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Antioxidants and Radical Scavenging Properties of Vegetable Extracts in Rats Fed Aflatoxin-Contaminated Diet

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The present study evaluated the protection role of garlic, cabbage, and onion extracts against the toxic effects of aflatoxin. One hundred and twenty mature male Sprague−Dawley rats were randomly assigned to eight experimental groups and treated for 15 days with extracts with or without aflatoxin. Blood samples were collected from all animals from the retro-orbital venous plexus at the end of the experimentation period for biochemical analysis. Livers and kidneys were removed at the end of the treatment period for determination of glutathione, malondialdehyde, and superoxide dismutase. The results indicated that animals treated with aflatoxin showed significant signs of aflatoxicosis. Extracts alone had insignificant effects on all parameters tested, whereas cotreatment with aflatoxin and extracts resulted in a significant improvement in all parameters; moreover, garlic extract was found to be the most effective in the prevention of aflatoxin-induced toxicity and free radical generation in rats.

KEYWORDS: Aflatoxin; garlic; cabbage; onion; free radicals; antioxidants

INTRODUCTION

Aflatoxins are secondary metabolites of certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* that have been shown to be toxigenic, carcinogenic, mutagenic, and teratogenic to different species of animals (*1*, *2*). Those aflatoxin-producing fungi are widely distributed in nature and can grow over a wide range of environmental conditions (*3*). Aflatoxins have been detected in cereal grains, whole wheat and rye breads, oilseeds, fermented beverages made from grains, milk, cheese, meat, nut products, fruit juices, and numerous other agricultural commodities (*4*). Therefore, the presence of aflatoxins or toxigenic fungi in foods presents a potential hazard to human and animal health.

Human epidemiology and experimental animal studies have provided the statistical association and biological information necessary to suggest that aflatoxins are risk factors for human liver cancer (*5*, *6*). The degree to which aflatoxins contribute to this disease will be influenced by a number of human health factors, including hepatitis B virus infection, nutritional status, and age as well as the extent of aflatoxin exposure. Aflatoxin B_1 has been reported to exert its liver-specific carcinogenicity by inducing a guanine (a purine) to thymine (a pyrimidine) substitution at coden 249 on the p53 gene (*7*, *8*). Aflatoxin also is metabolized by the mixed-function oxidase system to a number of hydroxylated metabolites and to aflatoxin 8,9-epoxide, which binds to DNA, forming covalent adducts (*9*), and disturbs DNA replication, causing chromosomal aberrations (*10*). Moreover, Galvano et al. (*11*) reported that aflatoxin also is known to produce membrane damage through increased lipid peroxidation. As liver cancer causes at least 200,000 deaths worldwide per year, prevention measures must be developed to reduce the incidence of this largely fatal disease. Preventive strategies will be facilitated by the identification of individuals at high risk (*12*). Moreover, direct exposure to aflatoxin-contaminated food commodities may impose a great risk to the consumer. Consequently, to reduce and/or prevent human and animal exposure, a practical and effective method for the detoxification of aflatoxin-containing foodstuffs is an urgent requirement.

The role of oxidative stress in the initiation of neoplastic processes through DNA chain scission leading to cell injury was discussed in relation to components of the diet (*13*). Consequently, increased emphasis should be placed on the use of chemopreventive agents that are natural constituents of our diet, to inhibit the carcinogenic process during its early stages (*14*, *15*). Non-nutritive compounds in a number of food of plant origin modulate aflatoxin B_1 biotransformation, binding to DNA, and carcinogenesis. Of the cruciferous vegetables that have been studied most widely, broccli (*16*), cabbage (*17*), and Brussels sprouts (*18*) have been found to be protective in rats and modulate biotransformation enzymes activities measured in vitro. Plants of the *Allium* genus have been also studied for their anticarcinogenic effects. Dwivedi et al. (*19*) reported that oil-soluble organosulfur compounds present in garlic induced an antiperoxidant effect. Moreover, Sumioka et al. (*20*) stated that *S*-allylmercaptocysteine (SAMC), one of the water-soluble organosulfur compounds in ethanol extracts of *Allium sativum*, protects mice against acetaminophen-induced liver injury. The objectives of the present study were to evaluate the protective effects of garlic, cabbage, and onion extracts against aflatoxin-induced toxicity in rats and its effects as antioxidants in vivo.

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MATERIALS AND METHODS

Chemicals. Aflatoxin (AF) standards were purchased from Sigma Chemical Co. (St. Louis, MO). The purity was confirmed by capillary GC−mass spectroscopy and UV spectrophotometry. Other commercially available chemicals were of the highest purity.

Kits. Transaminase (ALT and AST), cholesterol, and urea were obtained from BioMérieux SA. Lactate dehydrogenase (LDH), triglycerides, alkaline phosphatase (AP), albumin, createnine, creatine kinase (CK), total protein (TP), and total bilirubin (TB) kits were obtained from Sintinal CH. Glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD) were obtained from Eagle Diagnostics.

Aflatoxin Production. AF was produced via fermentation of rice by *A. parasiticus* NRRL 2999 as described by Shotwell et al. (*21*) and modified by West et al. (*22*). Fermented rice was autoclaved and ground to a powder, and the AF content was measured using HPLC (*23*). The AFs within the rice powder consisted of 40% B_1, 10% B_2, 35% G_1, and 15% G_2 based on total AFs in the rice powder. The rice powder was incorporated into the basal diet to provide the desired level of 3 mg of AF/kg of diet. The diet containing AF was analyzed, and the presence of parent AF was confirmed and determined as mentioned above.

Preparation of Garlic, Cabbage, and Onion Extracts. Fresh garlic bulbs, fresh onion, and cabbage seeds were purchased from a local market. Garlic bulbs, onion, and cabbage seeds were extracted with 95% ethanol in a Waring blender at room temperature as previously described by Fan and Chen (*24*). The extracts were purified and concentrated according to the method described in our previous work (*25*). In brief, the extracts were centrifuged at 11500*g* for 10 min at 4 °C. The supernatant was then extracted with diethyl ether three times, followed by separation into two portions: one each for the aqueous phase, which was soluble in diethyl ether. The aqueous portion was concentrated with a freeze-dryer (Freeze-Dry System, Mycotoxins Central Lab, NRC, Dokki, Egypt) to remove water, and then the water soluble extracts were obtained. The organic portions were dissolved by adding methanol and then filtered. The pooled filtrate was concentrated with a vacuum concentration apparatus to remove solvents, and then the oil soluble extracts were obtained. Subsequently, both extracts were stored at −20 °C until used. The oil soluble extracts were used in our study.

Experimental Animals. Three-month-old, sexually mature male Sprague−Dawley rats weighing 150−200 g (purchased from Animal House Colony, Giza, Egypt) were maintained on standard laboratory diet (protein, 16.04%; fat, 3.63%; fiber, 4.1%; and metabolic energy, 0.012 MJ) and water ad libitum at the Animal House Laboratory, National Research Center, Dokki, Cairo, Egypt. After an acclimation period of 1 week, animals were divided into eight groups (15 rats/group) and housed individually in filter-top polycarbonate cages housed in a temperature-controlled and artificially illuminated room free from any source of chemical contamination.

Experimental Design. Animals within each treatment group were treated daily for 15 successive days as follows: group 1, untreated control; group 2, fed AF-contaminated diet (3 mg/kg of diet); group 3, orally given garlic extract [5 mg/kg of body weight (bw)]; group 4, orally given cabbage extract (5 mg/kg of bw); group 5, orally given onion extract (5 mg/kg of bw); group 6, fed AF-contaminated diet and treated orally with garlic extract; group 7, fed AF-contaminated diet and treated orally with cabbage extract; and group 8, fed AF-contaminated diet and treated orally with onion extract, using the same doses. Feed intake was recorded daily for each animal within each treatment group. Blood samples were collected from all animals from the retro-orbital venous plexus at the end of the experimentation period for biochemical analysis. The following biochemical methods were performed: ALT and AST (*26*), AP (*27*), TP (*28*), albumin (*29*), TB (*30*), createnine (*31*), urea (*32*), CK (*33*), LDH (*34*), cholesterol (*35*), and triglycerides (*36*).

All animals were sacrificed and dissected on day 15; livers and kidneys were removed for GSH, MDA, and SOD determination.

Measurement of Glutathione. Fifty milligrams of kidney or liver was homogenized in 5% sulfosalicyclic acid and centrifuged at 10000*g* for 20 min, and the supernatant was analyzed according to the method of Anderson (*37*).

Assessment of Lipid Peroxidation by Measurement of Malondialdehyde. Tissues were homogenized in 20 mM Tris-HCl (pH 7.4). Homogenates were centrifuged at 6000*g* for 30 min. The LPO levels in the supernatants were determined using a spectrophotometric assay kit according to the manufacturer's instructions. The LPO values are expressed as nanomoles of MDA production per milligram of tissue.

Measurement of Superoxide Dismutase Activity. Tissues were homogenized in 10 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA. Homogenates were centrifuged at 6000*g* for 30 min. SOD activities in the supernatant were measured using a spectrophotometric assay kit according to the manufacturer's instructions. The SOD activities are expressed as units per milligram of tissue.

Statistical Analysis. All data were subjected to statistical analyses using the General Linear Models procedure of the Statistical Analysis System (*38*). The significance of the differences among treatment groups with variable means was determined by the Waller−Duncan *k* ratio *T* test (*39*). All statements of significance were based on a probability level of $P \leq 0.05$.

RESULTS

The effect of different extracts (i.e., garlic, cabbage, and onion) and aflatoxin on feed intake of animals in the different treatment groups revealed that AF alone significantly ($P \leq 0.05$) decreased the feed intake, whereas that of the animals treated with garlic, cabbage, or onion extracts alone or in combination with aflatoxin were comparable to the control (**Figure 1**). No animal mortality was observed in any of the extracts alone or plus aflatoxin treated group. Three of 15 animals (20%) treated with aflatoxin died between days 11 and 14. A concentration of 3 mg of AF/kg of diet resulted in a significant increase in ALT, AST, AP, cholesterol, TB, LDH, CK, urea, and createnine, whereas it caused a significant decrease in triglycerides, TP, and albumin (**Table 1**). Treatment with garlic extract alone did not affect the different biochemical parameters except for cholesterol, which decreased significantly ($P \leq 0.05$). Treatment with cabbage extract alone caused a significant decrease in urea, whereas the other biochemical parameters were unaffected. Onion extract alone caused a significant ($P \leq 0.05$) increase in LDH and CK and an insignificant increase in urea, whereas the other parameters were comparable to the control values (**Table 1**). Treatment with garlic, cabbage, or onion extracts plus AF resulted in a significant improvement in the different biochemical parameters, and these extracts succeeded in reducing the elevation of ALT, AST, AP, cholesterol, TB, LDH, CK, urea, and createnine resulting from aflatoxin treatment. Moreover, the combination treatments caused a significant improvement in triglycerides, TP, and albumin levels as compared to treatment with AF-contaminated diet.

The effects of different treatments on GSH, MDA, and SOD activity in liver and kidney are depicted in **Table 2**. Aflatoxin alone decreased GSH and SOD in the liver and SOD in kidney, whereas it increased MDA in liver and kidney. Garlic extract alone increased GSH and MDA in the liver and MDA and SOD in the kidney, whereas it decreased SOD in the liver and GSH in the kidney. Cabbage or onion extract alone increased GSH in liver and MDA in kidney, whereas it had no significant effects on GSH in kidney or SOD activity in both liver and kidney.

Addition of garlic extract to aflatoxin caused a significant ($P \leq 0.05$) increase in GSH in liver and MDA in liver and kidney. GSH in kidney and SOD in liver and kidney in the animals treated with garlic plus AF were comparable to the controls. Treatment with cabbage or onion extract plus AF resulted in a significant ($P \leq 0.05$) increase in MDA in liver

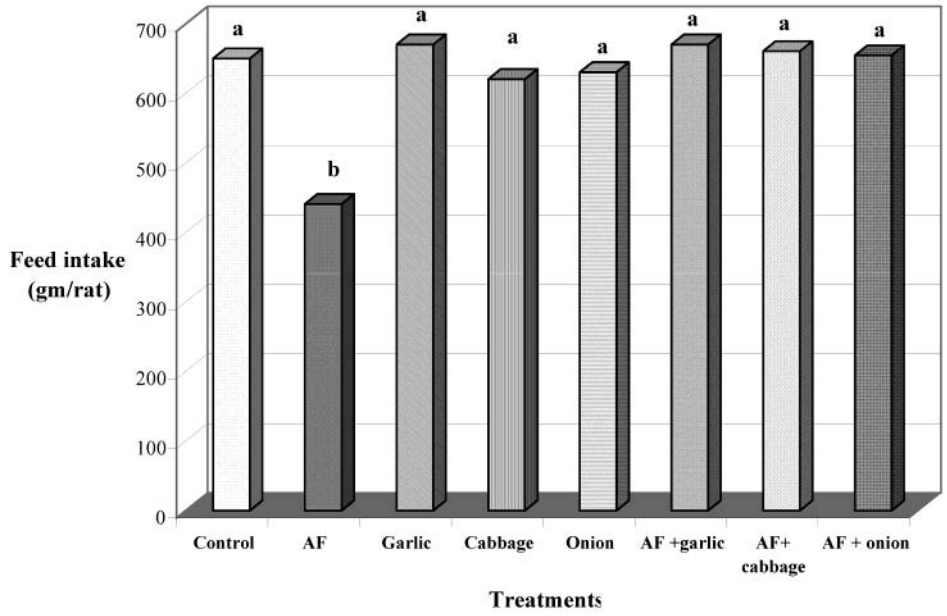

Figure 1. Effects of vegetable extracts on feed intake in rats fed aflatoxin-contaminated diet for 15 days.

Table 1. Effect of Garlic, Cabbage, or Onion Extract on Serum Biochemical Parameters in Rats Fed Aflatoxin-Contaminated Diet (3 mg/kg of Feed)[a]

parameter	control	AF	garlic	cabbage	onion	AF + garlic	AF + cabbage	AF + onion
ALT (units/L)	28.31a ± 1.58	145.18b ± 3.28	27.82a ± 1.23	27.23a ± 1.48	28.22a ± 1.47	35.22c ± 2.08	36.42c ± 1.92	39.05d ± 1.78
AST (units/L)	50.32a ± 2.31	188.32b ± 3.25	49.32a ± 1.22	52.42a ± 1.23	51.48a ± 2.33	55.28c ± 2.34	58.66d ± 1.78	60.08d ± 1.98
AP (units/L)	3.09a ± 0.26	20.33b ± 1.21	3.27a ± 0.86	3.06a ± 0.85	3.01a ± 0.75	6.83c ± 1.08	7.91c ± 1.21	8.22d ± 1.05
cholesterol (mg %)	55.87a ± 2.07	90.09b ± 3.86	50.12c ± 1.27	54.92a ± 2.33	55.92a ± 2.36	58.21a ± 2.39	60.02a ± 3.21	59.22a ± 3.48
TB (mg/dL)	0.33a ± 0.17	3.09b ± 0.19	0.32a ± 0.14	0.33a ± 0.15	0.31a ± 0.09	1.21c ± 0.36	1.32c ± 0.16	1.76c ± 0.22
triglyceride (mg/dL)	53.88a ± 4.25	48.78b ± 2.86	51.76a ± 3.65	52.33a ± 2.68	54.27a ± 2.38	52.08a ± 2.87	51.99a ± 2.86	53.18a ± 2.76
TP (g/dL)	7.09a ± 1.62	5.32b ± 1.44	7.12a ± 1.65	7.32a ± 1.46	7.07a ± 1.38	7.00a ± 1.48	7.53a ± 1.98	6.98a ± 1.99
albumin (g/dL)	2.53a ± 0.79	0.97b ± 0.02	2.55a ± 1.62	2.49a ± 0.61	2.48a ± 0.45	1.99c ± 0.36	1.78c ± 0.41	1.97c ± 0.42
LDH (units/L)	180.5a ± 16.31	650.23b ± 6.41	182.3a ± 4.51	179.14a ± 2.36	186.4a ± 7.23	195.32c ± 4.65	201.21c ± 6.21	199.26c ± 5.31
CK (units/L)	280.5a ± 31.32	720.30b ± 112.51	279.23a ± 25.18	277.15a ± 14.62	285.21a ± 16.31	311.2c ± 15.14	325.6c ± 17.21	350.4d ± 18.16
urea (mg/dL)	30.16a ± 2.61	52.16b ± 3.81	30.18a ± 2.19	35.42a ± 2.66	32.66a ± 2.31	39.27c ± 2.22	41.41c ± 2.33	40.15c ± 3.22
createnine (mg/dL)	0.612a ± 0.03	1.981b ± 0.09	0.601a ± 0.02	0.600a ± 0.03	0.592a ± 0.04	0.721a ± 0.03	0.762a ± 0.04	0.661a ± 0.05

[a] Within each row, means with the same letter are not significantly different ($P \leq 0.5$).

Table 2. Effect of Different Extracts on GSH, MDA, and SOD Activities in Liver and Kidney of Rats Fed Aflatoxin-Contaminated Diet (3 mg/kg of Feed)[a]

	liver			kidney		
group	GSH	MDA	SOD	GSH	MDA	SOD
control	6.52a ± 0.29	0.86a ± 0.03	0.42a ± 0.03	2.31a ± 0.04	0.88a ± 0.03	0.31a ± 0.02
AF	6.23b ± 0.15	4.95b ± 0.05	0.21b ± 0.02	2.68b ± 0.01	3.44b ± 0.07	0.18b ± 0.07
garlic	7.49c ± 0.16	0.89a ± 0.04	0.41a ± 0.01	2.30a ± 0.03	1.05c ± 0.02	0.32a ± 0.03
cabbage	7.12c ± 0.41	0.85a ± 0.02	0.42a ± 0.02	2.37a ± 0.02	1.07c ± 0.04	0.30a ± 0.05
onion	7.05c ± 0.23	0.87a ± 0.01	0.41a ± 0.03	2.35a ± 0.03	1.08c ± 0.06	0.29a ± 0.04
AF + garlic	7.44c ± 0.43	1.21c ± 0.14	0.41a ± 0.05	2.33a ± 0.07	1.11c ± 0.03	0.31a ± 0.01
AF + cabbage	7.03a ± 0.25	1.26c ± 0.18	0.39a ± 0.04	2.36a ± 0.08	1.23c ± 0.04	0.30a ± 0.01
AF + onion	6.82a ± 0.13	1.38c ± 0.21	0.37a ± 0.01	2.28a ± 0.07	1.41d ± 0.08	0.28a ± 0.05

[a] Within each column, means with the same letter are not significantly different ($P \leq 0.5$).

and kidney, whereas GSH and SOD activities in both tissues were comparable to the controls.

DISCUSSION

Several epidemiological studies indicated that AFB_1 intake is associated with a high incidence of primary liver cancer in man in Africa and Asia (*40*). Many reports have shown that AF content in some African and Asian foods is >10-fold the recommended maximum level (*41*). In view of the fact that this metabolite can induce several toxic effects in the human body (*42*), it is important to find a method of detoxification, because people consume this contaminant daily in some areas. Nutrition is likely to be important in this respect.

In the present study, we evaluated the protection role of garlic, cabbage, and onion extracts against the toxic effects of aflatoxin. The selected doses of AF and extracts were based on our previous work (*2*, *25*). Aflatoxin alone significantly decreased the feed intake of the treated animals. Animals treated with extracts (i.e., garlic, cabbage, or onion) alone or in combination with aflatoxin were comparable to those of the control with

regard to their feed consumption. The liver is considered to be the principal target organ for aflatoxin. The activity of ALT and AST are sensitive indicators of acute hepatic necrosis, and the AP level is known to be indicative of hepatobiliary disease (*43*). In the present study, AF-contaminated feed was found to cause a significant increase in ALT, AST, AP, cholesterol, TB, LDH, CK, urea, and createnine levels. Generally, these results may indicate degenerative changes and hypofunction of liver and kidneys (*43*). The results also showed that AF-contaminated diet significantly decreased serum levels of triglycerides, TP, and albumin. The increased levels of blood urea and creatinine with decreased levels of blood protein may indicate protein catabolism and/or kidney dysfunction (*2*). Moreover, the increased level of LDH and CK in the AF-treated group is an indication of AF-induced myocardial infraction and cardiac injury (*44*). These results clearly showed that aflatoxin has a harmful and stressful influence on the hepatic, renal, and cardiac tissue consistent with those reported in the literature of aflatoxicosis (*45*).

Treatment with garlic alone resulted in a significant decrease in cholesterol, whereas the other biochemical parameters were comparable to the control values. Biochemical parameters of animals treated with cabbage were comparable to the control levels; meanwhile, onion extract increased the serum level of LDH and urea. Combined treatment of aflatoxin with extracts resulted in a significant improvement in different serum biochemical parameters. Treatment with extracts succeeded in reducing the elevation of these parameters resulting from AF, although it failed to normalize them. It is of interest to mention that low cholesterol levels in the animals treated with extracts alone or in combination with AF suggest that these extracts may have antiatherosclerotic properties and may protect against the development of coronary diseases (*46*).

Aflatoxin treatment resulted in enhancement of lipid peroxidation in rats, which is directly related to free radicl mediated toxicity. The targets of oxidative damage are usually critical biomolecules such as nucleic acids, proteins, and lipids (*47*). In the present study, AF alone decreased GSH in the liver, whereas extracts alone or in combination with AF resulted in a significant increase in GSH. Aflatoxin B_1 is metabolized by the cellular cytochrome p450 enzyme system to form the reactive intermediate, aflatoxin B_1-8,9-epoxide, which in turn reacts with macromolecules such as lipid and DNA, leading to lipid peroxidation and cellular injury (*48*). The AF-induced alteration in hepatic antioxidant status may therefore be a manifestation of increased tissue oxidative stress caused by AF metabolism (*49*). The decrease in GSH activity in liver tissue of rats treated with AF may be due to the conjugation of GSH with aflatoxin-epoxide. Because the detoxification of AF can be mediated by GSH *S*-transferase-catalyzed conjugation with GSH in the liver (*50*), the increased hepatic GSH activity induced by plant extracts can therefore reduce the acute AF hepatotoxicity. In this regard, GSH *S*-transferase has been shown to play a critical role in preventing the binding of aflatoxin-epoxide to DNA in species resistant to AF toxicity (*51*). In addition, the increase in hepatic GSH activity, as shown in rats treated with plant extracts (**Table 3**), may be facilitated by the regeneration of GSH from its oxidized form. This postulation is consistent with the increase in hepatic GSH level in rats treated with plant extracts with or without AF challenge. It is of interest to mention that GSH in kidney tissue was increased in rats treated with AF. These findings may be explained by the fact that the liver is the target organ for AF, so that liver was affected more than kidneys. Generally, the organosulfur compounds in garlic, cabbage, or onion extracts enhance the defense mechanisms against AF and supported the earlier findings that organosulfur compounds enhanced the protection of liver against aflatoxin B_1 (*49*, *52*).

Reactive oxygen species (ROS), which induced cellular damage, were estimated by monitoring the lipid peroxidation (LPO), which is a well-known indicator of cellular damage by oxidative stress. In the present study, MDA in the liver and kidney showed a significant increase in AF-treated rats, whereas plant extracts resulted in a slight increase in the MDA concentration except for the cabbage extract, which induced a slight decrease in MDA concentration in the liver. These results supported the antioxidant properties of organosulfur compounds in plant extracts and their ability to scavenge free radical intermediates of lipid peroxidation (*53*).

Superoxide dismutase activity is an indicator of ROS production. SOD activities in the liver and kidney of rats treated with AF were decreased significantly ($P \leq 0.05$). Both tissues showed a significant improvement in SOD activity in the animals treated with extracts alone or plus AF. The decrease in SOD in the liver and kidney of AF-treated animals might indirectly lead to an increase in oxidative DNA damage (*54*). The addition of extracts to AF resulted in a significant improvement in SOD activity toward the control value in both hepatic and renal tissues. These results led to the conclusion that organosulfur compounds affect AF metabolism and epoxide−DNA binding by inhibiting phase 1 enzymes and can therefore be considered as potential chemopreventive agents (*52*). The organosulfur compounds in the extracts tested could scavenge peroxyl radicals and hydroxyl radicals and were able to react with hypochlorous acid at a rate sufficient to protect catalase and α-1-antiproteinase to facilitate hydrogen radical generation from H_2O_2 (*55*) and also inhibited oxidative modification of LDL (*56*).

In conclusion, the results of the present study have indicated that aflatoxin induced adverse effects in rats. It caused severe biochemical changes in the liver and kidney as well as harmful effects on cardiac tissues. Garlic, cabbage, and onion have a protective effect against the toxicity of AF. The protective role of these extracts may be due to their rich content of organosulfur compounds, which act as a precursor of GSH, which conjugates with aflatoxin-epoxide and results in the inhibition of epoxide binding to DNA. This conjugation between the reactive epoxide and GSH is considered to be the important detoxification mechanism of AF. Moreover, organosulfer compounds are the main antioxidant and free radical scavenger; they also play a role in increasing the antioxidant status and lowering the oxidative damage of nucleic acid in the body.

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