Effect of Kefir on the Gene Expression Profiles of GSTM1 and GSTT1 with Antioxidant Defence in the Environment of Detoxification of Aflatoxin an in Vivo Study*,**

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Abstract

This study has been achieved to observe the effects of low dose aflatoxin in mice on lipid peroxidation, antioxidant enzyme activity, some functional hepatic enzymes together with histopathological findings in tissue and gen expressions that codes the antioxidant enzymes and investigate the activities on reducing or completely eliminating these effects of the kefir contents. Study has been performed on four groups by applying 300 ppb AFB₁, 300 ppb AFB₁+30 ml/kg kefir, 30 ml/kg kefir together with standard diet during 45 days and control (standard diet) to Swiss albino mice which are average 12 weeks age.

In the study ALT, AST, LDH and albumin measurements were performed by auto-analyzer. MDA, GSH and GST activity by spectrophotometric method and GSTM1 and GSTT1 gen expressions were analyzed by using RT-PCR. Tissues were observed to evaluate the histopathological differences by haematoxylin-eosin (H.E.) staining.

When our findings have been considered, we observed a weight loss in AFB₁ group while weight gain was concordant with the control group in kefir consumed group together with AFB₁. We observed a significant difference (p<0.05) between kefir given group together with aflatoxin and aflatoxin given group in ALT and LDH values. While the lipid peroxidation (MDA) increased significantly in aflatoxin given group with respect to the control group, the decrease in MDA was significant in kefir given group together with aflatoxin (p<0.01). In parallel with this finding, we observed a statistically significant difference in GSH concentration and GST activity between two groups. GSTM1 gen expression profiles were statistically significant in aflatoxin and aflatoxin+kefir groups (p<0.05). In histopathological investigations of the liver and kidney, necrosis were recognized in AFB₁ given group while there was an evident decrease in the intensity of lesions in the kefir given group together with aflatoxin. These positive effects might depend on the possibility that the probiotic microorganisms and organic compounds in the kefir drink might support the antioxidant defence in the body by effecting the phase II reaction of the xenobiotic metabolism.

Key words: Aflatoxin, Kefir, Probiotic, Liver, Kidney

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Introduction

A mycotoxin is a toxic secondary metabolite produced by an organism of the fungus kingdom, including mushrooms, molds, and yeasts. Mycotoxins are non-volatile, relatively low-molecular weight secondary metabolites of certain fungi that are toxic to human beings, plants and animals. The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms. Mycotoxicosis is the poisoning by ingestion of mycotoxins through food contaminated by toxigenic fungi. Mycotoxins comprise a structurally diverse and chemically complex group of fungal metabolites and many of which have been implicated as significant health hazards on a world wide scale. Research suggests that mycotoxins can decrease the formation of glutathione due to decreased gene expression of the enzymes needed to form glutathione. Mycotoxin-related compromise of glutathione production can result in an excess of oxidative stress that leads to tissue damage and systemic illness (1,2).

Aflatoxins represent a relevant group of mycotoxins produced by Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius. These molds principally invade plant tissue, in particular when damaged, and mainly produce aflatoxin B1 (AFB1). International Agency for Research on Cancer (IARC) has classified AFB1 as Group 1 human carcinogen. Because it has been associated with hepatocellular carcinoma in humans. More risk for the occurrence of AFB1 is commonly associated with foodstuffs produced in warm climates (3-5). According to the results of Liu and Wu (2010) has found that aflatoxin exposure may contribute to 28.2% of all liver cancer case in the worldwide (6). Symptoms of chronic aflatoxin intoxication in cattle include decreased appetite, weight loss, milk yield, and feed efficiency and liver damage (7,8).

Probiotic bacteria have been identified as a potential means to reduce availability of AFB1 as well as other food contaminants. Intestinal mucus has been found to compete with AFB1 binding sites on the surface of bacteria. Furthermore, AFB1 induced hepatotoxicity was slightly reduced and weight loss was alleviated in rats dosed with probiotics (9). The name probiotic comes from the Greek 'pro bios' which means 'for life'. The history of probiotics began with the history of man; cheese and fermented milk were well known to the Greeks and Romans, who recommended their consumption, especially for children and convalescents. Probiotics are defined as the living microorganisms administered in a sufficient number to survive in the intestinal ecosystem. They must have a positive effect on the host (10).

Kefir-a traditional beverage whose consumption has been associated with health benefits-is a logical natural product to investigate for new probiotic strains. Kefir is a traditional fermented milk originating from the Caucasus mountains. It is a self-carbonated dairy product with a slightly acidic taste, yeasty flavor, creamy consistency, and low percentage of alcohol. Many health benefits have been attributed to kefir, including the enhancement of the immune system and improvement of digestive health, as well as antimicrobial, antitumoral, antiviral, antimutagenic, and antioxidant activity (11-13). Kefir has been assigned a variety of health claims in addition to its nutritional value. Many studies regarding
Kefir’s biological activities have established that kefir has anti-inflammatory activity, immune-modulating activity, antimicrobial activity and anti-proliferative activity, and it has the potential to become a type of functional food (14-15).

This study has been achieved to observe the effects of low dose aflatoxin in mice on lipid peroxidation, antioxidant enzyme activity, some functional hepatic enzymes together with histopathological findings in tissue and gen expressions that codes the antioxidant enzymes and investigate the activities on reducing or completely eliminating these effects of the kefir contents.

Materials and Methods
All experimental applications was carried out in the labs of Kafkas University, Faculty of veterinary medicine and and science faculty. Genetic studies was also carried out in molecular biology Laboratories of the Middle East Technical University under the control of expert personnel advice.

The animals used in the study were obtained from the Kafkas University Department of Biology, Faculty Sciences. According to the experimental practices and nutritional status of the four groups is as follows:

- **Group A**: Standard mouse bait + water (control group)
- **Group B**: Standard mouse bait + water + 300 ng/kg Aflatoxin B1
- **Group C**: Standard mouse bait + water + 300 ng/kg Aflatoxin B1 + 30 ml/kg of kefir
- **Group D**: Standard mouse bait + water + 30 ml/kg of kefir

Study has been performed on four groups by applying diet during 45 days and control (standard diet) to Swiss albino mice which are average 12 weeks age.

Standard mouse bait used in the study was provided from Bayramoglu feed mill Inc. (Erzurum). AFB1 was provided from Ankara Provincial Control Laboratory of the Ministry of Agriculture and Rural Affairs within the solution of 1 µg/ml acetonitrile/toluene (% 2). Kefir was obtained from kefir grains from Ege University, Faculty of Agriculture.

In the study, ALT, AST, LDH and albumin measurements were performed by auto-analyzer, MDA, GSH and GST activity by spectrophotometric method and GSTM1 and GSTT1 gen expressions were analyzed by using RT-PCR. Tissues were observed to evaluate the histopathological differences by haematoxylin-eosin (H.E.) staining.

Statistical analysis
Mean and standard deviation (SD) were calculated for continuous variables. The normality of the variables was analyzed by Kolmogorov–Smirnov test. Kruskall Wallis and Mann-Whitney of non-parametric test were used, because of non-normality distributions. Before and after each group, experiment with variations in weight, the weight is the only factor to compare changes between repeated measures ANOVA was used for two-factor model. Because of the complicated patterns for two-factor ANOVA, between the groups and the analysis of the measurements in the mixed together of the pattern within the groups frequently used a multifactorial analysis. Includes two factor mentioned in the pattern. In this study, experimental operations (control, aflatoxins, aflatoxin kefir and yogurt) the first factor; live weight is the measurements for preliminary and final (repeated measures) is the second factor. Two-sided p values were
considered statistically significant at P≤0.05. Statistical analyses were carried out by using the statistical packages for SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The distribution of body weight averages at the before and after experiment for the animals live in groups was shown in Table 1.

Table 1: The distribution of body weight averages at the beginning and the end of the experiment

<table>
<thead>
<tr>
<th>Experiment Groups</th>
<th>Weight before experiment (gr)</th>
<th>Weight after experiment (gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>(\overline{x})</td>
</tr>
<tr>
<td>Control (A)</td>
<td>10</td>
<td>34.74</td>
</tr>
<tr>
<td>Aflatoxin (B)</td>
<td>10</td>
<td>38.22</td>
</tr>
<tr>
<td>Aflatoxin+Kefir (C)</td>
<td>10</td>
<td>32.07</td>
</tr>
<tr>
<td>Kefir (D)</td>
<td>11</td>
<td>34.29</td>
</tr>
</tbody>
</table>

As shown in Table 1, the average weight of the three groups which are Control (A), Aflatoxin+Kefir (C) and Kefir (D) were increased. However the weight average in the group of Aflatoxin (B) was decreased. These changes were shown by Figure 1.

Figure 1. The weight changes for four groups
Two way ANOVA test were used to test Group x Weight effect. Different groups, the effects of the repeated measures with the weight has been found to be significant \( F(1,37) = 8.497, p<.01 \). Experimental operations on four group has created a significant differentiation on the weights \( F(1,37) = 3.027, p<.05 \). In addition, with regard to the effect of measuring, the weight before and after experiment was found significant \( F(1,37) = 4.310, p<.05 \).

In the study, ALT, AST, LDH and albumin measurements were performed by auto-analyzer, MDA, GSH and GST activity by spectrophotometric method and GSTM1 and GSTT1 gen expressions were analyzed by using RT-PCR. Tissues were observed to evaluate the histopathological differences by haematoxylin-eosin (H.E.) staining.

When our findings have been considered, we observed a weight loss in AFB1 group while weight gain was concordant with the control group in kefir consumed group together with AFB1.

We observed a significant difference (P<0.05) between kefir given group together with aflatoxin and aflatoxin given group in ALT and LDH values.

While the lipid peroxidation (MDA) increased significantly in aflatoxin given group with respect to the control group, the decrease in MDA was significant in kefir given group together with aflatoxin (P<0.01). In parallel with this finding, we observed a statistically significant difference in GSH concentration and GST activity between two groups. GSTM1 gen expression profiles were statistically significant in aflatoxin and aflatoxin+kefir groups (P<0.05).

The graphical view of the band fields according to the specific density measurements obtained as a result of the experimental groups, the comparison of the average density values and control genes were summarized in Figure 2 for GSTM1 and in Figure 3 for GSTT1.
Figure 2. The comparison the GSTM1 density curved line with the control genes.
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Aflatoxin</th>
<th>Aflatoxin+kefir</th>
<th>Kefir</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTT1</td>
<td>OD: 2984,173</td>
<td>OD: 2955,17</td>
<td>OD: 2986,651</td>
<td>OD: 2560,52</td>
</tr>
<tr>
<td>β-aktin</td>
<td>OD: 2774,651</td>
<td>OD: 3268,358</td>
<td>OD: 3.601</td>
<td>OD: 3.135</td>
</tr>
</tbody>
</table>

Figure 3. The comparison the GSTT1 density curved line with the control genes
Histopathological findings of four groups of rats were examined as follows by identified pictures. It has been not observed any macroscopic findings in all mice.

No any evidence of microscopic examination was detected of the liver in control (Picture 1) and just only kefir (Picture 2) groups.

Picture 1: A control group of mice with normal liver histology, x185, H.E.

Picture 2: Only kefir group of mice with normal liver histology, x185, H.E.
Picture 3. Liver has severe degree of hydropic degeneration with focal necrosis in the midzonal area in the group of just given aflatoxin, x185, H.E.

Picture 4. Hepatocytes with perisentral formations of the vacuole in the group of just given aflatoxin.

Picture 5. Focal necrosis in the midzonal area of the mice liver in the group of just given aflatoxin (N), x185, H.E.
Picture 6. Mild hydropic degeneration in the mice's liver in the group given aflatoxin with kefir, x92, H.E.

Picture 7. Close view of mild hydropic degeneration in the mice's liver in the group given aflatoxin with kefir, x185, H.E.

Picture 8. Normal histological appearance of a mice kidney in the control group, x370, H.E.
Picture 9. Normal histological appearance of a mice kidney in the group of just given kefir, x370, H.E.

Picture 10: Moderate hydropic dejanarations and rare necrosis in the epitels, hyperemia in intertubuler area in a mice kidney tubulus of the group just given aflatoxin, x370, H.E.

Picture 11. Mild hydropic degeneration and hyperemia in intertubuler area of the mice kidney in the group given aflatoxin with kefir, x370, H.E.
Discussion

This study has been achieved to observe the effects of low dose aflatoxin in mice on lipid peroxidation, antioxidant enzyme activity, some functional hepatic enzymes together with histopathological findings in tissue and gene expressions that code the antioxidant enzymes and investigate the activities on reducing or completely eliminating these effects of the kefir contents.

Morphological impairment of the liver was made by AFB1. It suggested that feeding 0.4 mg/kg AFB1-contaminated diet resulted in adverse effects on blood parameters and liver morphology. The main target of the most hazardous Aflatoxin B1 (AFB1) among the aflatoxins is liver. The results indicated the adverse effects to liver caused by AFB1 were obviously serious. One of the important reasons may be that mycotoxins cause oxidative stress in liver (16,17). Unfortunately, aflatoxins contaminate approximately 25% of agricultural products worldwide. According to the result of studies, they can cause liver failure and liver cancer. Aflatoxins have a variety of hepatotoxic and carcinogenic characteristics. Chronic exposure has been linked to hepatocellular carcinoma (18-20).

In the current study histopathological investigations of the liver and kidney, necrosis were recognized in the liver of rats with AFB1 (300 ng/kg) group. Hyperemia and hydropic degeneration was observed and hydropic degeneration, necrosis, glomerular epithelium and a small number of intertubuler were identified. These serious negative effects were determined with low dose of AFB1 as 300 ng/kg.

The effects of probiotic yeast Saccharomyces boulardii as a biotherapeutic agent is well known. In addition to their nutritive value, probiotic yeasts are generally resistant to gastrointestinal passage and to most antibiotics. They do not appear to alter or adversely affect the normal flora of the intestine and can be consumed with normal probiotic bacteria. S. boulardii reduces the growth of Clostridium albicans, Escherichia coli, Salmonella, Shigella dysenteriae, Vibrio cholerae, Salmonella enteritidis, and Clostridium difficile (21). One of the advantages of probiotics compared to antibiotics is to preserve commensal bacteria and not to select resistant bacteria. No adverse effect and especially no bacteremia caused by probiotics were reported. No adverse effect was reported after administration of the probiotic. The use of probiotics to prevent respiratory infections was the object of numerous studies (22).

Trials have shown that probiotics may be beneficial to patients with alcoholic cirrhosis by decreasing hepatic encephalopathy, improving liver biochemistry and decreasing the rate of infections after liver transplantation and other surgery (23).

A major instigate of liver disease is an anomaly in the gut flora. A balanced and healthy gut prevents a high percentage of harmful liver conditions. Probiotic administration is safe, inexpensive and a noninvasive strategy as compared to antibiotic therapy and surgery. The expanding usage of antibiotics has resulted in the emergence of drug-resistant strains which pose a serious threat to humankind survival. Furthermore, the probiotic therapy shows no severe side effects unlike antibiotic therapy. Although results from clinical trials performed on common liver diseases showed the positive effects of probiotics, there are two problems that limit the usage of probiotics as a routine therapy. Since functional mechanisms of
probiotic are specific to strain, recognize special strains with the highest prophylactic, and preventive properties on liver disease may be required. Also, engineering probiotics for specific, desirable properties might be useful. Lastly, to confirm the viability of bacteriotherapy, more clinical trials in various countries with disparate races, ethnicity, and lifestyles would be required (24).

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, both in adults and in children. NAFLD represents a spectrum of liver diseases that range from hepatic steatosis to steatohepatitis and cirrhosis. However, NAFLD is more prevalent in overweight and obese individuals. Evidences thus far suggest that hepatic triglyceride accumulation is not always derived from obesity; gut microbiota can also play a role in the development of insulin resistance, hepatic steatosis, necroinflammation and fibrosis. On the other hand, probiotics can strengthen the intestinal wall, reducing its permeability, bacterial translocation, and endotoxemia according to animal and human studies. They can also reduce oxidative and inflammatory liver damage, while improving the histological state in certain situations (25). Several studies have provided evidence that probiotics may reduce NAFLD progression and have preventive and therapeutic effects (26-30).

Kefir—a traditional beverage whose consumption has been associated with health benefits—is a logical natural product to investigate for new probiotic strains. Kefir contains live active, growing living cultures of very strong strains of normal flora. Statistical data show that people who consume kefir in their diet are longevous. The data suggest that probiotic bacteria in the gut of kefir consumers are abundant and diverse, and microbial communities in the gut are closely correlated with health (15,31). In our study, the positive effects of kefir’s were observed like the results of the study reported above. Liver lesions and reduction in hydropic degeneration were observed in the liver of rats with kefir group.

When our findings have been considered, we observed a weight loss in AFB1 group while weight gain was concordant with the control group in kefir consumed group together with AFB1. We observed a significant difference between kefir given group together with aflatoxin and aflatoxin given group in ALT and LDH values. The protective effect of kefir has been observed especially in terms of AST and LDH. While the lipid peroxidation malondialdehyde (MDA) increased significantly in aflatoxin given group with respect to the control group, the decrease in MDA was significant in kefir given group together with aflatoxin. In parallel with this finding, we observed a statistically significant difference in Glutathione (GSH) concentration and Glutathyon S transferaz (GST) activity. Glutatyon S transferaz M1 (GSTM1) gen expression profiles were statistically significant in aflatoxin and aflatoxin+kefir groups. Glutathione-S-transferase (GST) performed at the major role in genetic damage cellular detoxification.

The same effects of kefir were found and it has been explained that anti-hypercholesterolemic effect of kefir may be due to its antioxidative and antilipidemic effects. Kefir has played an inducible role on the glutathione GSH, glutathione peroxidase GSH-Px and MDA (32,33).

These positive effects might depend on the possibility that the probiotic microorganisms and organic compounds in
the kefir drink might support the antioxidant defence in the body by effecting the phase II reaction of the xenobiotic metabolism. It was concluded that kefir could play an antioxidant role, may prevent the oxidative damage and it has also a protection against intoxication from aflatoxin B1.

References


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