

# Khat induces premature differentiation and keratinization in organotypic models of normal oral mucosa



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Ochiba M. Lukandu

Division of Non-Communicable Diseases

**Ministry of Public Health and Sanitation, Nairobi, Kenya**

and

Department of Pathology, The Gade Institute

**University of Bergen, Bergen, Norway**



# Overview

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- Introduction
- General aim
- Materials and Methods
- Results
- Conclusion

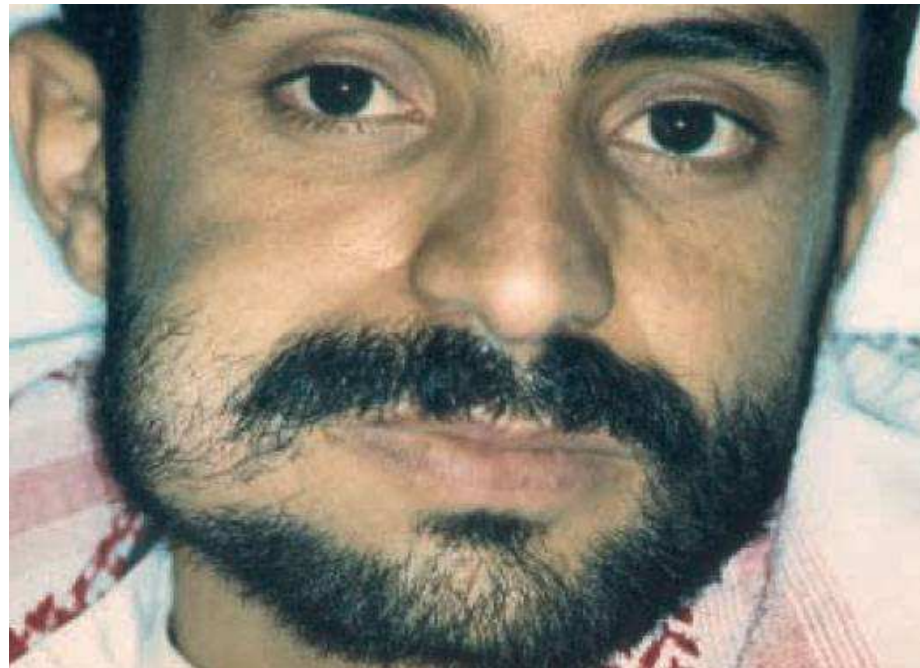
# Introduction

- Khat is an evergreen shrub grown in the Middle East and the horn of Africa



# Introduction

- Khat use is common in the regions where it is grown. (Over 70% of men in Yemen routinely chew khat)



A young man chewing khat (From Ali et al 2004)



# Introduction

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- Reasons for chewing khat:
  - Pyscho-stimulant
  - Medicinal
  - Recreational
- Khat is an important cash crop whose economic value in some regions exceeds coffee and tea.

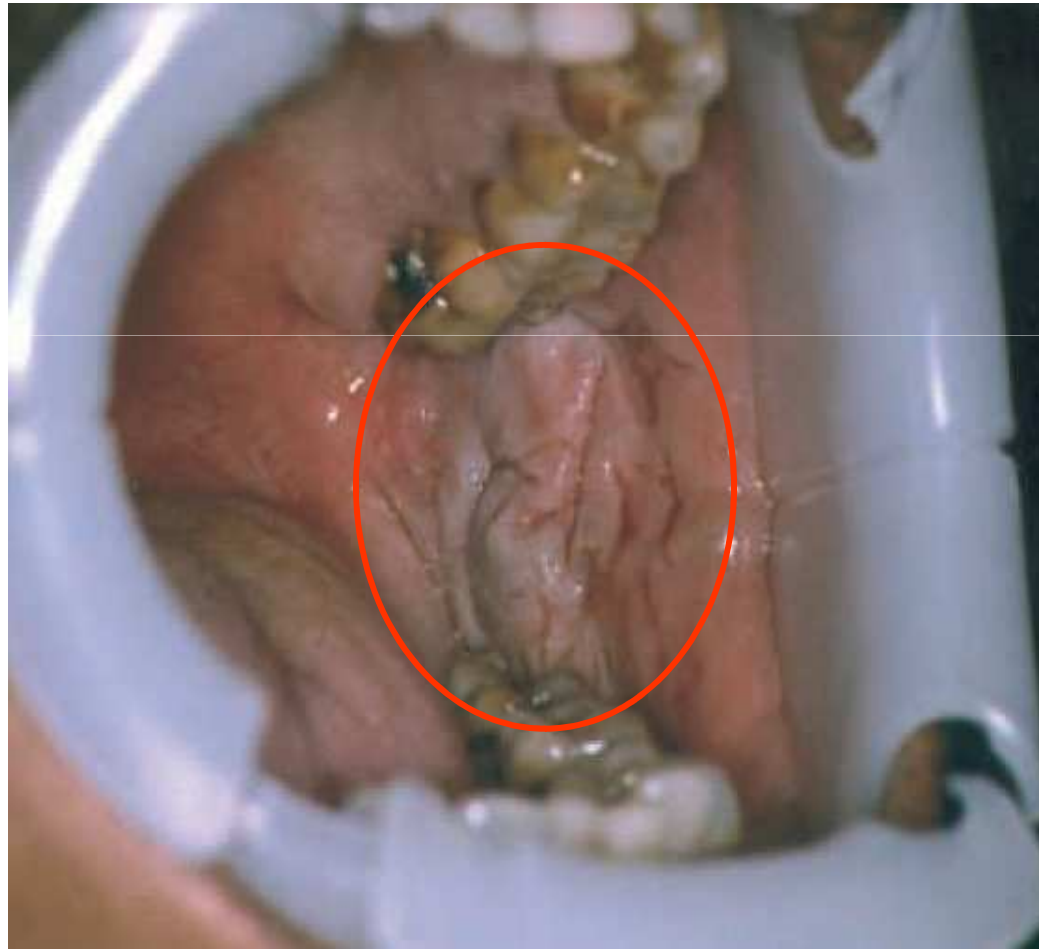


# Introduction

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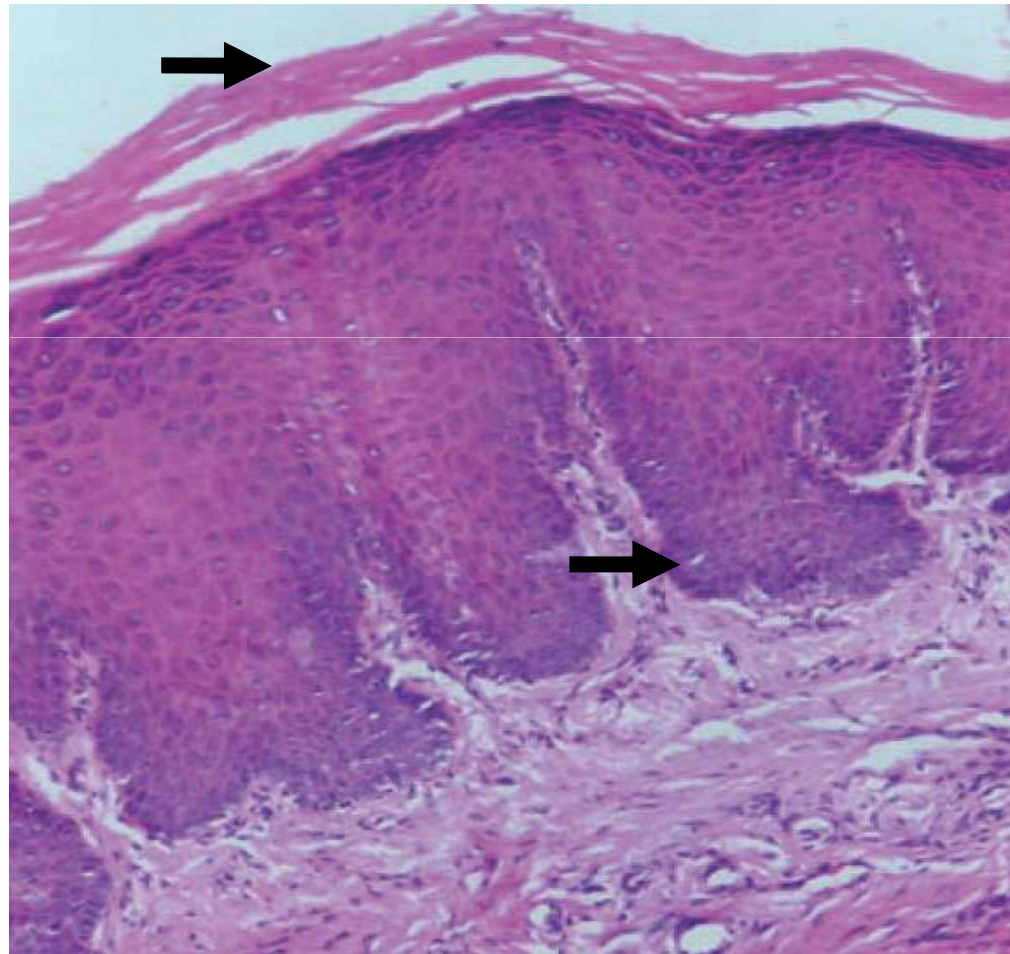
- Adverse effects of khat use – General
  - Psychotic disorders (Odenwald et al 2005)
  - Myocardial infarction (Al-Motarreb et al 2002)
  
- Adverse effects of khat use – Oral
  - Hyperkeratosis (Ali et al 2004, 2006, Gorsky et al 2004)
  - Genotoxicity (Kassie et al 2004)
  - Oral cancer?? (Soufi et al 1991, Nasr et al 2000)

# Introduction



White lesions (From Ali et al 2004)

# Introduction



Histopathology (Hyperkeratosis) (From Ali et al 2006)





## Rationale of study

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- Generally, studies on the toxicological potential of khat are still scarce (Carvalho F. 2003)
- Despite reports on the potential adverse effects of khat use on oral soft tissues, no previous study has looked at biological effects of khat in normal human oral cells *in vitro*



# General aim of the study

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- To investigate the toxic effects induced by an extract of khat on organotypic models of normal oral mucosa



# Materials

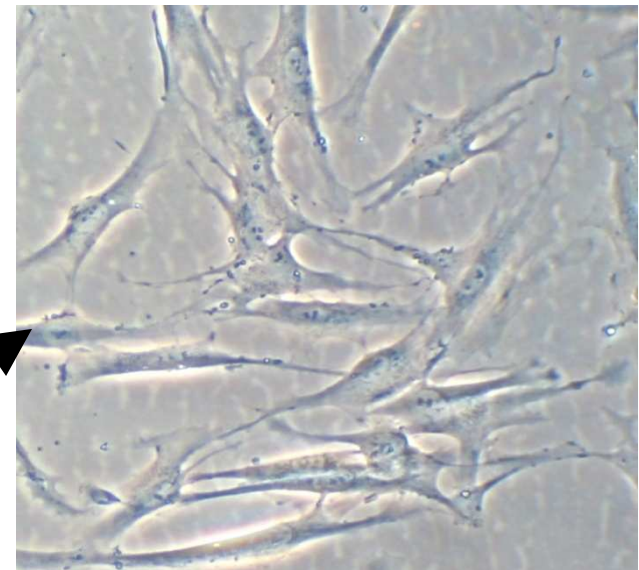
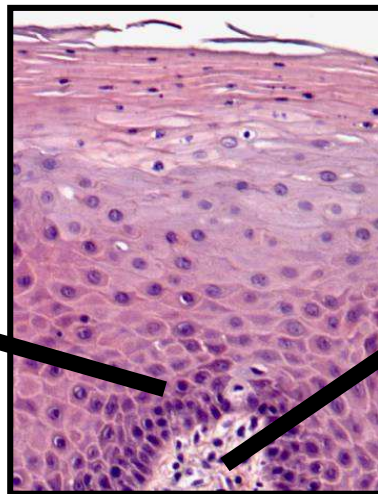
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- Khat

- Fresh khat from the Meru district of Kenya
- Methanolic extraction and dissolved in an organic solvent (DMSO) (Dimba et al, 2004)
- An aqueous extraction (Al Hebshi et al 2005) of khat was also tested for biological effects using fibroblasts
- Frozen stock solutions of khat were thawed and diluted in culture medium to the desired concentrations

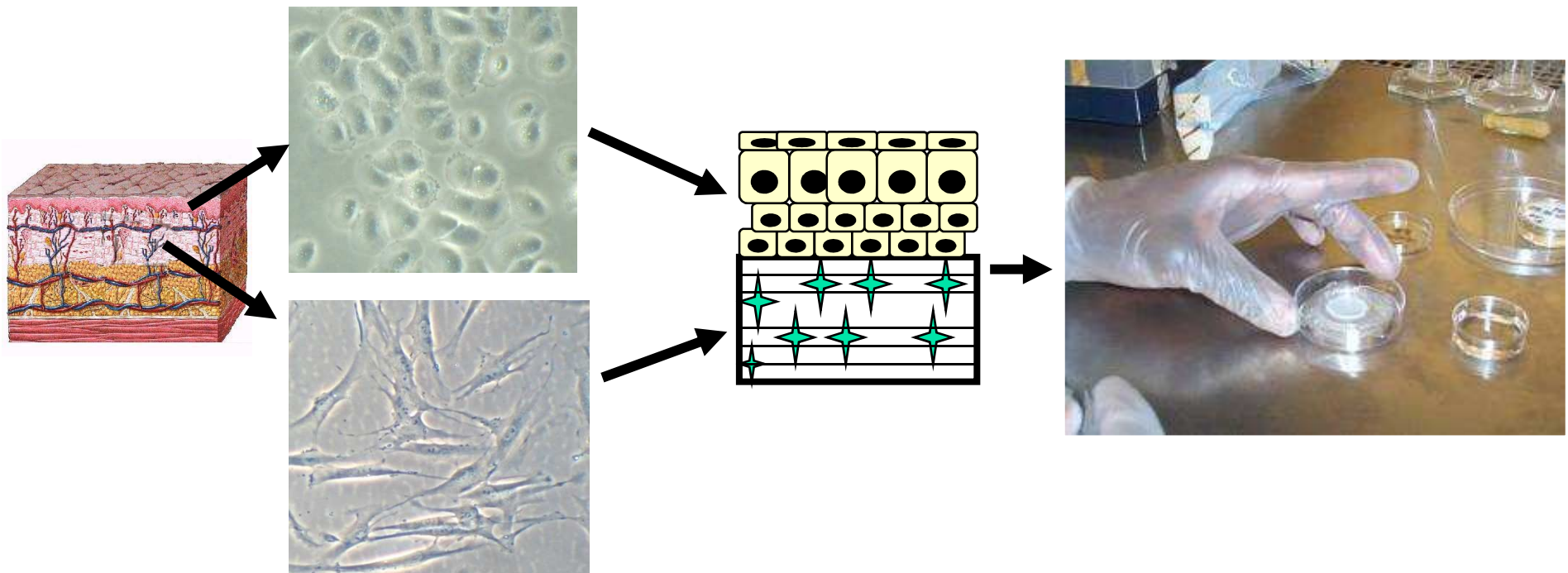
# Materials and methods

- Oral cells
  - Isolated from samples of normal buccal mucosa (Costea et al, 2002 and 2004). Cultured under standard conditions prior to treatment with khat



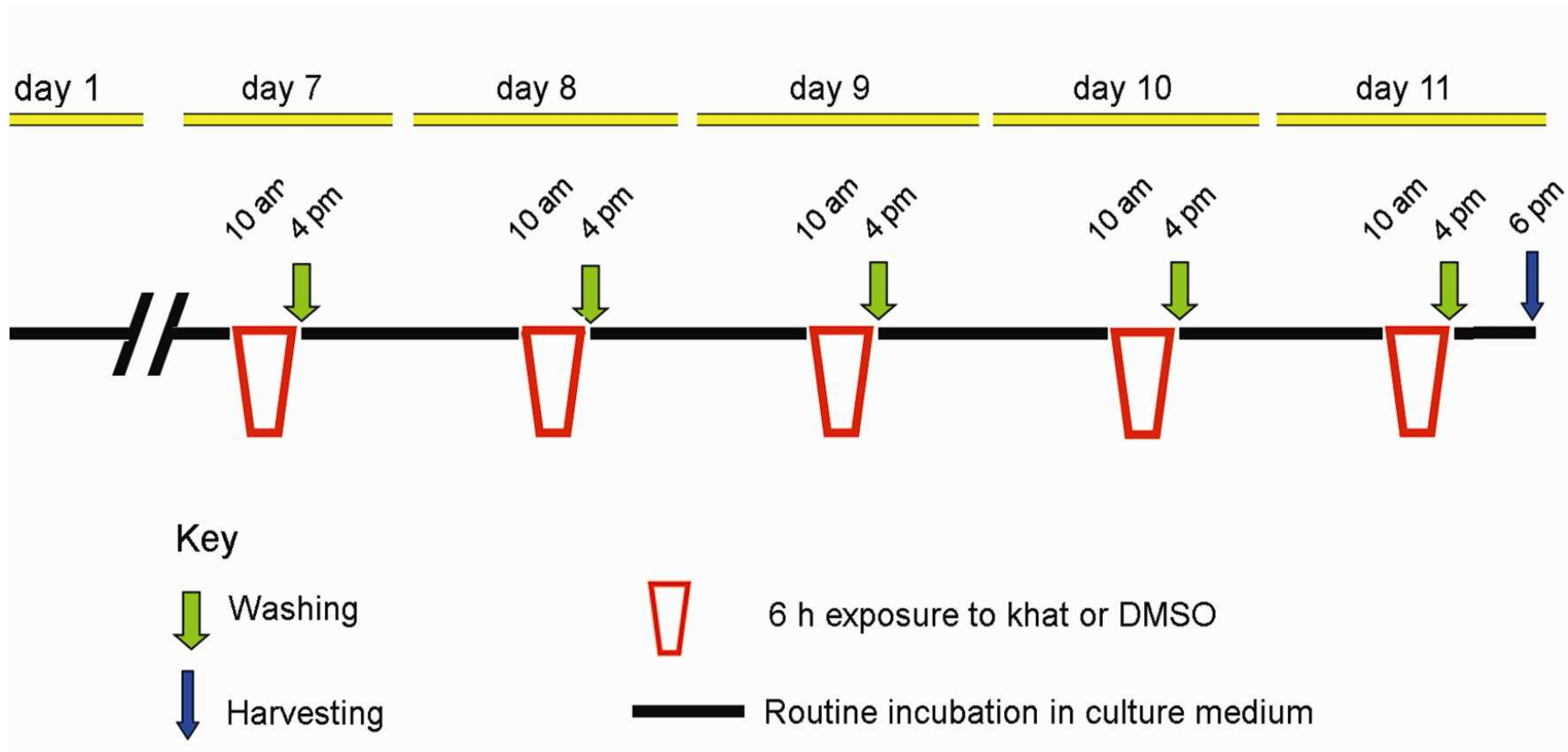
# Materials and methods

- Organotypic culture cells (Reconstitution)



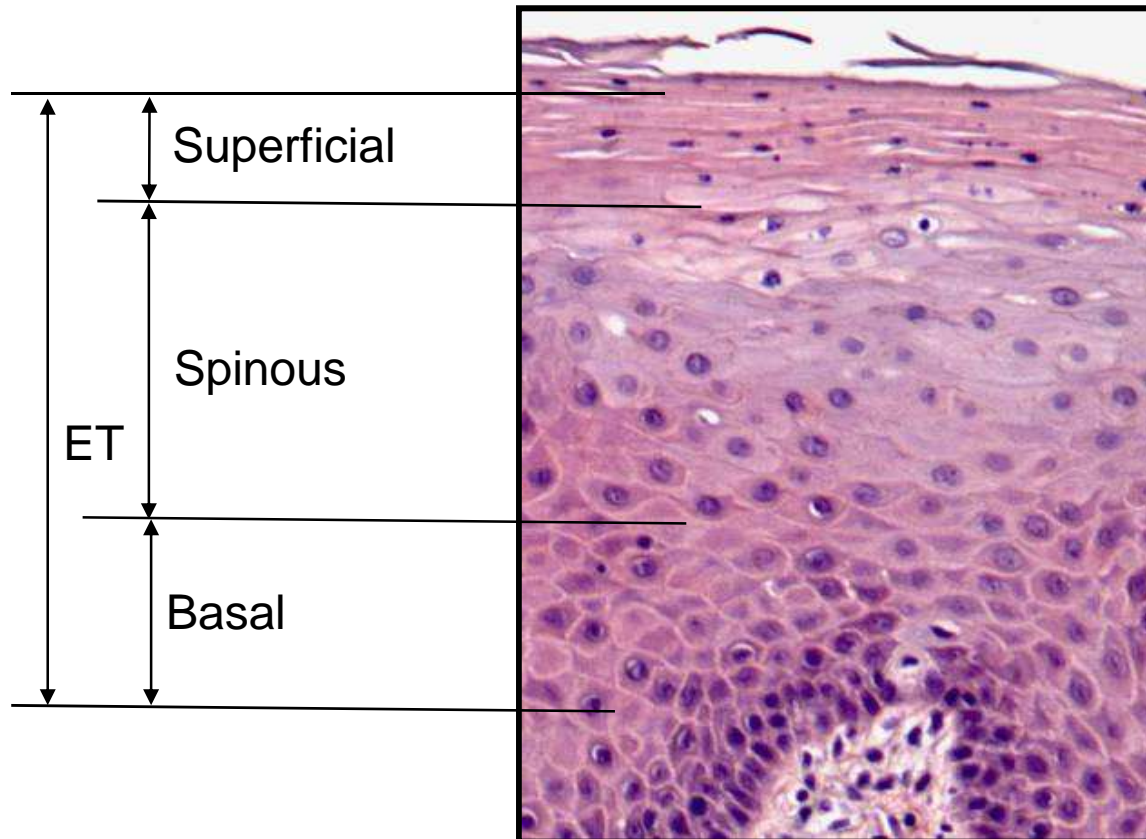
# Materials and methods

- Organotypic culture (Timing of exposure)



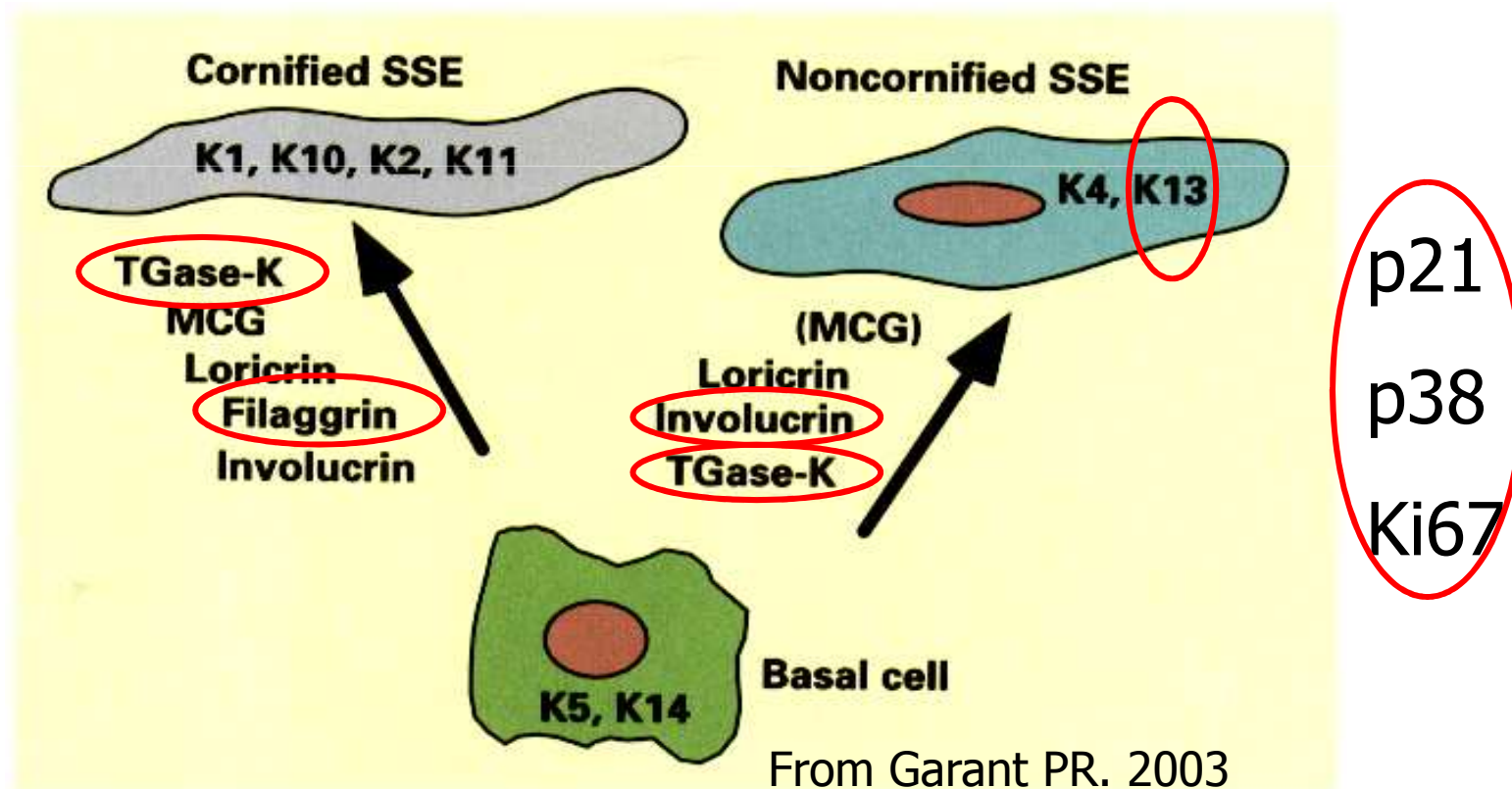
# Materials and methods

- Organotypic culture (Histomorphometry)



# Materials and methods

- Protein expression in oral epithelia

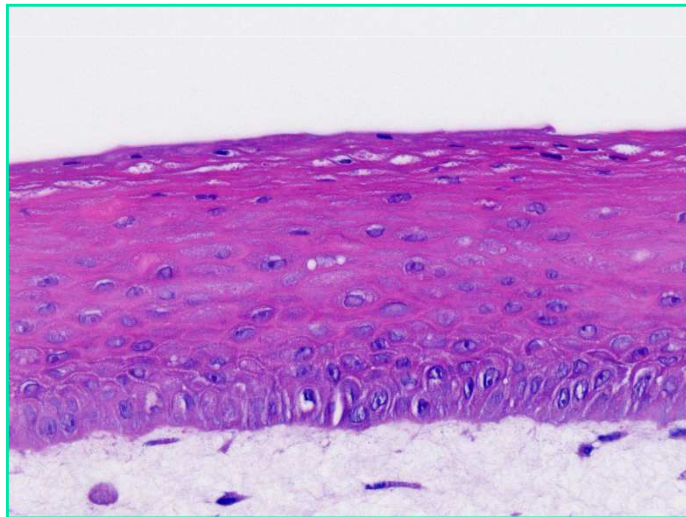




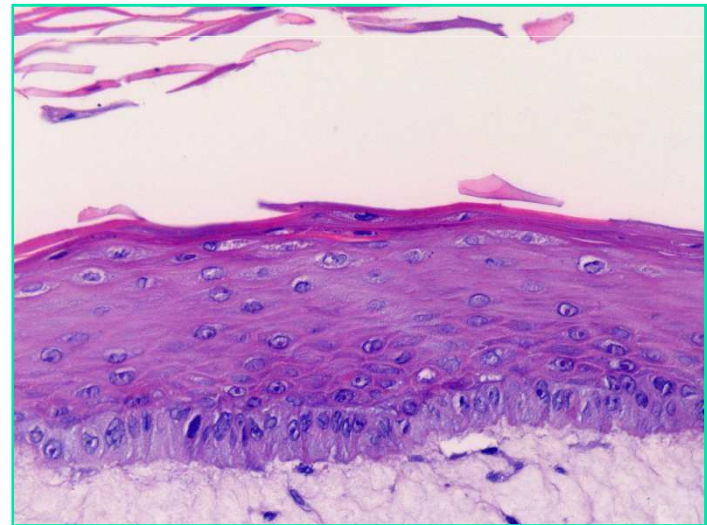
# Results

- Effect of khat on differentiation of oral cells

H & E staining



Control

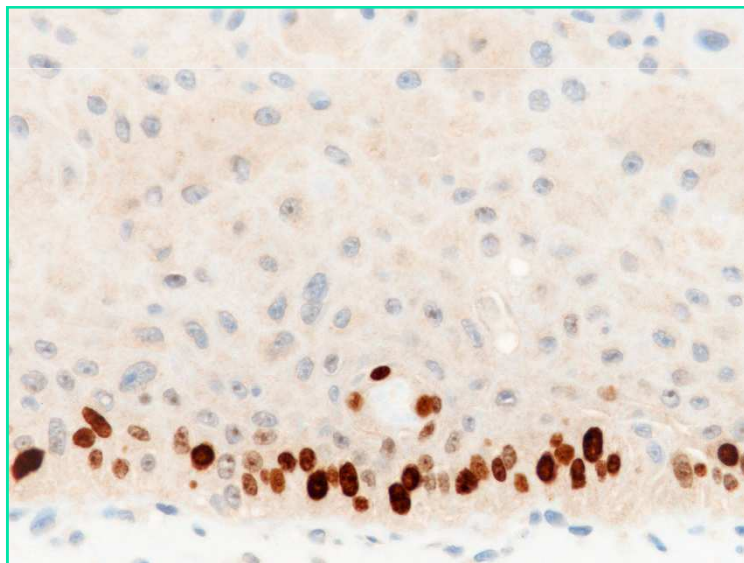


32µg/ml

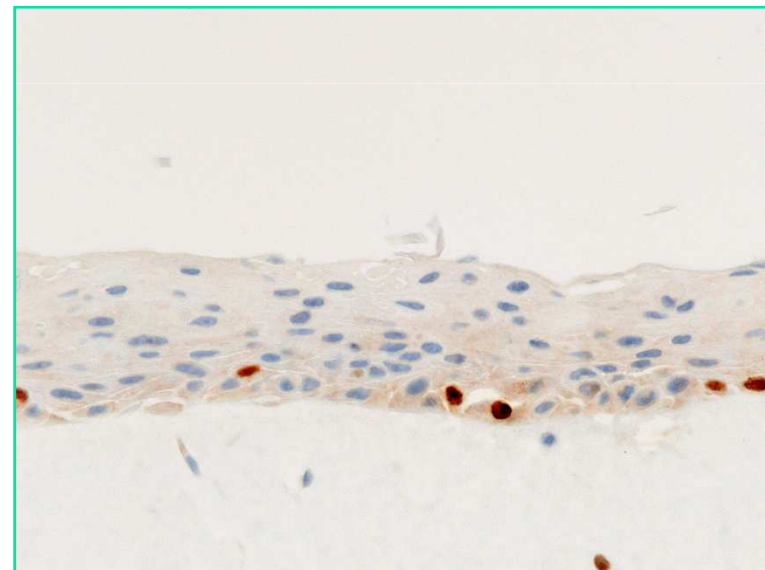
# Results

- Effect of khat on proliferation of oral cells

Ki67 staining



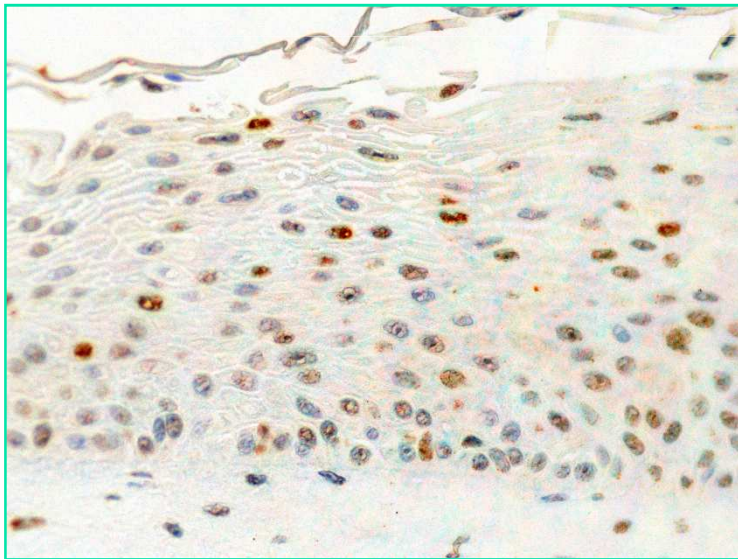
Control



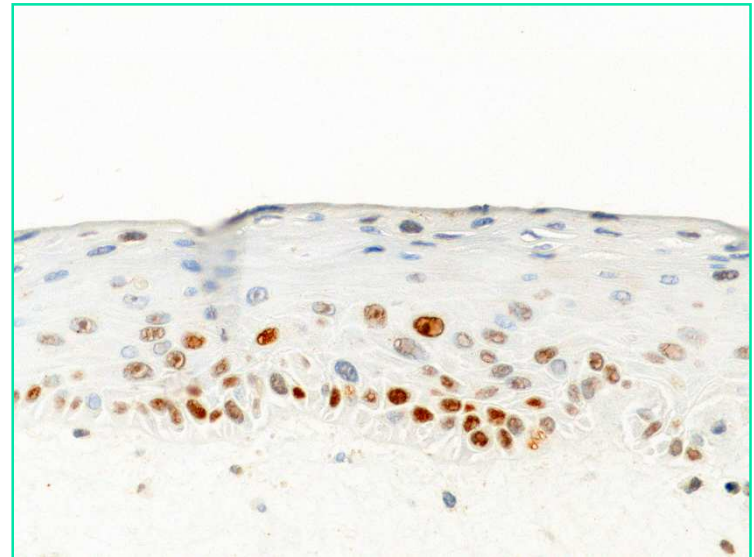
32µg/ml

# Results

- Effect of khat on differentiation of oral cells  
p21 expression



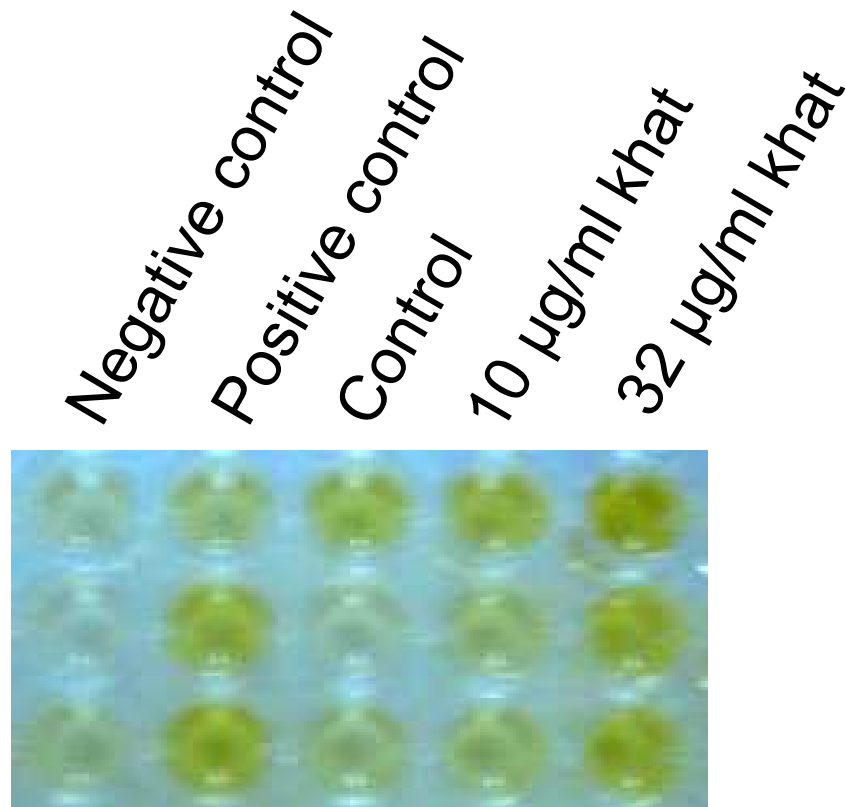
Control



32µg/ml

# Results

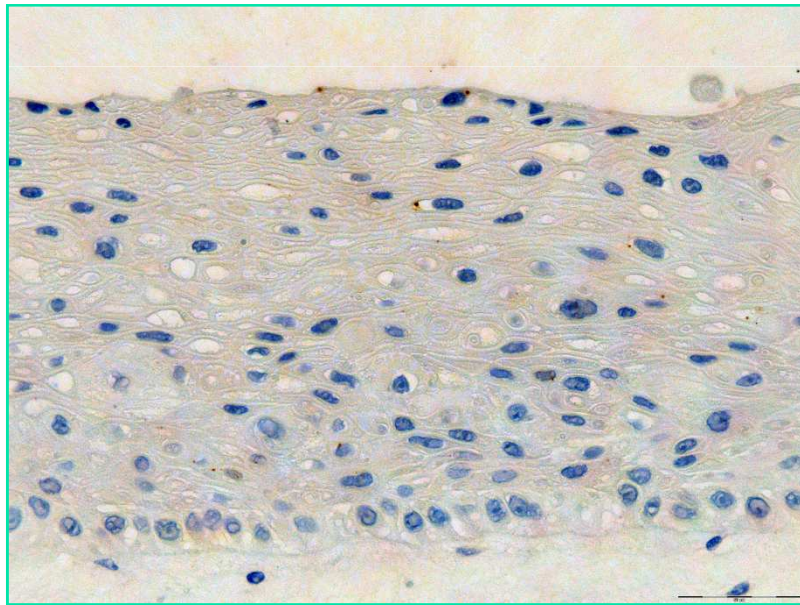
- Effect of khat on Transglutaminase activity



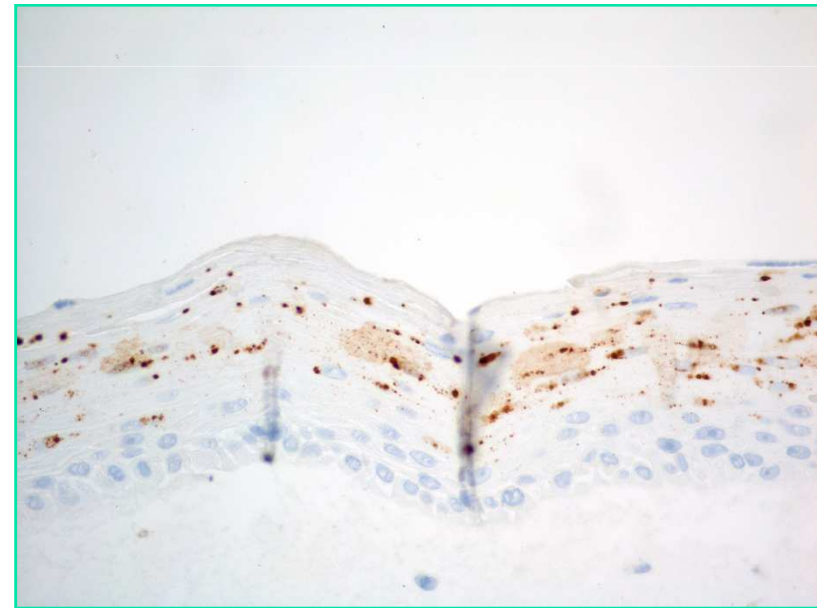
# Results

- Effect of khat on differentiation of oral cells

Fillaggrin expression



Control



32µg/ml



## Some issues of clinical relevance

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- Using low concentrations comparable to those in the oral cavity of khat chewers
- Using whole extract of khat rather than khat specific compounds or fractions (eg cathinone)
- Exposing organotypic cultures for six hour intervals daily



# Concluding remarks

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- Exposure to an extract of khat reduced
  - proliferation of cells within the tissues
  - total epithelial thickness
- Khat induced premature differentiation in oral keratinocytes



## Concluding remarks

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- Khat also induced a switch from nonkeratinising to keratinising phenotype in oral keratinocytes
- The effects of khat on organotypic tissues were possibly mediated through p38 MAP kinase signaling





## Conclusions of the study

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- This study shows that khat has toxic effects on human oral cells and tissues and raises concerns about khat use and the development of various oral lesions.
- Whether these findings could explain the whitening oral mucosa, and hyperkeratosis seen in khat chewers will become clearer with further studies



# Acknowledgement

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