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# Effects of kefir on liver function tests and histopathological changes in rats exposed to aflatoxin B1

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## Abstract

**Background:** Aflatoxin B1 (AFB1) is one of the most important mycotoxins that contaminate food worldwide. Long-term consumption of foods contaminated with AFB1 endangers human health. Detoxification of AFB1 from food improves community health. A Specific approach to aflatoxin reduction is the use of probiotics. Kefir drink is a strong probiotic. The purpose of this study was to investigate the protective effect of kefir drink on AFB1-induced hepatic injury in adult male rats

**Methods:** In this experimental study, 24 adult rats weighing between 150 and 200 g were used. The rats were randomly divided into 4 groups: 1) control, 2) AFB1 (50 µg/kg body weight), 3) kefir drink (10 mL/kg body weight), and 4) AFB1 + kefir drink. Aflatoxin and kefir drink received through oral gavage. At the end of the experiment (8 weeks), blood and liver samples were collected for different assays. Liver function tests and histopathological examinations were performed. Data were analyzed using 1-way analysis of variance (ANOVA) and at a significance level of $<0.05$.

**Results:** Aflatoxin B1 significantly increased serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (T.Bili), as well as decreased total protein (T.P) content, compared to the control group ($P < 0.05$). Aflatoxin B1 induced histological changes in the liver. The results obtained from the groups treated with kefir drink with and without AFB1 were not significantly different from the control group. Histopathological changes were not found in groups treated with kefir drink with and without AFB1.

**Conclusion**: The consumption of kefir drink reduced AFB1-induced disruptions in rats' livers.

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## Introduction

Aflatoxins are one of the most important types of mycotoxins produced as secondary fungal metabolites by *Aspergillus* genera (mainly *Aspergillus flavus* and *A. parasiticus*) that contaminate food and animal feed worldwide (1). More than 18 types of aflatoxin have been introduced. Among them are aflatoxin B1 (AFB1), AFB2, AFG1, and AFG2, which significantly contaminate food sources. In addition, AFM1 and AFM2 are synthesized in the animal body that consumes food contaminated with AFB1 and AFB2 (2). A published epidemiological study showed a strong association between AFB1 use and cancer incidence, and the International Agency for Research on Cancer (IARC) has classified AFB1 as a group 1 carcinogen (3). The main biological effects of aflatoxins are acute and chronic liver disease, but liver toxicity, carcinogenicity, mutagenicity, teratogenicity, and suppression of the immune system are also among their effects (4). Long-term consumption of foods contaminated with aflatoxins can damage liver cells, impair their function, and cause significant changes in biochemical parameters associated with liver activity.

Numerous physical, chemical, and biological methods have been used to detoxify and eliminate aflatoxins in animal feed and food. In most of these methods, by modifying or destroying the molecular structure or absorbing toxins, they eliminate or reduce the transfer of toxins in the gastrointestinal tract, inhibiting and reducing their access to target tissues. However, the use of these methods is limited due to nutrient degradation, loss of organoleptic properties, the presence of new residues, and the need for expensive equipment (5). An easy, cheap, and healthy way to eliminate or reduce aflatoxins is to use healthy foods. Healthy foods are products that, in addition to providing basic nutrition, increase people's health. Among healthy foods, foods containing probiotic microorganisms are of special importance. According to the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), probiotics are living microorganisms that, when consumed in sufficient quantities, promote health in the host (6). Kefir drink is a strong probiotic fermented dairy product that is produced by incubating kefir grains in milk. Kefir grains contain yeasts and bacteria of lactic acid and acetic acid, which are integrated into a polysaccharide background and form small masses similar to cauliflower. During fermentation, existing bacteria and yeasts grow and multiply to produce lactic acid, acetic acid, ethanol, aromatic compounds, essential amino acids, vitamins, minerals, and exopolysaccharides (7).

Antimicrobial, anti-cancer, anti-inflammatory, and immunosuppressive effects have been reported for kefir grains and their products. It is also used in the treatment of gastrointestinal disorders, hypertension, allergies, and cardiovascular diseases (8). Recently, probiotics have shown beneficial results in detoxifying and reducing the destructive effects of toxins, including aflatoxins, in and out of the body (9).
This study investigated the chronic effects of AFB1 on hepatic cell synthesis function in adult male Wistar rats, as well as the effect of kefir drink as a potent probiotic in reducing or eliminating these effects.

## Methods

Aflatoxin B1 from *A. flavus* was purchased from Sigma-Aldrich (CAS number: 1162-65-8, Aryatajhiz Company, Tehran, Iran). Human biochemical kits, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (T.P), and total bilirubin (T.Bili) were purchased from Pars Azmun Company (Tehran, Iran), and kefir grains were obtained from a local traditional market of probiotic dairy products (Gorgan, Iran).

Kefir drink was manufactured by pasteurized fresh cow milk inoculated with 5% (w/v) kefir grains and incubated in a glass bottle at 25 °C for 24 hours. At the end of the fermentation process, kefir grains were separated from fermented milk by a sterile plastic sieve and stored for later use. The strained product, which is called kefir drink, was used to feed rats. According to The Codex Alimentarius standards for kefir, the minimum number of bacteria per mL should be $10^7$ $10^7$ and $10^4$ $10^4$ for yeasts (10).

In the present study, 24 adult male Wistar rats weighing between 150 and 200 g were obtained from the Pasteur Institute of Iran and transferred to the animal house of Gorgan University of Medical Sciences. The rats were housed in a controlled unit under standard temperature and humidity conditions (22 ± 3 °C, 12-hour light/dark cycle). During the experimental period, standard commercial rat feed (pellet feed) and tap water ad libitum were provided to the rats (basal diet). All of the animal experiments were carried out in accordance with the guideline for the care and use of laboratory animals in Iran (11).
After a 1-week adaptation period, the animals were randomly divided into 4 experimental groups (n = 6 for each group) as follows:

Group N (Control group): The rats were fed on a basal diet.
Group A: The rats were fed on a basal diet and orally administered with AFB1 (50 µg/kg body weight).
Group K: The rats were fed on a basal diet and orally administered with kefir drink (10 mL/kg body weight).
Group AK: The rats were fed on a basal diet and orally administered with AFB1 (50 µg/kg body weight), followed by a kefir drink (10 mL/kg body weight).

The doses of AFB1 used in this study were selected according to previous studies (12).

At the end of the experiment (8 weeks), the rats were fasted overnight and anesthetized by intraperitoneal injection of ketamine (40 mg/kg) and xylazine (10 mg/kg). Then, blood samples (5 mL) from each rat were collected directly from the heart and poured into normal tubes. The sera were carefully transferred to clean tubes, analyzed, and stored at -20 °C until use for the determination of the biochemical parameters. At the beginning and end of the experimental period, the weight of the rats was measured in all groups.

After dissection, liver tissues were collected for histopathological evaluation, fixed in 10% formalin solution, and sent to the pathology laboratory for the preparation of tissue sections using a Shandon Citadel 1000 Tissue Processor (England) and hematoxylin and eosin (H&E) staining.

The measurement of aminotransferase enzyme activity levels was performed according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and ALP activity was measured by the photometric method according to the German Society for Clinical Chemistry. The concentration of T.P was measured by following the Biuret method. Total bilirubin was measured photometrically using 2, 4 dichloroaniline (DCA) (13).

Data were statistically analyzed using SPSS version 16 (SPSS Inc, Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to examine the significant difference between the different means at $P < 0.05$.

## Results

The results after 8 weeks of treatment showed that the weight gain (%) of the rats in group A (35.2%) was lower than the control group (46.7%), while the weight gain of the rats in K (44.9%) and AK (45,9%) groups was not different compared to the control group (Table 1).

**Table 1**. The results of changes in weight gain of the rats in all groups after 8 weeks of treatment with or without aflatoxin B1 (50 µg/kg body weight)
percentage weight gain = (final weight - initial weight)/initial weight × 100.

| Rat groups | Initial weight (g) | Final weight (g) | Weight gain (g) | Weight gain % |
|---|---|---|---|---|
| Control (group N) | 166.8 ± 20.3 | 244.8 ± 33.7 | 78 | 46.7 |
| AFBI (group A) | 174.0 ± 20.8 | 235.4 ± 21.8 | 61.4 | * 35.2 |
| Kefir (group K) | 175.5 ± 6.3 | 254.3 ± 22.8 | 78.8 | 44.9 |
| AFB1+ kefir (group AK) | 178.5 ± 18.3 | 260.6 ± 28.1 | 82.1 | 45.9 |

* Significant as compared to other groups

In group A, AST, ALT, ALP, and T.Bili increased significantly compared to the control group and groups treated with or without kefir ($P < 0.05$). T.P levels were significantly reduced ($P < 0.05$) in group A compared to the other groups (Table 2).

The liver sections of group A showed a disrupted lobular architecture and mild to severe degenerative changes, which were characterized by swelling and a vacuolar appearance of hepatocytes (Figure 1B). Kefir administration decreased liver failure caused by AFB1 intoxication (Figure 1D). The liver of rats fed on a basal diet (Figure 1A) or supplemented with kefir (Figure 1C) showed a normal liver structure characterized by normal hepatocytes arranged as cords.

**Table 2**. The results of Liver Function Tests in all groups after 8 weeks of treatment with or without aflatoxin B1 (50 µg/kg body weight)

| | Control group | Group A | Group K | Group AK |
|---|---|---|---|---|
| AST (U/L) | 129.6 ±15.6 | 179.8 ± 56.3 * | 123.3 ± 11.2 | 118.1 ± 9.7 ** |
| ALT (U/L) | 70.3 ± 7.3 | 90.3 ± 9.7 * | 70.5 ± 7.1 | 75.0 ± 5.2 ** |
| ALP (U/L) | 457.6 ± 80.5 | 758.5 ± 154.9 * | 413.5 ± 57.2 | 489.3 ± 72.4 ** |
| T. Bili (mg/dL) | 0.29 ± 0.04 | 0.62 ± 0.15 * | 0.30 ± 0.03 | 0.36 ± 0.03 ** |
| T. P (g/dL) | 7.23 ± 0.33 | 5.60 ± 0.87 * | 7.03 ± 0.31 | 6.53 ± 1.20 ** |

Data are expressed as means ± SD for 6 rats per group
Significance was calculated at $P < 0.05$; * Significant as compared to control animals; ** Significant as compared to AFB1 treated animals
Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; T.Bili, Total bilirubin; T.P, Total protein

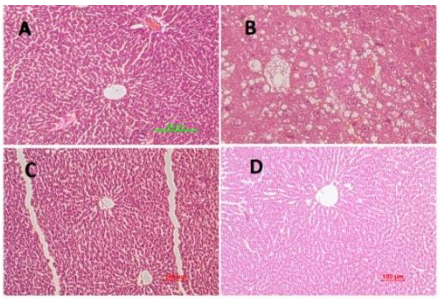


**Figure 1**. The hepatic sections of the control group (A), AFBI group (B), kefir group (C), and AFB1 + kefir group (D) following hematoxylin and eosin staining. Histopathological changes in the liver of AFB1-treated rats show swelling and vacuolar cytoplasm (arrows).

## Discussion

Previous studies have confirmed that AFB1_causes serious damage to the liver, leading to significant changes in liver function tests. Mosaad et al. (2020) showed that treatment with AFB1 resulted in a significant decrease in body weight and T.P and increased ALT, AST, creatinine, uric acid, and urea compared to the control group (14).

Aflatoxin B1 is mainly biotransformed in the liver (15) through the action of the cytochrome P450 enzyme family (CYP450), resulting in the conversion of AFB1 into other metabolites. It is important to note that several of these metabolites are highly toxic (such as AFB1-8,9-epoxide [AFBO]). AFBO has oxidative activity and can cause oxidative damage to hepatocytes directly or via promoting reactive oxygen species (ROS) production. AFBO is a highly reactive compound that forms additives with amino acids and nucleic acids. DNA adducts are relatively resistant to DNA repair processes, causing gene mutations that lead to cancers, especially liver cell carcinomas. Other metabolites can cause acute toxicity, liver necrosis, and inhibition of cellular metabolizing enzymes, resulting in impaired metabolism of substances in the liver (13). Gaspar et al (2019) showed that kefir could moderate the activity of antioxidant enzymes and remove free radicals like a scavenger, thus reducing oxidative stress (16). Lactobacilli and bifidobacteria strains found in kefir could reduce oxidative stress by scavenging free radicals and reducing lipid peroxidation (17). Intracellular ALT, AST, and ALP are released into the peripheral blood, increasing the concentration of these enzymes in the serum when hepatocytes undergo damage. In addition, the ability of hepatocyte to ingest, transform, and excrete bilirubin is reduced, resulting in elevating levels of serum bilirubin. Therefore, serum ALT, AST, ALP, and bilirubin can serve as biomarkers of liver dysfunction. In this study, the increase of AST, ALT, ALP, and T. Bili, as well as the decrease of T.P in group A, provided strong evidence of serious injury in the hepatobiliary system. However, the lack of significant differences in AST, ALT, ALP, T. Bili, and T.P in group AK compared to the control group gives a clue that kefir has an obvious positive effect in alleviating AFB1-induced liver damage. Sahar AbdEl-Mogheith et al. (2017) reported similar results in an experimental model of carbon tetrachloride-induced liver damage, in which kefir-treated animals maintained normal liver function tests (18).

Aflatoxin is known to impair protein biosynthesis AFB1 by forming additional compounds with RNA and DNA and proteins by inhibiting RNA synthesis, decreasing the synthesis of DNA-dependent RNA polymerase, and inhibiting the transfer of amino acids and mRNA transcription. It disrupts the synthesis of proteins (19). The reduction of T.Ps in group A compared to the control group showed that the oral administration of AFB1 caused disruption in liver synthesis, while T.Ps in group AK were not significantly different from the control group. These biochemical findings were similar to those reported by Ozturk and Guven (2014) (20). The results obtained from several studies show that kefir grains, kefir drink microorganisms isolated from kefir, and substances extracted from kefir are able to eliminate or reduce the effects of aflatoxin toxicity through different mechanisms (21). In a study for optimizing the amount of AFB1 reduction in pistachio nuts by kefir grains, it was found that kefir grains could significantly reduce the amount of AFB1, and the cell viability of kefir grains was not necessary for its decontamination effect (22). Eiri et al isolated *Saccharomyces cerevisiae* and *Kluyveromyces marcianus* from kefir and showed that they absorbed 46% and 53%, respectively, in a broth medium (22, 23). Taheur et al. (2017) demonstrated that kefir could adsorb 82% to 100% of AFB1 when cultivated in milk and consequently reduce their toxicity (23, 24). Furthermore, the reduction of oxidative stress biomarkers could be attributed to the non-availability of a part of AFB1 in rat organisms.

## Conclusion

Our study showed that kefir drink could reduce the damage caused by AFB1 in adult rats. Previous studies have also confirmed these results. Regular consumption of kefir drink appears to have potential benefits for human health.

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## Ethical statement

The study was approved by the Ethics Committee of Ferdowsi University of Mashhad (Code: IR.UM.REC.1398.108).

## Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## Author contributions

A.S performed the experiments. H. J supervised the research. F.N supervised the research and planned the experiments. Z.M contributed to data analysis of histopathology. All authors contributed equally to writing the final paper.

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