Anti-cytotoxic strategies: The individual role of vitamin C and synergistic role of glutathione in improving biomarkers of liver and kidney functions and reducing AFB1-induced genetic damage in a rabbit.

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Abstract

Aflatoxin B1 (AFB1) is characterized by ability to cause liver toxicity, Demonstrated in the Occurrence of multiple pathological Impacts in humans and animals, Spanning from growth inhibition And Stunting, to negative impact on the Efficacy of the immune system, and Compromised Procreative capacity. Therefore, it is essential to implement Stringent Tracking programs for food and Feed Elements, along with the development of effective therapeutic and preventive Protocols, to assure Reduced exposure levels and mitigation of the toxic impacts of AFB1. The toxic effects of AFB1 have Been studied by assessed its biological effects and its impact on the liver cellular structures and kidney Of rabbits treated with it, The effect of vitamin C and glutathione and their role in promoting the fight Against AFB1 toxicosis and their synergistic effect with each other. The results showed the harmful Impact of AFB1 on rabbits, as exposure to AFB1 led to an increase in the activity of the enzymes Aspartate transaminase (AST) and Alanine transaminase (ALT), and led to a decrease in total serum Protein levels (TP). AFB1 also increased the production of reactive oxygen species (ROS) and Malondialdehyde (MDA), while inhibiting the activity of Glutathione (GSH), Glutathione peroxidase (GSH-PX), Superoxide dismutase (SOD), and Catalase (CAT) in liver tissues.

Keywords: AFB1, Glutathione, Vitamin C, Oxidative Stress, ROS, Antioxidant Defense.

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Introduction

Mycotoxins are secondary metabolites that can be produced by filamentous fungi such as Aspergillus, Alternaria, and Penicillium during the harvesting, processing, storage, and/or handling of spices. [1] They are produced by fungi and have been shown to have a negative impact on consumer health by exhibiting mutagenic, carcinogenic, nephrotoxic, neurotoxic, and teratogenic activities. [2] Therefore, it is essential to understand the mechanisms of action of these toxins and develop strategies to mitigate them, in order to ensure consumer safety and reduce economic losses. [3, 4] Aflatoxins are mycotoxins typically produced by Aspergillus and Penicillium fungi, Aflatoxins occur naturally and are characterized by their heat resistance, which has been linked to many harmful effects on human and animal health, such as immunosuppression, genetic mutations, cancer, and malformations. Aflatoxins are considered among the most dangerous mycotoxins [5] and their types are Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) , and Aflatoxin G2 (AFG2) [6]. AFB1 is the most prevalent toxin, representing 75% of the aflatoxins produced in food products contaminated with fungi [1]. Its harmful effects on humans range from liver necrosis and bleeding to neurological disorders, and in extreme cases, death [3], The International Agency for Research on Cancer (IARC) classifies it as a Group 1 carcinogen [7], In the liver, AFB1 undergoes bioactivation by cytochrome P 450 (CYP 450) enzymes, leading to the formation of AFB1 exo-8,9-epoxide (AFBO) [8] This epoxide (AFBO), when subsequently bound, forms addition products with DNA, leading to cytotoxicity, mutations, and DNA damage.[9], There are types of mineral and organic adsorbents that can partially or completely mitigate the harmful effects of dietary AFB1.[10], However, these absorbent materials may also retain essential nutrients such as minerals and vitamins, and biodegradation, which is characterized by the microbial enzymatic conversion of AFB1 into less harmful metabolites, is considered a promising approach due to its high specificity, efficiency, and environmental sustainability [11].

Materials and Methods

Chemicals and Reagents: AFB1 with (a purity of $\geq$ 98%) and Glutathione was obtained from (Macklin, China); Vitamin C from (Reagent World, USA).

Experimental Animals and Housing: White rabbits were purchased with an average weight of 1 kg ± 100 g, free from diseases and parasites, and placed in cages, The temperature in ambient was maintained at 25°C throughout the

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45-day feeding experiment, The animals were divided into four groups: 1. the control group (A basic diet only), as; 2. AFB1 at 4 µg/kg of body weight and A basic diet group 3. A basic diet with AFB1 at a concentration of 4 µg/kg of body weight and vitamin C at a concentration of 100 mg/kg of body weight. 4. A basic diet with AFB1 at a concentration of 4 µg/kg of body weight and vitamin C at a concentration of 100 mg/kg of body weight, in addition to glutathione at a concentration of 20 mg/kg of body weight.

Serum Biochemical Analysis

Blood samples were collected and blood count indices such as red blood cells (RBC), white blood cells (WBC), and hemoglobin (Hb) were measured, The blood samples were then centrifuged to collect serum (for 15 minutes at 3000 rpm and 4°C) to measure biochemical parameters, including aspartate aminotransferase (AST) ,alanine aminotransferase (ALT), and alkaline phosphatase (ALP), Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), glutathione (GSH), and catalase (CAT) in serum using ELISA kits according to the manufacturer's instructions (Shanghai Ideal Medical Technology, Shanghai, China).

Statistical Analysis

The data were analyzed using One-Way Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test using SPSS statistical software at a probability level of $P \leq 0.05$. All data were expressed as mean ± standard error.[12]

Results

Impact of Glutathione and Vitamin C on Hematological Parameters

The results showed that exposure to AFB1 led to a decrease in white blood cells, and treatment with glutathione, vitamin C, or both resulted in a significant increase in WBC values ($p < 0.05$). These results are consistent with those found in [13] that AFB1-contaminated diets reduced white blood cell values when consumed alone. The results differed for vitamin C, which did not show a significant difference in the groups exposed to AFB1.

Exposure to AFB1 led to a significant decrease in the level of red blood cells (RBCs) in the serum of laboratory rabbits ($p < 0.01$) compared to the control group, Treatment with glutathione and vitamin C resulted in a significant increase in RBC values ($p < 0.01$), and the synergistic effect between glutathione and vitamin C was

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the best treatment, as its values approached those of the control group, These results were consistent with what (Imbabi et al. 2024) [14] found, that AFB1 caused a decrease in the number of (RBCs) and (WBC) blood cells. And Treatment with AFB1 significantly reduced the percentage of hemoglobin in the blood serum of laboratory rabbits ($p < 0.01$) compared to the control group, The synergistic Treatment of glutathione and vitamin C showed a significantly high protective response ($p < 0.01$) in protecting blood hemoglobin and preventing the breakdown of red blood cells compared to treatment with glutathione or vitamin C alone, This agrees with (Nabi et al. 2022) [15] that AFB1 causes the breakdown of red blood cells and reduces the level of hemoglobin. Platelet values were also significantly reduced due to AFB1 ($p < 0.05$), and glutathione treatment was superior to the synergistic effect or vitamin C alone in raising platelet values, The study agreed with what (Uluışık et al. 2020) [16] found, that exposure to AFB1 causes a decrease in platelet values.

Table (1): Effect of Glutathione (GSH) and Vitamin C treatment on hematological parameters in rabbits exposed to AFB1.

Treatments	WBC X 109/L		R B C X 109/L		H G B %		P L T X 109/L	
Control	6.130 ±0.280	bc	5.830 ±0.277	a	12.233 a±0.115	a	616.00 a±4.58	
AfB1 (4 µg/day)	5.127 ±0.146	c	4.403 ±0.150	c	9.933 c±0.586	c	436.30 23.7	c±
AfB1 + GLU(4 µg/day+20 mg)	7.440 ±2.040	b	5.467 ±0.222	b	11.900 ab±0.608	ab	594.70 58.0	a±
AfB1 + VIT C(4 µg/day+100 mg)	7.693 ±0.635	b	5.537 ±0.397	b	11.967 ab±0.231	ab	405.70 24.8	c±
AfB1 + VIT C + GLU(4 µg/day+100 mg+20 mg)	7.010 ±1.578	b	5.603 ±0.486	ab	11.667 b±1.206	b	556.00 ±29.1	b

* Values are expressed as Mean ± Standard Deviation (Mean ± SD).

** Different superscript letters within the same column indicate significant differences at the $p < 0.05$ level.

Effect of Glutathione and Vitamin C on Liver Function Biomarkers

The results showed that exposure to AFB1 significantly increased the serum

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activity of Aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in laboratory rabbits ($p < 0.01$) compared to control groups, However, treatment with vitamin C and glutathione or both in the AFB1-exposed groups significantly reduced Aspartate transaminase (AST) activity ($p < 0.01$), Vitamin C treatment was recorded as the best treatment that contributed to reducing Alanine transaminase (ALT) activity, followed by treatment with glutathione and vitamin C synergistically, then glutathione alone, In the case of alkaline phosphatase (ALP), the synergistic case of glutathione and vitamin C together represented the best treatment case, followed by glutathione alone, then vitamin C alone, significantly ($p < 0.01$), The study was consistent with the findings of [17] that exposure to AFB1 led to an increase in ALT, Aspartate transaminase (AST), and alkaline phosphatase (ALP) compared to the control group, The results of vitamin C treatment were also consistent with a study (Saad-Hussein et al. 2019) [18] showing that vitamin C significantly reduced the activity of the liver enzymes Aspartate transaminase (AST) and alkaline phosphatase (ALP) in workers exposed to AFB1 after taking antioxidant supplements, The reason is attributed to the ability of antioxidants, including vitamin C, to reduce Aspartate transaminase (AST) and alkaline phosphatase (ALP) levels in the blood due to their protective role against cell necrosis [19]. The results of glutathione treatment were consistent with what was found (Qota et al. 2009) [20]. The use of glutathione in a diet containing AFB1 showed a significant protective effect (20-28%) for the Aspartate transaminase (AST) and Alanine transaminase (ALT) enzymes. The results showed no significant differences in total protein compared to the control group, and the study was consistent with the findings of (Abaskhroun et al. 2010)[21] that there was no significant change in total protein levels in the groups exposed to AFB1.

Table (2): Impact of Glutathione (GSH) and Vitamin C on liver function biomarkers in rabbits exposed to AFB1.

Treatments	AST IU/ml	ALT IU/ml	ALP IU/ml	Total protein mg/dl
Control	10.00 c ± 0.00	26.33 f ± 0.58	65.67 a ± 8.39	6.633 a ± 0.057
AfB1 (4 µg/day)	68.67 a ±3.06	96.33 a ± 4.16	71.33 a ± 2.52	6.000 a ± 0.100
AfB1 + GLU(4	24.00 b ±4.36	72.30 b ±9.81	49.67 c ±	6.367 a ±

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µg/day+20 mg)		9.22	0.473
AfB1 + VIT C(4 µg/day+100 mg)	10.33 c ± 2.31	44.33 e ±3.06	57.00 b ± 8.20	6.367 a ± 0.306
AfB1 + VIT C + GLU(4 µg/day+100 mg+20 mg)	15.33 c ± 3.71	57.00 d± 3.61	48.33 c ± 9.45	6.533 a ± 0.611

* Values are expressed as Mean ± Standard Deviation (Mean ± SD).

** Different superscript letters within the same column indicate significant differences at the $p < 0.05$ level.

Impact of Glutathione and Vitamin C on Antioxidant Enzyme Activities and Lipid Peroxidation

The results showed that AFB1 significantly increased MDA values ($p < 0.05$) compared to the control group, Treatment with the synergistic effect of glutathione and vitamin C was the most effective in reducing MDA values, followed by vitamin C alone, and then glutathione alone, Exposure to AFB1 also significantly reduced SOD, GSH, GSH-PX, and CAT values ($p < 0.05$) compared to the control group, Treatment with glutathione alone was significantly superior ($p < 0.05$) to treatment with glutathione synergistically with vitamin C and treatment with vitamin C alone. Treatment with glutathione and vitamin C synergistically recorded the highest value, followed by vitamin C alone, and then glutathione alone ($p < 0.01$), Treatment with glutathione alone and with glutathione synergistically with vitamin C significantly increased GSH-PX values ($p < 0.01$), followed by vitamin C, As for CAT, all treatments had the same level of effect, and the CAT values significantly increased ($p < 0.01$) compared to those in the AFB1 group.

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Table (3): Influence of Glutathione (GSH) and Vitamin C on antioxidant enzyme activities in rabbits challenged with AFB1.

Treatments	M D A µmol/L	S O D µmol/L	G S H µmol/L	G S H - P X µmol/L	C A T µmol/L
Control	21.05 c ± 2.57	16.007 a± 1.234	212.0 a ± 24.1	0.4040 b ± 0.0541	65.71 a ± 1.99
AfB1 (4 µg/day)	33.52 a ± 4.65	12.803 c ± 0.267	114.1 d ± 18.0	0.2930 c ± 0.0467	53.29 c ± 2.31
AfB1 + GLU(4 µg/day+20 mg)	32.84 a ± 3.51	14.760 b ± 2.171	162.8 c ± 21.0	0.6540 a ± 0.1100	62.78 b ± 2.79
AfB1 + VIT C(4 µg/day+100 mg)	29.68 ab ± 7.76	12.303 c ± 0.795	182.5 b ± 28.1	0.3503 b ± 0.1193	61.32 b ± 1.07
AfB1 + VIT C + GLU(4 µg/day+100 mg+20 mg)	25.71 bc ± 6.70	14.287 b ± 0.701	223.4 a ± 25.2	0.6207 a ± 0.1718	61.67 b ± 4.54

* Values are expressed as Mean ± Standard Deviation (Mean ± SD).

** Different superscript letters within the same column indicate significant differences at the $p < 0.05$ level.

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Discussion

AFB1 contamination poses a serious toxic threat and leads to decreased body weight, reduced feed intake, and liver damage [22]. Therefore, developing and implementing effective AFB1 detoxification strategies is crucial, Many ways, including chemical, physical, and biological techniques, exist to relieve the harmful effects of AFB1, with biological methods emerging as particularly promising, Since it offers the possibility of treatment without leaving negative side effects, the liver is the main target of AFB1's pathological effects because it is the site of its metabolism. Histological examination revealed an increase in liver morphological changes and structural disturbance in rabbits fed a diet containing AFB1, and in line with the analysis of histopathological images, The groups exposed to AFB1 showed an increase in serum liver enzyme activities Alanine transaminase (ALT) , Aspartate transaminase (AST), and alkaline phosphatase (ALP) higher than the control group, The results show that AFB1 may have caused significant liver damage, Vitamin C, alone or in synergy with glutathione, works to relieve the harmful effects of AFB1 and reduce liver damage, It has the ability to restore tissues to normal and control the activity of liver enzymes in the serum, Thus, dietary antioxidant intake shows a protective effect against liver abnormalities resulting from exposure to AFB1, Adding Vitamin C to the diet containing AFB1 led to an improvement in negative indicators and tissue abnormalities, Because of the ability of vitamin C to activate the phagocytosis process, which enhances the function of phagocytic cells and thus eliminates pollutants [13], The defense mechanism of glutathione is shown through its binding to AFB1 metabolites, thus contributing to reducing their toxicity by preventing them from interacting and binding to large cellular components [23], Exposure to AFB1 leads to a decrease in red and white blood cells, This effect is attributed to the damage that AFB1 can cause to liver tissue, which in turn leads to weakness in blood-forming tissues [24], The increase in liver MDA levels along with the decrease in antioxidant enzymes GSH-Px, GSH, SOD and CAT, It indicates that oxidative stress may be the initial trigger for the exacerbation of AFB1-induced liver damage [25], Liver damage occurs due to a decrease in the antioxidant enzymes Superoxide dismutase SOD, Glutathione peroxidase GSH-Px and CAT, which are characterized by their ability to eliminate free radicals and peroxides [26].

The results indicate that vitamin C has a strong ability to break down AFB1 within the digestive tract of rabbits, thereby reducing the concentration of AFB1 absorbed by intestinal cells and keeping the liver in a stable state, which is reflected in normal liver functions [27].

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Conclusions

The study demonstrated the protective and therapeutic role of both GSH and Vitamin C in reducing the toxic effects of AFB1 in rabbits, as the antioxidants used proved to have a high ability to neutralize free radicals resulting from metabolism of AFB1, leading to a reduction in (MDA) levels and an increase in the efficiency of the enzyme defense system and restoring oxidative balance within the body's cells, and protecting the liver through maintaining the integrity of the liver cell membrane, which was evident in the control of liver enzymes: Alanine transaminase (ALT), Aspartate transaminase (AST), alkaline phosphatase (ALP) and their return to their normal ranges, indicating the ability of these substances to repair tissue damage caused by AFB1, It improves blood parameters by protecting red blood cells from oxidative degeneration, and saving hemoglobin levels and other blood parameters within normal physiological limits.

Summary

In summary The results indicate that AFB1 exerts deleterious impacts on hepatic tissues and systemic physiological functions, However, antioxidant supplementation proved to be highly effective in attenuating the negative consequences of AFB1 on liver function markers, hematological profiles, and oxidative stress indicators, The Synergy of Glutathione and Vitamin C as dietary additives or therapeutic agents represents a strategic and safe approach for controlling poisoning by AFB1.

Ethical Approval

The experimental protocols and animal handling procedures in this study were reviewed and formally approved by the Research Ethics Committee of the College of Agriculture, University of Tikrit (Approval No. AgTUA-022-2025). All procedures were conducted in strict accordance with international ethical standards for the protection and welfare of laboratory animals used in scientific research.

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