even more inflammation and itching. This is called itch-scratch cycle. This novel treatment represents for how Atopic dermatitis will be treated and opens the door to future targeted therapeutics.

MATERIALS AND METHODS

The herbs was identified,collected,grinded and dried into fine powder. The authors decided the ratio of the poly herbal drug and wound healing analysis was done by Scratch Wound Healing Assay at Biogenix Laboratory, Trivandrum.

L929 cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen).

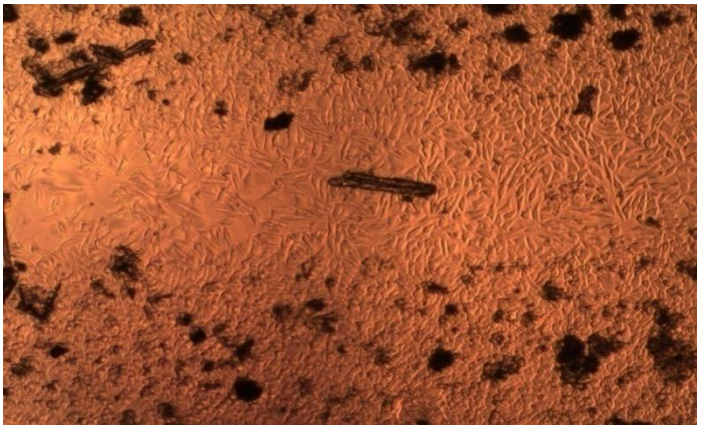
The cell lines was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, , L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

Scratch wound healing assay

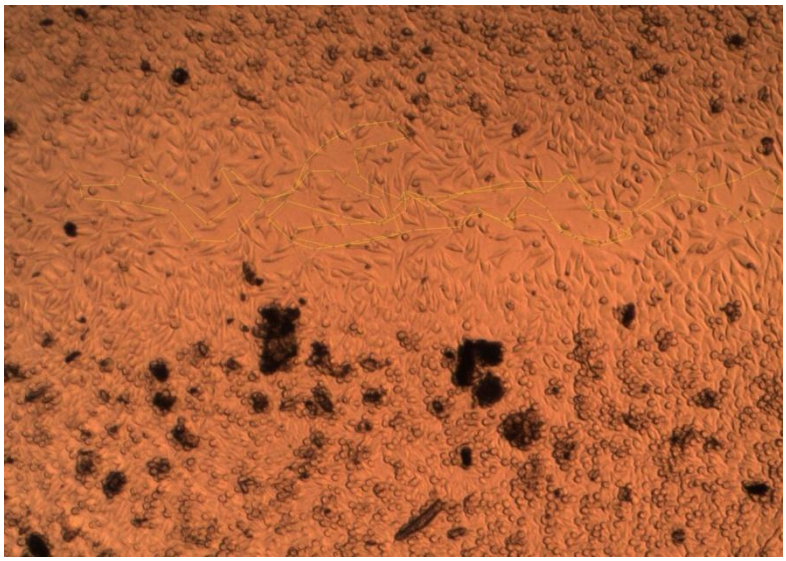
Exponentially growing cells were trypsinized and seeded at a density of 200,000 cells per well into 12-well plate for 24 h incubation (~90% confluence). The scratch wounds were made by a sterile 1 mL pipette tip through a pre-marked line. After removal of the resulting debris from five lineal scratches, the cell monolayer was subsequently rinsed three times with PBS followed by incubation with treatment of wound with extract containing medium in the concentration of 50ug/ml and 100ug/ml.. The wound areas were displayed by taking images just above the interchanges between scratched wound areas and pre-marked lines and the effect of extracts on wound closure was determined microscopically (20X magnification, Olympus CKX41) after 24 hours of incubation. The effect of material on wound closure was measured in terms of area using MRI-ImageJ analysis software

Sample preparation – 1mg/ml in distilled water

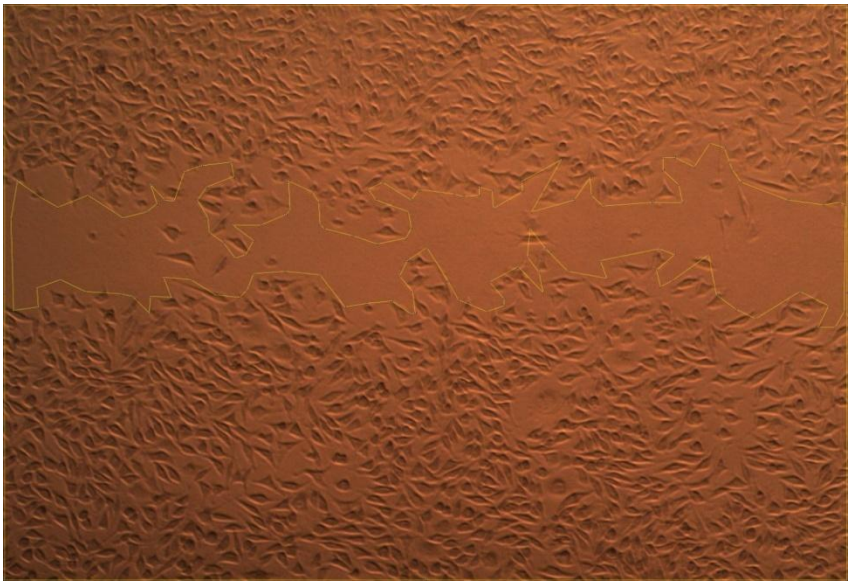
STAGE 1



STAGE 2



STAGE 3



RESULTS

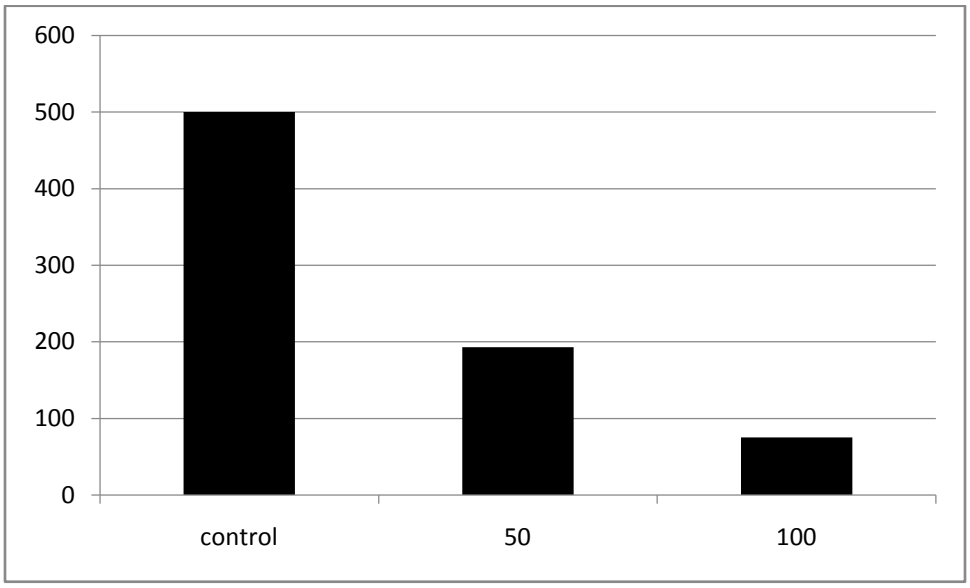


Fig: Wound healing effects of drug extracts on fibroblast cells. Along Y axis area of wound in arbitrary units as measured using MRI wound assay software , Along X axis samples such as Untreated control samples (area 500.264 arbitrary units), 50ug/ml (193.21 arbitrary units), 100ug/ml (75.12 ug/ml arbitrary units).

The preliminary studies has shown considerable effect of extracts on cell infiltration to wound area leading to closure of wound and in 100ug/ml an approximate 85% healing effect was obtained which can be considered significant.

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