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Effects of Red Cabbage and Garlic Extracts on Oxidative Stress Induced by Fumonisin B1

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Abstract

This study was designed to investigate the protective effects of red cabbage and garlic extracts on the oxidative stress induced through treatment with fumonisin B1 in male mice. The study was conducted on sixty male mice that were divided randomly into six groups. Group 1included control mice, mice in group 2 received a daily oral dose (200 µg/kg.b.w) of fumonisin B1 for one month to induced mycotoxicosis, mice in group 3 received 500 mg/kg.b.w red cabbage extract plus fumonisin B1, mice in group 4 received only red cabbage extract, mice in group 5 received 500 mg/kg.b.w garlic extract plus fumonisin B1, and group 6 received garlic extract alone. After finishing the experiments, samples of blood were used for biochemical examination. The results indicated that group 2 mice had significantly increased ($p < 0.05$) serum levels of malondialdehyde, which is a biomarker of lipid peroxidation, in addition to significantly decreased levels of catalase and the antioxidant enzymes glutathione peroxidase , glutathione , and superoxide dismutase , as compared to the normal control group. Oral administration of garlic and red cabbage extracts produced significantly decreased levels of serum malondialdehyde and significantly increased levels of superoxide dismutase, catalase, glutathione peroxidase, and glutathione, as compared to group 2. These results indicated that the experimental treatment with fumonisin B-1 caused an elevated oxidative stress in addition to the consumption of the antioxidant enzymes in response to the induced oxidative aggression.

Keywords: Red cabbage; Garlic; Oxidative stress; Fumonisin B1;Enzymatic antioxidant.

تأثير مستخلصات اللهانة الحمراء والثوم على مستويات الاجهاد التأكسدي المستحث بوساطة فيومونيسين ب 1

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الخلاصة

صممت هذه الدراسة لتقصي التأثير الوقائي لمستخلص اللهانة الحمراء و مستخلص الثوم على حالة الاجهاد التأكسدي المستحث بوساطة فيومونيسين ب 1, وقد اجريت الدراسة على 60 من ذكور الفئران, قسمت حيوانات التجربة بصورة عشوائية الى ست مجاميع, الاولى هي مجموعة الفئران السليمة والمجموعة الثانية هي الفئران المعاملة بالسم فيومونيسين ب 1, اما المجموعة الثالثة فشملت الفئران التي عوملت بمستخلص اللهانة

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الحمراء زائد فيومونيسين ب 1, والمجوعة الرابعة شملت الفئران المعاملة بمستخلص اللهانة الحمراء فقط, اما المجموعة الخامسة شملت الفئران المعاملة بمستخلص الثوم زائد فيومونيسين ب 1 , بينما المجموعة الاخير شملت الفئران المعاملة بمستخلص الثوم فقط .تم تجريع الحيوانات من فيومونيسين ب 1 بجرعة 200 مايكروغرام/كيلوغرام من وزن الحيوان يوميا و عن طريق الفم و لمدة اربعة اسابيع. اخذت عينات الدم بعد انتهاء التجربة من جميع الحيوانات وتم قياس مؤشرات الاجهاد التأكسدي حيث شملت المالونالديهايد والكلوتاثايون المختزل و انزيم السوبراوكساید دسميوتيز و الكلوتاثايون بيروكسيديز و الكتليز. بينت النتائج ان هنالك زيادة معنوية في مستوى المالونالديهايد في مجموعة الفئران المعاملة ب فيومونيسين ب 1 فقط في حين وجد هنالك انخفاضا في مستويات الكلوتاثايون و انزيمات سوبراوكساید دسميوتيز و كلوتاثايون بيركسيديز وكتيليز. ان تناول 500 ملغم من مستخلص اللهانه الحمراء والثوم ادى الى انخفاض مستوى المالونالديهايد بالاضافة الى ارجاع مستويات كلا من الكلوتاثايون المختزل و فعالية انزيمات السوبراوكساید دسميوتيز وكلوتاثايون بيروكسيديز وكتليز الى مستوياتها الطبيعية مقارنة بمجموعة السيطرة. هذه النتائج تقترح ان فيومونيسين ب 1 يكون مصاحب مع زيادة الجهد التأكسدي و مع استهلاك انزيمات مضادات الاكسدة .

Introduction

Mycotoxins have been considered as natural secondary metabolites with low molecular weight that are produced by certain strains of species that belong to different filamentous fungi, such as *Fusarium*, *Aspergillus*, and *Penicillum*. These species invade kernels in the fields and grow on foods in favorable circumstances of temperature and moisture [1].

Fusarium verticillioides, *Fusarium proliferatum*, and some other species produce the secondary metabolite fumonisin [2]. Moreover, *Aspergillus niger* also produces fumonisin in the crops of maize, peanut, and grape [3]. Fumonisin is also commonly produced by these species in other plants, including millet, wheat, rye, rice, maize, oat, and barley, as well as grains products (chips, corn flasks, tortillas) [4,5], which induces massive impacts on health. There are over fifteen homologues of fumonisin which are classified as fumonisins A, B, C, and P [6]. The major toxic form is the FB1, which might be coexisting with other Fumonisin forms, such as FB-2 and FB-3 [7].

Studies have shown that fumonisin is competitive inhibitors of sphingolipid biosynthesis and metabolism. Structurally, fumonisin have been found to be comparable to the sphingoid bases, like sphingosin, which act components of sphingolipid molecules. They have the ability of inhibiting sphingosin-sphinganin-transferase and ceramide synthase [8].

Studies, both in vitro [9,10] and in vivo [11,12], indicated the capability of FB-1 of inducing oxidative stress with the resulting reactive oxygen species (ROS) generation, cytotoxic impacts, as well as apoptosis. FB1 actions on the generation of ROS was specified as a consequence instead of being a mechanism regarding its toxicity [13]. Yet, few researches indicated that FB1 has the ability of increasing oxidation rate, promoting free radical production, as well as accelerating chain reactions related to lipid peroxidation in the membranes [12].

Antioxidants have the ability of competing with the other oxidizable substrates at low concentrations and, therefore, considerably delaying or inhibiting the substrates' oxidation [14]. The physiological role of antioxidants is to prevent the damage to the cellular components resulting from the chemical reactions, including free radicals. The protective properties of antioxidants are caused by their capability of acting as free radical scavengers, thus protecting DNA, cell proteins, and lipids from the mycotoxin-induced damage. Numerous natural substances were utilized for their capability in modulating oxidative stress resulting from mycotoxins, including flavonoids, tocopherol (vitamin E), ascorbate (vitamin C), as well as carotenoid (vitamin A) [15,16].

Red cabbage (*Brassica oleracea*) has a remarkable popularity due to the fact that it is rich with vitamins (K, E, C, and A), antioxidants, phytochemicals, and minerals (potassium, magnesium, calcium, iron, and manganese), along with its low contents of cholesterol and saturated fatty acids. Furthermore, B vitamins, such as thiamine (B-1), riboflavin (B-2) and folate (B-2) were reported to be produced in red cabbage. In addition to these minerals and vitamins, cabbage contains some proteins [17]. Additionally, the protective actions associated with the cruciferous vegetables were attributed to the existence of antioxidant phytochemicals, particularly β-carotene, ascorbic acids, anthocyanine, polyphenols, flavonoids, gluosinolate, and α-tocopherol [18].

Garlic (*Allium sativum* L.) is one of the members of the Amaryllidaceae family, which is generally specified as a spice of high importance and a remedy for different ailments and physiological disorders. Furthermore, garlic contains natural antioxidants having the ability of removing ROS as well as reducing lipid peroxides along with low density lipoprotein (LDL) oxidation [19]. Since garlic contains diallyl sulfides, allicin, in addition to other compounds of sulfur, it shows a lot of physiological impacts and activities in different metabolic pathways [20]. To date, there is no study on the protective effects of red cabbage and garlic extracts, which are known potent antioxidants and free radical scavengers, against the oxidative stress induced by Fumonisin B1 (FB1). This study aims to investigate the antioxidant activity of red cabbage and garlic extracts against oxidative stress induced by FB1.

Materials and methods

Preparation of red cabbage extract

The plant material was prepared based on a conventional approach [21]. The leaves were sliced into small-sized pieces. Afterwards, the extraction was conducted with the use of dried plant material (100gm) and flowing solvent (800ml) acidified with HCL ((1 ml, 1 molar) ethanol (EtOH), and methanol (MeOH),. The extract was filtered with a gauze, and water was evaporated under decreased pressure at a temperature of 50 Celsius with the use of evaporator. Following evaporation, the dried samples were placed in a desiccator over CaCO3 for removing the remaining water. The subsequent dried violet-red pigment was utilized for further research. Also, the dried extract was dissolved in distilled water to a concentrations of 500 mg/ ml prior to administration to mice.

Preparation of garlic extract

Plant material was prepared based on a conventional approach [22], in which dried garlic powder (100g) was subjected to mixing with 800ml of flowing solvent acidified with 1ml of HCL (1molar), along with EtOH, and MeOH, as well as water (W). The mixture was then kept on a water bath shaker at a temperature of 40 Celsius for 12 hours. Then, it was filtered and the filtrate was collected and utilized for the preliminary chemical analysis. Furthermore, dried extract was subjected to dissolving in distilled water to a concentration of 500 mg/ml prior to administration to mice [22].

Preliminary phytochemical screening

Phytochemical screening was conducted on of crude extracts which were screened for the absence or existence of secondary metabolites, including tannins, flavonoids, steroidal compounds, alkaloids, phenolic compounds, saponins, glycosides, terpenoids, and anthraquinones, with the use of standard protocols [23].

Antioxidant activity of plants extracts

The percentage of antioxidant activity (AA%) of all substances was evaluated through the use of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. The measurement of DPPH free radical scavenging activity was conducted based on a previously described method [24]. The samples were subjected to reaction with DPPH radicals in a reaction mixture that contained ethanol (0.5mM), absolute ethanol (3mL), sample (0.5mL), and DPPH radical solution (0.3mL). DPPH reacts with the antioxidant compound that might donate hydrogen, it has been decreased. Color changes (deep violet to light yellow) were read by means of absorbance (Abs) at 517nm, following a reaction of 100min, using UV-VIS spectrophotometer . The mixture of ethanol (3.3mL) and the sample (0.5mL) served as blank. Also, a control solution was prepared through mixing 0.3mL DPPH radical solution and 3.5mL ethanol. The scavenging activity percentage (AA%) was evaluated based on the following equation [25]:

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank})x100}{Abs_{control}}\right]$$

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, were adapted for two weeks before

starting the experiments. They were maintained under laboratory environment with diet, water, and temperature in the animal house of the Biotechnology Researches Center/ Al- Nahrain University .

Experimental test of LD-50

The 36 mice were divided in to 6 groups, each was gavaged orally with various FB-1 concentrations; 50µg, 100µg, 150µg, 200µg, 250µg and 300µg. Following 24 hours, all the treated mice were studied for determining the concentration that caused the killing of 50% of animals, which was specified as the median lethal dose (LD-50) [26].

Experimental design

For the purpose of studying the possible significance of the extracts to prevent FB1 toxic impacts, 60 male albino mice were divided randomly into the following groups; animals receiving neither FB-1 nor extract medications **(G1)**, animals treated with only FB-1 (100µg/Kg /day) for one month on a daily basis **(G2)**, animals treated with red cabbage extract (500mg/animal/day) and FB-1 (100µg/Kg /day) for one month on daily basis **(G3)**, animals treated with red cabbage extract (500mg/animal/day) for one month on daily basis **(G4)**, animals treated with garlic extract (500mg/animal/day) and FB-1 (100µg/Kg/day) for one month on daily basis dose **(G5)**, and animals treated with garlic extract (500mg/animal/day) for one month on daily basis **(G6)**.

Measurement of serum malondialdehyde (MDA)

MDA concentration in the serum was evaluated based on Buege and Aust approach [27]. MDA is created from the breakdown of poly-unsaturated fatty acids and can serve as a possible index of peroxidation reactions. The approach of thiobarbituric acid (TBA) was utilized for estimating MDA, which reacts with TBA to provide pink color that can be read at λ max of 535nm [27].

Measurement of serum Glutathione GSH

Serum thiol concentration was evaluated based on Ellman and Lysko [28].

Determination of serum superoxide dismutase (SOD) activity

The superoxide dismutase activity assay kit (Colorimetric, ab65354) is considered as a sensitive and robust kit to measure the activity of SOD in tissue/cell lysates, plasma, serum, as well as the other biological fluids. SOD inhibition activity was evaluated through an colorimetric assay at OD 450nm.

Determination of Serum glutathione peroxidase (GPX)

Glutathione peroxidase assay kit (Colorimetric, ab102530) was used in this assay, in which GPX reduces cumene hydroperoxide, whereas it oxidizes GSH to glutathione reductase (GSSG). The formed GSSG is reduced to e GSH with the consumption of NADPH through GR. NADPH reduction (simply evaluated at 340 nm) is proportional to the activity of GPX.

Determination of Serum catalase (CAT)

Catalase activity assay kit (Colorimetric/Fluorometric, (ab83464) is a direct, simple, and extremely sensitive assay to measure catalase activity in various biological samples, including tissues, cell lysates, and biological fluids. Catalase that exists in the sample reacts with hydrogen peroxide (H_2O_2) to produce oxygen and water. The unconverted H2O2 reacts with the probe to form a product that can be calorimetrically measured at OD of 570nm or fluorometrically at Ex/Em of 535/587nm.

Statistical analysis

Data analysis was achieved with the use of SPSS for Windows, version 22 (SPSS Inc. Chicago, Illinois, United States). Bonfferoni Post Hoc test for multiple comparisons was utilized following the ANOVA test [29].

Results and discussion

Phytochemical screening of red cabbage

The phytochemical screening was achieved with the use of color formation in addition to the precipitating chemical reagents on red cabbages' dried whole leaves. The acquired results from the tests are indicated in Table-1. The results show that the extract of red cabbage contains phenolic compounds, flavonoids, as well as anthocyanins that are glycosylated with mono or dimolecules of saccharides. The main constituents of red cabbage extract are anthocyanins, such as cyanidin 3-5 diglycoside, cyanidin 3-glucoside, cyanidin 3-galactoside. The extract also contained acetylated anthocyanins, along with sinapic acids, coumaric acids, ferulic acids, isothiocyanates glucosinolate, vitamins C, B, A, and β-carotenes [30] Anthocyanins, which are considered as a group of phenolic natural pigments existing in the RCE, have been indicated for having strongest antioxidizing power of 150 flavonoids [31].

Table 1 - Results of phytochemical screening of aqueous RCE along with the tests and reagents used.

Test	Reagent	Result
Test for alkaloids	Red-orangeprecipitate	-
Test for steroids	Greenishcolor	-
Test for phenolics	Greenishcolor	+
Test for flavonoids	Yellow-orange color	+
Test for saponins	Persistent frothing	-
Test for tannins	Green, purple, blue or black color	-
Test for anthraquinones	Purple-blue	+
Test for glucosides	Brick redcolor	+
Terpenoids	Brown colour ring formation	+

Flavonoids, as well as the anthocyanins, have been considered as effective antioxidants *in vitro,* acting on quenching free radicals and terminating chain reactions which cause oxidative damage [32]. A study conducted by Reinisa [33] showed the antioxidant impacts of anthocyanins *in vitro* on human colon cancer cell line ($CaCO_2$). Cyanidin and cyanidin-3-glucoside treatment decreased the proliferation and growth of cells in a dose-dependent manner. The author reported reduced levels of ROS in all Cyanidin concentrations used, while the this effect was observed only at lowest concentrations of cyanidin-3-glucoside. Elevated cell cycle/stress proteins expression was also recorded **[**34]. These two compounds impact the fragmentation of DNA, showing their antioxidant activities. Also, the study indicated that anthocyanins exert antioxidant properties and might thus be of high importance in the treatment of diseases in which the production of free radicals is of high importance [35].

Phytochemical screening of garlic

The results of the phytochemical screening of garlic extract (GE) are presented in Table-2. The GE phytochemical screening revealed the existence of volatile oil, saponin, terpenoid, flavonoids, amino acid, protein, and phenol . However, tannin, alkaloids, carbohydrates, and glycosides were absent in garlic extract.

Table 2- Results of phytochemical screening of aqueous GE

Test	Color of the test	Results
Saponin	Persistent frothing	+
Tannin	Green, purple, blue or black color	-
Phenol	Greenish color	+

Alkaloids	Red-orange precipitate	-
Terpenoid	Brown colour ring formation	+
Flavonoids	Yellow-orange color	+
Amino acid and protein	Violet colour	+
Carbohydrate	Red precipitate	-
Glycosides	Brick red color	-

These findings are consistent with those of Mikail (2010) who reported the presence of saponin, carbohydrates, and cardiac glycoside, in garlic; however, alkaloids, flavonoid and glycoside were reported to be absent [36]. Similar results were also indicated by Pavni *et al*. (2011), as well as Huzaifa *et al*., (2014), who reported that garlic bulb had flavonoids, saponin, and cardiac glycosides. On the contrary, Abdullahi *et al*. (2014) reported that tannin and phlobatannin were present in garlic extract [37, 38, 39].

Saponins are considered as steroids or triterpenoid glycosides with bitter or astringent tastes as well as properties of foaming and hemolytic impacts on the red blood cells. Furthermore, they have useful cholesterol-lowering properties, showing structure-dependent biological activities [40]. Also, they cause a decrease in blood cholesterol through preventing its reabsorption [41]. Flavonoids are considered as water soluble molecules that are belonging to the polyphenol family. Flavonoids have certain antioxidant activities in addition to health promoting impacts, such as the anti-thrombotic, anti-oxidant, anti-cancer, anti-allergic, anti-inflammatory, vasoprotective, tumor inhibitory, and antiviral impacts. Such impacts were related to the impacts of flavonoids on arachidonic acid metabolisms. A few of the flavonoids consist of plants, which have anti-spasmodic (liquorice), diuretics (buchu), in addition to other antimicrobial properties [42].

Antioxidant activities of red cabbage and garlic extracts

The results shown in Table-3 demonstrate that all the extracts showed antioxidant activities (AA%) at varying degrees (18), The antioxidant activities for methanolic, ethanolic, and aqueous extracts of red cabbage were 36.6%, 61%, and 96%, whereas for garlic extracts they were 36%, 72%, and 93%, respectively .

Table 3- Results of the antioxidant activities of garlic and red cabbage extracts

	Methanolic extra	Ethanolic extract	Aqueous extract
AA% of RCE	36.6%	61%	96%
AA% of RCE	36%	72%	93%

The scavenging of the stable DPPH radical has been an extensively applied approach for evaluating free radical scavenging ability in different samples.

All the determinant parameters of the antioxidant activity show an almost similar trend when different extracts of garlic were used. According to Ryan and Prescott (2010), when phenolic compounds are subjected to *in vitro* digestions, they are transformed into various structural forms, with different functions and chemical properties [43]. Such functions and properties might provide various results of antioxidant activity which are estimated through various approaches. Thus, antioxidant capacity

measurement through more than one approach was suggested by Akillioglu and Karakaya (2010). Phyto-nutraceuticals have been utilized since time in-memorial for improving the health of humans [44]. Individuals consuming diet abundant in bioactive components are at low risks of chronic disparities, thus decreasing the rates of morbidity and mortality [45]. Such phytochemical compounds are rich in antioxidants that have the ability of neutralizing free radicals via donating electrons, thus converting them into certain harmless compounds. Hence, they can assist in preventing or decreasing different physiological threats caused by free radical formation[46].

Determination of LD50 for male mice treated with FB1

LD50 resulting from FB1 treatment was determined through the dose that caused the death of 50% of the tested animals. Following the oral gavaging of FB1 to mice, death occurred at 200 mg, whereas no death was observed in the concentrations of 50 and 100Mg, as shown in Table-4. Therefore , the concentration of 100 Mg was used for studying all biochemical and immunological parameters.

Table 4- Percentage of mice that died after the oral gavage with FB1.

Groups	FB1concentration Mg	No. of mice	No. of death after 24 hr.	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

Mycotoxins are classified into three classes: extremely toxic (with lethal dose below 1ppm), very toxic (lethal dose of 1-10 ppm), and toxic (lethal dose of 10-100 ppm) [47]. According to this classification, the LD50 of FB1 obtained in this study is ranked within the very toxic group of toxins.

Impacts of red cabbage and garlic extracts on oxidative stress parameters

The results in Table-5 indicate the fact that the mice treated with FB1 alone (100 µg/Kg) for 4 weeks had a significantly increased ($p < 0.05$) MDA level (2.47±0.20 µmol/L) in comparison to the control 1.54±0.10 µmol/L) and other groups. Also, there was a significant decrease in MDA level among mice treated with FB-1 plus red cabbage, red cabbage alone, FB1 plus garlic, and garlic alone, as compared to mice treated with FB1 alone. GSH level was also significantly decreased ($p \leq 0.05$) in mice treated with FB1 alone (123.41±6.46 µmol/L) as compared to the control (211.16±10.10 µmol/L) and other groups. Additionally, there were significant increased levels of GSH among mice treated with FB1 plus red cabbage, red cabbage alone, FB1 plus garlic, and garlic alone, as compared to the control group.

Table 5- Effects of red cabbage and garlic extracts on the serum levels of GSH and MDA in mice orally administrated with FB-1 (mean ± SD).

Parameter	**G1 (n=10)**	**G2 (n=10)**	**G3 (n=10)**	**G4 (n=10)**	**G5 (n=10)**	**G6 (n=10)**	***P* value**	**Significance**
S. GSH (µmol/L)	211.16 (10.10)^{a}	123.41 (6.46)^{b}	298.33 (24.11)^{c}	291.75 (55.63)^{c}	316.50 (54.04)^{c}	309.50 (43.27)^{c}	0.00	Significant
S. MDA (µmol/L)	1.54 (0.10)^{a}	2.47 (0.20)^{b}	1.53 (0.19)^{a}	1.77 (0.09)^{a}	1.74 (0.14)^{a}	1.68 (0.08)^{a}	0.00	Significant

Different small letter(s) denote significant differences.
P≤0.05 = 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.

The results regarding the increased lipid peroxidation levels might be associated to those of a research conducted by Qiaoling Yuan *et al*. (2019), who indicated that FB-1 treatment of 50µg/mL for a period of 48 hours caused an elevated lipid peroxidation in pigs, indicating that FB-1 promoted cell membrane damages [48]. Also, the destruction of the intracellular redox balance was specified as a significant factor in animal diseases. ROS overproduction is a major property of oxidative damage. Also, MDA can be considered as an ultimate product of lipid peroxidation in the cell membrane through oxygen free radicals [49]. Malonedialdehyde is described as an organic molecule that is the ultimate product of lipid metabolism, which results in toxic stress in cells. Its mutagenic impact is conferred through reacting with deoxy guanosine and deoxyadenosine in DNA molecules, which ultimately causes their damage. Our results are in accordance with those of former work. Authors of a previous study made comparisons between 3 mycotoxins (ZEN, DON, and FB-1) and showed their ability to induce MDA production (lipid peroxidation), with the magnitude of the effects of these toxins being in the sequence of FB1 > DON > ZEN [50]. Aziza *et al*. (2010) reported elevated levels of serum MDA in FB-treated rats, associated with a significant reduction in GSH [51] . Such reduction in serum GSH could lead to increment in the oxidative damage of DNA, which might be specified through the relation between GSH and FB or its metabolites [52].

Impacts of garlic and red cabbage extracts on antioxidant enzyme activities

The activities of the antioxidant enzymes catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD) revealed different conditions following the administration of FB1, as compared to healthy mice. The current study showed a significant reduction ($p < 0.05$) in SOD, GPX, and CAT serum levels in mice treated with FB1 alone in comparison with the control and other group. There was a significant increase in serum SOD level in mice treated with garlic alone as compared to the control group (Table-6).

Table 6-Impacts of garlic and red cabbage extracts on serum levels of antioxidant enzymes in mice given FB1 orally (mean ± SD).

Parameter	G1 (n=10)	G2 (n=10)	G3 (n=10)	G4 (n=10)	G5 (n=10)	G6 (n=10)	*P* value	Signifi cance
SOD (µU/ml)	3456.66 (245.57) [a]	2062.50 (134.45) [b]	3600.16 (111.10) [a]	3476.83 (297.22) [a]	3352.50 (194.95) [a]	4118.33 (141.05) [c]	0.00	Signifi cant
GPX (nmol/ml)	21.66 (1.03)[a]	10.33 (2.25)[b]	17.83 (1.16)[c]	21.83 (0.98)[a]	20.33 (2.25)[a]	20.66 (1.50)[a]	0.00	Signifi cant
Catalase (nmol/ml)	25.91 (1.19)[a]	9.55 (1.93)[b]	24.7 (1.77)[a]	24.31 (1.54)[a]	26.62 (0.64)[a]	27.73 (0.46)[a]	0.00	Signifi cant

Different small letter(s) denote significant differences.
$P \leq 0.05$ = Significant.

Enzymatic antioxidant defense systems have been accountable to protect from ROS like O2 ·−, HO· as well as H_2O_2 [53]. Furthermore, the primary antioxidant enzymes have been described to be represented by GPX, CAT, and SOD. Such antioxidant enzymes might be serving as redox biomarkers due to the fact that they are first in indicating the antioxidant state via the processes of oxidation/reduction [53]. GSH is considered to be the major abundant intracellular antioxidant involved in cell protection against oxidative damage as well as in different mechanisms of detoxification [54].

Antioxidant enzymes have been considered as significant mediators related to immunotoxicity that is induced via FB-1. Following treatment of mice daily (for 4 weeks) with oral gavage of FB-1 (100 µg/kg), the results of a previous study indicated that the exposure to the mycotoxin resulted in increased lipid peroxidation, IL-4, and IL-10 mRNA levels, yet reduced GSH content as well as down-regulated the expression of GPX, SOD, and CAT [55]. FB-1 could significantly increase the levels of intracellular reactive oxygen metabolites (ROM) and reduces cytoplasmic SOD. In addition, it can lead to GPX mRNA abundance, indicating that FB-1 could induce cytotoxicity via the impairment of the oxidative status of peripheral blood mononuclear cells (PBMCs) [56]. Antioxidant activities during oxidative stress have been specified as mechanisms associated with FB-1 neurotoxicity [57]. Also, the imbalance in the defense system of antioxidant enzymes was indicated *in vivo* when animals were administered with FB-1. A study conducted by Abbes *et al*. (2015) indicated that FB-1 reduced the content of GSH and down-regulated GPX and SOD expressions in the spleen in Balb/c mice daily treated (for 2 weeks) with an oral gavage of FB-1 (100 µg/kg) [55].

References

1. Iheshiulor, O. O. M., Esonu, B. O., Chuwuka, O. K., Omede, A. A., Okoli I. C. and Ogbuewu, I. P. **2011** . Effect of mycotoxins in animals nutrition: A review. *Asian J. Anim. Sci*., **5**: 19-33.
2. Rheeder, J.P., Marasas, W.F. and Vismer, H.F. **2002** .Production of fumonisin analogs by Fusarium species. Appl. Environ. *Microbiol*. **68**: 2101–2105.
3. Kumar, P., Mahato, D.K., Kamle, M., Mohanta, T.K. and Kang, S.G.**2017**. Aflatoxins: A global concern for food safety, human health and their management. Front. *Microbiol.* **7**: 2170.
4. Dall'Asta, C. and Battilani, P.**2016**. Fumonisins and their modified forms, a matter of concern in future scenario? *World Mycotoxin J*. **9**: 727–739.

5. Cendoya, E., Chiotta, M.L., Zachetti, V., Chulze, S.N. and Ramirez, M.L.**2018**. Fumonisins and fumonisin-producing Fusarium occurrence in wheat and wheat by products: A review. *J. Cereal Sci*. **80**: 158–166.

6. Braun, M.S. and Wink, M. **2018**.Exposure, occurrence, and chemistry of fumonisins and their cryptic derivatives. Compr. Rev. *Food Sci. Food Saf.* **17**: 769–791.

7. Damiani, T., Righetti, L.,Suman, M., Galaverna, G. and Dall'Asta, C. **2019**. Analytical issue related to fumonisins: A matter of sample comminution? *Food Control* . **95**: 1–5.

8. Merrill, J.R., Sullaeds, A.H., Wang, M.C., Voss, K.A. and Riley, R.T. **2001**. Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins". *Environmental Health Perspectivves*, **109**: 283-289.

9. Domijan, A.M., Gajski, G., Jovanovic, I.N., Geric, M. and GarajVrhovac, V. **2015**. In vitro genotoxicity of mycotoxin ochratoxin A and fumonisin B1 could be prevented by sodium copper chlorophylin-implication to their genotoxic mechanism. *Food Chemistry*, **170**: 455-462.

10. Mary, V.S., Theumer, M.G., Arias, S.L. and Rubinstein, H.R. **2012**. Reactive oxygen species sources and biomolecular oxidative damage induced by aflatoxin B1 and fumonisin B1 in rat spleen mononuclear cells. *Toxicology*, **302**: 299-307.

11. Abbes, S., Ben Salah-Abbes, J., Jebali, R., Younes, R.B. and Queslati, R. **2016**. Interaction of aflatoxin B1 and fumonisin B1 in mice causes immunotoxicity and oxidative stress: possible protective role using lactic acid bacteria. *Journal of Immunotoxicology***, 13**: 46-54.

12. Hassan, A.M., Abdel-Aziem, S.H., El-Nekeety, A.A. and AbdelWahhab, M.A. **2015**. Panax ginseng extract modulates oxidative stress, DNA fragmentation and up-regulate gene expression in rats subcronically treated with aflatoxin B and fumonisin B. *Cytotechnology***, 67**: 861-871.

13. Wang, X., Wu, Q., Wan, D., Liu, Q., Chen, D., Liu, Z., MatinezLarranaga, M.R., Martinez, M.A., Anadon, A. and Yuan, Z. **2016**. Fumonisins: oxidative stress-mediated toxicity and metabolism in vivo and in vitro. *Archives of Toxicology***, 90**: 81-101.

14. Diplock, A.T., Charleux, J.L., Grozier-Willi, G., Kok, F.J., Rice-Evans, C. and Roberfroid, M. **1998**. Functional food science and defence against reactive oxidative species. *British Journal of Nutrition*, 80, **1**: S77-S112.

15. Sorrenti, V., Di Giacomo, C., Acquaviva, R., Barbagallo, I., Bognanno, M. and Galvano, F. **2013**. Toxicity of ochratoxin A and its modulation by antioxidants: a review. *Toxins*, **5**: 1742-1766.

16. Strasser, A., Carra, M., Ghareeb, K., Awad, W. and Bohm, J**. 2013**. Protective effects of antioxidants on deoxynivalenol-induced damage in murine lymphoma cells. *Mycotoxin Research*, **29**: 203-208.

17. Hasan, M.R. and Solaiman, A.H.M. **2012**. Ecacy of organic and organic fertilizer on the growth of Brassica oleracea L. (Cabbage). *Int J Agric Crop Sci*, **4(3):** 128-138.

18. Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., and Mainland, C. M. **2000**. Antioxidant Capacity As Influenced by Total Phenolic and Anthocyanin Content, Maturity, and Variety of Vaccinium Species. *J. of Agricultural Food Chemistry* **,46**: 2686-2693.

19. Saravanan, G. and Prakash, J. **2004**.Effect of garlic (Allium sativum) on lipid peroxidation in experimental myocardial infarction in rats. *J Ethnopharmacol*.;**94**(1):155–158.

20. Amagase, H., Petesch, B.L., Matsuura, H., Kasuga, S. and Itakura, Y. **2001**. Recent advances on the nutritional effects associated with the use of garlic as a supplement: intake of garlic and its bioactive components. *J. Nutr*;**131**(3):955S–962S.

21. Hazem, A. H., Kataya, and AlaaEldin, A. Hamza, **2008**. Red Cabbage (Brassica oleracea) Ameliorates Diabetic Nephropathy in Rats.*J. eCAM* ;**5**(3): 281–287.

22. Toryali, A., Ezhilvalavan,S., Varun, A.,Sundaresan,A. and Manimaran,K.. 2018. Qualitative phytochemical analysis of garlic (Allium sativum) and nilavembu (Andrographis paniculata).*J. International Journal of Chemical Studies* ; **6**(3): 1635-1638.

23. Harborne, J. B. **1989**. General procedures and measurement of total phenolics.*Methods in Plant Biochemistry*. , 1-28.

24. Brand-Williams, W, Cuvelier, M.E. and Berset, C. **1995**.Use of a free radical method to evaluate antioxidant activity. *Lebenson Wiss Technol*; **28**: 25-30.

25. Mensor, L.L., Menezes, F.S., Leitao, G.G., Reis, A.S., dos Santos, T.C.and Coube, C.S. **2001**. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother Res*;**15**:127-130.

26. Makun, ,H.A.; Gbodi, T.A.; Akanya, .H.,Salako,A.E.;Ogbadu, G.H. and Tifin, U.I. **2010**. Acute toxicity fumonisin content of the culture material of Fusarium verticilloides Nirenberg (CABI-MI 392668) isolated from rice in Nigeria, Agric.*Biol.j.N.Am*,**1**:103-112.

27. 27. Buege, J.A., and Aust,S.D.**1978**."microsomal lipid peroxidation, *Methods- Enzymol*, **52**: 302- 310.

28. Ellman, G. and Lysko, H. 1967. Disulfide and sulfhydryl compounds in TCA extracts of human blood and plasma. *The Journal of laboratory and clinical medicine*, **70**: 518.

29. Glover, T.and Mitchell, K.**2008**.An introduction to Biostatistics, 2nd ed. Waveland press .Inc. Third Edition.

30. Jagdish, Singh, A.K., Upadhyay ,A., Bahadur ,B., Singh ,B., Singh, K.P., Mathura ,and Rai, A.K. **2006**.Antioxidant phytochemicals in cabbage (Brassica oleracea L. var. capitata). *Scientia Horticulturae* , **108**: 233–7.

31. Sterling, M.**2002**. Got anthocyanins. These plant pigments are more than coloring agents for fruit juices, wine and other beverages; they also contain an array of health-romoting benefits. *NSN*; **5**: 231–4.

32. Li, Y., Qin ,C.G.,Niu,W.N., Zhang , R.J.,and Shang,X.Y.**2009**.Comparison of free radical capacity among several plant anthocyanins in vitro.*Food Sciencve*,**30**: 91-94.

33. Renisa, M., Calandraal, L., Scifoa1, C., Tomaselloa, B., Cardilea, V., Vanellaa, L., Beia, R., Faucia, L., and Galvanoa, F. **2007**. Response of cell cycle/stress related protein expression and DNA damage upon treatment of CaCo2 cells with anthocyanins. *British Journal of Nutrition*, **100**(01): 27.

34. Hayes, J.D., Kelleher, M.O., and Eggleston, I.M .**2008** The cancer chemopreventive actions of phytochemicals derived from glucosinolates. *Eur J Nutr***, 47**(Suppl 2): 73-88.

35. Kong, J.M.,Chia,L.S.,Goh,N.K.,and Chia,T,F.**2003**.Analysis and biological activities of anthocyanins.*Phytochemistry*,**64**: 923-933.

36. Mikail, H.G.**2010**. Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of (Allium sativum L) bulbs in experimental rabbits. *J Med Plant Res*. **4**(4): 322-326.

37. Pavni, K., Esha, B., Neha, J. and Tushar A.**2011**. Phytochemical screening of developing garlic and effect of its aqueous extracts on viability of cardiac cell line. *J Pharm Res*. **4**(3): 902-904.

38. Huzaifa, U., Labaran, I., Bello, A.B. and Olatunde, A.**2014**. Phytochemical screening of aqueous extract of garlic (Allium sativum) bulbs. *Rep Opinion*. **6**(8): 1-4.

39. Abdullahi, D.K., Michael, O.O. and Indabawa, I.**2014**. Antibacterial activities and phytochemical screening Of Aloe vera (A. Babardensis), garlic (A. Sativum) and Ginger (Z. Officinale). *Journal of Emerging Trends in Engineering and Applied Sciences***. 5**(3): 172-178.

40. Osagie, A.U. and Eka, O.U.**1998**. Mineral elements in plant foods. In: Nutritional quality of Plant foods. *Ambik press*, Benin City, Edo State, Nigeria. **8**(14): 43- 86.

41. Prohp, T.P. and Onoagbe, I.O.**2012**. Acute toxicity and dose response studies of aqueous and ethanol extracts of Triplochton scleroxylon K. Schum (Sterculiaceae). Intern. *J Appl. Biol. Pharm. Technol* , **3**(1): 400-409.

42. Trease, G.E. and Evans, W.C.**2002**. Phytochemicals. In: *Pharmacognosy*. **15**th ed. Saunders Publishers, London ; 42-44, 221- 229, 246- 249, 304-306,331-332, 391- 393.

43. Ryan, L. and Prescott, S.L.**2010**. Stability of the antioxidant capacity of twenty-fivecommercially available fruit juices subjected to an in vitro digestion. *Int JFood Sci Tech* ,**45**: 1191-1197.

44. Akillioglu, H.G. and Karakaya, S.**2010**. Changes in total phenols, total flavonoids,and antioxidant activities of common beans and pinto beans aftersoaking, cooking, and in vitro digestion process. *Food Sci Biotechnol* ,**19**: 633-639.

45. Perveen, R. Suleria, H.A.R. Anjum, F.M. Butt, M.S. Pasha, I. Ahmad, S.**2015**. Tomato (Solanum lycopersicum) Carotenoids and Ly-copenes Chemistry; Metabolism, Absorption, Nutrition, and Allied Health Claims-A Comprehensive Review. Crit. Rev. *Food Sci. Nutr*, **55**(7): 919-929.

46. Capasso, A. **2013**.Antioxidant action and therapeutic efficacy of Allium sativum L. *Mol*., **18**(1): 690-700.

47. Makun, H.A.,Ghodi, T.A.,Akanya, H.O.,Salako, A.E., Ogbado, G.H. and Tifin, U.I. **2010**. Acute toxicity and total fumonisin content of the culture material of Fusarium verticilloides Nirenberg (CABI-IMI 392668) isolated from rice in Nigeria, *Agric. Biol, J.N. Am*, **1**: 103-112.

48. Qiaoling, Y. , Yancheng, J. , Ying, F. , Yingfeng, M. , Hongyu, L. and Jianming, S.**2019**. Fumonisin B1 Induces Oxidative Stress and Breaks Barrier Functions in Pig Iliac Endothelium Cells.*J.Toxins*. **11**(7): 387.

49. 49.Hannalien, M., Zanele, D., Skhosana, D., Mamsy, M.,Wiana, L. and Egmont, R. **2019**.Long Term Monitoring (2014–2018) of Multi-Mycotoxins in South African Commercial Maize and Wheat with a Locally Developed and Validated LC-MS/MS Method. *Toxins* , **11**: 271.

50. Kouadio, J.H., Mobio, T.A., Baudrimont,I., Moukha,S., Dano,S.D. and Creppy,E.E.**2005**. "Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line Caco-2," *Toxicology*, **213**(1–2): 56–65.

51. Aziza, M., Hassana, Sherif, R., Mohamedb, Aziza, A., El-Nekeety, Nabila, S. ,Hassanc, Mosaad ,A. and Abdel-Wahhab.**2010**. Aquilegia vulgaris L. extract counteracts oxidative stress and cytotoxicityof fumonisin in rats. *J.Cancer Prevention Research. Toxicon*, **56**(1): 8-18.

52. Circu, M.L. and Aw,T.Y.**2010**. Reactive oxygen species. Cellular redox system and apoptosis. Free radic. *Biol.Med*. **48** (6):749-762.

53. Yang, H.Y. and Lee, T.H. **2015** Antioxidant enzymes as redox-based biomarkers: a brief review. *BMB Rep*, **48**(4): 200–208.

54. Shi, J., Sun, B. and Shi, W. **2015**. Decreasing GSH and increasing ROS in chemosensitivity gliomas with IDH1 mutation. *Tumour Biol* **, 36**(2): 655–662.

55. Abbes, S., Ben, Salah-Abbes, J., Jebali, R., Younes, R.B.and Oueslati, R. **2016**. Interaction of aflatoxin B and fumonisin B in mice causes immunotoxicity and oxidative stress: possible protective role using lactic acid bacteria. *J Immunotoxicol*,**13**(1): 46-54.

56. Bernabucci, U., Colavecchia, L. and Danieli, P.P. **2011**. Aflatoxin B1 and fumonisin B1 affect the oxidative status of bovine peripheral blood mononuclear cells. *Toxicol In Vitro***, 25**(3): 684–691.

57. Domijan, A.M. and Abramov, A.Y. **2011**. Fumonisin B1 inhibits mitochondrial respiration and deregulates calcium homeostasis—implication to mechanism of cell toxicity. *Int J Biochem Cell Biol***, 43**(6): 897–904.