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Lowering Effect of Selenium and Yogurt on Nuts Contaminated With Aflatoxins Induced Hepatotoxicity in Rats

AMNAH M. A. ALSUHAIBANI

Nutrition and Food Sciences Dept, Princess Nourah Bint Abdulrhman University, Riyadh, Saudi Arabia.

Abstract

Nuts which contaminated with aflatoxins are potent to hepatotoxic and hepatocarcinogenic agents. Herein, we were assessed the ability of selenium and yogurtto ameliorateaflatoxin-contaminated nut-induced hepatotoxicity in experimental rats. Relative to the control group, the aflatoxin-contaminated nut-fed rats has been reduced body weight gain and feed efficiency ratio (FER), whereas those rats given selenium or yogurt, or both, and consumed 3% aflatoxin-contaminated nuts showed no significant decrease in body weight gain or decrease in FER. Food intake did not vary significantly between the groups. After 60 days,alanine and aspartate aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase activities were increased in the serum of rats fedaflatoxin-contaminated nuts, suggesting hepatic damage.The 3% aflatoxin-contaminated nut-fed group has been reduced total protein and serum, liver glutathione peroxidase and superoxide dismutase(GPX and SOD) enzymes but elevated creatinine, urea, uric acid, bilirubin and malondialdehyde (MDA) levels, as well as liver MDA, compared to the control group. Moreover, we were found that feeding of the ratsby selenium,yogurt or both could be normalize of liver and antioxidant enzyme levels (GPX, SOD,and MDA), as well as total protein, albumin, globulin, and uric acid contents. Based on our findings, we were proposed that selenium and yogurt could reduce the side effects of hepatotoxicity in experimental rats that have consumed aflatoxin-contaminated nuts.

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Introduction

Nuts are rich in an unsaturated fatty acids and various bioactive compounds, which have been including high-quality vegetable protein, minerals, fiber, phytosterols, tocopherols and phenolic compounds. Almonds (*Prunusamigdalis*), hazelnuts (*Corylusavellana*), walnuts (*Juglansregia*), caju (*Anacardiumoccidentale*), and pistachios (*Pistachiavera*) are among popularly consumed edible tree nuts[1,2].

CONTACT Amnah M. A. Alsuhaibani amalsuhaibani@pnu.edu.sa Nutrition and Food Sciences Dept, Princess Nourah Bint Abdulrhman University, Riyadh, Saudi Arabia.

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Fungi have growing on nuts which were formed the mycotoxin and can be decrease the quality of nuts by reducing their nutritive quality[3]. Both of the mycotoxins and aflatoxin have been formed by the molds *Aspergillusflavus* and *Aspergillusparasiticus.* Blue 1 (B1), B2, Green 1 (G1), and G2 are the most common aflatoxin strains that were found in foods, thus named because of their fluorescence properties and chromatography patterns[4]. Optimal aflatoxin production occursat temperatures nearly to 30 °C[5].

Mammals convert aflatoxins into the M1 and M2 metabolites, which are carcinogenic[6,7].Aflatoxin contamination usually occurs either by slowly acquiring aflatoxin over time in smaller quantities or by consuming large amounts at once. Aflatoxin contamination can lead to a variety of health problems, including cancers, mental and digestive problems, hemorrhages, and malabsorption[8].

Aflatoxins reduce growth and suppress immune functions in animals. Aflatoxin induces hepatic and renal tumors in rodents, and has been implicated inesophageal cancer. Humans are exposed to mycotoxins through the consumption of contaminated foods, as well as by exposure to dust and air containing these toxins. Aflatoxins are hepatocarcinogenic, predominantly in conjunction with chronic hepatitis B virus infection, and form aflatoxicosis in episodic poisoning outbreaks[9,10].

Here we have been evaluated the potential for yogurt and selenium to ameliorate aflatoxin-contaminated nut-induced hepatotoxicity in rats.

Materials and Methods

Nuts

Five kilograms of commonly consumed nuts(pistachio, caju, walnut, almond, and hazelnut) were obtained from a local market of Riyadh in the Kingdom of Saudi Arabia. These nuts are more susceptible tomold growth.

Reagents

All the materials used for this experiment were of analytical grade and used without further purifications. Bio Meriuex Kits were purchased from Alkan Co. for Chemicals and Biodiagnostics. Selenium was obtained from Sigma-Aldrich.

Probiotic Bacteria

Lactobacillus delbrueckii subspecies bulgaricus CH-2 and *Streptococcus thermophilus* ST-36 were obtained from the Hansen Lab(University of Denmark).

Experimental Animals

Fifty adult male Sprague-Dawley strain (albino) rats, weighswithin 130–140 g, were provided by the experimental animals of the center in Research Center in Prince Sultan Military Medical City, Riyadh. Rats were housed as groups in wire cages under laboratory conditions and, during a 1-week adaption period, were fed a standard diet. Food and water were provided *adlibitum*. Animal management ethical guidelines were followed throughout the study and permission was obtained from the concerned department.

Standard Diet

The standard experimental diet was composed of cornstarch(598 g kg^{-1}), casein(200 g kg^{-1}), soybean oil(100 g kg^{-1}), vitamin mixture (10 g kg^{-1}), salts mixture (40 g kg^{-1}), cellulose (50 g kg^{-1}), and choline chloride (2 g kg^{-1}), according to Ref.[11].

Preparation of Ordinary Yogurt

L.delbrueckii subspeciesbulgaricus CH-2 was cultured in MRS broth at 37 °C for 24 h. *S.thermophilus* ST-36 was grown in M17 broth at 40 °C for 24 h. Whole milk was heated to boiling temperature to reduce its volume by approximately 20% after cooling; this milk was then heated to90 °C for 5 min and then cooled to 42 °C and inoculated with 1% of *L.delbrueckii* sub-speciesbulgaricus CH-2 and *S. thermophilus* ST-36, thenincubated at 40 °C until coagulation (about 4 h)[12].

Nuts Storage and quantification of Aflatoxin Content

Raw nutswere stored in glass dishes at 25 °C and 60% relative humidity for 6 months. Then, these nuts were crushed to estimate their aflatoxins content, as previously described[13,14]. The total aflatoxins were 23.25, 23.66, 22.07, 26.02 and 28.6 µgkg^{-1}in the pistachios, caju, walnuts, almonds, and hazelnuts, respectively. Mixed crushed nuts were added as 3%(w/w) to the standard diet, with consideration of the nutritional value of the nuts.

Treatment Schedule

Rats were divided into five groups, 10 rats per group, as follows: (1) normal control group fed the standard diet; (2) positive control group fed the standard diet plus aflatoxin-contaminated nuts to induce hepatotoxicity, as reported in previous work;[9,10] (3) selenium group fed the standard diet plus aflatoxin-contaminated nuts plus selenium by stomach tube (3 mg kg^{-1} body weight); (4) yogurt group, fed the standard diet plus aflatoxin-contaminated nuts plusyogurt by stomach tube (160 $mlkg^{-1}$ body weight); (5) yogurtplus selenium group, fed the standard diet plus aflatoxin-contaminated nuts plus selenium and yogurt by stomach tube (3 mg kg^{-1} and 160 $mlkg^{-1}$ body weight, respectively).

The daily food intake and weekly body weight of the rats were recorded.The feed efficiency ratio (FER) was calculated using the method of Ref.[15] After completion of the experimental period (60 days), rats were fasted overnight and sacrificed to obtain blood and liver samples for biochemical analyses.

Serum Analyses

From these samples, we quantified the serum alanine and as partate aminotransferase (ALT +AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (γGT) enzyme activities. Also, serum total protein, globulin, albumin, creatinine, uric acid, urea, and bilirubin contents were enzymatically determined as mentioned in the method of Ref.[16]. The activity of glutathione peroxidase (GPX), malondialdehyde (MDA), and superoxide dismutase(SOD) enzymes were determined as previously described[17–19].

Liver Biochemical Estimations

Livers of rats were rapidly removed and parts of them perfused with 50 to 100 mlof ice-cold 0.9% NaCl solution for estimation of the liver glutathione peroxidase (GPX), superoxide dismutase (SOD) and malondialdehyde (MDA) activities according to Refs[18,20,21].

Statistical Analysis

Data were subjected to ANOVA. Comparison of means was performed using Duncan's multiple-range test with level of significance 0.05, complemented by Kruskal-Wallis correlation method to analyze correlations between parameters at significance levels of 0.05.

Results and Discussion

Here in, we found that rats fed aflatoxin-contaminated nuts have been decreased the body weight gain and FER,but that selenium or yogurt, or both,could ameliorate these effects. Food intake did not vary among the five test groups(Table 1). It has been well-documented nut contamination with aflatoxins (from *A.flavusand* and *A.parasiticus*) is a major health concern, especially in hot and humid regions. Under conventional storage conditions, aflatoxin-producing molds are able to grow exponentially[23]. Aflatoxins have a low molecular weight therefore quickly absorbed in the gastrointestinal tract and appear as metabolites in the blood[24,25]. Following exposure to aflatoxins, they can be detected covalently bound to DNA, there by reducing protein synthesis; this effect can be persist for up to 5 days. Also, animals exposed to aflatoxinare less efficientat food use anddietary animals exposed to aflatoxinhave a reduced growth rate and productivity.These effects are likely due to an increased degradation of lipids and proteins[26,27].

Co-administration of selenium and yogurt has made improvement in the nutritional results. Indeed, Navarro-Alarcon and Cabrera-Viquehave been detected the increasing in growth and development of the selenium supplementation[28]. Fermented milk and yogurt are considered safe and nutritious. Yogurt is related to its component and proteolytic bacteria. Milk is a source of protein, calcium, and the B-group vitamins, as well as vitamin A, vitamin C, magnesium, and zinc[29]. The consumption of fermented dairy products containing lactic acid bacteria, as well as the probiotic bacteria which was found in yogurt. These can be proposed to reduce the risk of liver cancer by binding to the mutagens produced by intestinal bacteria[30,31].

Because of the liver's role in the detoxification of environmental xenobiotics, consuming aflatoxins-contaminated nuts can cause liver injury and induce hepatotoxicity, which is agreement with our results. The group fed aflatoxins-contaminated nutshad increased serum ALT, AST, ALP, and γGT enzyme activities, compared to the control group. Supplemental selenium or yogurt, or both, could significantly lower these enzyme activities to control levels (Table 2). Multiple studies reported that aflatoxins, poten the patotoxics, and hepatocarcino genic mycotoxin can induce lipid peroxidation. These

compounds have been also associated with various diseases, including aflatoxicosis and hepatocellular carcinoma[27,32]. Here, we demonstrate that the consumption of aflatoxin-contaminated nuts by rats forced a marked elevation in liver enzyme activities, demonstrating hepato cellular damage, as previously reported[33]. Selenium tended to alleviate serum liver enzymes[34,35]. The improvement in the liver enzyme levels followed by the consumption of yogurt, that is likely related to the yogurt's probiotic organisms. Therefore, directly affect multiple processes, including digestion and immune function[36,37].

Table 1: Mean values ± standard deviation (SD)of body weight gain, food intake, and feed efficiency ratio (FER) of the control group, rats fedaflatoxin-contaminated nuts, and those supplemented with selenium, yogurt or both selenium and yogurt

Groups Variables	Normal Control	Rats fed aflatoxin-contaminated nuts (3%)			
		Positive control	Selenium	yogurt	Yogurt with selenium
Body weight gain (g)	130.20± 12.51ab	100.21± 8.99d	115.51± 11.33bc	116.81± 12.45bc	126.11± 12.24bc
Food intake (g/w)	15.41± 1.40a	14.50± 1.69a	15.77± 1.57a	15.86± 1.37a	15.93± 1.28a
FER	0.140± 0.003a	0.114± 0.002d	0.121± 0.005c	0.121± 0.004c	0.131± 0.001b

Values with the different letters indicate significant difference (P<0.05) and vice versa.

Table 2: Mean values ± standard deviation (SD)of serum ALT, AST, ALP and γ GT enzymesof the control group, rats fedaflatoxin-contaminated nuts, and those supplemented with selenium, yogurt or both selenium and yogurt

Groups Variables	Normal Control	Rats fed aflatoxin-contaminated nuts (3%)			
		Positive control	Selenium	yogurt	Yogurt with selenium
AST (μ ml^{-1})	45.87± 5.41bc	75.755± 8.13a	54.21± 6.41b	47.74± 3.45bc	46.53± 6.21bc
ALT (μ ml^{-1})	39.55± 5.22c	61.95± 9.67a	42.11± 5.60bc	45.83± 5.75bc	48.25± 6.52b
ALP (μ ml^{-1})	40. 55± 3.99c	68.88± 9.50a	48. 88± 4. 66b	46.37± 4.85bc	43. 41± 7.15bc
γGT (μ ml^{-1})	6.11± 0.55bc	9.63± 1. 65a	6.87± 1.07bc	7. 53± 1. 15b	7. 76± 1. 12b

Values with the different letters indicate significant difference (P<0.05) and vice versa.

Compared to the control group, the consumption of aflatoxin-contaminated nuts has been reduced the total protein and elevated the creatinine, urea, uric acid, and bilirubin levels. Supplemental selenium decreased globulin and increased creatinine, urea, and bilirubin levels,whereas yogurt increased urea compared to the control group. Compared to the group fed aflatoxin-contaminated nuts, selenium or yogurt, or both, significantly increased serum total protein anddecreasedcreatinine, urea, uric acid, and bilirubin levels(Table 3).Uric acid is the metabolic end product of purine metabolism. The observed decreasing level of uric acid in the treated groups is likely a result of an increased utilization of uric acid, thereby inhibiting the generation of free radicals[27]. Selenium can reduce nephrotoxicity by attenuating oxidative-stress-associated kidney injury through the reduction of oxygen free radicals and lipid peroxidation in rats treated with gentamicin. Selenium is a co-factor of several enzymes that participate in the regulation of enzymatic antioxidant defenses[38,39]. Here we show that improvement of this renal parameter is related to the bioactive peptides thatare generated during the production of fermented dairy products,which have antioxidative and growth promoting properties[40–42].

Table 3: Mean values ± standard deviation (SD) of serum total protein, albumin, globulin, creatinine, urea, uric acid and bilirubin of the control group, rats fed aflatoxin-contaminated nuts, and those supplemented with selenium, yogurt or both selenium and yogurt

Groups Variables	Normal Control	Rats fed aflatoxin-contaminated nuts (3%)			
		Positive control	Selenium	yogurt	Yogurt with selenium
Total protein (gdl^{-1})	6.99± 0.80a	5.66± 0.52b	6.41± 0.55a	6.75± 0.63a	6.25± 0.90a
Albumin (gdl^{-1})	3.63± 0.49a	2.70± 0.49ab	2. 81± 0.26ab	3.16± 0.40a	3.02± 0.34a
Globulin (gdl^{-1})	3.36± 0.52a	2.96± 0.23ab	2.15± 0.24b	3.24± 0.33a	3.23± 0.25a
Creatinine ($mgdl^{-1}$)	0.66± 0.21c	1.91± 0.43a	0.88± 0.10b	0.73± 0.19bc	0.69± 0.13c
Urea (μmg^{-1})	35.65± 3.51c	65. 50± 6.81a	48.87± 4. 16b	49. 69± 4.51b	41.40± 5. 91bc
Uric acid ($mgdl^{-1}$)	3.99± 0.39c	6.09± 0.80a	4.59± 0.34bc	4.62± 0.54bc	4.01± 0.44bc
Bilirubin ($mgdl^{-1}$)	0.65± 0.14cd	1.76± 0.53a	1.09± 0.32b	0.88± 0.25c	0.79± 0.21c

Values with the different letters indicate significant difference ($P<0.05$) and vice versa

Also, the aflatoxin-contaminated nut-fed group had decreased serum and liver GPX and SOD enzyme activities and increased serum and liver MDA, as compared to the control group. Selenium or yogurt, or both, was able to normalize serum and liver GPX, SOD, and MDA levels (Table 4). In the current study, consumption of aflatoxin-contaminated nuts induced oxidative damage through the generation of reactive oxygen species. This was accompanied by an increase in the expression of liver enzymes and decreased biological activities of some liver antioxidant enzymes.Glutathione is exhausted by glutathione-related enzymes to detoxify the peroxides formed from increased lipid peroxidation under oxidative stress. The increase in hepatic MDA level might be because aflatoxins are metabolized

by the cellular cytochrome P450 enzyme system to form the reactive intermediate, aflatoxin-8,9-epoxide, which in-turn reacts with macromolecules, such as lipids and DNA. This leads to cellular injury and lipid peroxidation[43]. Also, MDA was elevated in the treatment groups compared to the control group, most possibly reflecting an adaptive reaction towards free radical damage in the liver[44].

Table 4: Mean values ± SD of serum and liver GPX, SOD and MDA of the control group, rats fedaflatoxin-contaminated nuts, and those supplemented with selenium, yogurt or both selenium andyogurt

Groups Variables	Normal Control	Rats fed aflatoxin-contaminated nuts (3%)			
		Positive control	Selenium	yogurt	Yogurt with selenium
GPX	8.27±	4.11±	7.51±	7.50±	7.70±
(mmol L blood^{-1})	1.47a	0.64b	0.54a	0.67a	0.68a
SOD	35.20±	22.95±	32.51±	31.31±	33.86±
(mmol L blood^{-1})	4.51a	2.93c	3.29a	3.91ab	3.59a
MDA	4.02±	7.80±	5.07±	5.14±	5.15±
(mmol L blood^{-1})	0.45bc	1.34a	0.67b	0.83b	0.54b
GPX	65.01±	31.85±	59.91±	58.91±	61.14±
(µ mg liver^{-1})	6.24a	4. 71c	5.84ab	6.32ab	6.39a
SOD	44.91±	25. 89±	45.01±	42. 87±	45. 41±
(µ mg liver^{-1})	5.16a	2. 16b	4. 12a	4.27a	3.16a
MDA	13.97±	27.91±	15.51±	14. 73±	15. 17±
(mmolg liver^{-1})	2.53bc	2.99a	2. 91b	2.41b	3. 16b

Values with the different letters indicate significant difference (P<0.05) and vice versa

Selenium's ability to enhance GPX activity might be because of increased selenium bioavailability, thereby preventing the formation of free radicals and protecting both integrity and functions of tissues, thus protecting the liver from peroxidation of lipid and changes in glutathione and antioxidant enzyme activities[45,46]. The administration of the lactic acid bacteria inyogurt results in increased antioxidative enzyme activity and modulated circulatory oxidative stress, thereby protecting the cells against damage[31,42,47].

Conclusion

Selenium and yogurt reduce the hepatotoxicity in rats caused by consuming gaflatoxin-contaminated nuts. Further studies are now needed to better understand the *in vivo* mechanism(s) by which selenium and yogurt reduce aflatoxin toxicity.

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Competing Interest

I declare that there is no conflict of interest regarding the publication of this paper which titled "Lowering effect of selenium and yoghurt on nuts contaminated with aflatoxins induced hepatotoxicity in rats."

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