UNIVERSITI PUTRA MALAYSIA

EFFECT OF PROBIOTIC LACTOBACILLUS CASEI SHIROTA STRAIN ON AFLATOXIN B1 LEVEL IN AFLATOXIN-INDUCED RATS

ELHAM NIKBAKHT NASRABADI

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By

ELHAM NIKBAKHT NASRABADI

Thesis submitted to School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the requirement of the Degree of Master of Science

February 2013

DEDICATIONS

This thesis is dedicated to my beloved husband, who has been a great source of inspiration and motivation,

To my dear father and mother who have supported me all the way since the beginning of my study,

And to all my friends and families who have believed my abilities, and helped me to make some of my dreams come true.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chair: Rosita Binti Jamaluddin, PhD

Faculty: Medicine and Health Sciences

Aflatoxin B_1 (AFB_1) is considered as the most toxic food contaminant with harmful impact on human and animal health produced by *Aspergillus* species of fungi namely *Aspergillus flavus (A. flavus)* and *Aspergillus parasiticus (A. parasiticus)*. It is classified as group one carcinogen by the International Agency for Research on Cancer that is linked to the etiology of liver cancer. Intervention and intercession approaches concentrate on pre-and post-harvest measures to reduce AFB_1 levels in crops or on the individual level to modulate bioactivation and excretion of AFB_1 or reduce its bioavailability. Microorganisms, especially bacteria, have been studied for their potential to reduce the bioavailability of aflatoxins as well as other food contaminants. Among them, *lactic acid bacteria* are known to have the ability to reduce the bioavailability of aflatoxins *in vitro*.

This research aims to investigate the effect of probiotic *Lactobacillus casei* Shirota strain (LcS) on AFB_1 level in aflatoxin induced rats through the weight, liver and kidney function tests, and also AFB_1 blood serum level based on two separate studies

of acute and chronic aflatoxicosis. Finally a comparison was done to observe the effect of probiotic LcS supplementation in different duration in acute and chronic exposure to AFB_1. To achieve this purpose, an experimental study was conducted, and a total of 48 animals were divided into two groups (n=24) to conduct two studies of acute and chronic aflatoxicosis. Animals in acute aflatoxicosis experiment were divided into three subgroups of A_a, B_a, and untreated control (n=6). Group A_a (n=9) received LcS (10^8 CFU) by oral gavage daily for 7 successive days, and group B_a (n=9) received medium which was normal saline (1 ml) daily for 7 successive days. Immediately after the fourth probiotic and medium dose, animals of both groups of A_a and B_a were induced with a single oral dose of AFB_1 in amount of 1.5 mg/kg body weight. Animals of chronic aflatoxicosis study were divided into three subgroups of A_c, B_c, and untreated control group as well. Group A_c was given LcS (10^8 CFU) by oral gavage daily for 20 successive days, and group B_c received normal saline (1 ml) again daily for 20 successive days. Immediately after the fourth probiotic and medium dose and from the 4^{th} day, rats of both groups of A_c, and B_c started to receive multiple oral dose of AFB_1 in amount of 25 µg/kg body weight daily for 5 days per week over the next 2 weeks.

Based on the analysis of rats' body weight in acute aflatoxicosis, a significant difference was found between control group with the other two groups dosed with AFB_1 (groups A_a and B_a) in days 6 and 7 ($p<0.05$). However there was no significant difference between groups A_a and B_a, but the mean of rat's body weight was higher in the group A_a. On the other hand there was a significant difference between control group and groups A_a and B_a in terms of blood liver and kidney biomarkers in acute aflatoxicosis ($p<0.05$). However there was no significant difference between groups

A_a and B_a, the mean values of these biomarkers (ALT, AST, CREA and UREA) were greater in the group B_a in comparison to group A_a. To investigate the effect of LcS on AFB_1 absorption, blood serum level of AFB_1 was measured in all groups and then compared together. As expected aflatoxin B_1 was detected from all of the serum samples expect for untreated control blood serum samples. The mean of AFB_1 blood serum level in the group B_a was higher than group A_a. In the chronic aflatoxicosis study different results were obtained. With regard to rats' body weight; there was a significant difference between group B_c and the other two groups (untreated control and group A_c). This significant difference was found from day 9 to the end of the chronic aflatoxicosis study. Analysis of blood liver enzymes in chronic aflatoxicosis study revealed that there was a significant difference between untreated control group with groups A_c and B_c. A significant difference was also found between group A_c and group B_c ($p<0.05$). With regards to the level of creatinin and uric acid, similarly to acute aflatoxicosis study, analysis indicated that there was a significant difference between untreated control animals and animals of groups groups A_c and B_c ($p<0.05$). However no significant difference was found between groups A_c and B_c. AFB_1 was detected from rats serum sampels of groups A_c and B_c, but compared to the acute aflatoxicosis study, there was a significant difference between groups A_c and B_c ($p<0.05$).

According to the results of this study it can be concluded that probiotic LcS supplementation could improve the adverse effect of AFB_1 induction more effective and significantly in chronic aflatoxicosis study compared to the acute aflatoxicosis study. Therefore longer duration of probiotic LcS supplementation with more

number of animals is suggested for future studies to confirm the ability of probiotic Lcs to reduce the bioavalibilty of AFB_1 *in vivo*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PROBIOTIK *LACTOBACILLUS CASEI* STRAIN SHIROTA TERHADAP TAHAP AFLATOKSIN B_1 DALAM TIKUS YANG DIARUH DENGAN AFLATOKSIN

Oleh

ELHAM NIKBAKHT NASRABADI

Februari 2013

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Aflatoksin B_1 (AFB_1) dianggap sebagai bahan pencemar makanan yang paling toksik dengan kesan berbahaya kepada kesihatan manusia dan haiwan yang dihasilkan oleh spesies kulat *Aspergillus (A.)* iaitu *A. flavus* dan *A. parasiticus*. Ia dikelaskan sebagai karsinogen oleh satu kumpulan Agensi Antarabangsa untuk Penyelidikan Kanser yang dikaitkan dengan etiologi kanser hati. Intervensi dan pendekatan pencegahan dengan menumpukan lebih tumpuan kepada langkah-langkah pra dan pasca penuaian bagi mengurangkan tahap AFB_1 dalam tanaman atau pada peringkat individu untuk memodulasi integrasi biologi dan penyingkiran AFB_1 atau mengurangkan kebolehserapan biologi tanaman tersebut. Mikro organisma, terutamanya bakteria, telah dikaji potensi mereka untuk mengurangkan kebolehserapan biologi aflatoksin serta bahan cemar makanan yang lain. Antara mereka, *lactic acid bacteria* dikenalpasti mempunyai keupayaan untuk mengurangkan kebolehserapan biologi aflatoksin *in vitro*.

Penyelidikan ini bertujuan untuk menyiasat kesan bakteria probiotik *Lactobacillus casei* strain Shirota (LcS) terhadap tahap AFB_1 dalam dalam tikus yang diaruh oleh aflatoksin melalui ujian berat, hati dan fungsi buah pinggang, dan juga AFB_1 dalam serum berdasarkan pada dua kajian yang berasingan iaitu akut dan kronik aflatoksin. Akhirnya perbandingan telah dilakukan untuk melihat kesan daripada suplemen probiotik dalam tempoh yang berbeza antara kajian akut dan kronik AFB_1. Untuk mencapai tujuan ini, satu kajian eksperimen telah dijalankan, dan sejumlah 48 ekor tikus telah dibahagikan kepada dua kumpulan (n=24) untuk menjalankan dua kajian iaitu akut dan kronik aflatoksin. Tikus-tikus dalam kajian akut aflatoksin telah dibahagikan kepada tiga kumpulan kecil iaitu A_a, B_a, dan kawalan yang tidak dirawat (n=6). Kumpulan A_a (n=9) menerima probiotik LcS (10^8 CFU/ml) melalui mulut setiap hari selama 7 hari berturut-turut, dan kumpulan B_a (n=9) menerima kandungan air masin yang sederhana (1 ml) setiap hari selama 7 hari berturut-turut. Sejurus selepas dos yang keempat probiotik dan air masin yang sederhana, tikus di kedua-dua kumpulan A_a dan B_a telah diberikan dengan satu dos AFB_1 sejumlah 1.5 mg/kg berat badan melalui mulut. Tikus-tikus dalam kajian kronik aflatoksin juga telah dibahagikan kepada tiga kumpulan kecil A_c, B_c, dan kumpulan kawalan yang tidak dirawat. Kumpulan A_c telah diberikan probiotik LcS (10^8 CFU/ml) melalui mulut setiap hari selama 20 hari berturut-turut, dan kumpulan B_c menerima kandungan air masin yang sederhana (1 ml) sekali lagi setiap hari selama 20 hari berturut-turut. Sejurus selepas dos yang keempat probiotik dan kandungan air masin yang sederhana serta dari hari ke-4, tikus dikedua-dua kumpulan A_c, dan B_c telah mula menerima pelbagai dos daripada AFB_1 pada setiap hari sejumlah 25 μg/kg berat badan untuk 5 hari seminggu selama lebih 2 minggu melalui mulut.

Berdasarkan kepada analisis berat badan tikus dalam kumpulan aflatoksin akut, perbezaan yang signifikan didapati di antara kumpulan kawalan dengan dua kumpulan yang lain yang telah diberikan dengan dos AFB_1 (kumpulan A_a dan B_a) pada hari ke-6 dan ke-7 ($p<0.05$). Walau bagaimanapun, terdapat tiada sebarang perbezaan yang signifikan antara kumpulan yang A_a dan B_a tetapi purata berat badan tikus adalah tinggi dalam kumpulan A_a. Sebaliknya terdapat perbezaan yang signifikan antara kumpulan kawalan dan kumpulan A_a dan B_a dari segi status darah dari hati dan penanda biologi dari buah pinggang dalam kumpulan aflatoksin akut ($p<0.05$). Walaupun tidak terdapat perbezaan yang signifikan di antara kumpulan A_a dan B_a, purata nilai penanda biologi tersebut (ALT, AST, CREA dan UREA) adalah lebih besar dalam kumpulan B_a berbanding dengan kumpulan A_a. Untuk menyiasat kesan probiotik LcS terhadap penyerapan AFB_1, tahap serum AFB_1 telah diukur dalam semua kumpulan dan kemudian dibandingkan bersama-sama. Seperti yang dijangka, aflatoksin B_1 dikesan daripada semua sampel serum darah untuk kawalan. Purata AFB_1 pada tahap serum darah dalam kumpulan B_a adalah lebih tinggi daripada kumpulan A_a. Dalam kumpulan kronik aflatoksin keputusan yang berbeza telah diperolehi. Dengan mengambil kira berat badan tikus, terdapat perbezaan yang signifikan antara kumpulan B_c dan dua kumpulan lain (kawalan yang tidak dirawat dan kumpulan A_c). Perbezaan yang signifikan ini didapati didapati dari hari ke-9 hingga hari terakhir kajian aflatoksin kronik. Analisis enzim hati dalam darah dalam kajian aflatoksin kronik mendedahkan bahawa terdapat perbezaan yang signifikan antara kumpulan kawalan yang tidak dirawat dengan kumpulan A_c dan B_c. Perbezaan yang signifikan juga didapati antara kumpulan A_c dan kumpulan B_c ($p<0.05$). Merujuk kepada tahap kritinin dan asid urik, begitu juga kajian aflatoksin akut, analisis menunjukkan bahawa terdapat perbezaan yang signifikan antara tikus-tikus

dalam kumpulan kawalan yang tidak dirawat dan tikus-tikus dalam kumpulan A_c dan B_c (p<0.05). Walau bagaimanapun, tiada perbezaan yang signifikan didapati antara kumpulan A_c dan B_c. AFB_1 telah dikesan dari sampel serum tikus dari kumpulan A_c dan B_c, tetapi berbanding dengan kajian aflatoksin akut, terdapat perbezaan yang signifikan antara kumpulan A_c dan B_c (p<0.05).

Menurut hasil kajian ini dapat disimpulkan bahawa suplemen probiotik LcS boleh mengurangkan kesan buruk akibat aruhan AFB_1 lebih berkesan dalam kumpulan kajian aflatoksin kronik berbanding kajian aflatoksin akut. Oleh itu, tempoh yang lebih lama untuk pemberian suplemen probiotik LcS dengan bilangan haiwan yang lebih banyak dicadangkan untuk kajian masa depan bagi mengesahkan keupayaan LcS untuk mengurangkan kebolehserapan biologi daripada AFB_1 secara *in vivo*.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of requirement for degree of Master of Science. The members of Supervisory Committee were as follows:

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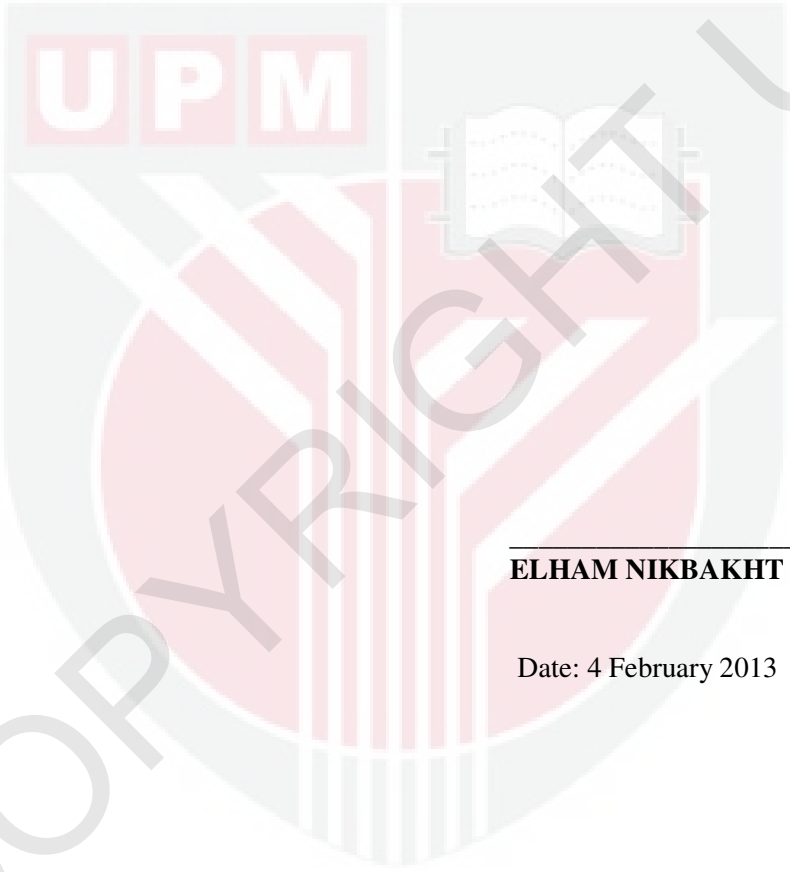
Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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ELHAM NIKBAKHT NASRABADI

Date: 4 February 2013

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LIST OF ABBREVIATIONS

ACUC	Animal Care and Use Committee
AFB_1	Aflatoxin B_1
AFB_1GSH	Aflatoxin B_1Glutathione conjugate
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFL	Aflatoxicol
AFM_1	Aflatoxin M_1
AFM_2	Aflatoxin M_2
AFP_1	Aflatoxin P_1
AFQ_1	Aflatoxin Q_1
ALA	Alpha linolenic acid
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
Bhd	Berhad
CFU	Colony forming unit
CYP	Cytochrome P450
EDTA	Ethylenediaminetetraacetic acid
GG	*Lactobacillus rhamnosus* strain GG
HCA	Heterocyclic Amines
GGT	Gamma glutamyl transpeptidase

HPLC	High performance liquid chromatography
IAC	Immunoaffinity column
IARC	International Agency for Research on Cancer
LAB	Lactic acid bacteria
LC705	*Lactobacillus rhamnosus strain* LC705
LD50	Lethal dose, 50%
PJS	*Propionibacterium freudenreichii* ssp. *Shermanii* JS
USA	United State of America
WHO	World Health Organization
α	Alpha
β	Beta
γ	Gama
ω	Omega
Δ	Delta
℃	Degree centigrade
<	Lower than
>	More than
±	Plus and mines
%	Percentage
µ	Micro
µl	Microliter
L	Liter
dl	Deciliter
d	Day
cc (ml)	Milliliter
cm	Centimeter
M	Molar

m	Meter
mm	Millimeter
mg	Milligram
mmol	Millimol
nm	Nanometer
kg	Kilogram
g	Gram
w (Wt)	Weight
v	Volume
U (IU)	International unit
Min	Minute
p	*p* value
mAU	Peak area
C_{18}	Carbon 18
H_2O	Water
NH_4SCN	Ammonium thiocyante
O_2	Oxygen
RCOOH	Carboxylic Acids

CHAPTER 1

INTRODUCTION

1.1 Background

Food is the fuel of life, and everyone is concerned about the quality and safety of food. Harmful components in plant derived foods can be produced by the plant itself, or contaminants derived from either man-made sources or microorganisms. Among these microorganisms, toxin producing fungi are ubiquitous in the environment and can invade the crops and produce toxic secondary metabolites known as mycotoxins. Mycotoxins can appear in the food chain as a result of fungal infection of crops, which are either eaten by humans or through those used as livestock feed. Low-level chronic exposure to mycotoxins can induce chronic toxic effects in human, such as carcinogenic and estrogenic effect (Bennett & Klich, 2003), mutagenicity (Lehmann et al., 2006), teratogenicity (Creppy, 2002), and high-level exposure can cause acute disease (Robbins et al., 2000), which may result in death.

Aflatoxins are the most important mycotoxins. They can be produced by four toxic species of *Asperagillus (A.),* which are *A. flavus, A. flavus ssp. parasiticus, A. nomius and A. pseudotamarii* (Pitt, 2000) as secondary metabolites. Aflatoxins commonly contaminate maize and groundnuts, and are categorized as group one human carcinogens by the International Agency for Research on Cancer which is linked to the etiology of liver cancer (IARC, 2002).

At least 13 different types of aflatoxin are produced in nature. Aflatoxin B_1 (AFB_1) is considered as the most toxic and is produced by both *A. flavus* and *A. parasiticus*. Aflatoxins G_1 and G_2 are produced by *A. parasiticus*. Aflatoxins M_1 and M_2 were originally discovered in the milk of cows, which were fed on moldy grain. These compounds are products of a conversion process in the animal's liver. However, aflatoxin M_1 is present in the fermentation broth of *A. parasiticus* (Boutrif, 1998).

Microorganisms, especially bacteria, have been studied for their potential to either degrade mycotoxins or reduce their bioavailability. Among these bacteria and probiotics, *lactic acid bacteria* (LAB) have been known to have the ability to reduce the bioavailability of aflatoxins *in vitro* (Gratz et al., 2005). Furthermore, probiotic bacteria exert a number of other beneficial health effects, which make them even more suitable as additives for both food and feed.

Probiotics are dietary supplements of live microorganisms shown to be healthy for the host organism. According to the currently adopted definition by FAO/WHO (2001), probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". LAB and *Bifidobacteria* are the most common types of microbe used as probiotics; however, certain yeasts and bacilli might also be helpful (Srividya & Vishnuvarthan, 2011).

The term "probiotics" indeed was first introduced by Kollath in 1953 (Hamilton-Milleret, 2003). At that time, probiotics were defined as microbially derived factors

that can stimulate the growth of other microorganisms. Fuller (1989) suggested a definition of probiotics that has been widely used: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". In the following decades, intestinal LAB species that were considered to have beneficial health properties were introduced as probiotics, including *Lactobacillus (L.) rhamnosus*, *L. casei*, and *L. johnsonii* (Tannock, 2003).

When discussing probiotic bacteria, the term LAB is often used. LAB constitute a heterogeneous group of gram-positive, low-GC (G and C refer to the guanine and cytosine content in their genomes), acid-tolerant, generally non-sporing, non-respiring cocci or rods, producing lactic acid as the major end product of carbohydrate fermentation and include strains from the genera *Aerococcus, Alliococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Lactoshaera, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* (Axelsson, 2004). As probiotics, only strains from the genus *Lactobacillus* (e.g.*, L. acidophilus, L. bulgaricus, L. casei, L. plantarum, L. reuteri, and L. rhamnosus*) are used.

Probiotics have been successfully incorporated in a number of foods including the traditional vehicle of fermented milks and yogurts, and increasingly in other fermented and non-fermented foods beyond the dairy sector. Dairy products include fermented milk, yogurt, frozen yogurt, kefir, matured cheese, fresh cheese, ice cream, dairy desert, fermented soymilk, fermented cereal products, dry products, such as infant formula and nutritional powders, some juices, fermented meats, and

confectionary products. Furthermore, probiotics can be added to fresh service products, such as sandwiches, sushi, and smoothies, which can be refrigerated.

1.2 Statement of problem

Toxins are regularly ingested. A number of toxins come from foods eaten every day. Mycotoxins represent one of the important classes of naturally occurring toxicants in food, which poses a considerable health risk (Shetty & Jespersen, 2006). Mycotoxins heavily resist decomposition or breaking down in digestion, and, therefore, will remain in the food chain. They cannot even be destroyed by temperature treatments such as cooking and freezing (Bennett & Klich, 2003). Low levels of exposure to mycotoxins occur in some parts of the world where food is available in higher quality and variety; whereas high level exposure exists in areas where populations depend on a single staple food commodity (Wild & Gong, 2010).

As mentioned earlier, the most important mycotoxins are aflatoxins, and since no animal species has resistance to the acute toxic effects of aflatoxins, it is reasonable to assume that humans may be correspondingly affected. The chronic exposure to aflatoxins of farm and laboratory animals compromises immunity and interferes with protein metabolism and multiple micronutrients that are crucial to health. The prevalence and level of human exposure to aflatoxins on a global scale have been reviewed, and the result indicates that approximately 4.5 billion persons living in

developing countries are chronically exposed to largely uncontrolled amounts of the toxin (Williams et al., 2004).

An outbreak of food poisoning resulting in 13 deaths in children occurred in Malaysia during the Chinese Festival of the Nine-Emperor Gods in 1988. The offending food was a Chinese noodle called 'Loh See Fun' (LSF). The source was traced to a factory where a banned food preservative was added to make the LSF. The food poisoning was attributable to aflatoxins and boric acid (Chao et al., 1991). Moreover several studies reported aflatoxin contamination in foodstuffs in Malaysia (Arzandeh et al., 2010; Leong et al., 2011).

Furhteremore there is no non-drug supplement to reduce the level of aflatoxin in human body. The use of non-nutritional aflatoxin adsorbents such as clays or activated carbons can cause toxicity and pose harmful effect (Kabak & Ozbey, 2012).

On the other hand, there are several hypotheses suggesting the mechanisms of probiotics and toxin. Fermentation of food has been used as a method of preservation for centuries, and LAB are reported to reduce mold growth and aflatoxin production (Mokoena et al., 2006). Several bacterial strains, of food or human origin, have been tested for their ability to bind aflatoxins and other mycotoxins to the bacterial cell wall (El-Nezami et al., 2002a; El-Nezami et al., 2002b; Styriak & Conkova, 2002). Under *in vivo* conditions, it is expected that

probiotic bacteria bind AFB_1 as soon as they interact with each other inside the intestinal tract, and, also, numerous studies reveal the benefits of probiotics to human health. Thus, the goal of this study is to identify the potential of a probiotic, specifically, *Lactobacillus casei* Shirota strain (LcS), on toxin reduction in acute and chronic aflatoxicosis rats.

The following are the research questions that are addressed in this study:

1. Is there any difference in the body weight of rats that are dosed with aflatoxin and those given probiotic LcS plus aflatoxin in acute and chronic aflatoxicosis?
2. In aflatoxin-induced rats, does probiotic LcS have any impact on hepatotoxic effect of AFB_1 and also the elevation of kidney biomarkers, in acute and chronic aflatoxicosis?
3. What is the efficacy of probiotic LcS in reducing the absorption of aflatoxin B_1 in acute and chronic aflatoxicosis rats?
4. Is there any difference in probiotic LcS supplementation efficacy in terms of duration between acute and chronic aflatoxicosis rats?

1.3 Significance of study

Biological decontamination of mycotoxins using microorganisms is one of the renowned strategies for the management of mycotoxins in foods and feeds. Among

the different potential decontaminating microorganisms, LAB represents unique groups, which are widely used in food fermentation and preservation.

Aflatoxin B_1 as mentioned previously is an important food contaminant with a detrimental effect on human and animal health. Intervention approaches concentrate on pre- and post-harvest measures to reduce AFB_1 levels in crops or on the individual level to regulate bioactivation and excretion of AFB_1 or reduce its bioavailability (Khlangwiset & Wu, 2010). Probiotic bacteria have been known to have the capacity to reduce the bioavailability of AFB_1 as well as other food contaminants (Styriak & Conkova, 2002).

This study compared the effect of probiotic LcS in two different durations of toxin induction namely acute and chronic aflatoxicosis. No study has been done before to compare the effect of probiotic LcS in acute and chronic aflatoxicosis. Furthermore, probiotics and specially LcS propose a wide range of potentially beneficial medicinal uses. Therefore, it is hoped that this research can be useful in contributing to the knowledge of reducing the level of toxins *in vivo* by applying probiotics. Hence, it may provide knowledge for nutritionist and health practitionoures concerning the importance of probiotics LcS for human health. This exclusive bacterium, which is also called Shirota strain has been tested for its beneficial role in the digestive tract, such as improving bowel movement, maintaining balance of intestinal flora, Boosting the immune system, and finally reducing toxins in the intestines (Spanhaak et al., 1998).

1.4 Objective of study

1.4.1 General objective

To investigate the effect of probiotic *Lactobacillus casei* Shirota strain on aflatoxin B_1 level in acute and chronic aflatoxicosis rats.

1.4.2 Specific objectives

1. To determine the effect of LcS on the rats body weight that are dosed with AFB_1 in acute and chronic aflatoxin exposure.
2. To determine the effect of LcS on hepatotoxic effect of AFB_1 induction through liver function test and also kidney biomarkers in acute and chronic aflatoxicosis rats.
3. To determine the effect of LcS on AFB_1 absorption through AFB_1 blood serum level of acute and chronic aflatoxicosis rats.
4. To compare the effect of probiotic LcS supplementation in the same daily dosage, but different duration between acute and chronic aflatoxicosis rats.

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