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

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Overview

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

- Reasons for chewing khat:
  - Psycho-stimulant
  - Medicinal
  - Recreational

- Khat is an important cash crop whose economic value in some regions exceeds coffee and tea.
Introduction

- 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
  - Genotoxicity (Kassie et al 2004)
Introduction

White lesions (From Ali et al 2004)
Introduction

Histopathology (Hyperkeratosis) (From Ali et al 2006)
Rationale of study

- Generally, studies on the toxicological potential of khat are still scarce (Carvalho F. 2003)

- Despite reports on the potential adverts effects of khat use on oral soft tissues, no previous study has looked at biological effects of khat in normal human oral cells \textit{in vitro}
General aim of the study

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

- 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)

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 am</td>
<td>Washing</td>
</tr>
<tr>
<td>7</td>
<td>4 pm</td>
<td>Washing</td>
</tr>
<tr>
<td>8</td>
<td>10 am</td>
<td>Washing</td>
</tr>
<tr>
<td>9</td>
<td>4 pm</td>
<td>6 h exposure to khat or DMSO</td>
</tr>
<tr>
<td>10</td>
<td>10 am</td>
<td>6 h exposure to khat or DMSO</td>
</tr>
<tr>
<td>11</td>
<td>4 pm</td>
<td>6 h exposure to khat or DMSO</td>
</tr>
<tr>
<td></td>
<td>6 pm</td>
<td>Routine incubation in culture medium</td>
</tr>
</tbody>
</table>

Key:
- Green down arrow: Washing
- Red triangle: 6 h exposure to khat or DMSO
- Black line: Routine incubation in culture medium

Day 1: 10 am, 4 pm
Day 7: 4 pm
Day 8: 10 am, 4 pm
Day 9: 4 pm
Day 10: 10 am, 4 pm
Day 11: 6 pm
Materials and methods

- Organotypic culture (Histomorphometry)
Materials and methods

- Protein expression in oral epithelia

From Garant PR. 2003
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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Image of Plates</th>
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<tbody>
<tr>
<td>Negative control</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>10 µg/ml khat</td>
<td></td>
</tr>
<tr>
<td>32 µg/ml khat</td>
<td></td>
</tr>
</tbody>
</table>
Results

- Effect of khat on differentiation of oral cells

Fillaggrin expression

Control

32µg/ml
Some issues of clinical relevance

- 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

- 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

- 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

- 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.
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