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Histopathological studies on kidney and liver of albino rat infected with toxigenic *Aspergillus flavus* after treatment with isolated *Lactobacillus* species from Kunu

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Abstract

Background: Aflatoxin is a metabolic product of *Aspergillus flavus* that causes several injuries to vital organs in the body.

Methods: The liver and kidney tissue of healthy rats challenged with toxigenic *A. flavus* after treatment with *Lactobacillus plantarum* and *Lactobacillus delbrueckii* were examined.

Results: The weight of the liver (3.61 g) and kidney (11.33 g) of infected rats with toxigenic mould were significantly reduced ($P < 0.05$) when compared to the group treated with *Lactobacillus* spp.; BD+AP+LP, BD+AP+LD and BD+ AP+LPD. The rats fed basal diet and *Lactobacillus* spp. have a normal histological structure. Necrotic lesions, thickening of the glomerular basement membrane and collapse of the glomerulus were observed in the liver and kidney of rats induced with *A. flavus*. The rats infected with *Lactobacillus* spp. regained their strength and activity after treatment but showed mild necrosis in the liver and thickening of glomerular basement in the kidney.

Conclusions: The use of *Lactobacillus* species suppressed the growth and eliminated the potential risk of toxigenic *A. flavus* in the infected rats. This showed that *Lactobacillus* spp. possess some therapeutic properties due to their ability to secrete secondary metabolites. The bioactive compounds can be exploited and used in food products to inhibit the growth of food borne pathogens.

Keywords: Aflatoxins, Lactic Acid Bacteria, Bio-control, Fermented beverage.

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Études histopathologiques sur les reins et le foie de rats albinos infectés par *Aspergillus flavus* après un traitement avec des espèces isolées de *Lactobacillus* de Kunu

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Abstrait

Contexte: L'aflatoxine est un produit métabolique d'*Aspergillus flavus* qui provoque plusieurs lésions des organes vitaux du corps

Méthodes: Les tissus hépatique et rénal de rats sains mis au défi par *A. flavus* toxigénique après un traitement par *Lactobacillus plantarum* et *Lactobacillus delbrueckii* ont été examinés.

Résultats: Le poids du foie (3,61 g) et du rein (11,33 g) des souris infectées atteintes de moisissure toxigénique était significativement réduit ($P < 0,05$) par rapport au groupe traité par *Lactobacillus* spp. BD + AP + LP, BD + AP + LD et BD + AP + LPD. Les rats nourris avec un régime alimentaire de base et *Lactobacillus* spp. avoir une structure histologique normale. Des lésions nécrotiques, un épaississement de la membrane basale glomérulaire et un collapsus du glomérule ont été observés dans le foie et les reins de rats induits par *A. flavus*. Les souris infectées par *Lactobacillus* spp. ont retrouvé leur force et leur activité après le traitement, mais ont présenté une légère nécrose du foie et un épaississement du socle glomérulaire dans le rein

Conclusions: L'utilisation d'espèces de *Lactobacillus* a inhibé la croissance et éliminé le risque potentiel de toxine *A. flavus* toxigénique chez les souris ingérées. Cela a montré que *Lactobacillus* spp. possèdent certaines propriétés thérapeutiques en raison de leur capacité à sécréter des métabolites secondaires. Les composés bioactifs peuvent être exploités et utilisés dans des produits alimentaires pour inhiber la croissance d'agents pathogènes d'origine alimentaire

Mots-clés: Aflatoxines, Bactéries Lactiques, Bio-contrôle, Boisson fermentée

Introduction

Mycotoxins are secondary metabolites produced by some fungi. The fungal toxins; aflatoxins, fumonisins, ochratoxins, patulin, trichothecenes and zearalenone cause suppression of immune system, acute and chronic hepatocellular injury in animal or human (1). Nowadays, there is public concern about the type of mycotoxin produced by moulds in food. Aflatoxin is an important mycotoxin, commonly produced by *Aspergillus flavus* and *Aspergillus parasiticus* in food, unlike *Aspergillus nomius*, *Aspergillus bombycis*, *Aspergillus pseudotamari* and *Aspergillus ochraceoroseus* that are aflatoxigenic but less encountered in food. Biosynthesis of fungal toxin is highly influenced by fungal species, humidity, temperature, inadequate drying of the crops and type of foods (2). Food crops may come in contact with any of the phytopathogenic fungi on the field, during harvesting, post-harvest, storage or when processing and thus, adversely affect the quality of food products (3).

Consumption of food containing aflatoxigenic fungi or fungal toxins cause injuries in animals by decreasing their productivity due to chronic damage to their vital tissues and organs (4), Aflatoxin B1 produced by fungi is metabolized in the liver by the cellular cytochrome p450

microsomal enzymes to form an intermediate called aflatoxin B1-8, 9-epoxide, which thus, reacts with macromolecules such as lipid and DNA. The consequence of the reaction leads to disruption of transcription, lipid peroxidation, cellular impairment and abnormal cell proliferation (5). The acute intoxication of aflatoxin has devastating effects on the body. Hepato-cellular carcinoma, a liver cancer is also a primary disease associated with aflatoxin intake (6, 7).

However, the application of non-pathogenic microorganisms and their metabolites to prevent fungal infection will minimize public health hazards. Lactic Acid Bacteria (LAB) inhibit the growth of mould by secreting antimicrobial compounds, which bind with fungal toxins to eliminate their pathogenicity (8). The present study was therefore undertaken to reveal the protective role of LAB against the pathogenicity of aflatoxigenic *A. flavus* on the liver and kidney of induced albino rats.

Materials and Methods

Source of Toxigenic *Aspergillus flavus*

The studied toxigenic *A. flavus* had been screened for aflatoxins B1, B2, G1 and G2 in the previous studies of Jeff-Agboola (9). The fungus was sub-cultured

into Petri dishes with Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) and incubated at 28 ± 1 °C for 7 days. After fungal full sporulation, their spores were harvested into sterile peptone water (0.2%) and filtered using sterile cotton filter to avoid the presence of conidia or mycelial debris (10). The fungal spore in peptone water was serially diluted and adjusted to the dilution 10^6 *A. flavus* spores/ml as the final concentrations.

Source of Lactic Acid Bacteria

Lactobacillus plantarum and *L. delbrueckii* were isolated from "Kunu", a traditionally fermented beverage. These *Lactobacilli* have been reported to have pronounced inhibitory effect against toxigenic fungus in our previous study (11).

Experimental Design

The male and female Wistar albino rats for the experiment were obtained from the Department of Animal Production and Health, The Federal University of Technology, Akure. The rats were 12-16 weeks old and weighing between 140 to 147 g. The animals were kept in a cage for 7 days to acclimatize them to the environmental conditions at 25 ± 2 °C, 12 h light-darkness cycle with adequate access to feed and water *ad libitum*.

Five rats were randomly assigned into each group and labeled as follows; BD: rats fed with basal diet; BD+AF: rats fed basal diet and injected with toxigenic fungus; BD+LP: rats fed basal diet and injected with *L. plantarum*; BD+LD: rats fed basal diet and injected with *L. delbrueckii*; BD+AF+LP: rats fed basal diet, injected with toxigenic fungi and *L. plantarum*; BD+AF+LD: rats fed basal diet, injected with toxigenic fungi and *L. delbrueckii*; and BD+AF+LPD: rats fed basal diet, aflatoxigenic fungi, *L. plantarum* and *L. delbrueckii*.

In this study, the experiment was performed in accordance with the institutional ethics and international standard of animal welfare described by National Research Council (12). The experiment was conducted using a completely randomized design.

Healthy rats challenge with toxigenic *A. flavus* and treatment with LAB

Five hundred microliter (500 μ l) of the infectivity dose of the test fungi (10^6 spores/ml) was orogastrically administered into the rats, and after signs of infection were observed, 500 μ l of suspension containing LAB (10^8 cfu/ml) was administered into the animals.

Histopathological Examination

All animals were sacrificed by cervical decapitation. Livers and kidneys were dissected out, washed with ice-cold saline and weighed using a digital scale (KERRO BL 200001, MxRady Lab Solutions Pvt. Ltd., Delhi, India). Thereafter, samples of liver and kidney tissues of each animal were excised and processed according to the methods of Drury et al. (13). Briefly, the tissue specimens were fixed with 10% neutral buffered formalin solution, dehydrated in alcohol and embedded in paraffin wax. Sections were cut at 5 μ m thickness and stained with hematoxylin and eosin (H&E, Thermo Shandon, USA).

Statistical Analysis

Data obtained were analyzed by one-way analysis of variance (ANOVA). Means were compared by Duncan's New Multiple Range Test and considered statistically significant when $P < 0.05$, using Statistical Package for Social Sciences (SPSS) software version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

The rats fed basal diet (BD) have liver and kidney weight of 6.0 g and 16.7 g respectively. The weight of liver (3.61 g) and kidney (11.33 g) in infected rats with toxigenic mould were significantly different ($P < 0.05$) when compared to treated group of rats with LAB; BD+AF+LP, BD+AF +LD, BD+AF +LDP (Table 1). The rats fed basal diet and *Lactobacillus* spp. have a normal histological structure without visible lesions in their liver and kidney. Histopathological analysis of the infected albino rats with toxigenic fungus shows generalized ballooning, lesions, necrosis of hepatocytes

and congested central vein in the liver (Plate 1b). There were histological changes (Plate 3b) in the kidney of rats injected with aflatoxigenic *A. flavus*. The treated group of rats with LAB after infection caused by *A. flavus* showed mild necrosis of

hepatocytes, less thickening of glomerular basement membrane and no gross change in their hepatic structures (Plates 2 and 4).

Table 1: Weight (g) of liver and kidney of experimental rats

Group of rats	Liver	Kidney
BD	6.00 ^d ±0.30	16.67 ^d ±0.41
BD+AF	3.61 ^a ±0.21	11.33 ^a ±0.50
BD+LP	5.60 ^c ±0.15	15.33 ^c ±0.35
BD+LD	5.60 ^c ±0.22	15.67 ^c ±0.51
BD+ AF+ LP	4.67 ^b ±0.15	13.67 ^b ±0.42
BD +AF +LD	4.67 ^b ±0.23	13.67 ^b ±0.36
BD +AF +LPD	4.67 ^b ±0.31	13.67 ^b ±0.43

Values are mean±sd of triplicates (n=3). Value with the same alphabet along column are not significantly different (P<0.05). The rats were fed;

BD=Basal diet

BD+AF= Basal diet and toxigenic *A. flavus*

BD+LP= Basal diet and *Lactobacillus plantarum*

BD+LD= Basal diet and *Lactobacillus delbrueckii*

BD+AF+LP = Basal diet, *A. flavus* and *Lactobacillus plantarum*

BD+AF+LD = Basal diet, *A. flavus* and *Lactobacillus delbrueckii*

BD+AF +LPD= Basal diet, *A. flavus*, *Lactobacillus plantarum* and *Lactobacillus delbrueckii*

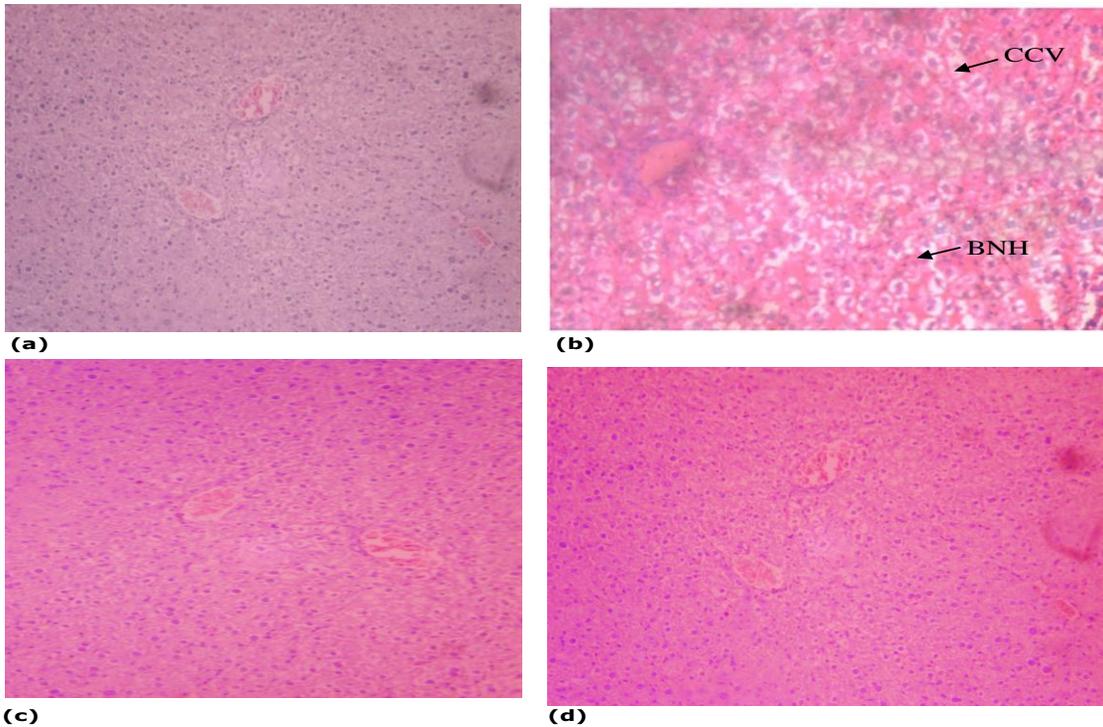


Plate 1: Photomicrograph of liver of albino rat fed (a) Basal diet (BD), (b) Basal diet with toxigenic *A. flavus* (BD+AF), (c) Basal diet with *L. plantarum* BD+LP and (d) Basal diet with *L. delbrueckii* (BD+LD). CCV = Congested central vein and BNH = Ballooning and necrosis hepatocytes.

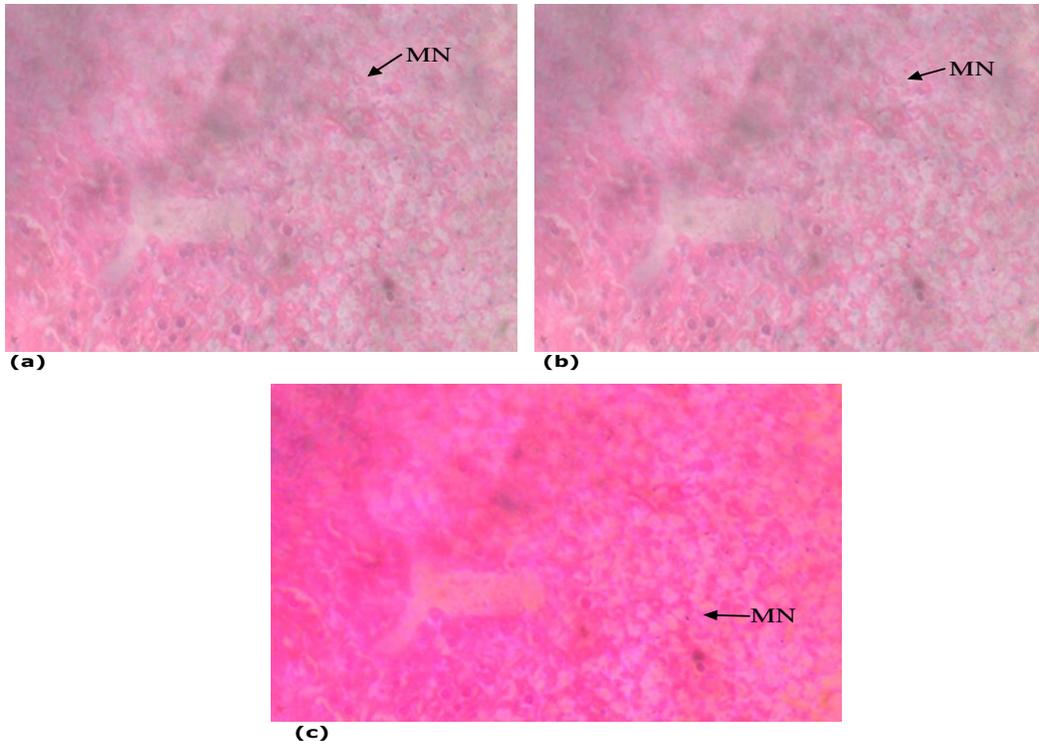


Plate 2: Photomicrograph of treated liver of albino rat fed (a) Basal diet, toxigenic *A. flavus* with *Lactobacillus plantarum* (BD+AF+LP), (b) Basal diet, toxigenic *A. flavus* with *Lactobacillus delbrueckii* (BD+AF+LD) and (c) Basal diet, toxigenic *A. flavus*, *Lactobacillus plantarum* with *Lactobacillus delbrueckii* (BD+ AF +LPD). MN: mild necrosis.

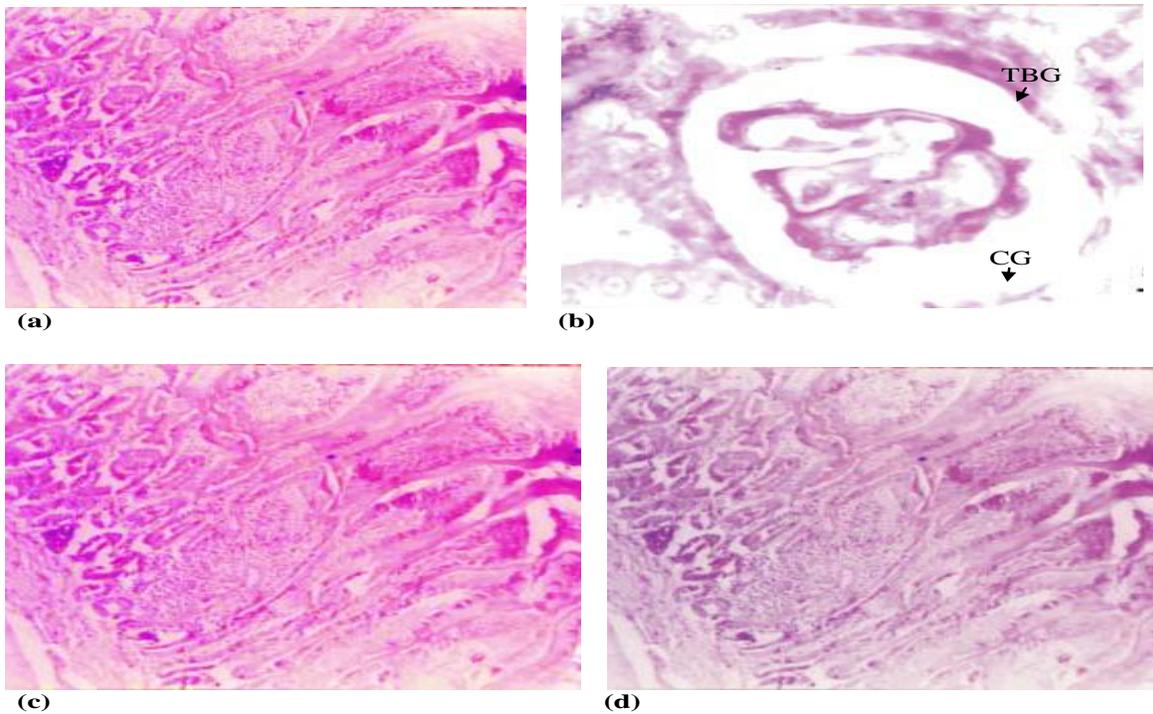


Plate 3: Photomicrograph of kidney of rat fed (a) Basal diet (BD), (b) Basal diet with toxigenic *A. flavus* (BD+AF), (c) Basal diet with *L. plantarum* BD+LP and (d) Basal diet with *L. delbrueckii* (BD+LD), TBG = thickened glomerular basement and CG = Collapse of glomerulus

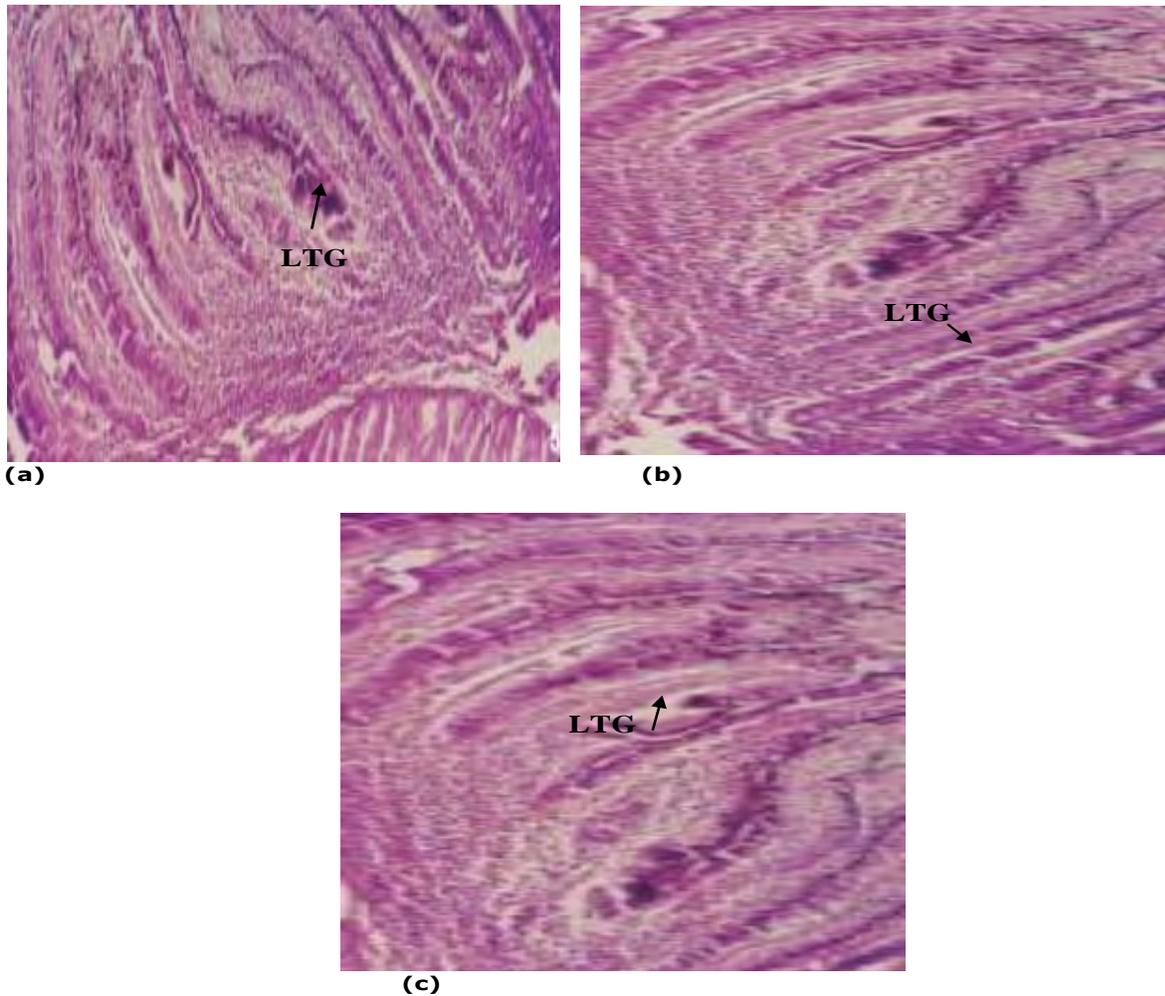


Plate 4: Photomicrograph of treated kidney of rat fed (a) Basal diet, toxigenic *A. flavus* with *Lactobacillus plantarum* (BD+AF+LP), (b) Basal diet, toxigenic *A. flavus* with *Lactobacillus delbrueckii* (BD+AF+LD) and (c) Basal diet, toxigenic *A. flavus*, *Lactobacillus plantarum* with *Lactobacillus delbrueckii* (BD+ AF +LPD). LTG = less thickened of glomerular basement membrane.

Discussion

Aflatoxigenic fungi, notably, *Aspergillus* spp. produced a secondary metabolite during hyphal growth in tissues, which causes inflammation or infections. The protective effect of LAB against the toxigenic activity of *A. flavus* on the liver and kidney of the infected rats was investigated. In an earlier study of Olonisakin et al. (11), the body weight of infected rats with toxigenic *A. flavus* was reduced to 120.23 g, while those treated with LP, LD and LPD after infection maintained their body weight as 150.43 g, 148.60 g and 155.84 g respectively. The reduction of feed intake and loss of

bodyweight in infected rats conformed to the finding of Abdel-Wahhab *et al.* (14) who indicated those signs as adverse effects and toxicity in rats injected with fungal toxin. The reduction in feed intake observed in tested rats has been reported to lead to protein catabolism, thereby causing impaired glomerular filtration and other kidney injuries (15).

The treated groups with LAB recovered with no symptoms of infection. A mixture of *Lactobacillus* species has been found to reduce fungal growth as well as aflatoxin production by *Aspergillus flavus* subsp. *parasiticus* (16). Hence, *Lactobacillus* spp. are probiotics, non-

pathogenic microorganisms with some therapeutic metabolites, which are essential for potential health benefits like maintaining normal intestinal microflora, modulating the immune system, detoxifying colonic contents, lowering serum cholesterol levels and promoting lactose tolerance in intestine (17).

The colour of the liver in infected rats with aflatoxigenic fungus was slightly pale and grey. This agreed with Yener *et al.* (18) who reported slightly pale, enlarged and grayish mottled in the liver of aflatoxin-induced rats, which indicated significant damage to the vital organs of the albino rats. The histopathological changes observed were in accordance with the studies of Yener *et al.* (18) and Gelderblom *et al.* (19) who stated that induced AFB₁ resulted to predominant lesions, extensive hydropic degeneration, necrosis, dysplastic and swollen hepatocytes. In the study of El-Nekeety *et al.* (20), they revealed that liver injury such as necrosis began to occur when the stored glutathione is almost exhausted due to their important role in the detoxification of toxic metabolites produced by fungi. Aflatoxigenic fungi generate Reactive Oxygen Species (ROS) and consequently caused lipid peroxidation, which lead to hepatotoxicity (21). Abdel-Wahhab *et al.* (22) and El-Mahalaway (23) had reported necrosis of tubules, degeneration of granular with cloudy swelling in the epithelial cell of proximal tubules and enlarged pale vacuolated cytoplasm. Hence, aflatoxigenic fungi are known to have a deleterious effect on immune system, cellular component, tissue and organs.

The bio-protective potential of LAB against the toxigenic fungus can be attributed to the production of antifungal compounds such as benzoic acid, methyl hydantoin, mevalonolactone, short-chain fatty acids, proteinoous compounds and secretion of de-conjugated bile acids from bile salt synthesized by the host (24, 25). The detoxification of aflatoxins and elimination of their symptoms by LAB is a promising task toward an immunostimulatory property.

Conclusively, the protective effect of *Lactobacillus* spp. on the organs of rats induced with aflatoxigenic fungi can be attributed to some bioactive metabolites. The presence of natural antimicrobial products in *Lactobacillus* spp. can be used for competitive exclusion of pathogens, which will be a biological approach to eliminate food borne pathogens as well as decontaminating fungal toxin in foods.

Conflict of Interest

None

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