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The toxicity of fumonisin B1 on some biochemical and immunological parameters and its detoxification by red cabbage and garlic

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Abstract

The protective effects of red cabbage and garlic extracts against liver, kidney and thyroid gland damage induced by fumonisin B1 (FB1) in male mice were studied. Sixty mice divided in to six groups. Group one are the healthy mice, Group two are mice that received a daily oral dose of only FB-1 (100 µg/kg.b.w) for 1 month, Group three: are mice that received red cabbage extract (500 mg/kg.bw) plus FB1, Group four: are mice that received red cabbage extracts, Group five: are mice that received garlic extract (500mg/kg.bw) plus FB1, group 6: are mice that received only garlic extract. After finishing the experiments, samples of blood were used for biochemical examination. The results indicated that group (2) mice treated with fumonisin B1 had significantly increased levels of immunoglobulins (IgG and IgM), kidney function parameters (urea and creatinine), proteins (albumin and total protein (TP)), and thyroid hormones (T3 and T4), along with significantly decreased level of TSH ($p < 0.05$). In the LD50 experiment, we are choose concentration (100 µg/kg.b.w) gavage to the mice. Oral administration red cabbage garlic extracts produced significantl reduction the levels serum IgG, IgM, T3, T4, urea, creatinine, TP and albumin and with a significant increase in TSH.

Keywords: Fumonisin B1; Red cabbage; Garlic; Immunoglobulins.

تأثير سمية فيومونيسين ب 1 على بعض المعاملات البايوكيميائية و المناعية وأزالة التأثير السمي بواسطة مستخلصات نباتية

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الخلاصة

مستخلص الملفوف الاحمر والثوم لديهم تأثير وقائي على ضرر الكبد بواسطة فيومونيسين ب في ذكور الفئران. عشوائيا 60 فارة قسمت الى ست مجاميع. المجموعة الاولى هي مجموعة الفئران الاصحاء, والمجموعة الثانية عوملت ب فيومونيسين ب 1 بجرعة 100 مايكروغرام/ كيلوغرام يوميا لمدة شهر, اما المجموعة الثالثة عوملت بمستخلص الملفوف الاحمر 500 ملغم/كيلوغرام زائدا فيومونيسين ب 1, والمجموعة الرابعة استقبلت مستخلص الملفوف الاحمر فقط, والمجموعة الخامسة عوملت بمستخلص الثوم 500 ملغم/كيلوغرام زائدا فيومونيسين ب 1, بينما شملت المجموعة الاخيرة الفئران المعاملة بمستخلص الثوم فقط.

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بعد انتهاء التجربة، نماذج الدم استخدمت للفحوصات البايوكيميائية . النتائج تشير ان مجموعة الفئران الثانية التي عوملت ب فيومونيسين ب 1 لديها زيادة معنوية في الكوليبرولينات المناعية (اي جي أم و اي جي جي)، وايضا في هذه الدراسة وجدت هنالك زيادة معنوية في هرمونات الغدة الدرقية، تي3، تي4 و نقصان معنوي في تي أس أش، وايضا هنالك زيادة معنوية في وظائف الكلية، يوريا و كرياتينين، بالاضافة الى زيادة معنوية في بروتينات السيرم، البروتينات الكلية و الالبومين. في تجربة تحديد التركيز نصف القاتل للحيوانات، أختارنا 100 مايكروغرام/ كيلوغرام من التوكسين لأعطائه الى الفئران. التطعيم الفموي المتعلق بمستخلص اللهانة الحمراء والثوم انتج أختزال معنوي في اي جي أم ، اي جي جي، تي3، تي4، يوريا و كرياتينين، البروتينات الكلية و الالبومين وسبب زيادة معنوية في تي أس أش.

Introduction

The mycotoxins have been considered as natural secondary metabolites that have low molecular weight. They are created via specific strains of species that belongs to various filamentous fungi including *Penicillium*, *Aspergillus*, and *Fusarium*, through invading the kernels in the field. These fungi also grow on the foods through the storage within favorable conditions of moisture and temperature [1]. In addition, fungi produce such biochemical metabolites for a lot of purposes; several of them are not yet recognized [2]. The production of mycotoxin tends to be increased in the case when the fungal growth rates are slowing down, i.e. while the fungi are moving towards dormancy; in these examples, the production of mycotoxin is a defensive reaction [3].

The target of mycotoxins could be compacting the factors decreasing fungi's growth rate. Otherwise, fungi might be producing mycotoxins for protecting the fungal spores and dormant molds, to survive fungal species. Mycotoxins possibly contribute in protecting molds from opposing environmental conditions (extremely dry or cold), or from the absence of certain significant nutrients in substrates on which the mold has been growing on [4].

Fumonisin can be defined as the secondary metabolites that are created in cereals by pathogenic fungi, including as *Fusarium proliferatum*, *Fusarium verticillioides*, and associated species [5]. Furthermore, *Aspergillus niger* produces fumonisins in crop plants of grapes, maize, and peanut [6]. Maize, as well as maize-based products, were reported to be majorly infected with fumonisins, along with their existence in many other grains (e.g. millet, rye, barley, rice, maize, wheat, and oat) and grain products (e.g. chips, corn flasks, and tortillas) [7, 8]. Such interactions are considered to be of high risk on health. Over fifteen fumonisin homologues were recognized, such as fumonisin A, B, C, and P [9]. Furthermore, among fumonisin B, FB-1, -2, and -3 are the most abundant, with FB-1 is the major toxic form co-existing with the other forms [10].

Research indicated that fumonisins are competitive inhibitors of sphingolipid biosynthesis and metabolism. In addition, fumonisins are structurally comparable to sphingolipid bases, which have the ability of inhibiting ceramide synthase and sphingosin-sphinganine-transferase [11].

In 1995, the International Agency for Research on Cancer (IARC) reported that fumonisins cause various diseases in animals and humans [12]. They are hepatotoxic and nephrotoxic for animals (Sharma and Sharma, 2004). In 2002, IARC defined these toxins as 2B group of carcinogens [13]. Kidneys and liver are the organs in danger as a result of fumonisin toxicity. Genus, sex, and dose differences are efficient determinants of the toxin's rate of toxicity. Rabbits were reported to have high sensitivity to FB-1. In nephrotoxicity that results from FB-1, female mice have high sensitivity in comparison to male ones [14].

Reduced activity and immune responses were indicated in calves consuming FB-1 [15]. The administration of FB-1 in mice induces caspase-8 enzyme activities. Caspase-8 can be specified as one of the intracellular downstream signaling molecules included in tumor necrosis factor-alpha (TNF- α) apoptotic pathway, also having a role in the FB-1-induced apoptosis [16].

Red cabbage is regarded as an important plant for its rich content of anti-oxidants, phytochemicals, vitamins (K, E, A, and C), and minerals (potassium, magnesium, manganese, calcium, and iron), along with low contents of cholesterol and saturated fats. The B vitamins, for instance folate (B2), riboflavin (B2), and thiamine (B1), are located within the crop tissues. In addition to the minerals and vitamins, cabbage contains small amounts of protein [17]. Epidemiological data, in addition to in vitro researches, propose that cabbage has anti-oxidant phytochemical compounds, with strong protective impacts against the main degenerative diseases, such as cancer [18] and cardiovascular diseases. They

were also reported to have antihyperglycemic [19] and hypocholesterolemic [20] properties. Furthermore, the RC extract prevents oxidative stress which is induced in the brain and liver of animals exposed to paraquat [21] and N-methyl-D-aspartate [22]. The protective action that is related to the cruciferous vegetable was due to the existence of antioxidant phytochemicals, particularly β -carotene, ascorbic acid, anthocyanine, flavonoids, polyphenolic glucosinolate, and α -tocopherol [23]. Garlic (*Allium sativum* L.), which belongs to family Alliaceae is considered as one of the important sources of remedies for different ailments as well as physiological disorders. The name (garlic) might be taken from the Celtic word 'all', meaning pungent. Garlic is practically cultivated all over the world and spreads in China, being originated in Central Asia and the Mediterranean region before moving west to Southern and Central Europe, Mexico, and Northern Africa (Egypt) [24].

The medicinal effects of garlic and its extracts on cardiovascular diseases have been widely examined. In addition, garlic preparations and chemical constituents have been examined for possible impacts on cardiovascular diseases, including blood fibrinolytic activities, platelet aggregation, hypertension, and hyperlipidemia [25].

Garlic is recognized for containing natural anti-oxidants which might act in removing reactive oxygen species (ROS), reducing lipid peroxide types and low-density lipoprotein (LDL) oxidation [26]. Since garlic contains diallyl sulfides, allicin, and other sulfur compounds, it exerts various physiological activities in different metabolic pathways [27].

Materials and methods

Preparation of red cabbage extract

The plant material was prepared based on a conventional approach [28]. One Kilogram of leaves was sliced into small pieces. The extraction was achieved with the use of 100gm of plant material dried by exposure to the sun for one week. The material was mixed with 800 ml of water and left on magnetic bar shaker for temperature of 40 celsius and period of 12 hours. Using the dry plants might be efficient for minimizing the enzymatic degradations of phenolic compounds. Following overnight-maceration, the extract was filtered with double filter paper, after the water was evaporated by oven at temperature of 50 celsius for 24 hours. Following evaporation, the dried samples were placed in a desiccator over CaCO_3 for removing water residues. The resultant dried violet-red pigments were utilized for further experiments. The dried extract was dissolved in distilled water at a concentration of 500 mg/ml prior to being administrated to mice.

Preparation of garlic extract

The plant material was prepared based on the method described by Toryali et al., (29), as follows: . 100 g of dried commercial garlic powder from local market was mixed with 800 ml of water on a water bath shaker for 12 h at 40 °C . Thereafter, the mixture was filtered via with Whatman No. 1 filter paper, and the filtrate was gathered and utilized for preliminary chemical analysis. Furthermore, the dried extract was dissolved in distilled water to a concentration of (500 mg/ml) before being administrated to mice.

Determination of the median lethal dose of FB1

Laboratory Animals:

Thirty-six male Swiss albino mice (1 month-old, 24±2 gram weight), obtained from the Biotechnology Researches Center / Al- Nahrain University, Baghdad, Iraq, were adapted to experimental conditions for two weeks before starting the experiments. They were maintained under the laboratory environment of diet, water, and temperature at the animal house in the Center.

The experimental test of LD50

A total of 36 mice were divided into 6 groups, each received an oral gavage of various FB1 concentrations, which included: 50 μg , 100 μg , 150 μg , 200 μg , 250 μg and 300 μg . Throughout 24 hours after oral administration, the treated mice for all groups were monitored to determine the concentrations that killed 50% of the animals, which was specified as the median lethal dose (LD50) [30].

Experimental design :

To study the possible significance of the extracts to prevent FB1 toxic impacts, 60 male mice were divided randomly into six groups. G1 group included animals receiving neither FB-1 nor extract medications and served as negative control. G2 group included animals treated with 100 $\mu\text{g}/\text{Kg}$ /day of FB-1 only for one month and served as a positive control. G3 group included animals treated with

500mg/animal/day of red cabbage extract for one month and 100µg/Kg /day of FB-1 for one month (G4 group included animals treated with 500mg/animal/day of red cabbage extract for one month. G5 group included animals treated with 500mg/animal/day of garlic extract for one month) and 100µg/Kg/day of FB-1 for one month. G6 group included animals treated with 500mg/animal/day of garlic extract for one month.

Determination of serum immunoglobulin G

The quantitative determination of mouse IgG was achieved with the use of enzyme-linked immunosorbent assay (ELISA) mouse IgG Kit (Catalog No: 501240) (EIA- Cayman company, USA). Such immunological assay is based on the double-antibody sandwich method. Each of the wells of the microtiter plate provided in the kit was coated with the antibody that is specific for the mouse IgG. Such anti-body should bind to any mouse IgG introduced in the well. The second antibody which recognizes various epitopes of mouse IgG (Antibody/ horseradish peroxidase (HRP) Conjugate) was added to the well. This enables the 2 antibodies for creating a sandwich through binding 2 different epitopes on mouse IgG molecule. The sandwich was immobilized on the plate and the excess reagents were washed. Antibody/HRP conjugate was labeled with the HRP, after mouse IgG's quantitation. Adding HRP substrate TMB, succeeded via stop solution is producing a product with a yellow color which can be estimated spectrophotometrically. Color intensity is considered to be directly proportional to the amount of the bound anti-body/HRP conjugate, which is proportional to IgG concentration.

Determination of serum immunoglobulin M

The quantitative determination of mouse IgM was achieved with the use of mouse IgM ELISA Kit, (Catalog No: 0801181, Zeptometrix company). In this assay, IgM exists in samples reacting with anti-IgM antibodies that are adsorbed to the surface of polystyrene microtitre wells. Following the removal of the unbound portion through washing, the anti-IgM antibodies were conjugated with HRP. Such enzyme-labeled antibodies form complexes with formerly bound IgM. After another step of washing, the enzyme which is bound to the immunosorbent was assayed via the addition of the chromogenic substrate, TMB. The bound enzyme's level is proportional directly to IgM concentration in the tested samples; therefore, absorbance at 450 nm was used as a measure of IgM concentration in the tested sample. IgM level in the test sample was interpolated from the standard curve that is constructed from standards, then corrected for the dilution of the sample.

Determination of serum triiodothyronine (T-3), thyroxine (T-4), and thyroid stimulation hormone (TSH)

The levels of T3, T4, and TSH in the serum samples of mice from different experimental groups were determined by using Beckman coulter AU analyzer.

Determination of serum total protein :

Serum total protein was measured with the use of a colorimetric approach based on Tietz [31], using commercially available kits (Randox,France). This kit depends on the Biuret method to determine total protein in serum. Cupric ion in the alkaline medium interacts with the protein-peptide bond, causing the creation of a colored complex.

Determination of serum albumin

The concentration of albumin in serum was determined with the use of a colorimetric approach through a commercially available kit (CliniChem, India). The approach is based on the specific binding of bromocresol green (BCG), an anionic dye, with the protein in an acidic pH to create a complex of green color. The formed color's intensity is considered to be proportional to albumin's concentration in the sample.

Determination of globulin concentration

Total globulin level was estimated via subtracting the level of albumin from that of total protein [32]:
Globulin concentration (g/dl) = TP (g/dl) – Albumin (g/dl).

Determination of serum creatinine

A commercially available kit (BIOLABO, France) was used for colorimetrically determining creatinine concentrations in the urine and serum. A colored creatinine picrate complex that contains ionic bonds was formed via creatinine with alkaline picrate (1:1 ratio). The formation rate of the colored complex is considered to be proportional to creatinine concentrations.

Determination of serum urea level

Serum urea was colorimetrically estimated based on Fawcett and Scott [33], with the use of a commercially available kit (Randox, France).

Statistical analysis

Data analysis was performed by utilizing SPSS for Windows, V22 (SPSS Inc. Chicago, Illinois, United States). Bonferroni Post Hoc test for multiple comparisons was applied after the analysis of variance (ANOVA) test [34].

Results and discussion

Determination of the LD50 for male mice treated with FB1

The LD50 of FB1 was detected by determining the dose that caused 50% of death in laboratory animals. After oral gavage of FB1 toxin (100 µg/kg) to mice, death occurred at 200 Mg, and no death was observed in male mice using the concentrations of 50 Mg and 100Mg as shown in table (1). Therefore, 100 Mg was used for studying all biochemical and immunological parameters.

Table 1-Percentage of died mice after 24 hours oral gavage of FB1

Groups	FB1 concentration µg	No. of mice	No. of deaths after 24hr.	Percentage of death %
1	300	6	6	100
2	250	6	5	83.3
3	200	6	3	50
4	150	6	1	16.7
5	100	6	0	0
6	50	6	0	0

Consumption of FBs has been associated with increased incidence of esophageal cancer The (IARC) classified FB1 and FB2 in group 2B (possible carcinogenic to humans). Regulatory limits have been established in many countries. In the EU, the maximum level for total FBs (FB1 + FB2) exposure limit ranges from 200 µg/kg of body weight (bw) for processed maize-based foods and baby foods for infants and young children to 2000 µg/kg of bw for unprocessed maize. Recently the European Food Safety Authority (EFSA) established a tolerable daily intake (TDI) for FBs of 1.0 µg/kg bw per day. In animals, high concentrations of FBs cause various clinical signs. FBs elicit nephrotoxicity, hepatotoxicity, immunotoxicity, disturbances of the intestinal barrier function, and microbiota dysbiosis [35].

Serum immunoglobulin levels in treated mice groups

Effects of FB1 on antibody titers were confirmed by these results. Levels of IgM showed a significant increase in mice treated with only FB1 in comparison with the control and other groups. Also, these levels were decreased significantly in mice treated with FB1 plus red cabbage, red cabbage alone, FB1 plus garlic, and garlic alone, when compared with mice treated with FB1 alone, while increased significantly compared with the control group. As related to IgG, there was a significant increase in mice that were treated with only FB1 in comparison with the control and other groups. In mice treated with only RC extract, IgG level had a significant decrease in comparison with the mice with FB1 only and the control group. In the group of mice treated with FB1 plus garlic, IgG level was decreased significantly compared with mice treated with FB1 alone and increased significantly compared with the control group, as shown in table (2).

Table 2-Impacts of garlic and RC extracts on immunoglobulin levels in mice given oral FB1 (100µg/kg body weight) for 4 weeks (mean ± SD).

Parameters	G-1 (n=10)	G-2 (n=10)	G-3 (n=10)	G-4 (n=10)	G-5 (n=10)	G-6 (n=10)	P value
IgM (ng/ml)	169.66 (9.20) ^a	302.33 (10.81) ^b	195.50 (12.78) ^c	195.33 (5.46) ^c	218.83 (23.74) ^c	202.66 (5.95) ^c	0.00
IgG (ng/ml)	1287.16 (11.85) ^{acd}	1819.33 (54.20) ^b	1114.00 (233.20) ^{ace}	960.00 (139.15) ^e	1404.66 (175.51) ^d	1325.16 (45.91) ^{cd}	0.00

Different small letter(s) denote significant differences.

$P < 0.05$ = Significant.

a, a; b, b; c, c; d, d; a, ab mean no significant.

a, b; a, c; a, d; b, c; c, d mean significant.

G1, control group; G2, FB1 (toxin) group; G3, FB1 + red cabbage (RC) group; G4, RC group; G5, FB1 + garlic (G) group; G6, garlic (G) group.

Mice exposure to foods contaminated with 1, 5, and 10mg FB1/kg for up to four months was reported to have no considerable impacts on their antibody titers against the Aujeszky's disease virus [36].

Another research provided results that are in accordance with the results of this work. They showed decreased thymus weight, thymus necrosis, as well as increased IgM in the rats following intraperitoneal administration of 7.50 mg FB1/kg body weight for a period of 4 days [37]. In another investigation, FB1 had no considerable impacts on humoral and cellular specific and nonspecific immune responses in animals fed with high doses for short periods (100mg/animal/day for 8 days) or even with low doses for longer periods (1ppm, 5ppm and 10ppm for 3–4 months) [37]. There is a possibility that the mycotoxins have more pronounced impact on the mucosal lymphoid tissue in comparison to systemic immunity. For instance, a study conducted by Smith *et al.* [38] demonstrated that FB1 might be inhibiting the actions of the pulmonary intravascular macrophages in removing pathogens from pigs' circulation, which could make these animals more susceptible to diseases.

In our experiments of the immunological parameters, considerable differences among the groups were found. It can be indicated that FB1 had a considerable impact on immunoglobulin levels when the animals were fed a dose of 100 $\mu\text{g}/\text{Kg}$ for four weeks. The mode of action of FB1 is not completely understood yet. Wang *et al.* reported that the toxin disrupts the sphingolipid metabolism by inhibiting the sphingosine N-acyltransferase (ceramide synthase) enzyme. It results in the accumulation of sphingoid bases, alteration of signaling, and disruption of normal cell cycling [11]. The explanation of this result is that the gavage of the mice with FB1 alone causes an increasing oxidative stress, leading to tissue inflammation and thereby an increased antibody response to this inflammation.

Thyroid hormones' levels in the experimental groups

The impact of FB1 on thyroid hormones was investigated by oral administration of 100 $\mu\text{g}/\text{Kg}/\text{day}$ for four weeks. The levels of T_3 , T_4 , and TSH in treated and control animals are provided in table 3. In mice treated with only FB1, T_3 and T_4 were significantly increased ($p < 0.05$) compared with the control and the other groups, whereas TSH level was decreased significantly ($p < 0.05$) compared with control group. T_4 level in mice treated with FB1 plus garlic was decreased significantly compared with mice treated with only FB1, while significant increased compared with the control group. TSH level in mice treated with red cabbage extract alone showed a considerable increase compared with mice treated with only FB1 and the control group.

Table 3- Thyroid hormones levels in all experimented groups (mean \pm SD).

Parameters	G-1 (n=10)	G-2 (n=10)	G-3 (n=10)	G-4 (n=10)	G-5 (n=10)	G-6 (n=10)	P value
T3 (ng/ml)	7.43 (0.16) ^a	24.20 (1.20) ^b	7.10 (1.14) ^a	7.06 (1.11) ^a	8.68 (0.78) ^a	8.00 (0.26) ^a SD D	0.00
T4 (mg/dl)	25.44 (1.69) ^a	88.33 (7.08) ^b	29.55 (4.07) ^{ad}	26.65 (2.53) ^{ad}	45.66 (5.98) ^c	34.78 (3.23) ^d	0.00
TSH ($\mu\text{IU}/\text{ml}$)	0.13 (0.02) ^a	0.002 (0.001) ^b	0.09 (0.01) ^a	0.65 (0.22) ^c	0.07 (0.03) ^a	0.05 (0.02) ^a	0.00

Different small letter(s) denote considerable differences.

$P < 0.05$ = Significant.

The extracts of RC have a considerable anti-hyperthyroid activity which might, at least partially, modulate the oxidative stress that is resulting from hyperthyroid induced generation of free radicals. In addition, natural health products with vegetable origins, e.g. the day-to-day ingestions of 500 mg/kg. body weight RC extracts, was reported to ameliorate oxidative stress and hyperthyroidism condition.

At the same time, a study conducted by Suchetha *et al.* [39] revealed a pro-oxidant impact of thyroid hormone. It was also shown to increase oxygen free radical production and, therefore, lead to a reduction in the antioxidant state in the case of hyperthyroidism, in comparison with hypothyroidism and normal conditions. Garlic's protective effect on the thyroid function might be originating from its specific components, particularly flavonoids and allicin. Allicin is considered as one of angiotensin II inhibitors with vasodilating effect [40]. Also, saponins and flavonoids regulate the thyroid function, in addition to their general characteristics regarding inflammatory inhibition and anti-oxidation [41]. There are yet no studies concerning the regulation of thyroxine-induced mycotoxicosis. Thus, this work is considered as the first one showing the impacts of plant extracts in regulating hyperthyroidism as well as thyroxine-induced mycotoxicosis.

Kidney function in experimental groups

Table (4) showed the effect of FB1 on kidney functions (Creatinine and blood urea) and neutralized it by plant extracts. The results revealed that mice treated with FB1 alone caused considerable increase (P less than 0.05) in the creatinine level in comparison to the control group and other groups

Table 4- Effects of red cabbage and garlic extracts on kidney function parameters in mice orally treated with FB1 (100µg/kg body weight) for 4 weeks (mean ± SD).

parameters	G-1 (n=10)	G-2 (n=10)	G-3 (n=10)	G-4 (n=10)	G-5 (n=10)	G-6 (n=10)	P value
Urea (mmol/L)	65.06 (3.77) ^a	75.66 (6.18) ^b	52.51 (4.33) ^c	39.31 (1.28) ^d	37.42 (5.37) ^d	40.91 (3.57) ^d	0.00
Creatinine (µmol/L)	0.20 (0.06) ^a	0.40 (0.03) ^b	0.28 (0.04) ^a	0.21 (0.02) ^a	0.29 (0.07) ^a	0.22 (0.05) ^a	0.00

Different small letter(s) denote significant difference levels.

P<0.05 = Significant.

Sinai *et al.* [42] reported that FB1 induced elevations of blood urea and creatinine levels in mice. András *et al.* [43] demonstrated that the concentrations of plasma creatinine were increasing in parallel with the increase of mycotoxin dose, with maximum plasma concentrations of 100 ppm group. When comparing differences associated with exposure time, ten-day treatment was shown to induce considerably high concentrations of creatinine, in comparison with five-day treatment with 100 ppm. The results indicate that the plasma urea concentrations are gradually increasing in the 100 ppm group, with minimum concentrations estimated in the control. A study conducted by Guoqing *et al.* [44] indicated a significant increase in blood urea and creatinine concentrations with FB1 treatment in comparison with the positive control. In addition, the RC polar extracts were reported to prevent polyuria and renal enlargement. A study conducted by Hazem *et al.* [45] revealed an increase in serum levels of urea and creatinine in STZ-diabetic rats, which were normalized to the control values following sixty days of the ingestion of RC polar extracts. Also, garlic's aqueous extract was shown to decrease oxidative stress in diabetic rats' kidneys [46]. Garlic's aqueous extract enhanced the plasma levels of the renal biochemical factors that are induced through alloxan in Wistar rats [47].

Levels of serum protein in experimental groups

Table (5) shows the effects of red cabbage and garlic extracts on the level of serum protein in mice given oral FB1 (100µg/Kg) for four weeks. There was a significant increase (p < 0.05) in the serum level of total protein in mice treated with FB1 alone (G2) compared to the control (G1) and other groups. Also, the current study reports a significant increase (P < 0.05) in albumin and globulin levels in mice treated with FB1 alone (G1) in comparison with the control group (G1). The results revealed no significant differences in globulin levels of mice treated with red cabbage plus FB1(G3), red

cabbage alone(G4), garlic plus FB1 (G5), and garlic alone (G6) compared with mice treated with FB1 alone.

Table 5-Effect of red cabbage and garlic extracts on serum proteins in mice given oral FB1 (100µg/kg body weight) for 4 weeks (mean ± SD).

parameters	G-1 (n=10)	G-2 (n=10)	G-3 (n=10)	G-4 (n=10)	G-5 (n=10)	G-6 (n=10)	P value
TP (g/dl)	4.16 0.04) ^a (5.13 0.31) ^b (4.36 0.10) ^{ac} (4.54 0.18) ^{ac} (4.68 0.17) ^c (4.64 0.35) ^c (0.00
Albumin (g/dl)	2.82 0.07) ^{ab} (3.15 0.26) ^a (2.53 0.22) ^b (2.83 0.09) ^{ab} (2.77 0.22) ^b (2.70 0.23) ^b (0.00
Globulin (g/dl)	1.34 0.09) ^a (1.98 0.56) ^b (1.83 0.30) ^{ab} (1.71 0.10) ^{ab} (1.90 0.17) ^b (1.94 0.23) ^b (0.008

Different small letter(s) denote significant difference values.

P<0.05 = Significant.

Satheesh *et al.* [48] revealed a considerable increase in the values of total serum proteins, as well as a considerable reduction in serum albumin values, in Fumonisin B1 toxin-treated groups of birds. While Ewuola and Egbunike [49] reported that the serum levels of total protein and albumin were decreased significantly with increasing Fumonisin levels in diets, which might be due to experimental diets' toxin. A study conducted by Merrill *et al.* [50] showed that Fumonisin causes negative impacts on normal epithelial morphology, that might be resulting in poor absorption of proteins and performance of animal's gastrointestinal tract. Ricardo *et al.* [51] published results that are in agreement with our results, as they reported that the serum levels of total proteins and albumin were increased in broilers which received FB-contaminated food for fourteen and twenty-eight days. Protein levels in broilers that received a hepatotoxic substances were reported to occasionally decrease [52].

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