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ORIGINAL ARTICLE

Study on Chicory Effect of Aflatoxin in Kefir Probiotic Contain Chicory and Lactobacillus acidophilus or *Bifidobacterium bifidum*

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ABSTRACT
The aim of the study was to develop a new probiotics product using the best combination of probiotics and medicinal plant(chicory) and low level Aflatoxin M1 on the production.In this study in addition to produce probiotic products of kefir containg chicory Extract and Lactobacillus acidophilus or Bifidobacterium bifidum we compared them together on the decrease of Aflatoxin M1 .To the 1,2,3,4 samples different doses(0, 2, 4 and 6 gr) of Peppermint Extract had injected to the kefir and to the 5,6,7 samples 4 gr chicory and different doses(0.1, 0.3, and 0.6 gr) of Lactobacillus acidophilus and to the 8,9,10 samples 4 gr chicory and different doses(0.1, 0.3, and 0.6 gr) of Bifidobacterium bifidum and to the 11,12,13 samples 4 gr chicory and different doses(0.1, 0.3, and 0.6 gr) of Lactobacillus acidophilus and Bifidobacterium bifidum together and then they put in to the 38 degree incubation. The AFM1 concentration of samples was determined by a competitive ELISA method at the day of 9 of refrigeration.
Keywords: *Aflatoxin M1, chicory, Probiotic, Lactobacillus acidophilus, Bifidobacterium bifidum.*

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INTRODUCTION

Chicory plant (Doremaaucheri) belongs to Asteraceae family and contains flavonoids. Probiotics are foods that contain live bacteria, which are beneficial to health [26]. According to Gorbach definition, a probiotic is a live microbial food supplement that beneficially affects the host animal by improving the microbial balance and they are used in fermented dairy products [14]. The term 'probiotic' dates back to 1965 when it referred to any substance or organism that contributes to intestinal microbial balance (18), primarily of farm animals. At the beginning of this century, the basic probiotic concept was first conceived by Metchnikoff, he had long believed that the complex microbial population in the colon was having an adverse effect on the host through autointoxication. It was later revised to insist on the notion of a live microbial feed supplement, rather than any substances and became more relevant for humans [10]. Up until then, fermented milks had been a common source of food [11]. Most recently, probiotics is defined as 'living organisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition' [28]. Kefir is a natural probiotic.

Lactobacillus acidophilus and *Bifidobacterium bifidum* are generally considered safe for most people. Gas, upset stomach, and diarrhea are potential side effects in some people who take L. acidophilus B. bifidum cells daily.Kefir is a fermented milk product which has acidic properties and has a slightly alcoholic flavor [3, 9]. Kefir is a traditional popular Middle Eastern beverage.

produced from kefir grains which are contain a mixture of the complex microflora such as lactic acid bacteria, yeasts and sometimes acetic acid bacteria as well as a polysaccharide matrix "kefiran". Several studies have investigated the antitumor activity of kefir [1, 12,13] and of kefir grains [20, 29] and antimicrobial activity in vitro against a wide variety of gram-positive and gram-negative bacteria and against some fungi [1, 32].

Aflatoxins are a kind of mycotoxin mostly produced by a variety of moulds such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*. These moulds are common contaminants of foodstuffs, particularly in the tropical regions [27, 30]. Crops may be contaminated by one or more of the following four sub-types of aflatoxin: B 1 , B 2 , G 1 , and G 2 . Aflatoxin B 1 is the most toxic aflatoxin [22]. In the

liver, part of ingested AFB1 is bio-Transformed into the hydroxy derivative M1 (AFM1), which is then excreted into the milk of lactating mammals, including dairy animals [6].

The occurrence of AFM1 in milk and its derivatives is a serious problem of food safety, and many countries defined specific limits for AFM1 in the milk and for AFB1 in the feed of dairy animals [8]. In spite of the strict selection of raw materials used for the manufacturing of feeds, production of AFM1-free milk is not always achieved and many surveys reported high levels of contamination of milk and milk derived food for humans and infants [7, 17, 23, 25].

The European Commission has determined the legal limit for AFM1 in raw milk, treated milk, and dairy products at 50 ng/kg, and for infant formulae, infant milk, and special food products should not exceed 25 ng/kg [4]. Aflatoxins may cause human health disorders including toxic hepatitis and liver fibrosis, hepatocellular carcinoma, aflatoxicosis, and Reye's syndrome [5, 15]. Exposure of infants to AFM1 is worrisome, because their capacity for biotransformation of carcinogens is generally slower than that of adults and they are more susceptible to adverse effects of mycotoxins [19].

Toxicological studies on aflatoxins have shown that they are highly toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic compounds. Although AFM1 has been found to be about 10 times less mutagenic and carcinogenic than AFB1, this toxin classified as a Group 2B agent, by the International Agency for the Research on Cancer (IARC) [16, 31].

MATERIALS AND METHODS

In the present study, the potential of producing foods containing probiotic bacteria with the bases of kefir and chicory, anti-Aflatoxin M 1 activity was evaluated. The goal was to determine the effects of chicory on the Aflatoxin M 1, in kefir during 9 day refrigerated storage for production of probiotic kefir

The Effect of chicory on the Aflatoxin M 1 in Production of Probiotic Kefir: In order to produce Kefir containing the chicory, four containers; each containing 1 liter of low-fat sterilized milk (1.5% fat) were considered as four groups. The Kefir (4 gr)was added directly to all the containers, followed by adding dried chicory (0 gr to the control), 2, 4 and 6 gr to the other three containers.

Preparation of probiotic Lactobacillus acidophilus Kefir containing chicory

In order to produce Kefir containing the probiotic bacterium Lactobacillus acidophilus three containers each containing 1 liter of low-fat sterilized milk (1.5% fat) were considered as our four groups 4gr Kefir and 4 gr of chicory was added directly to all the containers, followed by adding Lactobacillus acidophilus of 0.1, 0.3, and 0.6 gr to containers.

Preparation of probiotic *Bifidobacterium bifidum* Kefir containing chicory

In order to produce Kefir containing the *Bifidobacterium bifidum*, three containers each containing 1 liter of low-fat sterilized milk (1.5% fat) were considered as our four groups 4 gr Kefir and 4 gr of chicory was added directly to all the containers, followed by adding *Bifidobacterium bifidum* of 0.1, 0.3, and 0.6 gr to containers.

Preparation of probiotic Lactobacillus acidophilus Kefir containing chicory

In order to produce Kefir containing the probiotic bacterium (Lactobacillus acidophilus and *Bifidobacterium bifidum*) together three containers each containing 1 liter of low-fat sterilized milk (1.5% fat) were considered as our four groups 4 gr Kefir and 4 gr of chicory was added directly to all the containers, followed by adding Lactobacillus acidophilus of0.1, 0.3, and 0.6 gr to containers.

Then the samples were incubated in the incubator at 38°C for 9 hours. Having produced the above-mentioned products, we stored each product in a disposable container placed in a refrigerator at 4°C for 9 days.

For evaluated AFM1, we stored 500 Cc of each product in a container then first, samples contaminated artificially with aflatoxin M1 (AFM1) at a level of 232(pg) and then The AFM1 concentration of samples was determined by a competitive Enzyme-Linked Immune Sorbant Assay (ELISA) method at the day of 9 of refrigeration.

RESULT

TABLE 1: Aflatoxin for different Group's kefir at refrigerator during storage in the refrigerator B: Mean and Std. Error of Mean Aflatoxin

group	Aflatoxin	N Valid	Mean	Std. Error of Mean
Kefir 4 gr	174.3	3	174.3000	.00577
chicory 2 gr	185.5	3	162.9000	.00577
chicory 4 gr	172.3	3	152.6000	.00577
chicory 6 gr	172	3	153.0000	.00577
Lactobacillus acidophilus 0. 1 gr and chicory 4 gr	166.4	3	166.4000	.00577

Lactobacillus acidophilus 0. 3 gr and chicory 4 gr	188.1	3	162.3000	.00577
Lactobacillus acidophilus 0. 6 gr and chicory 4 gr	167.7	3	153.0000	.00577
Bifidobacterium bifidum 0. 1 gr and chicory 4 gr	164.7	3	169.1000	.00577
Bifidobacterium bifidum 0. 3 gr and chicory 4 gr	119.3	3	159.3000	.00577
Bifidobacterium bifidum 0. 6 gr and chicory 4 gr	156.2	3	136.7000	.00577
Lactobacillus acidophilus and *Bifidobacterium bifidum* 0. 1 gr and chicory 4 gr	156	3	149.0000	.00577
Lactobacillus acidophilus and *Bifidobacterium bifidum* 0. 3 gr and chicory 4 gr	168.7	3	151.0000	.00577
Lactobacillus acidophilus and *Bifidobacterium bifidum* 0. 6 gr and chicory 4 gr	156	3	161.0000	.00577

TABLE 2: Kruskal-Wallis Test

	group	N	Mean Rank
chicory	Kefir 4%	3	11.00
	chicory 2 gr	3	8.00
	chicory 4 gr	3	2.00
	chicory 6 gr	3	5.00
	Total	12	

Test Statistics [a,b]

		Aflatoxin
chicory	Chi-Square	10.385
	df	3
	Asymp. Sig.	.016

TABLE 3: Kruskal-Wallis Test

	group	N	Mean Rank
chicory	*Lactobacillus acidophilus* 0. 1 gr and chicory 4 gr	3	8.00
	Lactobacillus acidophilus 0. 3 gr and chicory 4 gr	3	5.00
	Lactobacillus acidophilus 0. 6 gr and chicory 4 gr	3	2.00
	Total	9	

Test Statistics[a,b]

		Aflatoxin
chicory	Chi-Square	7.200
	df	2
	Asymp. Sig.	.027

TABLE 4: Kruskal-Wallis Test

	group	N	Mean Rank
chicory	*Bifidobacterium bifidum* 0. 1 gr and chicory 4 gr	3	8.00
	Bifidobacterium bifidum 0. 3 gr and chicory 4 gr	3	5.00
	Bifidobacterium bifidum 0. 6 gr and chicory 4 gr	3	2.00
	Total	9	

Test Statistics[a,b]

		Aflatoxin
chicory	Chi-Square	7.200
	df	2
	Asymp. Sig.	.027

TABLE 5: Kruskal-Wallis Test

	group	N	Mean Rank
chicory	*Lactobacillus acidophilus* and *Bifidobacterium bifidum* 0. 1 gr and chicory 4 gr	3	2.00
	Lactobacillus acidophilus and *Bifidobacterium bifidum* 0. 3 gr and chicory 4 gr	3	5.00
	Lactobacillus acidophilus and *Bifidobacterium bifidum* 0. 6 gr and chicory 4 gr	3	8.00
	Total	9	

Test Statistics [a,b]

		Aflatoxin
chicory	Chi-Square	7.200
	df	2
	Asymp. Sig.	.027

DISCUSSION

Checking the results of production of probiotic kafir of chicory according to measurement of aflatoxin, sample extract of chicory 6, 4, 0 and 2 gr had the minimum reduction in aflatoxin respectively. Therefore probiotic kafir of chicory 6 gr because of demonstrating the minimum aflatoxin had the maximum influence on the reduction of aflatoxin. Checking the results of production of probiotic kafir of Lactobacillus acidophilus and chicory according to measurement of aflatoxin, sample Lactobacillus acidophilus 0.1, 0.6and0.3gr had the minimum reduction in aflatoxin respectively. Therefore probiotic kafir of chicory 4 gr and Lactobacillus acidophilus 0.1gr because of demonstrating the minimum aflatoxin, had the maximum influence on the reduction of aflatoxin.Checking the results of production of probiotic kafir of *Bifidobacterium bifidum* and chicory according to measurement of aflatoxin, sample *Bifidobacterium bifidum* 0.3, 0.6and0.1gr had the minimum reduction in aflatoxin respectively. Therefore probiotic kafir of chicory 4 gr and *Bifidobacterium bifidum* 0.3 gr because of demonstrating the minimum aflatoxin, had the maximum influence on the reduction of aflatoxin.Checking the results of production of probiotic kafir of Lactobacillus acidophilus and *Bifidobacterium bifidum* and chicory.According to measurement of aflatoxin, sample Lactobacillus acidophilus and *Bifidobacterium bifidum* 0.1, 0.6and0.3gr had the minimum reduction in aflatoxin respectively. Therefore probiotic kafir of chicory 4 gr and Lactobacillus acidophilus and *Bifidobacterium bifidum* 0.1, 0.6gr because of demonstrating the minimum aflatoxin, had the maximum influence on the reduction of aflatoxin.

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