Kefir administration reduced progression of renal injury in STZ-diabetic rats

Cristina Stewart Bogsan
Pharmaceutical-Biochemical Technology Department
Outline

- Diabetic Mellitus;
- Kefir;
- Oxidative stress;
- Aim;
- Protocol;
- Results;
- Conclusion.
Diabetes Mellitus

Diabetes Mellitus (DM) is a group of metabolic disorders that have in common the hyperglycemia.

DM has become a serious public health problem that affects millions of individuals worldwide (Shaw, 2010).

30% of patients develop nephropathy, the main cause of morbidity and mortality in diabetic patients (Molitch et al., 2004).
Non Diabetic people

1. The TGI processes the food intake in small peptides, lipids and sugars. The glucose promotes the release of incretins hormones.

2. The incretins promote the insulin liberation.

3. The glucose goes to blood and cells.

4. The liver stops to produce glucose.

5. The insulin helps to glucose be internalized by cells.

6. The blood are filtered by kidney and the urine don’t have glucose.

Diabetic people

1. Less incretin hormone is produced than the insulin signaling was not activated.

2. The pancreas work hard to try maintain the homeostasis

3. Glucose are accumulated in the blood.

4. The liver continues to produce glucose.

5. Without insulin the glucose input to cell are compromised

6. The kidneys try to eliminate excess glucose increasing the diuresis and water intake. Glucose are found in the urine.
HYPERGLYCEMIA

Increased ROS production

Impaired NO bioavailability

Endothelial dysfunction

Lipid peroxidation (LPO)

Melandialdehyde (MPA)

Thiobarbituric acid substances (TBARS)

NF-kB Activation

Pro-inflammatory biomarkers

Increase C-reactive protein (CRP)
Kefir

• Kefir is a fermented milk that contains a complex symbiotic mixture of Lactic Acid Bacteria (LAB) and Molds.

• The main microorganisms are:
  o Lactobacillus,
  o Lactococcus,
  o Leuconostoc,
  o Streptococcus,
  o Kluyveromyces,
  o Saccharomyces,
  o Torula.
Kefir properties

Kefir is known for providing benefits to human health through its anti-inflammatory, immune-stimulatory and antioxidant properties.
AIM

This study aimed at assessing the effects of Kefir on oxidative stress in diabetic animals.
STZ - diabetic induction
45 mg/kg iv

Kefir preparation

1. **Skimmed Milk**
2. **20mg/ 100mL Kefir DA Inoculation**
3. **Fermentation until pH 4.6**
4. **Cooling in ice bath**
5. **Stiring (1 min)**
6. **Distribution in 50 mL cups**
7. **Storing (4 °C)**
8. **Kefir fermented milk**
Protocol

STZ → Kefir

5 days → 24 hs → 8 weeks → 24 hs → Sacrificed

Kefir groups intake 1.8 mL/day by gavage
Water to CTL and DM groups

Water and Chow intake
Diuresis, Weight
Fasting Blood tolerance
Creatinine, Proteinuria, T bars, NO

Water and Chow intake
Diuresis, Weight
Fasting Blood tolerance
Creatinine, Proteinuria, T bars, NO

Kidney histology
Metabolic profile, renal function, and oxidative stress

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CTL n=18</th>
<th>DM n=24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake (mL/24 h)</td>
<td>30.0 ±0.9</td>
<td>80.2 ±4.9c</td>
</tr>
<tr>
<td>Chow intake (g/24 h)</td>
<td>19.1 ±0.4</td>
<td>23.9 ±0.9c</td>
</tr>
<tr>
<td>Diuresis (mL/24 h)</td>
<td>13.0 ±0.7</td>
<td>61.1 ±4.6c</td>
</tr>
<tr>
<td>Weight (g/24 h)</td>
<td>269.8 ±5.2</td>
<td>253.7 ±4.0a</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>91.8 ±3.5</td>
<td>293.5 ±12.3c</td>
</tr>
<tr>
<td>NO plasmatic (µM)</td>
<td>89.7 ±12.2</td>
<td>58.9 ±9.0a</td>
</tr>
<tr>
<td>Excretion NO (nmol/24 h)</td>
<td>15.9 ±2.7</td>
<td>1.4 ±0.2c</td>
</tr>
<tr>
<td>TBARS plasmatic (nmol/mL)</td>
<td>3.03 ±0.06</td>
<td>3.17 ±0.06</td>
</tr>
<tr>
<td>Excretion TBARS (nmol/24 h)</td>
<td>86.9 ±5.3</td>
<td>192.4 ±10.4c</td>
</tr>
<tr>
<td>Urea plasmatic (mg/dL)</td>
<td>29.2 ±1.9</td>
<td>55.6 ±4.6c</td>
</tr>
<tr>
<td>Urea urinary (mg/dL)</td>
<td>7.556 ±444</td>
<td>2.403 ±129c</td>
</tr>
<tr>
<td>Creatinine plasmatic (mg/dL)</td>
<td>0.28 ±0.02</td>
<td>0.33 ±0.01</td>
</tr>
<tr>
<td>Creatinine urinary (mg/dL)</td>
<td>138.8 ±20.9</td>
<td>54.0 ±9.9b</td>
</tr>
<tr>
<td>Proteinuria (nmol/24 h)</td>
<td>11.2 ±0.6</td>
<td>21.4 ±1.0c</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. Student's unpaired t test. 
\(^{y}\)Corrected values per 100g weight; \(^{a}\)p<0.05; \(^{b}\)p<0.01; \(^{c}\)p<0.001
Metabolic profile, renal function, and oxidative stress of the groups after Kefir treatment.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CTL</th>
<th>CTLK</th>
<th>DM</th>
<th>DMK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake (mL/24 h)</td>
<td>24.8 ±1.8</td>
<td>25.8 ±3.7</td>
<td>124.4 ±12.4*</td>
<td>94.1 ±14.4 k,c</td>
</tr>
<tr>
<td>Chow intake (g/24 h)</td>
<td>17.3 ±0.6</td>
<td>19.1 ±1.0</td>
<td>36.9 ±2.0*</td>
<td>30.6 ±2.3*</td>
</tr>
<tr>
<td>Diuresis (mL/24 h)</td>
<td>13.1 ±1.0</td>
<td>13.7 ±0.7</td>
<td>90.9 ±9.0*</td>
<td>70.9 ±9.4* k,c</td>
</tr>
<tr>
<td>Weight (A)</td>
<td>67.5 ±4.4</td>
<td>69.3 ±1.6</td>
<td>25.3 ±4.5*</td>
<td>35.3 ±5.9 h</td>
</tr>
<tr>
<td>Plasmatic urea (mg/dL)</td>
<td>31.9 ±1.4</td>
<td>36.2 ±2.5</td>
<td>58.5 ±3.6*</td>
<td>47.6 ±2.4 k,c</td>
</tr>
<tr>
<td>Urinary urea (mg/dL)</td>
<td>8,691 ±343</td>
<td>8,423 ±229</td>
<td>2,006 ±142*</td>
<td>2,996 ±322 h k,c</td>
</tr>
<tr>
<td>Plasmatic creatinine (mg/dL)</td>
<td>0.71 ±0.05</td>
<td>0.72 ±0.04</td>
<td>0.75 ±0.03</td>
<td>0.78 ±0.05</td>
</tr>
<tr>
<td>Urinary creatinine (mg/dL)</td>
<td>131.7 ±92</td>
<td>127.9 ±4.5</td>
<td>33.2 ±5.6*</td>
<td>34.8 ±7.8 h</td>
</tr>
<tr>
<td>Proteinuria (nmol/24 h)</td>
<td>10.4 ±0.8</td>
<td>11.2 ±0.7</td>
<td>25.5 ±3.7*</td>
<td>21.0 ±2.8*</td>
</tr>
<tr>
<td>Plasmatic NO (μM)</td>
<td>66.6 ±4.3</td>
<td>77.8 ±6.6</td>
<td>79.2 ±5.0</td>
<td>76.5 ±5.4</td>
</tr>
<tr>
<td>NO excretion (μmol/24 h)</td>
<td>14.9 ±3.6</td>
<td>17.5 ±3.8</td>
<td>2.1 ±0.7*</td>
<td>16.4 ±4.9</td>
</tr>
<tr>
<td>Plasmatic TBARS (nmol/mL)</td>
<td>3.32 ±0.06</td>
<td>3.16 ±0.08</td>
<td>3.79 ±0.10*</td>
<td>3.58 ±0.17</td>
</tr>
<tr>
<td>TBARS excretion (nmol/24 h)</td>
<td>81.6 ±2.1</td>
<td>84.4 ±4.3</td>
<td>300.4 ±18.9*</td>
<td>248.9 ±19.2 h k,c</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. One-way ANOVA followed by Newman–Keuls Multiple Comparison post test. Control (CTL); control Kefir (CTLK); diabetic (DM); diabetic Kefir (DMK); n = 9–12/group.

* p < 0.001 vs CTL.
* p < 0.01 vs CTLK.
* p < 0.05 vs DM.
Glycemia levels in the 5th day after diabetes induction (0) and 2-4-8 weeks after Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=12; diabetic Kefir (DMK) n=12. Values are expressed as means ± SEM. One-way ANOVA followed by post test Student Newman Keuls. *** $p<0.001$ vs controls; ### $p<0.001$ vs DM
Glycemia levels during oral glucose tolerance test (OGTT) after 8 weeks of Kefir treatment. Control (CTL) n=4; control Kefir (CTLK) n=5; diabetic (DM) n=6; diabetic Kefir (DMK) n=4. Values are expressed as means ±SEM. One-way ANOVA followed by post test Student Newman Keuls. *p<0.05; **p<0.01; ***p<0.001 vs controls; #p<0.05 vs DM.
Renal function

Plasma urea concentration after 8 weeks of Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=12; diabetic Kefir (DMK) n=12. Values are expressed as means ±SEM. One-way ANOVA followed by post test Student Newman Keuls, **p<0.01, ***p<0.001 vs controls; ##p<0.01 vs DM.
Renal function

Urinary urea concentration after 8 weeks Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=10; diabetic Kefir (DMK) n=11. Values are expressed as means ±SEM. One-way ANOVA followed by post test Student Newman Keuls, *** \( p<0.001 \) vs controls; \# \( p<0.05 \) vs DM.
Excretion of nitric oxide (NO) in all groups after 8 weeks of Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=11; diabetic Kefir (DMK) n=12. Values are expressed as means ± SEM. One-way ANOVA followed by post test Student Newman Keuls. *p<0.05 vs CTL; #p<0.05 vs DM.
Oxidative Stress

Excretion of thiobarbituric acid reactive substances (TBARS) after 8 weeks of Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=10; diabetic Kefir (DMK) n=12. Values are expressed as means ±SEM. One-way ANOVA followed by post test Student Newman Keuls. ***p<0.001 vs controls, #p<0.05 vs DM.
Plasma C-reactive protein (CRP) levels after 8 weeks of Kefir treatment. Control (CTL) n=5; control Kefir (CTLK) n=5; diabetic (DM) n=6; diabetic Kefir (DMK) n=6. Values are expressed as means ±SEM. One-way ANOVA followed by post test Student Newman Keuls. *p<0.05 vs CTL.
Kidney histology

Renal histology after the 8th week of Kefir treatment. (A) Kidneys showed glycogen storage in diabetic rats tubules (shown by arrows). (B) Average glycogenated tubules represented graphically in each group (B). Control (CTL); control Kefir (CTLK); diabetic (DM); diabetic Kefir (DMK). Values are expressed as means ±SEM. n=3 per group. One-way ANOVA followed by post test Student Newman Keuls, ***p<0.001 vs controls; ###p<0.001 vs DM.
Conclusion

The results obtained in this study show that **Kefir treatment significantly reduced** the progression of STZ-induced **hyperglycemia and oxidative stress** in rats.

Kefir may play a role in **slowing the metabolic changes** that contribute to DM as a non-pharmacological adjuvant.
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cris.bogsan@usp.br