In-vitro cell migration activity of some lupeol esters

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Abstract

Modification of naturally occurring compounds with enhanced cell migration activity is a timely needed requirement for the treatment of wounds. Nine esters of lupeol were prepared by reacting with fatty acids of varying chain lengths (C-2 to C-18). Their structures were confirmed by ^{1}H and ^{13}C NMR spectroscopic data. Each lupeol ester was subjected to scratch wound assay (SWA). It is observed that the cell migration enhancement increases with the increase in the chain length of fatty acid moiety of the lupeol ester.

Introduction

Development of plant-based health care products with proven efficacy will enable rural communities to access them at an affordable cost. Wounds are a global health problem. Impairment to the natural wound healing process can be threat to the life. Hence search for promising plant-based wound healing compounds will lead to a low-cost wound treatment in many developing countries, which will in turn promote well-being for all at all ages (UN SDG 3).

Lupeol and lupeol esters are naturally occurring plant secondary metabolites. They are non-cytotoxic and capable of enhancing cell migration.[1,2] A long chain fatty acid ester of lupeol has been found to be active in *in-vitro* cell migration assay.[2] It is also known that lupeol acetate act as a pro-drug in the cell migration assay by undergoing slow hydrolysis.[3]

The current study is aimed at synthesis, characterization of some lupeol esters and evaluating their cell migration activity by employing the *in-vitro* scratch wound assay.

Methodology

Nine esters of lupeol were synthesized by the esterification of lupeol with different fatty acids of varying chain lengths (C-2 to C-18) in the presence of N,N'-dicyclohexylcarbodiimide (DCC), 4-pyrrolidinopyridine (4-PP) in dry CH_2Cl_2 (*Fig. 01*).

Synthesis of Lupeol esters (C-2 to C-18)

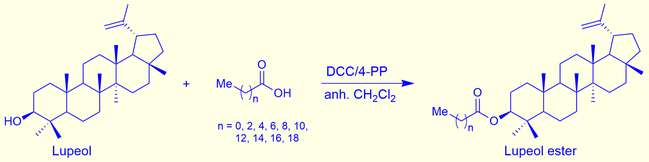

Fig.01: Reaction scheme for synthesis of lupeol esters.
(DCC = N,N'-dicyclihexylcarbodiimide, 4-PP = 4-pyrrolidinopyridine)

Purification and characterization of synthesized Lupeol esters

Each lupeol ester was purified by silica gel column chromatography.
The structures of these esters were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopic data.

Cell migration assay (CMA)

1. The MDCK cells were inoculated into wells (2.0×10^4 cells/well) in 24 well plates with DMEM and incubated in a humidified incubator for 24 h at 37 °C with 5% CO_2.
2. A monolayer of cells was observed under inverted tissue culture microscope (× 400).
3. A scratch was made along the vertical axis of each well and washed with phosphate buffered saline (PBS). Each test well was treated with each lupeol esters (25 μM). Initial width (at t=0 h) of the scratch was measured and taken as the mean of five readings. The plates treated with test samples and controls were incubated further for 24 h.
   The CMA was carried out in triplicate.
4. After 24 h, the width of the scratch (at t=24 h) was measured and taken as the mean of five readings.
   The cell migration enhancement was presented as mean percentage wound closure.

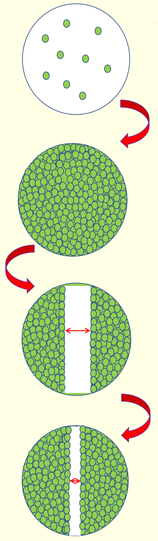

Fig.02: Key steps of cell migration assay

Statistical Analysis

Mean comparisons were performed using ANOVA and the least significant test (LSD) with significance level of $p \leq 0.05$ using the SAS Ver. 9 (1993).

Results and Discussion

- The results of the *in-vitro* scratch wound assay showed that the enhancement of cell migration occurred with the increase in the chain length of fatty acid part of the lupeol esters (*Fig: 03*) .

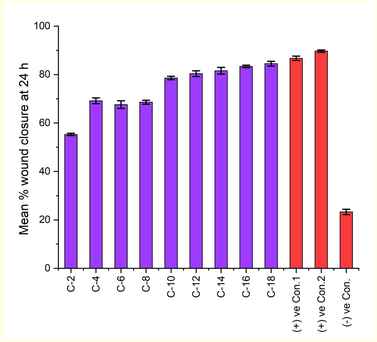

Fig.03: Mean % wound closure at 24 h for the synthesized lupeol esters with the controls. (-) ve Con. -1% DMSO in 20% DMEM, (+) Con. 1 &2 - Lupeol & Asiaticoside (25 μM)

- The cell migration activity of lupeol stearate with different concentrations was determined. The minimum concentration for maximum cell migration activity was found to be 12.5 μM. At this concentration, lupeol stearate showed 87.2% of wound closure (*Fig: 04* and *Fig: 05*).

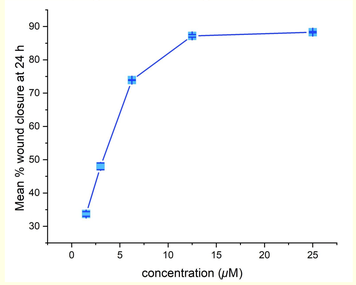

Fig.04: Dose- response curve for lupeol stearate

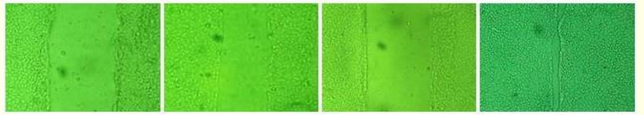

Fig. 05: Microscopic images (× 400) of the wounds in the monolayer of cells at the beginning (t=0 h) and after 24 h (t=24 h) treating with lupeol stearate (LS) (12.5 μM) with a negative control (-) Con.-1%DMSO in 20% DMEM)

Conclusion

It is revealed that the chemical modification of hydroxyl group of lupeol by esterification with long chain fatty acid allows obtaining substances with enhanced cell migration activity.

References

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Abbreviations: DMEM - Dulbecco's Modified Eagle Medium
DMSO - Dimethyl sulfoxide
MDCK- Mardin-Darby Canine Kidney