Evaluation of Alcohol Markers in Urine and Oral Fluid after Kombucha Consumption

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• The authors have no conflict of interest to disclose.
Kombucha

• Sweet and sour drink brewed from tea, sugar, bacteria/yeast

• Natural/ artificial flavorings
  • Juice, spices, extracts
Kombucha

• Potential health benefits
  • Probiotic supplement
  • Immune system booster
  • Antioxidant

• Home-brewing → commercially available
Kombucha

• Fermentation process naturally produces ethanol
  • “Hard” Kombucha
  • Regular Kombucha labeled as “non-alcoholic”

• US FDA Labeling Regulations (CPG Sec 510.400, 2015)
  • Beverages labeled as “non-alcoholic”: ABV < 0.5%

• Liu & Chan et al. (2019)
  • ABV level in “non-alcoholic” Kombucha drinks
  • 1.25 – 2.03%
Forensic Relevance

• DUI/workplace drug testing
  • Unintentional intoxication after consumption
  • “Kombucha” excuse
Purpose of Study

• Determine if alcohol markers, ethyl glucuronide (EtG) and ethyl sulfate (EtS) are present in urine and oral fluid after Kombucha consumption
Study Design

• Approved by SHSU IRB: #IRB-2019-249
  • Written informed consent

• 12 participants (N= 6 Males, 6 Females)
  • Age: 22-27

• Consumed one 16 oz. raw organic Kombucha within 20 min
  • Blueberry ginger (n=6, n=3/gender) or
  • Raspberry lemon ginger (n=6, n=3/gender)
  • Labeled “< 0.5% ABV”
Specimen Collection

**Oral-Eze®**
- n=10/person total

**Kombucha**

**Portable Breathalyzer Test**

**Urine**
- All voids on Day 1
- First void on Day 2 & 3
**Sample Analysis**

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Analysis</th>
<th>Cut off (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Fluid</td>
<td>Immunoassay: EtS</td>
<td>25</td>
</tr>
<tr>
<td>Urine</td>
<td>Immunoassay: EtG</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>LC-MS/MS: EtG,EtS</td>
<td>500, 100</td>
</tr>
</tbody>
</table>

- Positive sample: EtS w/ or w/o EtG
- Kombucha ABV determined by headspace GC
Results and Discussion

• Kombucha ABV: 0.6 – 1.0%
  • Blueberry Ginger > Raspberry Lemon Ginger

• All oral fluid specimens were negative for alcohol markers at all timepoints

• All breathalyzer tests were 0.000 within 1 h after drinking
Results and Discussion

• Positive urine specimen detected by LC-MS/MS:

<table>
<thead>
<tr>
<th>Voids</th>
<th>Positive Counts</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>5</td>
<td>41.7</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>10</td>
<td>83.3</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>6</td>
<td>50.0</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
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<tr>
<td>Day 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Day 3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Results and Discussion

• Urine specimen
  • EtS: 462 (100 – 3700) ng/mL; EtG: 1010 (530 – 2200) ng/mL
  • Of 27 positive samples, 16 were EtS only and 11 were EtS & EtG
Results and Discussion

• Performance of immunoassay vs LC-MS/MS confirm (N=105)

Confirm w/ EtG>500
- True Positive: 10
- True Negative: 94
- False Positive: 0
- False Negative: 1

• Sensitivity: 90.9%
• Specificity: 100.0%

Confirm w/ EtS>100
- True Positive: 10
- True Negative: 78
- False Positive: 0
- False Negative: 17

• Sensitivity: 37.0%
• Specificity: 100.0%
Conclusions

• No alcohol markers were detected in oral fluid

• No alcohol was detected in breath

• EtS and EtG detected above cutoff in urine specimen for 10/12 participants in Day 1
  • Negative on Day 2 & 3

• Kombucha consumption could lead to positive detection of alcohol markers in urine specimen

Questions

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