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 1 included control mice, mice in group 2 received a daily oral dose (200 µg/kg.b.w) of fumonisin B1 for one month to induce 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 اللحنة الحمراء والنثوم على مستويات الاجهاد التأكسدي المستحت بوساطة فيومونيسين 1

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الخلاصة

مصمت هذه الدراسة لتقديم التأثير الوقائي لمستخلصحات اللحنة الحمراء ومستخلص الثوم على حالة الأجهاد التأكسدي المستحت بوساطة فيومونيسين ب 1. وقد أجريت الدراسة على 60 من ذكور الفئران، فست حيوانات التجربة بصورة عشوائية إلى ست مجموعات. الأولى هي مجموعة الفئران السالمية والمجموعة الثانية هي الفئران المعاملة بالمستخلصات فيومونيسين ب 1, أما المجموعتان الثلاثة فشملت الفئران التي عُملت بمستخلص اللحنة حمراء ومستخلص الثوم. هذا كله أدى إلى نتائج مميزة. هذه الدراسة تقدم نظرة عامة حول التأثيرات العلاجية لمستخلصات الفئران. مع ذلك، فإن الدراسة تؤكّد على أهمية استخدام المستخلصات الطبيعية في علاج الأعداد الدوائية المهتمة.}

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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 *Penicillium*. 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 sphingosines-phosphatidyl-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 role of antioxidants is 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, and C), 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, gluinosinolate, 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, allin, 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]:

$$AA\% = 100 - \frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}}$$

**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 (H2O2) to produce oxygen and water. The unconverted H2O2 reacts with the probe to form a product that can be colorimetrically 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 glucosinolates, 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.

<table>
<thead>
<tr>
<th>Test</th>
<th>Reagent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for alkaloids</td>
<td>Red-orange precipitate</td>
<td>-</td>
</tr>
<tr>
<td>Test for steroids</td>
<td>Greenish color</td>
<td>-</td>
</tr>
<tr>
<td>Test for phenolics</td>
<td>Greenish color</td>
<td>+</td>
</tr>
<tr>
<td>Test for flavonoids</td>
<td>Yellow-orange color</td>
<td>+</td>
</tr>
<tr>
<td>Test for saponins</td>
<td>Persistent frothing</td>
<td>-</td>
</tr>
<tr>
<td>Test for tannins</td>
<td>Green, purple, blue or black color</td>
<td>-</td>
</tr>
<tr>
<td>Test for anthraquinones</td>
<td>Purple-blue</td>
<td>+</td>
</tr>
<tr>
<td>Test for glucosides</td>
<td>Brick red color</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Brown colour ring formation</td>
<td>+</td>
</tr>
</tbody>
</table>

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 (CaCO₂). 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

<table>
<thead>
<tr>
<th>Test</th>
<th>Color of the test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin</td>
<td>Persistent frothing</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>Green, purple, blue or black color</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>Greenish color</td>
<td>+</td>
</tr>
<tr>
<td>Compound</td>
<td>Reaction / Color Change</td>
<td>Result</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Red-orange precipitate</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>Brown colour ring formation</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Yellow-orange color</td>
<td>+</td>
</tr>
<tr>
<td>Amino acid and protein</td>
<td>Violet colour</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Red precipitate</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Brick red color</td>
<td>-</td>
</tr>
</tbody>
</table>

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, antioxidant, 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**

<table>
<thead>
<tr>
<th></th>
<th>Methanolic extract</th>
<th>Ethanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA% of RCE</td>
<td>36.6%</td>
<td>61%</td>
<td>96%</td>
</tr>
<tr>
<td>AA% of RCE</td>
<td>36%</td>
<td>72%</td>
<td>93%</td>
</tr>
</tbody>
</table>

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 Akkillioglu 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.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>FB1 concentration Mg</th>
<th>No. of mice</th>
<th>No. of death after 24 hr.</th>
<th>Percentage of death %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>6</td>
<td>5</td>
<td>83.3</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>6</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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 ≤ 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).

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

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]. Malondialdehyde 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).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G1 (n=10)</th>
<th>G2 (n=10)</th>
<th>G3 (n=10)</th>
<th>G4 (n=10)</th>
<th>G5 (n=10)</th>
<th>G6 (n=10)</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (µU/ml)</td>
<td>3456.66</td>
<td>3600.16</td>
<td>3476.83</td>
<td>3352.50</td>
<td>4118.33</td>
<td>0.00</td>
<td>Significant</td>
<td></td>
</tr>
<tr>
<td>(1245.57)</td>
<td>(111.10)</td>
<td>(297.22)</td>
<td>(194.95)</td>
<td>(141.05)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GPX (nmol/ml)</td>
<td>21.66</td>
<td>10.33</td>
<td>17.83</td>
<td>21.83</td>
<td>20.33</td>
<td>20.66</td>
<td>0.00</td>
<td>Significant</td>
</tr>
<tr>
<td>(1.03)</td>
<td>(2.25)</td>
<td>(1.16)</td>
<td>(0.98)</td>
<td>(2.25)</td>
<td>(1.50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase (nmol/ml)</td>
<td>25.91</td>
<td>9.55</td>
<td>24.7</td>
<td>24.31</td>
<td>26.62</td>
<td>27.73</td>
<td>0.00</td>
<td>Significant</td>
</tr>
<tr>
<td>(1.19)</td>
<td>(1.93)</td>
<td>(1.77)</td>
<td>(1.54)</td>
<td>(0.64)</td>
<td>(0.46)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Enzymatic antioxidant defense systems have been accountable to protect from ROS like O₂⁻, HO⁻ as well as H₂O₂ [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 downregulated 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


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-promoting benefits. NSV; 5: 231–4.


