



# Functional yogurt aims to protect against the aflatoxin B1 toxicity in rats

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## ABSTRACT

Recently, the rise of mycotoxin contamination in food materials was found to be due to several factors, including climatic changes' impact. Therefore, the aim of this study was to provide a novel food product that allows the reduction of aflatoxin impact in animal tissues. Chicory root-extract (CRE) was evaluated for its active components, antioxidant potency, and antimicrobial activity. The CRE was utilized to produce functional yogurt (FY) that was evaluated *in-vivo* using experimental rats. The CRE showed high antioxidant activity and recorded valuable content of the active components. Results also showed a high antimicrobial effect against toxigenic fungal strains. The results have reflected the efficiency of the FY to suppress aflatoxin impacts in the animal tissues and biochemical parameters of rats-serum. An enhancement was recorded in liver and kidney functions for rats taking FY with the presence of aflatoxin. It was concluded that consumption of the FY assisted in suppression of the oxidative stress in rats-tissues.

## 1. Introduction

Dairy food is a highly requested product for consumption by humans. Different kinds of milk and their related products are known as comprehensive and nutritive food [1]. Recently, milk was found to play significant functions to support diets with essential nutrients [2]. Dairy products are nutrient-rich food and are necessary to protect the bone [3]. Of those products, yogurt represents easily consumable and preferable food by several ages-. Due to the high consumption, it can be used as a provider of various supplements including probiotics [4], vitamins [5], and omega fatty acids [6]. Utilizing of yogurt supplements, like bioactive sources, refer to dual benefits occurring because of their adding. In this case, it could improve public health and reduce harmful contaminants like aflatoxins (AFs) [7].

Chicory as a bioactive source is as a significant fortifying nutrient. It is considerably rich in many nutrients and soluble fiber. It is a healthy herbal plant, widely grown in the Mediterranean basin by different varieties [8]. Chicory is named Cerise in the Egyptian countryside and consumed commonly as fresh food. Even chicory root extract (CRE) contains high oligosaccharides, mainly the inulin. It does not increase blood sugar and has a considerable value in food ingredients like a fat-replacer [9]. It also improves the food's contents of soluble dietary fibers that help the digestion process. Inulin of the CRE is a versatile

constituent in food processing, assists for mineral intake in the gut tract, and fermentable by probiotic bacteria [10].

Moreover, agro-food and agro-feed products are threatened by aflatoxin, and which is considered a risk factor. These factors give rise to critical problems in the tissue and living systems by the AFs that remain within the intestine tract. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is one of the main risks of cross-contamination in milk. Aflatoxin B<sub>1</sub> is metabolized to a reactive epoxide (aflatoxin 8,9-epoxide) primarily by the P450 cytochrome enzyme [11]. This fraction acts as a free radical trigger that may affect the cellular life of oxidative stress [12]. Various impacts of malfunctions and biochemical disorder on living tissues were reported [13].

The ability of several plant-bioactive and antioxidants to reduce AFs-toxicity was reported previously [7,14]. It was possible to utilize the natural extract or the by-product extract [14–16]. Therefore, the aim of this study is to add CRE to functional yogurt (FY) for the enhancing of the *in-vivo* resistance of aflatoxin toxicity. This will be achieved by the supplementation of the extract-bioactive molecules. The protection against AFs-contamination by fortified routine-food like yogurt will assist in the improvement of public health. Also, the CRE added to yogurt could achieve a nutritive and preserving function, primarily in the food of critical-age groups.

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## 2. Materials and methods

### 2.1. Chemicals, micro-organisms, and materials

Aflatoxin B<sub>1</sub> from *A. flavus* cat. number 1162-65-8, was purchased from Sigma-Aldrich™, the biochemical Kits were purchased from Biodiagnosics™, Cairo.

The Egyptian Chicory roots were obtained from a local market, Giza, Egypt. Fresh buffalo's milk was purchased from healthy lactating animals of a farm that has a known trade-mark, Egypt.

Bacteria and fungal strains were prepared and reactivated from the lyophilized medium. The bacterial strains used in this study were *Bacillus cereus* ATCC 10876, *Salmonella typhi* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 8739, and they were cultured on tryptic soy agar. The toxigenic fungi used in this study were *Aspergillus flavus* ITEM 698, *Aspergillus ochraceus* ITEM 5010, *Fusarium culmorum* KF846, and *Penicillium* sp., and they were cultured on potato dextrose agar media

### 2.2. Preparation of Chicory root extract

Use warm water, the Chicory root extract (CRE) was prepared according to the method described by Abdel-Salam et al. [17] with some modifications. In Brief, The roots were cleaned, cut into little parts, dehydrated in a hot air oven, and milled to a fine powder. They were extracted by a known quantity of distilled water (45 °C/2 h) using a hotplate/stirrer. A sterile cheese cloth was used to filtrate the aqueous extract, which was then filtered by Whatman paper (No.2) until a clear solution was obtained. The extract was stored in sterile dark bottles and refrigerated until analysis

### 2.3. Antimicrobial activity of chicory root extract

#### 2.3.1. Minimum inhibitory concentration

The minimal inhibitory concentration (MIC) was determined as follows: for bacterial strains; nutrient broth were prepared and sterilized in bottles, each containing 5 mL media. Graduated extract amounts were added to the broth media, followed by 50 µl of microorganism suspension, bottles were incubated (18 h/ 38 °C). The assay was carried out in triplicate. Azithromycin was utilized as a positive control antibiotic.

The minimal fungicidal concentration (MFC) was determined in the same way, while the applied medium was yeast extract sucrose broth (YES). Incubation conditions were carried out at 25 °C/ 72 h. The values were calculated at an extract concentration that caused non-fungal growth. Nystatin was applied as an antifungal positive-control.

#### 2.3.2. Disk diffusion assay

The potency of the CRE to inhibit the growth of pathogenic bacteria and toxigenic fungi was evaluated using Potato dextrose agar. The extract was applied at a concentration of 100 µL/well, whereas two wells were applied for each plate. Inhibition zones were determined and recorded in millimeters after incubation (24 h/bacteria & 5days/fungi). The assay was carried out in replicates. Azithromycin and Nystatin were used as a positive control.

### 2.4. Determination of the total phenolic content

The total phenolic and flavonoid contents of the extract were determined by the same methodologies utilized by Abdel Razek et al. [14]. Briefly, root extract (1.0 mL) was added to Folin–Ciocalteu reagent (5.0 mL) to determine the phenolic content. After 6 min, 5.0 mL of sodium carbonate solution (saturated solution) was added. The mixture was mixed thoroughly, then it was incubated for 2 h /25 °C, and the absorbance was measured at a wavelength of 770 nm. Total phenolic content was expressed as mg Gallic acid equivalent (GAE)/L. The same steps were done for yogurt extract. The absorbance was determined

using a UV-VIS Jenway 6850 spectrophotometer against a blank sample as a control. Quantitative determinations were performed based on 5 points standard calibration curve of methanolic solution of Gallic acid (80 %). The results were expressed as µg Gallic acid equivalents (GAE)/L of the yogurt.

### 2.5. Determination of the antioxidant activity

The scavenging activity of samples was estimated using DPPH radical according to the procedure described by Shimada et al. [18]. In brief; the DPPH solution was added to the extracts prepared from the CRE, yogurt extract, and the FY. The standard antioxidant solution was applied as a control. Each mixture was kept in the dark (30 min.). The absorbance of each solution was measured at 517 nm against a blank (in triplicates). The results were expressed as µM TE/L.

### 2.6. Preparation of functional yogurt from buffalo's milk

Globally, several regions have manufactured the yogurt from buffalo milk as a staple dairy product. It has a strong texture with a copious creamy-face, it tastes sweet and creamy with a mild to moderate acidity than is commonly known in other unsweetened plain yogurt. The functional yogurt (FY) from buffalo's milk was manufactured as the same method utilized by Abdel-Salam et al. [19]. Pasteurized buffalo's milk was inoculated by yogurt starter containing *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum* (at 10<sup>7</sup> - 10<sup>9</sup> CFU/g), incubated for 4 h /42 °C for the coagulation step, the curd pH was tested, then it was cooled and stored at refrigeration (4 °C).

### 2.7. Experimental animals

Forty-two male albino rats, of 152.4 ± 2.41 (Mean ± SD), were obtained from the National Research Centre animal-house, Giza, Egypt. The rats were housed in a controlled unit under standard temperature and humidity conditions. The basal diet was utilized to feed rats, according to AIN-93 guidelines, water ad-lib was provided during the experimental [20]. The balanced diet consisted of protein (21.6 %), maize oil (15 %), maize starch (58.4 %), salt mixture (4 %), and vitamin mixture (1 %). The vitamins that were soluble in oil were administered weekly to rats and separately from a diet.

### 2.8. The experimental design

The rats in this study were handled following the ethical committee's instructions of Egyptian NRC, and conformity to the instruction of the European Union Directive for animal use in scientific research [21]. Aflatoxin B<sub>1</sub> was dissolved in phosphate buffer saline (pH = 7.2) and administered at a concentration of (28 µg/kg BW) [14]. The CRE was dissolved in the same buffer using the same concentration which had no impact on plain yogurt (Data not shown). Randomly, the rats were divided into 7 groups (6 rats/group) according to the following;

**Group I:** (negative control) rats were fed on basal diet.

**Group II:** rats were fed on a basal diet and orally administered by AFB<sub>1</sub> (28 µg/kg BW).

**Group III:** Rats were fed on a basal diet and orally administered with 1 mL FY.

**Group IV:** Rats fed on a basal diet and orally administered with 0.1 mg of the CRE.

**Group V:** Rats were fed on a basal diet and administered with 1 mL of FY containing 0.1 mg CRE.

**Group VI:** Rats were fed on a basal diet, and orally administered by AFB<sub>1</sub> followed by 1 mL of FY.

**Group VII:** Rats were fed on a basal diet, and administered by AFB<sub>1</sub>, followed by 1 mL of FY containing 0.1 mg CRE.

At the end of the experiment (day 43), the rats were fasted overnight and anesthetized with an anesthesia solution, and blood samples were

collected using blood capillary tubes using retro-orbital puncture. At 4000 RPM/15 min. /5 °C, blood samples were centrifuged. Sera were collected, carefully transferred to clean tubes analyzed for biochemical parameters.

Hematological parameters included Red blood cell count (RBC) white blood cell count (WBC), hemoglobin (Hgb), hematocrit (Hct), platelet count (PLT), and total iron-binding capacity (TICB) determined by automated hematology analyzer [22]. Total cholesterol was determined according to Watson [23], high-density lipoprotein cholesterol (HDL) using a method of Demacker et al. [24], low-density lipoprotein cholesterol (LDL) according to Assmann et al. [25], and triglycerides according to the method of McGowan et al. [26]. Creatinine and urea were determined depending on Larsen [27] as indicators of kidney function.

The activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to Reitman and Frankel [28] with some modification. Briefly, 100 µL of serum was placed in each well of 96 well of the ELIZA plate followed by 100 µL of the standard kits (AST or ALT, ab234579 Rat ELISA kits, USA), and incubated for one hour, washed by phosphate buffer saline three times, then 100 µL of the development solution was added and incubated for 10 min. Finally, the stop-reaction solution was added at a 100 µL, and the optical density was recorded at 450 nm. Malondialdehyde (MDA) was determined according to Ohkawa et al. [29]. In brief, the MDA level was based on plasma reactions between MDA and TBA by reactive thiobarbituric acid (TBA) reactive substances (TBARS). Together with test samples, a standard MDA (100 N-MOL) solution (5 mL) was used. Then 1 mL of the serum sample, 1.5 mL TBA (0.8 %) were added. After that 0.4 ml of 8.1 % SDS and 1.5 ml of acetic acid were added. Finally, the mixture was made of distilled water up to 5 mL and was placed in a warm water bath at 95 °C/60 min. One milliliter of distilled water and 5 mL n-butanol and pyridine were added after cooling down (15:1, V / v). The mix was vortexed and read using the UV-vis spectrophotometer (Shimadzu) at 532 nm against the Blank after the centrifugation at 4000 Xg/15 min. Catalase activity was assayed according to the method of Beers and Sizer [30]. Superoxide Dismutase (SOD) activity was determined according to the method of Sun et al. [31]. Serum Glutathione-S-Transferase (GST) activity was determined according to Habig et al. [32].

Rats were dissected after blood sampling, and the liver, kidneys, and pancreas were immediately removed from each rat, measured, and then submerged in 10 percent formalin solution for histopathological analysis. This study was conducted in compliance with both the ethics of the National Research Center Committee and the guidelines of the National Institutes of Health for Experimental Animals Care and Use (Publication No. 85–23, revised 1985).

## 2.9. Histopathological examination

Liver and kidney samples were excised and fixed for 24 h, in formal saline (10 %). The specimens were washed with tap water, dehydrated in ascending graduated ethanol; xylene for clearance, and then was embedded in paraffin. The sections (5 µm) were sliced using the microtome. The sections were then de-paraffinized in xylene and re-hydrated in descending ethanol series and flushed with water. Using hematoxylin and eosin, the slides were stained, placed in the DPX, coverslipped, and investigated through a microscope connected to computer software (Leica, Germany) [33].

## 2.10. Statistical analysis

Data were statistically analyzed using SPSS software (version 16). Anova One-way was used to examine the significant difference between different means at  $P = 0.05$ .

## 3. Results and discussions

### 3.1. The antimicrobial properties of the CRE

Antibacterial activities of the CRE were determined against four pathogenic bacterial strains. The MIC value for *Salmonella typhi* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 8739 was recorded at a concentration of 70 mg/ mL of the medium, whereas for *Bacillus cereus* ATCC 10876 the MIC value was recorded at a concentration of 50 mg/ mL. The inhibition zone of the CRE extract (100 µL/well) against bacterial growth (*B. cereus*, *S. typhi*, *P. aeruginosa*, and *E. coli*) ranged between  $14.3 \pm 1.27$  to  $27.2 \pm 1.41$  mm, whereas the standard antibiotic (Azithromycin) showed an inhibition zone of  $32.4 \pm 1.15$  mm.

On the other hand, the MFC evaluated for the CRE against mycotoxigenic fungal strains (*A. flavus*, *A. ochraceus*, *F. culmorum*, and *Penicillium*), recorded at a concentration of 70 mg/mL, compared to the standard antifungal (Nystatin) that recorded 16 µg/ mL. The inhibition impact of the CRE was evaluated on agar-medium using a well-diffusion assay. The extract was found to inhibit the fungal growth at range between  $13 \pm 4.2$ – $25.3 \pm 2.17$  mm, while the diameter of the control recorded  $117.1 \pm 2.34$  mm. Regarding these results, it was confirmed that the CRE had a good antimicrobial impact to limit the contamination by both pathogenic and mycotoxigenic microorganisms.

### 3.2. Proximate analysis of the CRE

The proximate analysis of the FY and the CRE were determined, according to the AOAC [34] to characterize the factors that could affect their bioactivity. Data in Table 1 showed that there was no significant differences between the two types of FY (with/ without CRE) for total solids, protein, and ash. The significant differences were recorded for the values of crude fibers, carbohydrates, antioxidant activity, and total phenolic content. These differences could be due to the high oligosaccharides content of the CRE [35].

### 3.3. Animal experimental results

#### 3.3.1. The influence of CRE

The impact of the AFB<sub>1</sub> on the final-weight, feed intake, and body-weight gain were recorded in Table 2. The results showed a significant effect in the presence of AFB<sub>1</sub> orally administrated to the rats-group. The use of CRE at a dose of 0.1 mg/rats did not show any feed-refusal behavior in their group. Moreover, the presence of CRE beside AFB<sub>1</sub> showed ameliorating effect on feeding, even if the aflatoxin was present in the gastrointestinal tract (GIT).

#### 3.3.2. Biochemical parameters

Aflatoxin contamination could affect the protein content of serum; this influence was recorded for the Albumin content of rats-serum. The Aflatoxin group, and administrated with CRE, showed an enhancement in serum-protein values comparing the group just administrated by AFB<sub>1</sub>. The data showed in Fig. 1, demonstrates the amelioration resulted from the administration of CRE in the presence and absence of aflatoxin.

The AFB<sub>1</sub> treatment led to changes in the lipid profile of rats. Compared to the control; significant changes were noticed with a real decrease in a high-density lipoprotein level, and increases in cholesterol, triglycerides, and low-density lipoprotein levels (Fig. 2). The increment was recorded clear for the LDL level. While the administration of CRE directly or the yogurt-CRE displayed an enhancement closed to the control values. Whereas, the administration of AFB<sub>1</sub> the CRE and CRE-Yogurt gave results close to those of the lipid profile recorded for the control. The HDL values were increased to reach its neutral values in the control, with the decreases of LDL, cholesterol, and triglycerides. More or less, the CRE-treatment for rats that were administered AFB<sub>1</sub> seems to be able to suppress the lipid-profile changes that could occur due to

**Table 1**  
The proximate analysis of yogurt, CRE, and yogurt-CRE.

Parameters	Total Solids (%)	Fat content (%)	Protein (%)	Crude fiber (%)	Carbohy -drate (%)	Ash (%)	AA* (µM TE/L)	TP** (mg GA/L)
Functional Yogurt	12.25±1.22 <sup>a</sup>	6.03±0.93 <sup>a</sup>	4.15±0.79 <sup>a</sup>	ND	5.21±0.46 <sup>a</sup>	0.73±0.41 <sup>a</sup>	3.6±0.81 <sup>a</sup>	0.9±0.27 <sup>a</sup>
CRE	2.06±0.88 <sup>b</sup>	0.119±0.04 <sup>b</sup>	0.67±0.11 <sup>b</sup>	0.54±0.04 <sup>a</sup>	0.91±0.34 <sup>b</sup>	0.82±0.54 <sup>b</sup>	82.1±1.37 <sup>b</sup>	8.1±0.93 <sup>b</sup>
FY-CRE	12.79±1.39 <sup>a</sup>	6.08±0.67 <sup>a</sup>	4.19±0.22 <sup>a</sup>	0.16±0.02 <sup>b</sup>	5.73±0.71 <sup>c</sup>	0.78±0.67 <sup>ab</sup>	5.21±1.54 <sup>c</sup>	1.21±0.69 <sup>c</sup>

The data expressed as means ± S.D (n = 6).

CRE: chicory root extract.

AA: antioxidant activity.

TP: total phenolic.

The values of each column with superscript different letters had significant differences.

FY-CRE: yogurt with chicory root extract.

**Table 2**  
Food intakes and relative weight gain of rats administrated with the FY and chicory (with/without aflatoxin B<sub>1</sub>).

Treatment	Control	AFB <sub>1</sub>	FY	CRE	FY-AFB <sub>1</sub>	CRE-AFB <sub>1</sub>	FY- CRE- AFB <sub>1</sub>
Initial weight	152.4±2.41 <sup>a</sup>	153.3±2.35 <sup>a</sup>	154±2.84 <sup>a</sup>	157.4±1.17 <sup>a</sup>	155.7±2.23 <sup>a</sup>	155.1±2.51 <sup>a</sup>	155±1.58 <sup>a</sup>
Final weight	223±1.42 <sup>a</sup>	198.2±2.84 <sup>b</sup>	237±0.96 <sup>d</sup>	243.2±1.48 <sup>c</sup>	203.6±1.58 <sup>c</sup>	206.9±0.41 <sup>c</sup>	236±0.71 <sup>d</sup>
BWG	73.6±3.83 <sup>a</sup>	44.9±5.19 <sup>b</sup>	83±1.31 <sup>a</sup>	85.8±2.11 <sup>c</sup>	47.9±3.81 <sup>c</sup>	51.8±2.75 <sup>c</sup>	81±2.29 <sup>a</sup>
Relative WG %	48.29 <sup>a</sup>	29.28 <sup>b</sup>	53.89 <sup>a</sup>	54.51 <sup>c</sup>	30.76 <sup>c</sup>	33.39 <sup>c</sup>	52.25 <sup>a</sup>
Total food intake (g)	490.28±2.57 <sup>a</sup>	438.22±6.41 <sup>b</sup>	499.3 ± 3.22 <sup>a</sup>	498.63±4.22 <sup>c</sup>	468.44±5.72 <sup>d</sup>	482.71±4.56 <sup>e</sup>	496.7±3.22 <sup>f</sup>
Food intake (g/d)	17.51±0.13 <sup>a</sup>	15.65±0.24 <sup>b</sup>	17.83±0.1 <sup>a</sup>	17.81±0.15 <sup>a</sup>	16.73±0.18 <sup>c</sup>	17.24±0.11 <sup>d</sup>	17.74±0.11 <sup>a</sup>

The data expressed as means ± S.D (n = 6).

The values of each column with superscript different letters had significant differences compared to the control group.

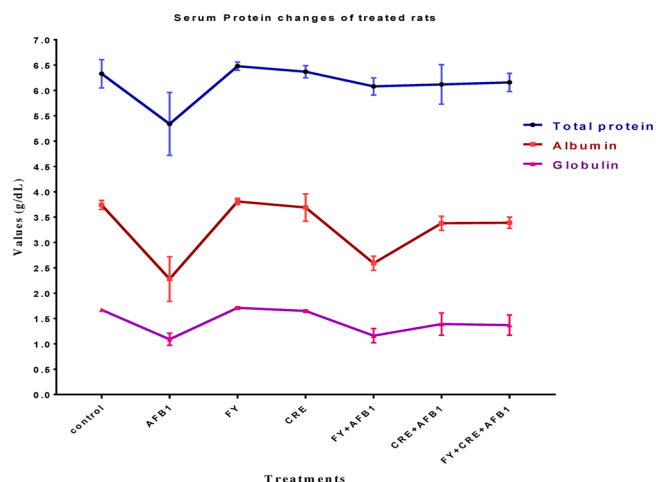
CRE: Rats were administrated by only chicory root extract.

FY: Rats were administrated just by yogurt.

FY-AFB<sub>1</sub>: Rats were administrated by yogurt plus aflatoxin.

FY-CRE – AFB<sub>1</sub>: Rats administration with yogurt + chicory root extract + aflatoxin.

FY-CRE: Rats administrated by yogurt with chicory root extract.



**Fig. 1.** Changes in serum-protein content due to aflatoxin B<sub>1</sub> and chicory extract.

aflatoxin toxicity.

The changes in liver and kidney function parameters were noticed in the serum of rats that were administrated by aflatoxin. The alanine transaminase (ALT) and aspartate transaminase (AST) enzymes represented the liver functions. The kidney functions were represented by urea and creatinine. The alkaline phosphatase (ALP) enzyme activity was reflected the liver malfunctions, where these changes considered as a tumor marker. The oral administrating of the CRE at a dose of 0.1 mg/mL may affect the changes in the liver and kidney enzymes caused by aflatoxins (Table 3). This means that the presence of the CRE in the biological system could play a pivotal function to limit aflatoxin-damages in the liver tissues.

The data for the level of enzymes in rat liver-tissues were shown in Fig. 3. The rats of the aflatoxin-treated group showed differences in the

level of tissue-enzymes compared to the negative control group. These changes were enhanced when rats were given an oral dose of 0.1 mg of the CRE after aflatoxin oral-administration. The enhancement was more effective using the CRE compared to the treatment of yogurt-CRE oral-dose. This could be described by the binding of the active sites of the CRE and its coating by yogurt components.

### 3.4. Histopathological investigations

Sections of the liver from normal control rats showed the normal architecture of the hepatic lobule. The central veins lie at the center of the lobules surrounded by cords of hepatocytes. Between the strands of hepatocytes, the hepatic sinusoids clearly were shown (Fig. 4A). In rats of positive control, examination showed congested central veins and central lobular necrosis of the hepatocytes that were associated with inflammatory cell infiltration (Fig. 4B). Also, positive control rats showed portal and periportal lymphocytic infiltration, congestion of the portal vessels, and periportal necrosis of the hepatocytes that surround the portal areas (Fig. 4C). In some rats, focal necrosis of the hepatocytes that were associated with lymphocytic infiltration and dilated hepatic sinusoids was seen (Fig. 4D).

In rats that were given the CRE plus AFB<sub>1</sub>, the architecture of the hepatic lobule appeared like the normal (Fig. 5D). However, in some rats, micro and macro vacuoles of fatty change were recorded (Fig. 5E).

The liver of rats given only the FY or the CRE showed the normal architecture of hepatic lobules (Fig. 4E, F respectively). In a case of rats given the FY plus AFB<sub>1</sub>, sections of the liver showed the architecture of the hepatic lobule appeared more or less like normal (Fig. 5A). While in some cases, focal necrosis associated with lymphocytic infiltration and congested blood sinusoids were found (Fig. 5B). In the rats given AFB<sub>1</sub>, the architecture of the hepatic lobule appeared like a normal (Fig. 5C). But in some rats, micro and macro vacuoles of fatty change were shown (Fig. 5D).

The diversity of AFB<sub>1</sub>-metabolic pathways were noted in several living organism's species, where the generality serious reaction is their

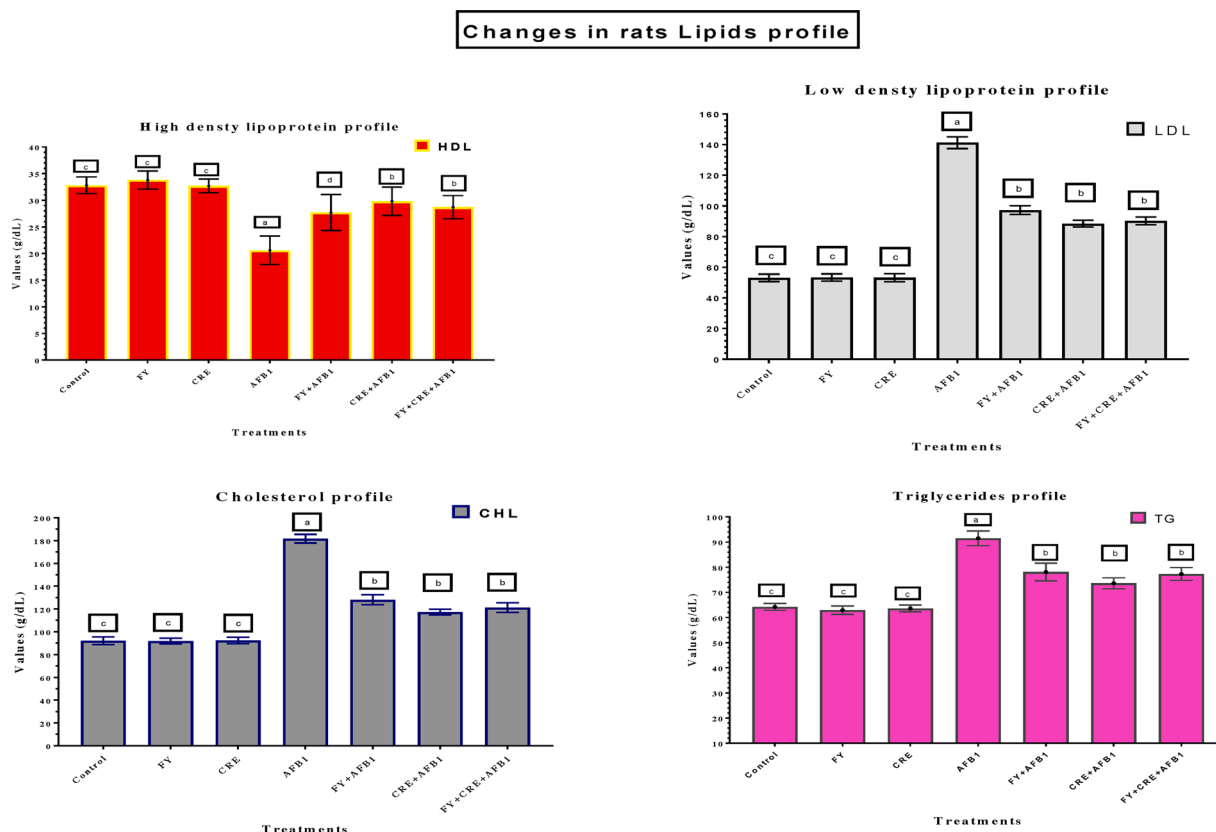


Fig. 2. The changes in the lipid profile of aflatoxin-treated rats due to the administrating with chicory extract and yogurt. The column with above different letters are significantly different at (P = 0.05).

**Table 3**  
Liver and Kidney function parameters for Aflatoxin-treated rats administrated by chicory extract.

Treatment	Control	AFB <sub>1</sub>	Yogurt	CRE	Yg-AFB <sub>1</sub>	CRE-AFB <sub>1</sub>	Yg + CRE + AFB <sub>1</sub>
ALT (U/L)	39.6 ± 1.18 <sup>a</sup>	119.8 ± 2.87 <sup>f</sup>	37.4 ± 1.73 <sup>a</sup>	40.3 ± 1.31 <sup>a</sup>	74 ± 2.39 <sup>d</sup>	49.8 ± 3.41 <sup>b</sup>	64.2 ± 4.25 <sup>c</sup>
AST (U/L)	158.2 ± 3.84 <sup>a</sup>	289.1 ± 7.19 <sup>f</sup>	159.6 ± 5.36 <sup>a</sup>	158.4 ± 3.52 <sup>a</sup>	207.9 ± 4.49 <sup>d</sup>	186.6 ± 2.88 <sup>b</sup>	191.1 ± 4.22 <sup>c</sup>
ALP (U/L)	52.8 ± 0.97 <sup>a</sup>	99.2 ± 1.86 <sup>f</sup>	53.1 ± 0.61 <sup>a</sup>	52.5 ± 0.73 <sup>a</sup>	68.4 ± 1.47 <sup>d</sup>	61.4 ± 1.08 <sup>b</sup>	70.7 ± 1.91 <sup>c</sup>
Urea (mg/dL)	6.91 ± 0.04 <sup>a</sup>	9.71 ± 0.81 <sup>f</sup>	6.97 ± 0.41 <sup>a</sup>	6.99 ± 0.24 <sup>a</sup>	7.94 ± 0.06 <sup>d</sup>	7.13 ± 0.06 <sup>b</sup>	7.24 ± 0.31 <sup>c</sup>
Creatinine (mg/dL)	0.97 ± 0.02 <sup>a</sup>	1.34 ± 0.21 <sup>f</sup>	0.99 ± 0.28 <sup>a</sup>	0.97 ± 0.05 <sup>a</sup>	1.03 ± 0.06 <sup>d</sup>	1.02 ± 0.06 <sup>b</sup>	1.07 ± 0.04 <sup>c</sup>

Data expressed as means ± SD; (n = 6; P = 0.05). (n = 6).  
The values of each column with superscript different letters had significant differences compared to the control group.

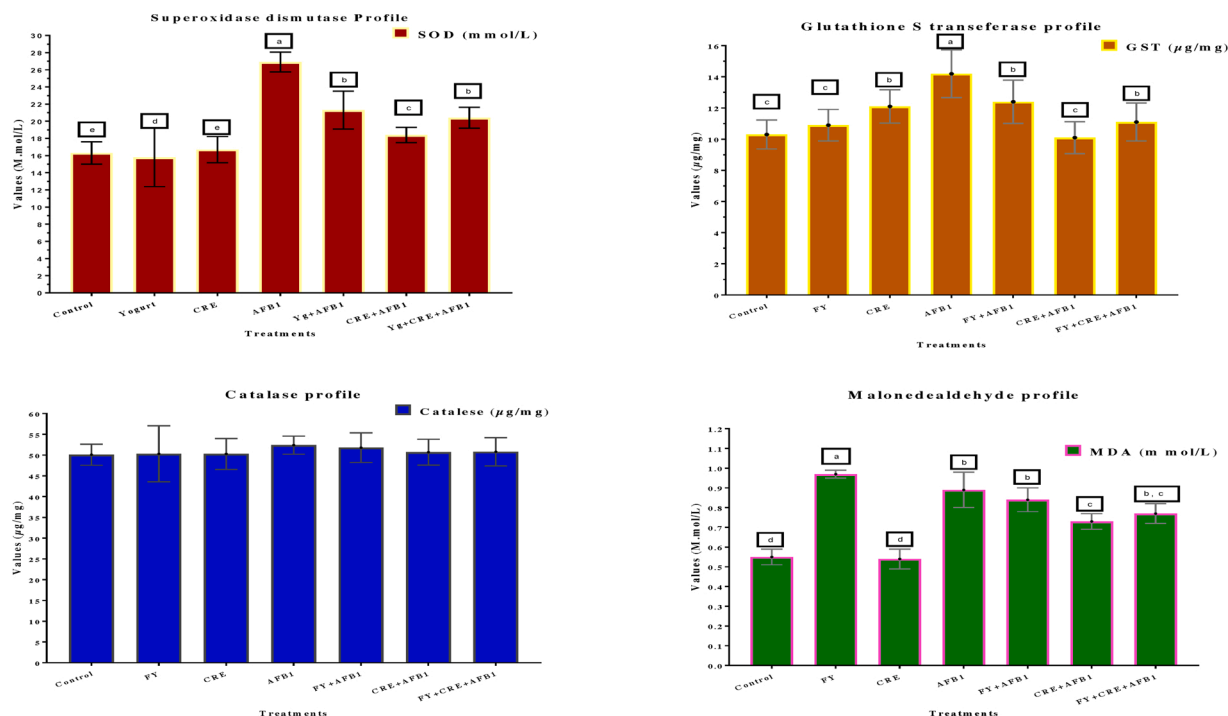
CRE: Rats were administrated by only chicory root extract.  
FY: Rats were administrated just by yogurt.  
FY-AFB<sub>1</sub>: Rats were administrated by yogurt plus aflatoxin.  
FY-CRE – AFB<sub>1</sub>: Rats administration with yogurt + chicory root extract + aflatoxin.  
FY-CRE: Rats administrated by yogurt with chicory root extract.

bioactivate to the epoxide fragment. The serious reactive metabolic change is joined to their ability to form DNA-adduct and induce mutations with the protein. The AFB<sub>1</sub>- residue in liver tissues is influenced by the cytochrome p53 and protein pathways in hepatocellular carcinoma [36,37]. Moreover, hepatitis diseases and carcinogenic effects, particularly the hepatitis B-virus, are influenced by human exposure to aflatoxins [38,39].

The present results revealed that CRE-efficiency as anti-aflatoxic material was determined. CRE demonstrated antibacterial and antifungal potency, particularly against the mycotoxigenic fungal strains. This potency recommended its application in food for enhancing safety and increasing their functionality. The proximate analysis for the CRE showed high content of the antioxidant activity and its content of total phenolic compounds recorded at a high level. The fortification of the FY using the CRE, significantly changed than those of FY alone, particularly in crude fiber content, carbohydrate content, and antioxidant activity.

The main components of the CRE activity were oligosaccharides, and the majority were reported to the inulin [40,41].

The bioactivity of the CRE was also evaluated by the *in-vivo* studies in experimental rats. The results were expressed by the changes in the liver and kidney enzymes. The mechanism of the reduction of aflatoxin is related to the ability of the extract components to enhance the antioxidant level in serum and tissues to avoid aflatoxin harm-impacts. Moreover, the oligosaccharides content of the extract (mainly inulin) was reported to affect aflatoxin toxicity [40,41]. The chicory extract was examined for their ability to reduce AFB<sub>1</sub> hazards, as aflatoxins are known to cause several damages in the liver tissues and are considered a pre-carcinogenic compound [42]. The CRE impacts were evaluated alone and added to the FY. The FY was reported to decrease aflatoxin-toxicity, which could be related to the binding effect of yogurt-starters [7]. The results revealed more potency of the CRE by the individual oral administration compared to its implication in the FY.



**Fig. 3.** The ameliorating effect of chicory extract on the enzymes of liver-tissues of aflatoxin treated rats. The column with the above different letters is significantly different at ( $P = 0.05$ ).

This behavior may be demonstrated through the active group of the extract that binds to the yogurt peptides [43,44]. The administration by the CRE for experimental rats reflects amelioration of feed intake, body weight gain, even if the aflatoxin was present in the GIT of rats. The CRE could decrease the harmful effect of aflatoxin on the protein fractions of rat-serum.

The admittance of the AFB<sub>1</sub> in rats' biological systems has existed clear alterations of lipid profile. The hepatic injury associated with dyslipidemia was sourced by the AFB<sub>1</sub> existence. These damages were initiated through the alterations of lipid expression and the metabolism of lipoproteins. Aflatoxin was reported occurring gene alteration that increases lipoprotein receptors [45]. The placement of lipid formation and their expression are regulated by gene expressions, leading to lipoprotein types formation. The AFB<sub>1</sub> adduct to the DNA could alter the lipoprotein formation process inside the cell, which results in abnormal changes. Where aflatoxin existence also leads to Inflammatory cells in the portal triad and a hepatic cellular-reaction named proliferation of bile duct-like structures. These results also were conjugated by histological liver failure, which congruent to the previous result [15,42].

As aflatoxin was recorded to cause changes in the lipoprotein formation cycle in rats' biological systems. This leads to changes in the lipids fractions, whereas lipoproteins are considered the principle lipid-receptors. The lipids are transferred or moved through the serum and tissues as lipoprotein form. This finding was emphasized by the ability of AFB<sub>1</sub> to induce harmful changes in liver and kidney [46]. The changes were reported in both lipid profiles; tissue lipids and serum lipids. The changes of lipids-profile were considered lower when these rats were administrated by the CRE or the CRE-Yogurt.

The utilization of the CRE as an oral administration in the rats have decreased the toxin behavior that may affect the liver and kidney functions, which was represented as an enhancement in the ALT, AST, and ALP enzyme levels (Table 3). Moreover, the liver-tissue enzymes in the AFB<sub>1</sub>-treated rats became so close to the negative control rats when administrated by the CRE as oral treatment (Fig. 3).

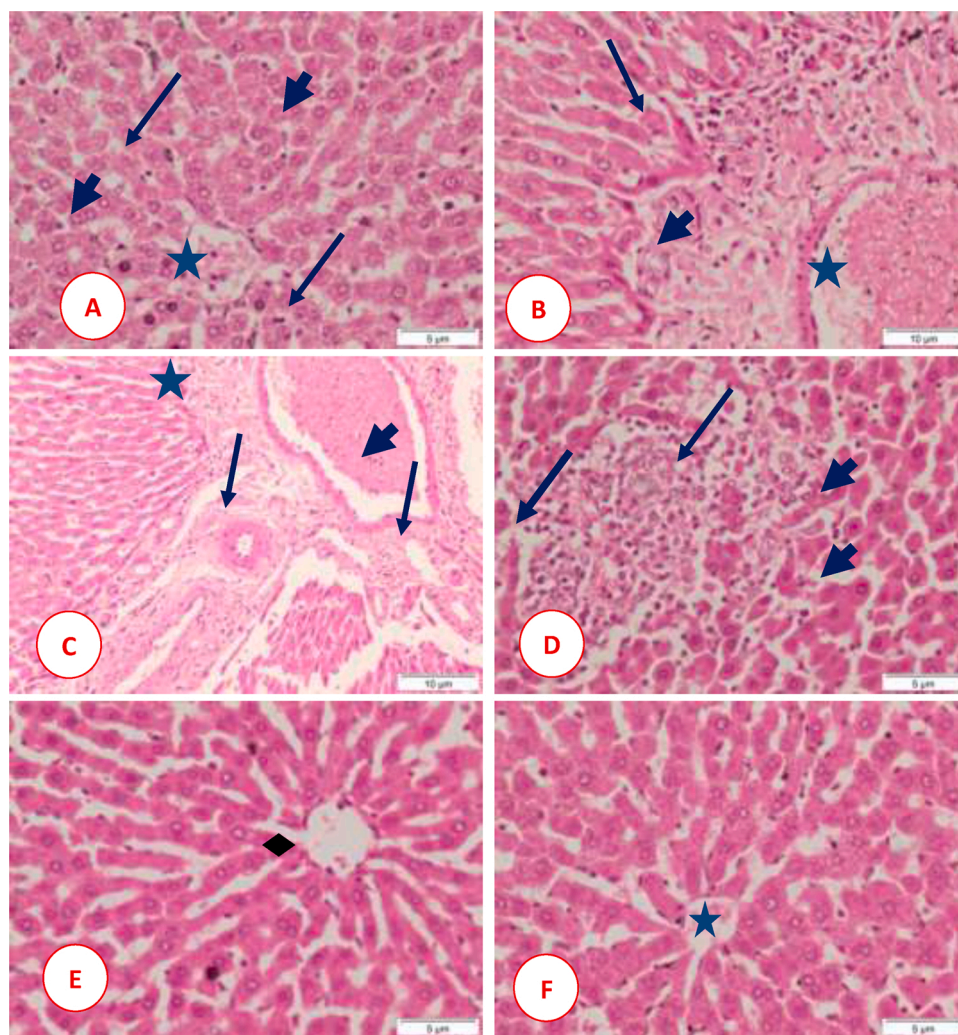
The histopathological examination of the liver tissues demonstrated partial normality of liver tissues of AFB- treated rats by CRE

administration. While the FY showed an enhancement in liver tissues of the AFB-treated rats (Fig. 4E), it was so close to the normal liver tissue in the case of CRE-administration (Fig. 4F).

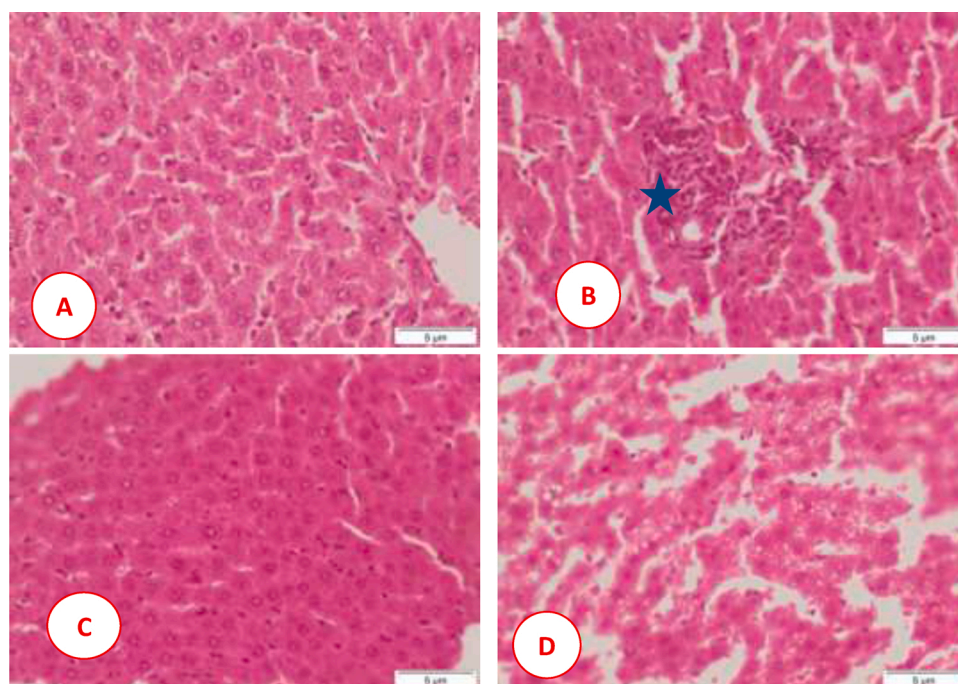
The oxidative stress, which is caused by the presence of aflatoxin e in the living cells, affected their viability and their functionality [40], but a utilizing of active components and natural extracts reported with a good reducing impact for the aflatoxin stress [14,47]. The oligosaccharides content of the CRE, partially inulin, and its fractions, play a major function against the oxidative stress influence of aflatoxins [48,49]. The more efficiency of the CRE as administrative individually than its joining with yogurt. This result had a slight difference of Roller et al. [50] which refer to enhancement activity of the bacterial growth by inulin against aflatoxin. The differences here may be affected by the strain-type of the starter, which is utilized to produce the yogurt. The corrective action of the CRE was indicated by the amelioration in the liver function and kidney function enzymes, also in liver tissue enzymes (Table 3; Fig. 3).

The oxidative stress impact of the AFB<sub>1</sub> on the liver cells was clearly shown on the pathogenic examination of liver-tissue (Fig. 4C and D). The administration, of CRE, demonstrated changes in the tissues nearly similar to that of control, (Fig. 5C and D). The ameliorative effect of the CRE is related to fructans and inulin content of the chicory root extract [51,52]. According to the result, the structure of the oligosaccharides (fructans, inulin, etc.) plays an important role in the decreasing effect of aflatoxin influences in the GIT. This could be explained by the behavior of these active components like dietary fibers, which could chelate aflatoxin by their active sites. The histopathological study on rats showed a lesser change in liver tissue of aflatoxin-treated rats due to the CRE administration, which supported this theory as experimental evidence.

AFB<sub>1</sub> is bio-produced into a highly reactive intermediate, AFB<sub>1</sub> 8–9 epoxides, by microsomal cytochrome P450 and attach to nuclear acids to make adducts. These adducts block transcript and translation, which affect the regulation and eventual hepatotoxicity of functional gene expression [53]. AFB<sub>1</sub>-prompts the hydroxyl-radicals, which interact with, and lead to mutations in DNA and result from the build-up of reactive oxygen species. In human hepatocytes (HepG2) AFB<sub>1</sub> also



**Fig. 4.** A micrograph of sections of the liver from A) normal control rat shows the normal architecture of the hepatic lobule. The central vein (asterisk) lies at the center of the lobule surrounded by cords of hepatocytes (arrow). Between the strands of hepatocytes, the hepatic sinusoids are shown (arrow-head), B) positive control rat (AFB<sub>1</sub>-treated) shows congested central vein (asterisk) and centrilobular necrosis of the hepatocytes (arrowhead) associated with inflammatory cell infiltration (arrow), C) positive control rat (AFB<sub>1</sub>) shows portal and periportal lymphocytic infiltration (arrow) and congestion of the portal vessels (arrowhead). Notice, the periportal necrosis of the hepatocytes that surround the portal area (asterisk), D) Aflatoxin-treated rat shows focal necrosis of the hepatocytes that are associated with lymphocytic infiltration (arrow). Notice dilated hepatic sinusoids foci of necrotic hepatocytes (arrow-heads), E) rat was given yogurt only shows the normal architecture of hepatic lobule (◆), F) rat given chicory root extract only shows the normal architecture of the hepatic lobule (asterisk) (H&Estain, Scale bar: 5 μm).



**Fig. 5.** A micrograph of sections of liver from B) rat given Yogurt and AFB<sub>1</sub> shows the architecture of the hepatic lobule appeared more or less like normal, C) rat given Yogurt and AFB<sub>1</sub> shows focal necrosis associated with lymphocytic infiltration and congested blood sinusoids, D) rat given chicory extract and AFB<sub>1</sub> shows the architecture of the hepatic lobule appeared more or less like normal, E) rat given chicory extract and AFB<sub>1</sub> show micro and macro vacuoles of fatty change (H&Estain, Scale bar: 5 μm).

causes apoptosis, cytotoxicity, and genotoxicity. The chronic aflatoxicosis caused by the exposure to low doses of AFB<sub>1</sub> in the diet has been involved in hepatocellular carcinoma over long exposure time [54]. Lipids are molecules possess with a key function in the metabolic pathways; fatty acids, triglycerides, cholesterol, and phospholipids are the lipids of clinical and physiological significance. These lipids are conveyed as lipoproteins in the blood, made of a hydrophobic core with a hydrophilic layer [55]. The AFB<sub>1</sub> has been reported to cause alterations in plasma and liver lipid levels. Lu et al. [45] reported that gene expression analysis and metabolite profiling are more sensitive than general toxicity studies for the detection of earlier hepatotoxicity induced by the AFB<sub>1</sub>. The active components, which presence in the media containing AFB<sub>1</sub> was previously showed with reduction impact [56]. The results demonstrate that AFB<sub>1</sub> induced hepatic damage with concomitant dyslipidemia, and this may occur in part through the alteration of expression of lipid and lipoprotein metabolizing genes. They also reported an increment in long-chain fatty acid concentrations subsequently to AFB<sub>1</sub> treatment in rats.

Nevertheless, the changes that occurred due to AFB<sub>1</sub>-adducts formed by DNA mainly depends on AFB<sub>1</sub>-oxidative stress in the biological system. These changes appeared in the lipid profile, the cellular tissue shape, serum, and tissue enzymes. The presence of the CRE in the biological system offers an alternative pathway for the AFB<sub>1</sub> to obtain its needs both electron (from phenolic compounds) and the antioxidant impact of the extract that stopped the harmful impact. The activity of AFB<sub>1</sub> molecules mainly depends on their lactone rings content, which contains unsaturated bonds that tend to react in the biological systems. The physiological impact of the CRE application in the biological systems was explained through its behavior as a functional bioactive extract. The presence of the CRE during the existence of AFB<sub>1</sub> in diet recorded a capability for stopping this adduct, subsequently, all the changes connected to it. The impact of the Cerise extracts relies upon their enrichment bioactive molecules, including the phenolic compounds and polysaccharides (mainly inulin). The availability of the Cerise extract that contains several biologically active substances helps in a highly effective way to reduce the changes resulting from the presence of aflatoxin in living tissues. The limitation of these changes mainly depends on converting the biological pathway of aflatoxin from its binding to the DNA strip to an ineffective binding form. The suggested mechanism is through suppressing the AFB<sub>1</sub> to the epoxide form, which resulted in gene alterations followed by the unbalancing of biological enzymes and hormone systems.

#### 4. Conclusion

The CRE analysis showed a functional impact due to its content of active components. The extract reflected an efficiency as an antioxidant and antimicrobial agent, particularly against toxigenic fungi. The implementation of the CRE in yogurt to produce the FY ameliorated its safety and nutritional potency. The rat experiment declared the ability of the FY to reduce the harmful impact of aflatoxin in liver tissue. Rats fed by the FY with aflatoxin existed as oral administration showed closed similarity to the control. However, the nutritive enhancement of the CRE was recorded. These results lead to the recommendation of the application of the CRE to produce functional products that may be able to ameliorate oxidative stress in live tissues caused by aflatoxins.

#### Authors' statement

**Ahmed M. Abdel-Salam** and **Ahmed H. Zaghoul** collected the raw materials, prepared the extract, and manufactured the functional yogurt. **Ahmed M. Abdel-Salam** and **Ahmed Noah Badr** designed experiments **Ahmed Noah Badr** performed the analysis, did the antimicrobial evaluation, performed the rat experiment, evaluated the biochemical parameters, statistically analyzed the data and discussed the final results, **Abdel Razik H. Farrag** performed the

histopathological evaluation, discussed the result of histology, and explained the CRE impact. **Ahmed Noah Badr** wrote the final manuscript, response to the correction and the revise copys. All authors reviewed, accept the manuscript, and approved the final version.

#### Declaration of Competing Interest

The authors report no declarations of interest.

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