

## Potentials protective role of yogurt against toxic effects of aflatoxin-contaminated *Prunus mahaleb* in rats

Mona Y. Mostafa<sup>1</sup>- <sup>2</sup>Samah A. Elsemelawy

<sup>1</sup>Home Economics Department, Faculty of Specific Education, Mansoura University, Egypt

<sup>2</sup>Home Economics Dept., Faculty of Specific Education, Tanta University. Egypt.

[monayasser300@gmail.com](mailto:monayasser300@gmail.com)



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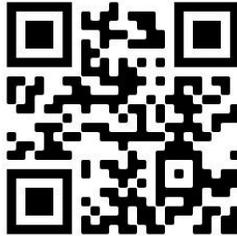
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## **Abstract**

**Background:** *Aspergillus* species contain a group of chemically toxic fungal metabolites known as aflatoxins. Fungi can contaminate nuts, leading to the development of mycotoxins. The study's objective was to inspect the ability of yogurt in helping experimental rats recover from the negative effects of eating stored *Prunus mahaleb* contaminated with aflatoxins. After one and two months of storage at room temperature, total aflatoxins concentrations in *Prunus mahaleb* were determined. Rats were divided into five groups (6 rats each); a negative control group (fed on a normal diet), group 2 (fed on a standard diet with 5% *Prunus mahaleb* was stored for one month), group 3 (fed on a standard diet with 5% *Prunus mahaleb* was stored for two months), group 4 (fed on a standard diet with 5% *Prunus mahaleb* was stored for one month with 180 cc<sup>2</sup> / kg body weight /day yogurt) and group 5 (fed on a standard diet with 5% *Prunus mahaleb* was stored for two months with 180 cc<sup>2</sup> / kg body weight /day yogurt).

**Results:** The findings demonstrated the yogurt's ability to reduce aflatoxin effects on biochemical parameters in rats' serum. An improvement was recorded in hemoglobin and packed cell volume, lipid profile, liver and kidney functions and antioxidant enzymes activities for rats taking yogurt with the presence of aflatoxin of stored *Prunus mahaleb*. Yogurt consumption aided in the reduction of oxidative stress in rats caused by feeding on aflatoxins-contaminated *Prunus mahaleb*.

**Keywords:** *Prunus mahaleb*, Aflatoxins, Yogurt, Antioxidant enzymes, Rats

## **Introduction**

*Aspergillus* mushrooms, such as *A. flavus* and *A. parasiticus*, contain aflatoxins, which are a form of mycotoxin. Aflatoxin refers to a group of four mycotoxins that are made, namely B1, B2, G1, and G2. Aflatoxin B1, the most toxic, is a potent carcinogen that has been linked to adverse health effects in many animal species, including liver cancer. Since mycotoxins are highly resistant to decomposition or digestion, they are found in

meat and dairy products and remain in the food chain. Some mycotoxins are not destroyed by heat treatments such as cooking or freezing (**Trucksess and Scott, 2008; Geiser, 2000**).

Mycotoxins, or Aflatoxins, are toxic carcinogens produced by some molds (*Aspergillus flavus* and *Aspergillus parasiticus*) that grow in soil, decaying vegetables, hay, and grains. It is naturally found in improperly stored staple foods, such as cassava, chili peppers, corn, cottonseeds, millet, peanuts, rice, sesame seeds, sorghum, sunflower seeds, nuts, wheat, and a variety of spices. Mycotoxins enter the general food supply when infected food is processed, and they can be present in human and pet foods, as well as raw materials given to farm animals. In turn, animals that feed on contaminated food transfer mycotoxins to eggs, dairy products, and meat (**Fratamico et al., 2008**). For example, in Pakistan, it has been found that poultry fed with feed contaminated with mycotoxins have high levels of these toxins in their meat and eggs (**Iqbalab et al, 2014**).

Mycotoxins have an especially negative impact on children, causing stunted growth, delayed development, liver damage, and liver cancer. As for adults, there is a difference in their exposure to mycotoxins, but they are also at risk. One of the most well-known carcinogens is mycotoxins. It is metabolized by the liver into epoxide or reactive hydroxylated compounds after entering the body, resulting in the less dangerous aflatoxin M1 (**Hamed, 2005**). Aflatoxins are often consumed with food. The most toxic type of aflatoxin, B1, can, however, penetrate the epidermis. (**Boonen et al, 2012**).

FDA-determined action levels for aflatoxins in food or feed are 20 to 300 ppb. The FDA has previously announced orders to withdraw food for humans and animals as a precaution to prevent exposure to aflatoxin (**Guidance for Industry, 2000**). There are 14 different mycotoxins that are naturally produced (**Boutrif, 1998**).

In the milk of cattle fed polluted feed, aflatoxin M2, a metabolite of aflatoxin B2. Aflatoxicol Aflatoxin Q1 (AFQ1), a

significant metabolite of AFB1 in other higher vertebrates' in vitro hepatic preparations (**Smith and Sivewright-Henderson, 1991**).

Mahlab cherry belongs to the pink (*Rosaceae*) family, and its scientific name is *Prunus mahaleb* L, and it is a small tree that has white roses and produces dark red edible seeds. A bitter flavor and edible, as for its seeds, they have a flavor close to almonds, and they have been used since ancient times in North Africa and the Middle East as a kind of spice to add flavor to baked goods, and recent studies have shown the benefits of eating mahlab seeds because they contain oils (**Popescu and Caudullo , 2020**). Mahlab seeds can be eaten raw or cooked in small quantities, and the dried seed germ is used as an additive flavor to baked goods and others, but it should not be eaten if it tastes too bitter. Because they contain a toxic substance, the seeds of mahlab contain phenolic compounds, flavonoids that are strong antioxidants, and they have an inhibitory effect on tyrosinase and xanthine oxidase, in addition to their anti-inflammatory properties (**Oskoueian et al, 2012**).

Some research has also been done on the health benefits of eating mahlab in small amounts. A laboratory study indicated that the seed extract of mahlab has an inhibitory effect on some types of bacteria (**Seyyednejad et al, 2008**). Another study indicates that the concentrated milkshake extract helps reduce the risk of cancer, as the study showed that the extract has properties that reduce the growth of breast cancer cells, in addition to its antioxidant properties thanks to its containment of polyphenol compounds Polyphenols (**Gerardi et al, 2016**). Concentrated extract of mahlab contributes to reducing the risk of ulcerative colitis because it is considered a rich source of flavonoids, which act as antioxidants (**Ferramosca et al, 2019**). A laboratory study conducted on mice indicates that the mahlab seed extract contributes to reducing the risk of kidney stones in mice (**Akbari et al, 2020**).

Over decades, the nutritional and therapeutic benefits of live *Lactobacillus acidophilus* in dairy products as a meal or supplement have been extensively researched (**Andrade and Borges, 2009**). Yogurt is a dairy product made from milk that has

been fermented by bacteria. Yogurt cultures are the bacteria that are used to produce yogurt. By fermenting sugars in milk, these bacteria produce lactic acid, which reacts with milk protein to give yogurt its texture and distinct tart flavour (**US Food and Drug Administration, 2016**).

*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* bacteria are used to make yogurt. Other lactobacilli and bifidobacteria are sometimes added during or after the culturing of yogurt. (**Lee et al, 2012**). Yogurt (plain yogurt made from whole milk) contains 81 percent water, 9% protein, 5% fat, and 4% carbohydrates, including 4% sugars. The energy content of a 100-gram serving is 406 kilojoules (97 kcal). A serving of yogurt contains a high amount of vitamin B12 (31 percent DV) and riboflavin (23 percent DV), as well as a moderate amount of protein, phosphorus, and selenium (14 to 19 percent DV). Yogurt is often associated with probiotics, which have been postulated to have beneficial effects on the immune, cardiovascular, and metabolic health. (**El-Abbadi et al, 2014; Astrup, 2014 and Gijbers et al, 2016**). To date, however, high-quality clinical evidence has been inadequate to suggest that eating yogurt reduces the risk of illness or improves overall health (**Rijkers et al, 2011**).

Yogurt is a food that is readily consumed and preferred by people of all ages. It can be used as a source of various nutrients, such as probiotics (**He et al., 2008**), vitamins (**Ethgen et al., 2016**), and omega fatty acids, due to its high intake (**Mc-Cowen et al., 2010**). Yogurt supplements, including bioactive sources, have two advantages that arise as a result of their addition. In this scenario, it has the potential to boost public health by lowering harmful pollutants such as aflatoxins (AFs) (**Badr et al., 2019**).

Some lactic acid bacteria found in yogurt have been shown to be able to remove or protect against AFB1. Lactobacilli have been shown to inhibit aflatoxin production as well as the growth of *Aspergillus spp.* in some studies. Lactobacilli were also found to be capable of rapidly removing AFB1 in vitro, with a removal

rate of approximately 50–80 percent (Chang and Kim, 2007 and Gerbaldo *et al.*, 2012).

Therefore, the study's aim is to promote the use of yogurt to improve aflatoxin toxicity tolerance *in vivo*. In addition to providing a nutritive and preservation feature, protecting against AFs contamination through the consumption of common foods such as yogurt would aid in the improvement of public health.

## **Materials and methods**

### **A-Materials:**

**The *Prunus Mahaleb***: Mahaleb were obtained from local market in Mansoura, Egypt. Mahaleb was authenticated in the Botany Department Faculty of Agriculture, and Cairo University.

**Rats**: Thirty meal albino (*Sprague dawley strain*) weighing 110 to 115 g provided from of National Research Center, Cairo, Egypt.

### **B- Methods:**

#### **Storage of experimental *Prunus mahaleb* and determination of aflatoxins:**

Experimental *Prunus mahaleb* samples were chemically analyzed for estimation of aflatoxins at first one month then stored for two months at room temperature. The determination of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> was conducted using HPLC method (A .O .A. C. 2000).

#### **Preparation of *Prunus mahaleb* powder:**

The highest values of estimated aflatoxine in *mahaleb* were appeared after two months of storage that was used for biological studies. *Mahaleb* was crushed into fine particals as far as possible and stored in poly ethylene bags in the refrigerator at 4°C during use (A.O.A.C. 2005).

**Yogurt**: Yogurt was generated using the same process as Abdel-Salam *et al* (2010). Yogurt has a therapeutic dosage of 2000 cc2 per day in humans. The diets were supplemented with 180 cc2 of rat yogurt per kg of body weight per day (Robinson 1991).

#### **Animals and diets:**

Thirty rats weighing  $110 \pm 5$  g were allocated in plastic cages with metallic stainless covers. They were kept under constant laboratory conditions room temperature  $25 \pm 2^\circ\text{C}$  and lighting (12L:12d). Rats were fed the basal diet for 7 days before the beginning of the experiment for adaptation. The standard diet comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg), cellulose (30 g/kg), corn oil (50g/kg), minerals mixture (100g/kg), vitamins mixture (20g/kg) and DL-methionine (3g/kg) prepared according to, (Reeves, et al., 1993) diet and water were provided ad libitum

### Experimental animal design:

Rats were adapted for 1 week before dietary manipulation under laboratory healthy conditions. Ethical guidelines were maintained in animal handling during the study and permission was obtained from Mansoura University. The rats were divided into five groups at random (6 rats each).

Group 1: (-ve control) fed on basal diet (BD)

Group 2: fed on BD containing 5% *Prunus mahaleb* was stored for one month.

Group 3: fed on BD containing 5% *Prunus mahaleb* was stored for two months.

Group 4: fed on BD containing 5% *Prunus mahaleb* was stored for one month with 180 cc<sup>2</sup> / kg body weight /day yogurt.

Group 5: fed on BD containing 5% *Prunus mahaleb* was stored for two months with 180 cc<sup>2</sup> / kg body weight /day yogurt.

During the study, feed intake was measured on a daily basis, and body weight gain was tracked every week. The feed efficiency ratio (FER) was calculated according to *Chapman et al., (1959)*.

### Biochemical evaluation

Serum aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were determined according to (Reitman and Frankel (1957), Kind and King (1954) and Henry, (1974). Weichselbaum's (1946) procedure was used to determine total protein in the serum. Serum

creatinine and urea were estimated according to **Bonsens and Taussky, (1984)**, and **Patton and Crouch, (1977)**, Serum cholesterol (CHO), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and total lipids were determined by using enzymatic colorimetric methods according to **Allain et al., (1974)**; **Buccolo and David 1973**; **Kostener (1977)** and **Schmitc (1964)**, respectively.

Blood Hemoglobin (HG) and packed cell volume (PCV) were estimated in heparinized blood according to **Drabkin (1949)**, **Mc Inory (1954)** and **Trinder (1969)**. Blood glutathione peroxidase (GPX) and superoxide dismutase (SOD) enzymes were estimated according to the methods of **Misra and Frisovich (1972)** and **Winterbourn, et al., (1975)**. The method of **Draper and Hadley (1990)** was employed in determining malondialdehyde (MDA). The level of liver glutathione S-transferase (GST), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity was determined by the method of **Habig et al., (1974)**, **Misra and Fridovich (1972)** and **Weiss et al., (1980)**, respectively.

### Statistical analysis:

Dunnet's t-test was used to examine differences between classes, accompanied by analysis of variance (ANOVA) (**Snedecor and Cochran, 1967**).

### Results and Discussion:

Table (1): Aflatoxins of stored *Prunus mahaleb*.

Aflatoxins Period of storage	B <sub>1</sub> (µg /kg)	B <sub>2</sub> (µg /kg)	G <sub>1</sub> (µg /kg)	G <sub>2</sub> (µg /kg)
One months	47.16± 7.14 b	2.22± 0.12 b	11.91± 2.31b	9.70± 2.11b
Two months	814.18± 9.761a	32.11±7.89 a	4021.11±21.51a	52.97± 8.61a

Each value represents the mean value  $\pm$ SD. Means with the different superscript letters under the same column were significant different at  $p \leq 0.05$ .

### *Aflatoxins of stored Prunus mahaleb*

As shown in Table 1; storing increased aflatoxins levels in *Prunus mahaleb*. Aflatoxins scored (47.16, 2.22, 11.91 and 9.70  $\mu$ g) for B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, respectively in the *Prunus mahaleb* after one month of storage. While storing it for two months increased aflatoxins levels as recorded (814.18 $\pm$  29.761, 32.11 $\pm$  7.89, 4021.11 $\pm$  21.51 and 52.97 $\pm$  8.61)  $\mu$ g for B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, respectively. Results agreed with those of **Bolarinwa et al. (2021)** who reported that further storage of samples, may increase the aflatoxin levels, and then become a health risk. Also, the increasing values of the aflatoxin show that the storage conditions favor the production of fungal toxins.

**Table (2): Body-weight gain, feed intake and food efficiency ratio of aflatoxin-treated rats administrated with yogurt.**

Groups Variables	Control (-ve)	One month SPM	Two months SPM	One month SPM + yogurt	Two months SPM + yogurt
Initial weight (g)	130.57 $\pm$ 5.50a	130.55 $\pm$ 5.41a	130.71 $\pm$ 6.71a	130.31 $\pm$ 3.71a	131.45 $\pm$ 3.71a
Weight gain (g)	150.20 $\pm$ 13.21a	100.21 $\pm$ 9.41bc***	104.51 $\pm$ 10.2bc***	138.65 $\pm$ 12.34a	135.24 $\pm$ 13.71a
Food intake (g)	16.31 $\pm$ 1.71a	15.49 $\pm$ 1.39a	15.87 $\pm$ 1.47a	16.57 $\pm$ 1.71a	16.17 $\pm$ 1.35a
FER	0.130 $\pm$ 0.003a	0.092 $\pm$ 0.004c***	0.093 $\pm$ 0.001c***	0.121 $\pm$ 0.002b*	0.123 $\pm$ 0.006b*

Each value represents the mean value  $\pm$ SD. Means with the different superscript letters under the same column were significant different at  $p \leq 0.05$ .

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$  FER: Food efficiency ratio SPM: Stored *Prunus mahaleb*

### *Body-weight gain, feed intake and feed efficiency ratio of aflatoxin-treated rats administrated with yogurt*

The impact of feeding on stored *Prunus mahaleb* on the body-weight gain, feed intake and FER were reported in Table 2. The findings demonstrated a significant influence of feeding on 5 percent SPM to the rats-group. In their group, the use of yogurt at a dose of 180 cc<sup>2</sup> / kg b.w did not result in feed refusal. Furthermore, even when aflatoxins were present in the rats' diets, the inclusion of yogurt improved feeding. Our results agreed with **Alsuhaibani et al., (2018)** which found that the positive control rat group that consumed aflatoxins contaminated nuts significant decrease the body weight compared to the negative control rat group which consumed safely mixed nuts. Aflatoxins infected nuts, combined with selenium-fortified yogurt, resulted in lower body weight and feed, as well as a lower feed efficiency ratio, when compared to the negative control group. Aflatoxin consumption can also cause weight loss (**Osborne et al., 1982**). In both chickens and turkeys, dietary exposure to AFB1 and other aflatoxins causes weight loss. During aflatoxicosis, nutritional efficiency is reduced, resulting in stunted development. (**Pandey and Chauhan, 2007 and Lee et al., 2012**). After eating yogurt with fat, proteins, lactose, and biogenic metabolites including peptides, vitamins, organic acids, and oligosaccharides, the rats' body weight recovered after being exposed to aflatoxin (**Santosa et al., 2006 and Junaid et al., 2013**). By increasing the excretion of orally administered aflatoxin in faeces, probiotic therapy avoided weight loss and decreased the hepatotoxic effects of a high dose of AFB1 (**Gratz et al., 2006**).

**Table (3): Hemoglobin and Packed cell volume for Aflatoxin-treated rats administrated by yogurt.**

Groups Variables	Control(-ve)	One month SPM	Two months SPM	One month SPM + yogurt	Two months SPM + yogurt
HG (g/dl)	13.78± 1.79a	10.36± 1.12bc**	11.45± 1.16b*	12.31± 1.11a	12.51± 1.14a
PCV %	37.24± 4.41a	28.35± 4.11b**	32.18± 4.99ab	35.51± 3.24a	37.21± 4.51a

Each value represents the mean value ±SD. Means with the different superscript letters under the same column were significant different at p≤0.05.

\* P&lt;0.05 \*\* P&lt;0.01 \*\*\* P&lt;0.001

HG: Hemoglobin PCV: Packed cell volume SPM: Stored *Prunus mahaleb****Hemoglobin and Packed cell volume for aflatoxin-treated rats administrated by yogurt***

As shown in Table 3; Feeding on stored *Prunus mahaleb* (SPM) caused changes in the rats' hemoglobin (HG) and packed cell volume (PCV) comparing the normal group. Aflatoxin contamination of one month SPM at (5 %) concentration could significantly (P<0.01) decrease the HG and PCV. When comparing the groups that received 180 cc<sup>2</sup> / kg b.w yogurt with SPM to the group that received SPM alone, the groups that received 180 cc<sup>2</sup> / kg b.w yogurt with SPM showed an increase in HG and PC values. This means that the presence of the yogurt in the rats' diet enhanced HG and PC changes that occurred by aflatoxin contamination of SPM. Decreased haemoglobin and PCV were observed by **Alsuhaibani (2018)** in the positive control rat group, which ate 3 percent aflatoxins-contaminated mixed nuts when opposed to the positive control group, intake of aflatoxins-contaminated mixed nuts combined with selenium-fortified yogurt resulted in substantial increases in haemoglobin and PCV. The drop in haemoglobin levels, according to **Mohiuddin et al. (1986)**, may be due to the degree of hepatic damage caused by aflatoxins. The drop in HG content may be linked to a drop in red blood cell count, which is a sign of anaemia.

**Table (4): Liver function parameters for aflatoxin-treated rats administrated by yogurt.**

Groups Variables	Control (-ve)	One month SPM	Two months SPM	One month SPM + yogurt	Two months SPM + yogurt
AST ( $\mu$ /ml)	49.67± 5.31c	81.35± 8.11a***	79.75± 8.61ab***	51.33± 6.11a	53.81± 6.14a
ALT ( $\mu$ /ml)	37.33± 3.45c	75.6± 7.36a***	65.38± 9.11b**	45.21± 5.14ab	49.31± 5.16a*
ALP ( $\mu$ /ml)	47.10± 4.95b	99.61± 10.31a***	95.60± 10.11a***	49.33± 5.17ab	52.61± 6.31ab

Each value represents the mean value  $\pm$ SD. Means with the different superscript letters under the same column were significant different at  $p \leq 0.05$ .

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$  SPM: Stored *Prunus mahaleb*

### ***Liver function parameters for aflatoxin-treated rats administrated by yogurt***

The changes in rats' serum liver function parameters were significantly ( $P < 0.001$ ) noticed in SPM groups comparing the normal control group. The liver functions were expressed by the enzymes alanine transaminase (ALT) and aspartate transaminase (AST). The activity of the alkaline phosphatase (ALP) enzyme was linked to liver dysfunction, and these changes were viewed as a tumour marker. The addition of 180 cc<sup>2</sup> / kg b.w yogurt to the 5% SPM diet may affect the changes in the liver enzymes caused by aflatoxins (Table 4). This means that the involvement of yogurt in the biological system can play a key role in limiting aflatoxin-induced liver tissue damage. The findings were consistent with those of **Abdel-Salam *et al.*, (2020)**, who found that yogurt reduced aflatoxin-toxicity activity that could affect liver and kidney functions, as measured by increased ALT, AST, and ALP enzyme levels. According to **Carvajal (2000)**, aflatoxins induce oxidative stress in animals by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidants. Lactic acid bacteria (*lactobacilli and streptococci*) in yogurt help the liver work more efficiently by reducing bacterial translocation, enhancing intestinal mucosa effects, and modifying intestinal microflora that affects the intestinal barrier (**Adawi *et al.*, 2001**).

**Table (5) Kidney function parameters for aflatoxin-treated rats administrated by yogurt**

Groups Variables	Control (-ve)	One month SPM	Two months SPM	One month SPM + yogurt	Two months SPM + yogurt
TP (g/dl)	7.69 $\pm$ 1.10a	5.81 $\pm$ 0.61b*	5.11 $\pm$ 0.55b*	7.15 $\pm$ 1.14a	7.11 $\pm$ 1.01a
Creatinine (mg/dl)	0.67 $\pm$ 0.10b	1.31 $\pm$ 0.14a**	1.35 $\pm$ 0.12a**	1.31 $\pm$ 0.14a**	1.35 $\pm$ 0.12a**

<b>Urea (<math>\mu</math> /mg)</b>	38.55± 4.31b	53.71± 5.41a**	54.66± 6.79a**	53.71± 5.41a**	54.66± 6.79a**
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Each value represents the mean value  $\pm$ SD. Means with the different superscript letters under the same column were significant different at  $p \leq 0.05$ .

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$  TP: Total protein SPM: Stored *Prunus mahaleb*

### ***Kidney function parameters for aflatoxin-treated rats administrated by yogurt***

Feeding on stored *Prunus mahaleb* caused changes in the rats' serum kidney function parameters which were represented by total protein, creatinine and urea comparing the normal group as shown in Table 5. The protein content of serum was significantly ( $P < 0.05$ ) affected by aflatoxin contamination of storing *Prunus mahaleb* for one and two months; this effect was only observed for the total albumin content of rats' serum. In comparison to the group that received only SPM, the group that received 180 cc<sup>2</sup> / kg b.w yogurt showed an increase in serum-protein values. On the other hand, urea and creatinine scores of all rats groups significantly ( $P < 0.01$ ) increased comparing the normal control one; however there was no significant improvement after the addition of yogurt to SPM diet. This means that the presence of the yogurt in the rats' diet enhanced total protein changes but had no effect on urea and creatinine changes occurred by aflatoxin contamination of SPM. As reported by **Williams et al. (2004)**, The four main forms of aflatoxins are B1, B2, G1, and G2. They are carcinogenic, teratogenic, hepatotoxic, immunosuppressive, and capable of inhibiting many metabolic processes, causing harm to the liver, kidneys, and heart.

**Table (6) Lipid profile parameters for aflatoxin-treated rats administrated by yogurt**

<b>Groups Variables</b>	<b>Control (-ve)</b>	<b>One month SPM</b>	<b>Two months SPM</b>	<b>One month SPM + yogurt</b>	<b>Two months SPM + yogurt</b>
<b>TG (mg/g)</b>	65.14± 7.25b	92.11± 10.11a**	95.14± 9.96a***	70.31± 7.30a	71.39± 7.22a
<b>CHO (mg/dl)</b>	93.17± 8.67b	171.14± 21.16a***	170.21± 19.16a***	120.36±12.44a*	118.14±11.88a*

<b>HDLc (mg/dl)</b>	31.41± 4.41a	19.75± 1.29b**	18.40± 1.67b***	25.67± 5.61a	23.41± 3.24b*
<b>LDLc (mg/dl)</b>	48.74± 65.66c	132.97± 13.14a***	132.79± 15.17a***	80.63± 9.11b**	80.49± 8.99b**

Each value represents the mean value ±SD. Means with the different superscript letters under the same column were significant different at  $p \leq 0.05$ .

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$  SPM: Stored *Prunus mahaleb*

### ***Lipid profile parameters for aflatoxin-treated rats administrated by yogurt***

The data in Table 6 shows that the administration of yogurt in the presence of aflatoxin from stored *Prunus mahaleb* improved the situation. The lipid profile of rats was altered after treatment with 5% SPM. Important differences ( $P < 0.001$ ) were observed in high-density lipoprotein levels, as well as cholesterol, triglycerides, and low-density lipoprotein levels, when compared to the control. For the LDL level, the increase was clearly registered. The addition of yogurt to the rats' diet resulted in an improvement that was similar to the control values. The administration of 180 cc<sup>2</sup> / kg b.w yogurt to rats' diets along with one or two months SPM produced results that were similar to the lipid profile observed for the control. HDL levels were raised to levels similar to those in the control group, while LDL, cholesterol, and triglyceride levels were reduced. The yogurt-treatment for rats given SPM appears to be able to suppress the lipid-profile changes that may occur as a result of aflatoxin toxicity in stored *Prunus mahaleb*. Results agreed with those of **Alsuhaibani (2018)** who found that, in comparison to the negative control group, rats who ate 3% mixed nuts contaminated with aflatoxins had significantly higher serum cholesterol, TG, LDLc, and VLDLc, as well as lower serum HDLc. When opposed to the negative control group, consuming 3% mixed nuts infected with aflatoxins along with selenium-fortified yogurt was able to somewhat normalise these values. **Agerholm-Larsen et al. (2000)**; **Pereira and Gibson, (2009)** have also demonstrated that by metabolising cholesterol and reducing its re-absorption in the gastrointestinal tract, lactobacilli and streptococci, two probiotic bacteria found in yogurt, can lower serum cholesterol levels.

Yogurt probiotics absorb cholesterol by injecting it through membranes and deconjugate and precipitate bile acids, resulting in bile acid excretion through the faeces.

**Table (7) Blood antioxidant enzyme activities and malondialdehyde for aflatoxin-treated rats administrated by yogurt**

Groups Variables	Control (-ve)	One month SPM	Two months SPM	One month SPM + yogurt	Two months SPM + yogurt
<b>GSP (mmol/l)</b>	8.35± 1.37a	4.11± 0.65b**	6.09± 0.54b**	7.51± 1.23a	8.21± 1.41a
<b>SOD (mmol/l)</b>	24.26± 8.01a	16.96± 1.91bc***	18.41± 2.21b***	21.41± 2.15ab	22.20± 2.10ab
<b>MDA (mmol/l)</b>	3.62± 0.25c	8.82± 1.15a***	9.97± 0.77b**	5.24± 0.48a**	4.20± 0.45b*

Each value represents the mean value ±SD. Means with the different superscript letters under the same column were significant different at  $p \leq 0.05$ .

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$

SPM: Stored *Prunus mahaleb*

GSP: Glutathione-peroxidase

SOD: Superoxide dismutase

MDA: Malondialdehyde

### ***Blood antioxidant enzyme activities and malondialdehyde for aflatoxin-treated rats administrated by yogurt***

Antioxidant enzymes GSH-Px, SOD and MDA plasma levels are shown in Table 7. Malondialdehyde plasma levels significantly increased ( $P < 0.001$ ) in SPM rats compared to the control. However, the MDA level significantly decreased ( $P < 0.01$ ) in response to yogurt administration to SPM rats comparing to the control and rats group fed on one or two months SPM only. Feeding on one and two months stored *Prunus mahaleb* decreased the concentration of GSP significantly ( $P < 0.01$ ); also SOD ( $P < 0.001$ ) in SPM serum blood rats' groups compared to the control. However, yogurt administration restored GSP and SOD values in one and two months SPM rats compared to untreated SPM rats. This means that the presence of the yogurt in the rats' diet enhanced blood antioxidant enzymes occurred by aflatoxin contamination of stored *Prunus mahaleb*. Several studies have

shown that viable *Lactobacillus* strains may increase Nrf2 expression under H<sub>2</sub>O<sub>2</sub> stress (Chauhan *et al.*, 2014), and that administering viable *Lactobacillus casei* and *Lactobacillus reuteri* cultures in aflatoxin-induced oxidative stress in a rat model improved TAC significantly (Hathout *et al.*, 2011). In comparison to AFB<sub>1</sub>-treated rats, rats fed AFB<sub>1</sub> + IC431 had significantly lower lipid peroxidation. This may be linked to increased CAT and GPx activity (Aguilar-Toalá *et al.*, 2019), as these antioxidant enzymes can play a key role in the protection against ROS generation or disrupt the lipid peroxidation process induced by AFB<sub>1</sub> exposure (Naaz *et al.*, 2014).

**Table (8): Serum Liver antioxidant enzyme activities and malondialdehyde for aflatoxin-treated rats administrated by yogurt.**

Groups Variables	Control (-ve)	One month SPM	Two months SPM	One month SPM + yogurt	Two months SPM + yogurt
GSH ( $\mu$ /mg)	12.18 $\pm$ 2.18a	7.48 $\pm$ 1.17b***	7.96 $\pm$ 1.04b***	10.49 $\pm$ 1.91ab	11.21 $\pm$ 2.61a
SOD ( $\mu$ /mg)	45.71 $\pm$ 6.11a	22.98 $\pm$ 2.61c***	25.91 $\pm$ 3.21bc***	38.11 $\pm$ 3.77ab	45.36 $\pm$ 5.16a
GPX ( $\mu$ /mg)	55.11 $\pm$ 6.24a	35.75 $\pm$ 5.17bc***	39.71 $\pm$ 4.81b**	40.14 $\pm$ 4.13ab	48.31 $\pm$ 5.16a
MDA (mmol/g)	20.77 $\pm$ 2.33c	27.91 $\pm$ 2.99a***	23.41 $\pm$ 2.19bc	24.31 $\pm$ 2.16ab	20.47 $\pm$ 1.87 c

Each value represents the mean value  $\pm$ SD. Means with the different superscript letters under the same column were significant different at  $p \leq 0.05$ .

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$

SPM: Stored *Prunus mahaleb*

GSP: Glutathione-peroxidase

SOD: Superoxide dismutase

MDA: Malondialdehyde

### ***Serum Liver antioxidant enzyme activities and malondialdehyde for aflatoxin-treated rats administrated by yogurt:***

Table 8 shows the GSH, SOD, GPX, and MDA concentrations. When compared to the control group, the concentration of GSH in SPM serum liver rats was significantly lower ( $P < 0.001$ ). In comparison to untreated SPM rats, yogurt

administration restored GSH levels in one and two months SPM rats. Glutathione-peroxidase and Superoxide dismutase activities were significantly reduced in rats fed stored *Prunus mahaleb* compared to the control group; however, the activities of these enzymes showed a substantial recovery in response to yogurt administration to SPM rats, reaching neutral values in the control group. Malondialdehyde values significantly increased ( $P < 0.001$ ) in the serum liver of SPM rats compared to the control. However, the MDA level significantly decreased in response to yogurt administration to SPM rats comparing to the control and rats group fed on one or two months SPM only. This means that the presence of the yogurt in the rats' diet enhanced serum liver antioxidant enzymes occurred by aflatoxin contamination of stored *Prunus mahaleb*. These findings are consistent with previous research that found a substantial increase in MDA levels in different tissues of rats fed aflatoxin (El-Nekeety *et al.*, 2011; Abdel-Wahhab and Aly, 2003 & Naaz *et al.*, 2014). Aguilar-Toalá *et al.*, (2019) determined that providing rats with *Lact. casei* CRL 431 intracellular material increased antioxidative enzyme activity and antioxidant potential in the blood and liver, reducing aflatoxin-induced lipid peroxidation. Antioxidant enzyme activities, such as SOD, catalase, GST, GPx, and GRase, were significantly reduced in the AFB1 treated population (liver 17 % -36 %; kidney 12 % -33 %). GSH and glycogen levels were also reduced as a result of AFB1 poisoning (liver 22 % -50 percent; kidney 10 % -24 %) (Rastogi *et al.*, 2001).

## Conclusions

The most important finding that was consuming yogurt decreases the negative effects of eating *Prunus mahaleb* contaminated with aflatoxins. Yogurt is recommended for its nutritional value as well as for reducing the adverse effects of aflatoxins in nuts.

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الملخص العربي

## الدور الوقائي المحتمل للزبادي ضد التأثيرات السامة للملح الملوث بالأفلاتوكسين في الفئران

1 منى ياسر عبد الخالق مصطفى - 2 سماح عبدالله السملوي

1 قسم الاقتصاد المنزلي - كلية التربية النوعية - جامعة المنصورة - مصر 2 قسم  
الاقتصاد المنزلي - كلية التربية النوعية - جامعة طنطا - مصر

**الخلفية:** تحتوي أنواع من فطر اسبرجلس على مجموعة من المستقلبات الفطرية السامة كيميائياً والمعروفة باسم الأفلاتوكسينات. والتي يمكن أن تلوث المكسرات، مما يؤدي إلى تطور السموم الفطرية. الهدف من الدراسة هو فحص قدرة الزبادي في مساعدة فئران التجارب على التعافي من الآثار السلبية لتناول الملح المخزن والملوث بالأفلاتوكسين. بعد شهر أو شهرين من التخزين على درجة حرارة الغرفة ، تم تحديد التركيزات الكلية للأفلاتوكسين في الملح. ثم تم تقسيم الفئران إلى مجموعات على النحو التالي:

- المجموعة 1: (الضابطة السالبة) تغذت على الوجبة القياسية
- المجموعة 2 : تم تغذيتها على الوجبة القياسية مع 5% من الملح الذي تم تخزينه لمدة شهر واحد على درجة حرارة الغرفة
- المجموعة 3 : تم تغذيتها على الوجبة القياسية مع 5% من الملح الذي تم تخزينه لمدة شهرين على درجة حرارة الغرفة
- المجموعة 4: تم تغذيتها على الوجبة القياسية مع 5% من الملح المخزن لمدة شهر على درجة حرارة الغرفة مع 180 سم 3 / كجم من وزن الجسم زيادي / يومياً
- المجموعة 5: تم تغذيتها على الوجبة القياسية مع 5% من الملح المخزن لمدة شهرين على درجة حرارة الغرفة مع 180 سم 3 / كجم وزن الجسم زيادي / يوماً.

**النتائج:** أظهرت النتائج قدرة الزبادي على تقليل تأثيرات الأفلاتوكسين على المعايير البيوكيميائية في مصل الدم لدى الفئران. تم تسجيل تحسن في الهيموجلوبين وحجم الخلايا ، الدهون ، وظائف الكبد والكلية ونشاط الإنزيمات المضادة للأكسدة للفئران

التي تناولت الزبادي مع وجود الأفلاتوكسين في المحلب المخزن. وساعد استهلاك الزبادي في تقليل الإجهاد التأكسدي في الفئران الناجم عن التغذية على المحلب الملوث بالأفلاتوكسين.

**الخلاصة:** إن أهم اكتشاف هو أن تناول الزبادي يقلل من الآثار السلبية لتناول المحلب الملوث بالأفلاتوكسين، ومن ثم ينصح باستخدام الزبادي لقيمه الغذائية وكذلك لتقليل الآثار الضارة للأفلاتوكسينات في المكسرات.

**الكلمات المفتاحية:** محلب ، أفلاتوكسين، زبادي، إنزيمات مضادة للأكسدة ، فئران.