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Antagonistic role of barley against bioaccumulation and oxidative stress of aflatoxin B1 in male rats

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Abstract

Background: This study aims to evaluate the protective effect of barley against the bioaccumulation and oxidative stress of aflatoxin B1 (AFB1) in male rats. The lethality percentile doses (LDs: LD1 to LD99 at 24, 48, 72, and 96 h) were measured. To achieve these goals during subacute treatments, one hundred rats were divided into five groups, each with twenty rats. The groups I, II, III, IV, and V throughout 21 days were daily given drinking water, DMSO, 2.0 g of barley/kg, and 7.49 mg/kg of AFB1 alone or in combination with 2.0 g of barley/kg, respectively.

Results: The results revealed that AFB1 was detected only in the liver, kidney, and serum of groups IV, in which the accumulated AFB1 exhibited a significant direct relationship with the experimental periods with a marked positive correlation coefficient. Additionally, the concentrations of AFB1 residue in the serum of rats given AFB1 alone exhibited a significant inverse relationship with the levels of GSH, activity of CAT, SOD, and GR, whereas the levels of MDA showed a significant positive relationship. In the serum of rats given AFB1 plus barley, all parameters were mostly recovered and didn't correlate with either the experimental periods or AFB1 in the serum.

Conclusions: The present data concluded that barley accelerated the biotransformation of AFB1 to a hydrophilic metabolite that is easily eliminated outside the body, leading to the recovery of all studied parameters to normal levels.

Keywords: Aflatoxin B_1, Antioxidants, Barley, Bioaccumulation, Oxidative stress, Toxicity

Background

Aflatoxins' (AFs) history started in the 6th decade of the twentieth century. The AFs were recognized as the main reason for several diseases, such as epizootic hepatitis in the U.S. and the turkey "X" in Britain (Wogan et al., 2012). These cases of illness were recently identified as aflatoxicosis and were reported in various mammalian and bird species with the same syndrome (Coppock et al., 2018). In animal models, the AFs are potent hepatocarcinogens, and they have the potential to be carcinogenic in humans. Aflatoxin B1 (AFB1) is the most important aflatoxin in terms of harmful potency and occurrence (Dohnal et al., 2014).

According to the International Agency for Research on Cancer (IARC) report (Ostry et al., 2017), AFB1 is classified as a group 1 carcinogen for humans. It can cause hemorrhage (Coppock et al., 2018), fibrosis (Zhang et al., 2019), and cirrhosis (Wang et al., 2018) in living organism. It is the main reason for hepatocarcinoma, with mutagenic, teratogenic, and carcinogenic impacts (Peltonen et al., 2000). Moreover, a relationship was reported regarding aflatoxin linkage with hepatitis viruses (Ramalho et al., 2018). Many strategies have been developed to reduce the effects of

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AFB1 on tissues, including the use of bioactive components derived from natural resources (Loi et al., 2020a, 2020b).

Barley is one of the cereal plants that is utilized in traditional medicine (Lim et al., 2019). It is rich in bioactive components like B-glucan and an assortment of phytochemical components (Malik, 2012), phenolic acids, lignin, cellulose, arabinoxylans, polysaccharides, hemicelluloses, and flavonoids (Tang et al., 2016). Clinical studies show that the bioactivity of cereal-flavonoids is responsible for the prevention of a variety of diseases, including cancer (Gani et al., 2012). Lignans have been suggested to induce biological effects, including antioxidants and antitumors (Rhee, 2016). Dietary factors, which reduce reactive oxygen species (ROS) impact, can protect against DNA damage and stimulate the immune system, thus lowering cancer risk (Nisha & Deshwal, 2011).

Furthermore, through boosting glutathione (GSH) synthesis, barley extract has been shown to protect liver cells from oxidative stress (Lee et al., 2017). In contrast, AFB1 is known to cause oxidative stress in tissues (Zhang et al., 2019). This recommends barley as a controlling factor or a regulator against the AFB1 toxicity. The effect of barley fiber and glucan on cancer prevention has been extensively studied (Shen et al., 2016). The tumor parameters were prevented by barley fibers more effectively than other cereal fibers (Kubatka et al., 2016).

To our knowledge, the full lethality percentile doses of AFB1 in rats had never been estimated, and this encouraged us to estimate them. Additionally, the present work aimed to evaluate the potential ameliorative and/or therapeutic role of barley against the bioaccumulation of AFB1 and its oxidative stress in the studied tissues during subacute experimentation.

Methods

Experimental animals

The healthy adult male rats weighing 115 ± 5 g were used as experimental animals. Rats were purchased from the National Research Centre (NRC), Cairo, Egypt. Animals were acclimatized to laboratory conditions for two weeks prior to the experiments at a temperature of 23 ± 1 °C, a relative humidity of 21%, and a light–dark cycle of 12 h per day. Animals have been fed ad libitum food and have had free access to drinking water. The food debris and feces were removed daily to keep the sawdust dry throughout the course of the experiments. Animals received human care in compliance with the guideline principles of living laboratory animals in scientific research (Sikes, 2016) and approved by the Ethical Committee of the National Research Centre, Cairo, Egypt (Registration No. 15-207).

Chemicals

Dimethyl sulfoxide (DMSO), a solvent of aflatoxin B1 (AFB1), was purchased from Sisco Research Laboratories (SRL) in Mumbai, India. The aflatoxigenic strain Aspergillus flavus ITEM 698 that is used for the production of AFB1 was obtained from the Toxicology and Food Contaminants Department, National Research Centre, Dokki, 12622 Cairo, Egypt. The AFB1 standard that was used to approve and estimate the amount of AFB1 production from the aflatoxigenic strain was purchased from Sigma-Aldrich in the United States.

Preparation of barely

Egyptian barley (*Hordeum vulgare L.*) was obtained from herbarium shops in Cairo, Egypt. Barley grains were ground until they became like powder, and because barley powder is hydrophobic, it was given as a suspended barley powder after mixing with distilled water. The dose of suspended barley used in the present experiment work for acute and subacute experimentation was equivalent to 2.0 grams per kilogram of body weight (2.0 g/kg b. wt.). This dose was chosen because our preliminary experiments indicated that the median lethal dose (LD_{50}) at 24 h of barley suspension was greater than 5.0 g/kg b. wt. and, consequently, the maximum dose of the watery barley was equivalent to 2 g/kg b. wt. that applied according to Megawati et al. (2022).

Preparation of aflatoxin B_1

In order to increase the sporulation of *Asperagellus flavus* (A. flavus), the fungus was cultivated on Potato Dextrose Agar (PDA) slants at 28 °C and then the spores were inoculated into 50 ml liquid cultures of Yeast Extract Sucrose (YES) media containing 2% yeast extract and 20% sucrose to facilitate, accelerate, and increase the production of aflatoxins (Koehler et al., 1975). Two weeks later, aflatoxin was extracted by adding 50 ml of chloroform and shaking it for 15 min in a shaker. After the separation in a 250 ml separatory funnel, the lower layer of chloroform was drained through Whatman No. 1 filter paper. This extraction procedure was repeated three times. The combined chloroform extracts were evaporated to a small volume on a rotary vacuum evaporator, quantitatively transferred to a volumetric flask, and adjusted to exactly 10 ml for quantitation by the thin-layer chromatography (TLC) technique. The concentrated extracts were spotted on 0.25 mm TLC plates along with standard aflatoxin solutions of known concentrations and developed in toluene: ethyl-acetate: formic acid (60v: 30v: 10v) as a mobile phase for separation (Kushiro et al., 2017). The produced extract was exposed to UV light for detection. Finally, the spots parallel to the AFB_1 standard have

Table 1 Experimental design for the subacute experimentation

Sources	Experimental groups				
	I	II	III	IV	V
Tap water	√	–	–	–	–
DMSO	–	√	–	–	–
Barley	–	–	2.0 g	–	2.0 g
Aflatoxin	–	–	–	LD_5 at 96 h (≡7.49 mg/kg)	7.49 mg/kg
Sample size (*n*)	20	20	20	20	20
Sampling time	1, 7, 14, and 21 days				

Table 2 Probit parameters that compute the levels of AFB_1 at the lethality periods 24, 48, 72, and 96 h

Parameter	Lethality periods (hours)			
	24 h	48 h	72 h	96 h
Alpha (*α*)	− 4.62 ± 3.86	− 5.23 ± 3.59	− 6.13 ± 3.77	− 5.18 ± 3.60
Beta (*β*)	8.27 ± 3.41	9.39 ± 3.25	10.42 ± 3.46	9.76 ± 3.35
Log LD_{50}	1.16 ± 0.04	1.09 ± 0.03	1.07 ± 0.03	1.04 ± 0.033

Data are represented as a mean ± SEM

α and *β*: constant values, Log LD_{50}: natural logarithm of (LD_{50}) of AB_1

been collected by scratching, solubilized by chloroform, and filtered using filter paper No. 1 to avoid any silica residues, and combined into a clean vial. Assays of AFB_1 have been carried out using the HPLC method (Gell & Carbone, 2019) with the AFB_1 standard.

Estimation of lethality percentiles doses of AFB_1

The lethality percentile doses (LDs: LD_1, …, LD_{50}, …, and LD_{99}) of AFB_1 to male rats at the lethality periods of 24, 48, 72, and 96 h post-oral administration were computed to identify an accurate subacute dose that was applied in the current study as well as to shed light, as novel toxicological data, on the full LDs of AFB_1 at various lethality periods of 24, 48, 72, and 96 h (Table 1).

In order to estimate the LDs of AFB1, thirty rats were divided into six sets, each with five rats. The first set was orally given tap water, whereas the second to sixth sets were given 8.5, 10.7, 12.8, 14.9, and 17.0 mg of AFB1/kg body weight, respectively. During each designated experimental period, the dead rats per each set were counted and recorded throughout the 96 h. The LDs were computed at 24, 48, 72, and 96 h on the basis of probit analysis using the Number Cruncher Statistical System Package Software (NCSS, version 2022). The LDs of AFB1 were expressed as an average of mg/kg ± standard error of mean (SEM) (Table 2).

Experimental design

The current design was constructed to assess the ameliorative and/or therapeutic role of barley against the accumulation of AFB1 in the liver, kidneys, and serum and the oxidative stress in the serum of rats during subacute experimentation.

To achieve this goal,a total sample size of one hundred male rats ($N=100$) was allocated randomly into five groups from I to V, each with twenty rats ($n=20$) as computed by G-power analysis. Animals of the first group were daily given drinking tap water (Group I), whereas groups II to V were daily administered DMSO, barley, and a subacute LD_5 of AFB_1 at 96 h (≡7.49 mg/kg b. wt.) alone or in combination with 2.0 g of barley/kg throughout twenty-one days every other day, respectively. The full design is summarized in Table 1.

Sampling

At the end of the experiments (1, 7, 14, and 21 days), rats' blood was collected in a dry clean tube to obtain serum, and then the rats were dissected to obtain the liver and kidney AFB1 assay.

Aflatoxin assay

The concentrations of AFB1 residue accumulated in the liver, kidney, and serum were analyzed by the High-Performance Liquid Chromatography (HPLC) technique according to a method described by El-Banna & Leistner (1989). The liver and kidney samples of 1.0 g were collected and homogenized by a porcelain mortar and pestle. Absolute chloroform of 5 ml is added to each sample of the homogenates, and also the serum of 1 ml, and separated in a separation funnel. The chloroform phase was filtered through Whatman No. 1 filter paper and concentrated to dryness under nitrogen steam. Now the samples of the liver, kidneys, and serum are ready to estimate the concentrations of AFB1 in them. In order to estimate the concentrations of AFB1 in the samples, 100 µl of trifluoroacetic acid (TFA) was added to the samples and mixed well for 30 s, followed by letting the mixture stand for 15 min. Then, 900 ml of a diluted solution (9 parts water to 1 part acetonitrile (9:1 v/v)) was added and vortexed for 30 s. The solutions of the samples and the standard are ready to be analyzed with the aid of Waters Alliance 2695 High-Performance Liquid Chromatography (HPLC), and a Phenomenex C18 (250 × 4.6 mm i.d.), 5 µm from Waters Corporation (USA) was applied. An isocratic system with water, methanol, and acetonitrile 240:120:40 (Deabes et al., 2011). The separation was performed at ambient temperature at a flow rate of 1.0 mL/min. The injection volume was 20 µl for both standard solutions and sample extracts. The fluorescence detector

was operated at a wavelength of 360 nm for excision and 440 nm for emission. The concentrations of AFB1 were computed as ng/g.

Antioxidants and oxidative stress assay

Malondialdehyde (MDA, nmol/ml), non-enzymatic antioxidant glutathione (GSH, mg/g), enzymatic Antioxidants Catalase (CAT, U/ml), superoxide dismutase (SOD, U/ml), and glutathione reductase (GR, U/ml) in the serum were determined using the enzyme-linked immunosorbent assay (ELISA) kits (Antibodies-online GmbH, Aachen Germany) on BioTek ELx800 ELISA reader. The measurements of MDA, GSH, CAT, SOD, and GR were executed according to the methods described by Shaker et al. (2019), Fan et al. (2018), Mohammed et al. (2018), Sarwar et al. (2021), and Alshabanah et al. (2011), respectively.

Statistical analysis

The G-power analysis was applied to compute the total sample size (N) of rats required for subacute experimentation. The data in this study were normally distributed, according to the Kolmogorov–Smirnov test, allowing for a parametric analysis. The parametric one-way analysis of variance (ANOVA) was applied to analyze the effect of the experimental periods (1, 7, 13, and 21 days) or the treatments (drinking water, DMSO, barley, AFB1 alone or in combination with barley) on the bioaccumulation of AFB1 in the liver, kidneys, and serum, oxidative stress (MDA), non-enzymatic (GSH), and enzymatic antioxidants (CAT, SOD, and GR) in the serum in male rats. The post hoc of Scheffe's test of homogenous subsets was applied to compare each of the desired two variables. Regression analysis and correlation coefficients were used to fit the relationships between the various analyzed variables. The mean ± standard errors of the mean (SEM) were used to represent the data. The current data were analyzed using IBM SPSS Statistics (Statistical Package for the Social Sciences, SPSS version 28).

Results

Lethality percentiles doses of AFB_1

The lethality percentile doses (LDs: LD_1, LD_5,..., LD_{50},..., and LD_{99}) of AFB_1 at 24, 48, 72, and 96 h were estimated to identify the accurate subacute dose of LD_5 at 96 h, which is equivalent to 7.49 mg/kg b. wt. that was applied in the present experiments (Table 3). As shown in Table 2 of the probit analysis, the lethal dose of AFB1 can be computed at any percentage at 24, 48, 72, and 96 h.

Table 3 The lethality percentile doses (LDs, mg/kg b. wt.) of AFB_1 at 24, 48, 72, and 96 h

LDs	24 h	48 h	72 h	96 h
LD1	7.61 ± 1.90	6.95 ± 1.51	7.00 ± 1.37	6.38 ± 1.42
LD_5■	9.20 ± 1.63	8.22 ± 1.35	8.13 ± 1.22	7.49 ± 1.28
LD_{10}	10.18 ± 1.44	8.98 ± 1.23	8.81 ± 1.12	8.16 ± 1.18
LD_{20}	11.51 ± 1.20	10.00 ± 1.07	9.71 ± 0.99	9.05 ± 1.05
LD_{25}	12.06 ± 1.13	10.42 ± 1.01	10.07 ± 0.94	9.42 ± 0.99
LD_{30}	12.57 ± 1.09	10.81 ± 0.97	10.42 ± 0.89	9.76 ± 0.95
LD_{40}	13.56 ± 1.10	11.56 ± 0.90	11.06 ± 0.83	10.40 ± 0.88
LD_{50}	14.55 ± 1.26	12.30 ± 0.89	11.70 ± 0.81	11.04 ± 0.84
LD_{60}	15.61 ± 1.58	13.08 ± 0.96	12.37 ± 0.85	11.72 ± 0.85
LD_{70}	16.83 ± 2.05	13.98 ± 1.13	13.13 ± 0.95	12.50 ± 0.94
LD_{75}	17.55 ± 2.37	14.51 ± 1.27	13.57 ± 1.05	12.95 ± 1.03
LD_{80}	18.39 ± 2.78	15.11 ± 1.46	14.08 ± 1.18	13.47 ± 1.16
LD_{90}	20.78 ± 4.06	16.84 ± 2.09	15.52 ± 1.64	14.94 ± 1.64
LD_{95}	22.99 ± 5.38	18.41 ± 2.77	16.82 ± 2.14	16.28 ± 2.16
LD_{99}	27.79 ± 8.57	21.75 ± 4.41	19.55 ± 3.35	19.12 ± 3.47

Data are represented as mean ± standard error of mean (SEM)

■ The dose of AFB_1 (LD_5 at 96 h) used in the current experiments

Bioaccumulation of AFB1

After the experimental periods of 1, 7, 14, and 21 days, aflatoxin B1 (AFB1) wasn't detected in the liver, kidneys, and serum of rats in groups I, II, III, and V but was observed only in those of group IV (Table 4), i.e., its concentrations were null, and for this reason they weren't included in the table.

The concentrations of AFB_1 in the liver, kidneys, and serum of rats in group IV, as affirmed by one-way ANOVA, were significantly affected by the experimental periods of 1, 7, 14, and 21 days (Table 4). According to the regression analyses and correlation coefficients, the experimental periods exhibited a significant direct power relationship with the levels of AFB_1 accumulated in the liver, kidneys, and serum of rats given AFB_1 alone, and this was associated with a significant positive correlation coefficient of +0.98, +0.97, and +0.99 (Table 4). Therefore, in rats given AFB_1 alone, the accumulation of AFB_1 was significantly elevated with increasing experimental periods.

In rats of group IV, according to the post hoc ANOVA of Scheffe's test of homogenous subsets, the concentrations of AFB1 accumulated by the liver, kidneys, and serum after twenty-one days were significantly higher than those after fourteen days, seven days, and lastly, one day of the experiments.

At all the experimental periods, as revealed by one-way ANOVA, the levels of AFB1 in the liver, kidneys, and serum of rats given AFB1 alone were significantly affected by the type of tissue (Table 4). As shown in this table, at all the experimental periods, the concentrations of AFB1 in the kidneys and serum were similar

Table 4 The levels of aflatoxin B_1 (AFB_1) accumulated in the liver, kidney (ng/g), and serum (ng/ml) of rats given a subacute LD_5 of AFB_1 alone at 96 h (≡ 7.49 mg/kg b. wt., Group IV), every other day, throughout 21 days

Tissues	Experimental periods (in days)				One-way ANOVA	Regression analysis
	1 day	7 days	14 days	21 days		
Liver	0.1068 ± 0.001^{aB}	0.2166 ± 0.008^{bB}	0.6496 ± 0.017^{cB}	1.3168 ± 0.008^{dB}	$F_{3,16(0.01)} = 8\,574$, $P < 0.01$	$y = 0.0654x^{-0.158}$, $r = +0.98$*
Kidneys	0.0041 ± 0.001^{aA}	0.0070 ± 0.0004^{bA}	0.0128 ± 0.0003^{cA}	0.0242 ± 0.0003^{dA}	$F_{3,16(0.01)} = 31$, $P < 0.01$	$y = 0.0009x^{0.9623}$, $r = +0.97$*
Serum	0.00394 ± 0.0001^{aA}	0.0082 ± 0.0003^{bA}	0.0210 ± 0.0006^{cA}	0.0366 ± 0.0007^{dA}	$F_{3,16(0.01)} = 215$, $P < 0.01$	$y = 0.001x^{1.1848}$, $r = +0.99$*
1-way ANOVA	$F_{2,12(0.05)} = 835.5$	$F_{2,12(0.05)} = 1086.0$	$F_{2,12(0.05)} = 8665.8$	$F_{2,12(0.05)} = 30{,}067.8$		
P-value	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$		

Data represented as an average ± SEM

[a, b, c, d]: in the same row (organ), values labeled with the same small letter are similar ($P \geq 0.05$)

[A, B]: in the same period, values labeled with the same capital letter are similar ($P \geq 0.05$)

and didn't differ, but at the same time, both of them were lower than those accumulated by the liver.

Oxidative stress and antioxidants

The levels of malondialdehyde (MDA), as an excellent biomarker of toxicity, glutathione (GSH) as a non-enzymatic antioxidant, as well as the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) as enzymatic antioxidants, were estimated in the serum of rats in groups I, II, III, IV, and V that were given drinking water, DMSO, barley, and aflatoxin B1 (AFB1) alone or in combination with barley, respectively.

As shown in Tables 5 and 6, the experimental periods (1, 7, 14, and 21 days) didn't affect the concentrations of MDA, GSH, and the activity of CAT, SOD, and GR in the serum of groups I, II, III, and V, whereas those of group IV were significantly influenced. This means that the levels of these parameters in the serum of rats in

Table 5 The levels of malondialdehyde (MDA, nmol/ml) and glutathione (GSH, mg/ml) in serum of rats in groups I to V, after 1, 7, 14 and 21 days of administration

Experimental groups	Experimental periods (in days)				One-way ANOVA	Regression analysis	*r*
	1 day	7 days	14 days	21 days			
MDA							
Group I	2.62 ± 0.037^{aB}	2.64 ± 0.037^{aA}	2.60 ± 0.031^{aA}	2.63 ± 0.031^{aA}	$F_{3,16(0.05)} = 0.065$, $P \geq 0.05$	NR	NC
Group II	2.32 ± 0.037^{aA}	2.44 ± 0.037^{aA}	2.40 ± 0.044^{aA}	2.43 ± 0.037^{aA}	$F_{3,16(0.05)} = 1.639$, $P \geq 0.05$	NR	NC
Group III	2.48 ± 0.037^{aAB}	2.48 ± 0.037^{aA}	2.52 ± 0.037^{aA}	2.52 ± 0.037^{aA}	$F_{3,16(0.05)} = 0.381$, $P \geq 0.05$	NR	NC
Group IV	3.28 ± 0.037^{aC}	3.56 ± 0.051^{aB}	3.9 ± 0.055^{bB}	4.38 ± 0.107^{cB}	$F_{3,16(0.05)} = 50.5$, $P < 0.05$	$y = 0.055x + 3.20$	+0.99*
Group V	2.65 ± 0.068^{aB}	2.49 ± 0.051^{aA}	2.54 ± 0.051^{aA}	2.69 ± 0.037^{aA}	$F_{3,16(0.05)} = 2.219$, $P \geq 0.05$	NR	NC
One-way ANOVA	$F_{4.20(0.05)} = 73.3$	$F_{4.20(0.05)} = 121.2$	$F_{4.20(0.05)} = 138.7$	$F_{4.20(0.05)} = 136.7$	–	–	–
P-value	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$			
GSH							
Group I	27.2 ± 0.37^{aBC}	27.8 ± 0.37^{aB}	28.2 ± 0.47^{aB}	28.4 ± 0.51^{aB}	$F_{3,16(0.05)} = 1.65$, $P \geq 0.05$	NR	NC
Group II	28.4 ± 0.51^{aC}	28.2 ± 0.55^{aB}	27.8 ± 0.27^{aB}	27.8 ± 0.37^{aB}	$F_{3,16(0.05)} = 0.38$, $P \geq 0.05$	NR	NC
Group III	27.8 ± 0.37^{aC}	28.8 ± 0.37^{aB}	28.6 ± 0.32^{aB}	28.0 ± 0.32^{aB}	$F_{3,16(0.05)} = 1.42$, $P \geq 0.05$	NR	NC
Group IV	23.4 ± 0.37^{dA}	20.6 ± 0.51^{cA}	17.8 ± 0.37^{bA}	15.4 ± 0.51^{aA}	$F_{3,16(0.05)} = 66.6$, $P < 0.05$	$y = -0.399x + 23.59$	−0.99*
Group V	25.8 ± 0.37^{aB}	26.2 ± 0.37^{aB}	27.4 ± 0.58^{aB}	26.4 ± 0.58^{aB}	$F_{3,16(0.05)} = 1.32$, $P \geq 0.05$	NR	NC
One-way ANOVA	$F_{4.20(0.05)} = 26.8$	$F_{4.20(0.05)} = 34.2$	$F_{4.20(0.05)} = 111.8$	$F_{4.20(0.05)} = 140.2$	–	–	–
P-value	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$			

Data represented as a mean ± SEM

NR no significant relationship; *NC* no significant correlation coefficient (*r*)

[A, B]: In the same column (Period), averages labeled with the same capital letter are similar (insignificant, $P \geq 0.05$), while others aren't ($P < 0.05$)

[a, b, c, d]: In the same row (Group), averages labeled with the same small letter are similar (insignificant, $P \geq 0.05$), while others aren't ($P < 0.05$)

Table 6 The activity of catalase (CAT, U/ml), superoxide dismutase (SOD, U/ml), and glutathione reductase (GR, U/ml) in serum of rats in groups I to V, after 1, 7, 14 and 21 days of administration

Experimental groups	Experimental periods (in days)				One-way ANOVA	Regression analysis	*r*
	1 day	7 days	14 days	21 days			
CAT							
Group I	2.8 ± 0.032^{aC}	2.88 ± 0.037^{aC}	2.82 ± 0.037^{aC}	2.88 ± 0.037^{aC}	$F_{3,16(0.05)} = 1.31, P \geq 0.05$	NR	NC
Group II	2.82 ± 0.037^{aC}	2.80 ± 0.032^{aC}	2.80 ± 0.032^{aC}	2.80 ± 0.032^{aC}	$F_{3,16(0.05)} = 0.91, P \geq 0.05$	NR	NC
Group III	2.86 ± 0.051^{aC}	2.86 ± 0.051^{aC}	2.92 ± 0.058^{aC}	2.88 ± 0.066^{aC}	$F_{3,16(0.05)} = 0.246, P \geq 0.05$	NR	NC
Group IV	2.28 ± 0.037^{dA}	1.98 ± 0.058^{cA}	1.7 ± 0.071^{bA}	1.38 ± 0.058^{aA}	$F_{3,16(0.05)} = 44.9, P < 0.05$	$y = -0.044x + 2.31$	−0.99*
Group V	2.42 ± 0.058^{aB}	2.47 ± 0.058^{aB}	2.39 ± 0.073^{aB}	2.49 ± 0.037^{aB}	$F_{3,16(0.05)} = 1.733, P \geq 0.05$	NR	NC
One-way ANOVA	$F_{4.20(0.05)} = 45.6$	$F_{4.20(0.05)} = 79.8$	$F_{4.20(0.05)} = 101.2$	$F_{4.20(0.05)} = 191.8$	–	–	–
P-value	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	–	–	–
SOD							
Group I	157.8 ± 0.37^{aBC}	156.0 ± 0.95^{aB}	156.6 ± 1.14^{aB}	157.0 ± 1.18^{aBC}	$F_{3,16(0.05)} = 0.691, P \geq 0.05$	NR	NC
Group II	155.0 ± 1.76^{aB}	157.8 ± 0.37^{aB}	157.8 ± 0.37^{aB}	157.8 ± 0.37^{aBC}	$F_{3,16(0.05)} = 0.091, P \geq 0.05$	NR	NC
Group III	160.4 ± 1.24^{aC}	158.6 ± 1.86^{aB}	157.4 ± 1.39^{aB}	160.6 ± 1.39^{aC}	$F_{3,16(0.05)} = 2.09, P \geq 0.05$	NR	NC
Group IV	141.0 ± 1.05^{dA}	135.6 ± 0.98^{cA}	128.6 ± 0.93^{bA}	119.6 ± 1.21^{aA}	$F_{3,16(0.05)} = 78.2, P < 0.05$	$y = -1.07x + 142.7$	−0.99*
Group V	156.0 ± 0.8^{aBC}	155.6 ± 0.84^{aB}	156.0 ± 0.66^{aB}	155.8 ± 0.80^{aB}	$F_{3,16(0.05)} = 0.056, P \geq 0.05$	NR	NC
One-way ANOVA	$F_{4.20(0.05)} = 54.1$	$F_{4.20(0.05)} = 113.0$	$F_{4.20(0.05)} = 276.1$	$F_{4.20(0.05)} = 421.4$	–	–	–
P-value	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	–	–	–
GR							
Group I	36.0 ± 0.84^{aB}	37.2 ± 1.16^{aB}	38.0 ± 0.95^{aB}	37.6 ± 0.98^{aBC}	$F_{3,16(0.05)} = 0.766, P \geq 0.05$	NR	NC
Group II	38.2 ± 0.86^{aB}	36.0 ± 0.84^{aB}	36.0 ± 0.84^{aB}	36.1 ± 0.84^{aB}	$F_{3,16(0.05)} = 1.704, P \geq 0.05$	NR	NC
Group III	40.0 ± 1.18^{aB}	39.8 ± 1.24^{aB}	40.4 ± 1.12^{aB}	40.4 ± 1.03^{aC}	$F_{3,16(0.05)} = 0.068, P \geq 0.05$	NR	NC
Group IV	31.6 ± 0.93^{cA}	27.0 ± 0.71^{bA}	23.8 ± 0.58^{aA}	21.2 ± 0.58^{aA}	$F_{3,16(0.05)} = 47.3, P < 0.05$	$y = 32.36e^{-0.0215}$	−0.98*
Group V	36.1 ± 0.68^{aB}	38.2 ± 0.84^{aB}	40.0 ± 0.84^{aB}	36.0 ± 0.51^{aB}	$F_{3,16(0.05)} = 0.672, P \geq 0.05$	NR	NC
One-way ANOVA	$F_{4.20(0.05)} = 8.76$	$F_{4.20(0.05)} = 26.2$	$F_{4.20(0.05)} = 50.1$	$F_{4.20(0.05)} = 73.02$	–	–	–
P-value	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	–	–	–

Data represented as a mean ± SEM

NR no significant relationship between experimental period and antioxidant enzyme activity; *NC* no significant correlation coefficient (*r*)

$^{a, b, c, d}$: in the same row (Group), averages labeled with the same small letter are similar (insignificant, $P \geq 0.05$), while others aren't ($P < 0.05$)

$^{A, B}$: in the same column (period), averages labeled with the same capital letter are similar (insignificant, $P \geq 0.05$), while others aren't ($P < 0.05$)

groups I, II, III, and V were similar and didn't differ at all the experimental periods.

According to the regression analysis and correlation coefficient, in the serum of rats in group IV, there was a significant direct linear relationship between the experimental periods and the concentration of MDA, and this relationship was associated with a significant positive correlation coefficient (Table 5). On the contrary, the levels of GSH and the activity of CAT and SOD exhibited a significant inverse linear relationship with the experimental periods, whereas the activity of GR showed a significant inverse exponential relationship, and all of them were accompanied by significant negative correlation coefficients (Table 5 and 6). In the serum of rats in group IV, the levels of MDA and GSH, as well as the activity of CAT, SOD, and GR differed significantly at most of the experimental periods, as affirmed by post hoc Scheffe's test of homogeneous subsets (Tables 5 and 6).

In addition, as shown in Table 5 and 6, the treatments with drinking water (Group I), DMSO (Group II), barley (Group III), and aflatoxin B1 alone (IV) or in combination with barley (Group V) caused a significant effect on

levels of MDA, GSH, and activity of CAT, SOD, and GR in the sera of rats in these groups (Table 5 and 6).

At all the experimental periods, the levels of MDA in the serum of rats in group IV were significantly higher than those of groups I, II, III, and V at the corresponding period (Table 5 and 6). On the other hand, the concentrations of GSH and activities of CAT, SOD, and GR in sera of group IV were significantly decreased when compared with those of groups I, II, III, and V (Table 5 and 6).

Generally, in the serum of rats in group V, the levels of MDA and GSH, the activity of CAT, SOD, and GR were recovered at all the experimental periods of 1, 7, 14, and 21 days and became similar to those of groups I, II, and III but significantly differed from those of group IV (Table 5 and 6).

As shown in Fig. 1, in the sera of rats in group IV, the levels of GSH and the activity of CAT exhibited a significant inverse exponential relationship with the levels of

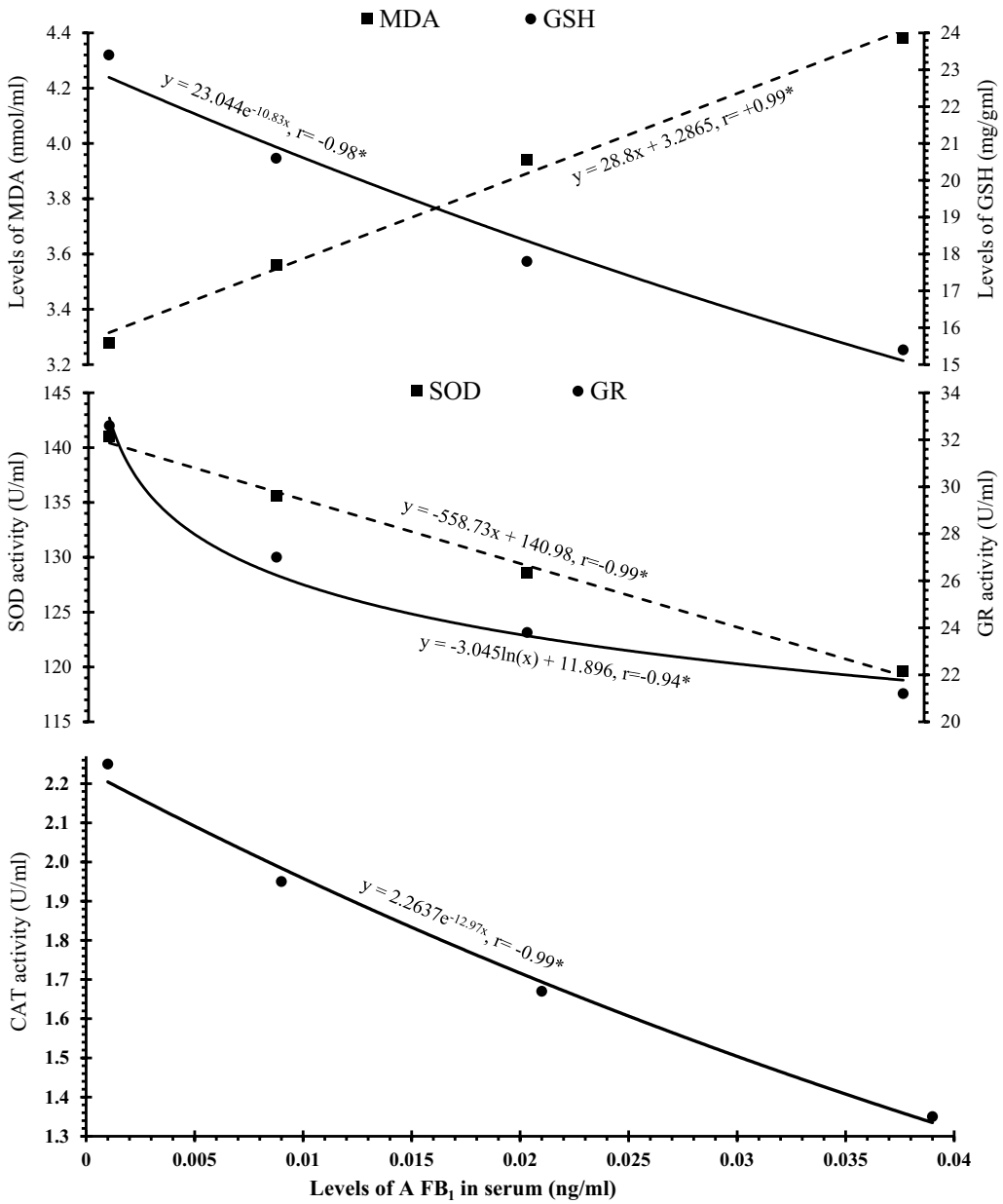

Fig. 1 The relationship between the levels of AFB_1 in the serum (ng/ml) of rats given a subacute dose of 7.49 mg of AFB_1 alone /kg every other day. *x* denotes serum AFB_1 levels, *y* denotes enzymes activity, and *r** denotes a significant correlation

AFB1 residue in serum, whereas the GR activity showed a significant inverse logarithmic relationship and the activity of SOD revealed a significant inverse relationship. On the contrary, the concentrations of AFB1 in the serum of group IV had a direct linear relationship with the levels of MDA throughout the experiments (Fig. 1).

Discussion

The current work is a part of an integrated study on the biological control of barley against the toxicokinetics (transportation, distribution, biotransformation, and excretion) and toxicodynamics (lethality, physiological, biochemical, and genotoxic effects) of AFB1 in male rats. In the present work, the lethality percentile doses of AFB1 (LDs: LD1 to LD99 including the LD50) at 24, 48, 72, and 96 h were estimated to identify the accurate subacute dose (LD5 at 96 h ≡ 7.49 mg/kg) of AFB1 that was applied in this research. As far as the author is aware, there are no published data on the full percentile doses of AFB1 in rats at 24, 48, 72, and 96 h, which gives this study a unique disposition. A literature search revealed an absence of studies focusing on the lethality percentiles dosages of AFB1 (LD1 to LD99 at 24, 48, 72, and 96 h) for mammalian models following oral intake (Pérez-Fernández & de la Escosura-Muñiz, 2022). This is of particular interest because this route of administration mimics the accidental oral intake by people who come into contact with materials, products, drugs, and commodities containing these compounds of aflatoxins within their components (Javed et al., 2022).

The impact of AFB1 interactions with the internal or external mammalian tissues and the possible effect on their accumulation inside them and subsequent toxicity has not been investigated in the breadth and depth they deserve. In rats of group IV, which were given AFB1 alone, the high levels of AFB1 uptake by the hepatic tissue and its high accumulation inside it reflect the vital role of the liver in the processes of trapping, fixation, and detoxification by biotransformation to convert the non-excretory lipophilic AFB1 to a hydrophilic form that is easily eliminatedoutside the body via the urine as a normal pathway for xenobiotic elimination (Chaharaein et al., 2021). Toxicologically, AFB1 is characterized by its lipophilicity (Budin et al., 2021), which facilitates its distribution to and accumulation in various tissues (Loi et al., 2020a, 2020b). In spite of the above-mentioned pathways to eliminate AFB1 from the tissues under accidental conditions, the significant accumulation of AFB1 in tissues in the present data can be attributed to the rate of phase I and II biotransformation of AFB1, which was not adequate to eliminate AFB1 due to the continuous repeated dosages over 21 days, leading to a massive accumulation of this toxin and triggering the abnormal pathological response such as oxidative stress (Shen & Singh, 2021). Therefore, as a response to the bioaccumulation of AFB1, a significant increase in lipid peroxidation as ensure by an excessive accumulation of MDA was recorded in association with a significant reduction of GSH levels and activity of CAT, SOD, and GR in the serum of group IV.

Extensive statistical analyses were applied to disclose the antagonistic biochemical interaction of barley with AFB1 during subacute dosages. This unprecedented study provides an insight into the ameliorative and/or therapeutic role of barley against the accumulation of the natural toxin AFB1 in the liver, kidney, and its residue in serum during subacute periods of experimentation (1, 7, 14, and 21 days). As shown in supplementary data, the results revealed that bioaccumulation of AFB1 in rats of groups I, II, and III was under detection limits and was not affected by the experimental periods (Additional file 1; Table S1). This indicated first that the laboratory environmental conditions were consistent and suitable for the preparations in the experimental protocol and eliminated any possibility of extraneous or superfluous sources of contamination with aflatoxins that might have questioned the integrity and credibility of the present results (Su et al., 2022). Second, oral intake of the drinking water, DMSO, and barley didn't induce a significant change to all of the studied parameters. Thus, in normal cellular metabolism, there is a balance between the generation of free radicals and the reactive oxygen species (ROS), leading to the safety and stability of cellular functions that are associated with normal oxidative damage. ROS are produced as a by-product of normal cellular metabolism, but they can also be produced as a result of xenobiotic exposure (Ray et al., 2012). Excessive liberation of ROS alters the cell organelles, causing a significant cellular dysfunction that induces lipid peroxidation (Mavrommatis et al., 2021). Toxicokinetics of AFB1, especially biotransformation by the liver, is the key that controls the elimination of this toxin outside the body under normal physiological conditions, i.e., the levels of ROS didn't change and were steady.

Toxicologically, the given single low dose of AFB1 is quickly converted to a hydrophilic metabolite that is easily excreted in urine by an effective and strong biotransformation by the cytochrome P450 (Wang et al., 2022). According to Deng et al., (2018), AFB1 was converted to AFB1-8-9-epoxyde by phase I of biotransformation that was catalyzed by cytochrome P450. The intermediate metabolite of AFB1-8-9-epoxyde is quickly conjugated with a sulfhydryl (-SH) group under the control of glutathione-s-transferase (Phase II biotransformation) to produce the hydrophilic AFB1-glutathione that is easily excreted and eliminated via urine. Alternatively,

the generated AFB1-8-9-epoxyde could be converted to AFB1-8,9-dihydrodiol under the control of the microsomal epoxide hydrolase (Phase I biotransformation) that reversibly produces the di-aldehydic phenolate that, in turn, under the control of AFB1 aldehyde reductase (Phase I), transforms into AFB1 di-alcohol (hydrophilic) that is easily via urine (Cao et al., 2022). The oxidative biotransformation of AFB1, by phase I, produces a reactive substrate that is ready for the phase II biotransformation detoxification enzymes. Generally, the main way to eliminate AFB1 is through the conjugation of AFBO with glutathione (GSH) (Marimón Sibaja et al., 2019). This mode of detoxification is the principal pathway of AFB1 elimination and excretion in mammalian species.

In mammals, including humans, cytochrome P450 (CYP) 1A2, CYP3A4, CYP3A5, and CYP3A7 in the liver and CYP2A13 in the lung are essential for the bioactivation of AFB1 to the extremely toxic exo-AFB1-8,9-epoxide (AFBO). In turkeys and ducks, CYP1A1, CYP1A2, CYP2A6, and CYP3A4 are important; in chickens and quail, CYP1A1 and CYP2A6 are important; in mice, CYP3A11 and CYP3A13; and in hamsters, CYP2A5 is important (Khalil et al., 2021). In humans, glutathione-S-transferase (GST) M1 and GSTT1 catalyze the conjugation of GSH to AFBO, whereas GSTM2 in nonhuman primates, GSTA3 in mice, GSTA5 in rats, and GSTA1, GSTA2, GSTA3, and GSTA4 in turkeys are important (Gützkow et al., 2021). Additionally, microsomal epoxide hydrolase (mEH) and aflatoxin-aldehyde reductase (AFAR) have also been shown to play key roles in AFB1 detoxification in humans, rats, and pigs (Javed et al., 2022).

In the present study, AFB1 residue in the serum of rats in group IV exhibited a significant direct linear relationship with the levels of MDA and a significant negative correlation with the concentrations of GSH. This can be attributed to AFB1 accumulated in the liver and kidneys that enhances and increases the formation and liberation of excessive abnormal levels of ROS (anion superoxide ($O_2^{\bullet\bullet}$), hydrogen peroxide (H_2O_2), hydroxy radical ($OH^{\bullet}$), and reactive nitrogen species such as nitric oxide (NO) and peroxynitrite ($ONOO^-$) that enhance and increase lipid peroxidation that causes a significant oxidative damage of tissues (Kodama et al., 1990). The main sites of ROS production in living organisms are the mitochondrial electron transport system, peroxisomal fatty acid, and cytochrome P450 (Ray et al., 2012). It is not clear if AFB1 promotes lipid peroxidation through the direct increase in ROS generation or if the tissue's high susceptibility to peroxidation is the result of the compromised antioxidant defense, but it may be a result of both processes being involved. Lipid peroxidation is started by the attack of any ROS that has a high reactivity to displace a hydrogen atom from a methylene group onto a polyunsaturated fatty acid (Abdulbaqi et al., 2018). The peroxidation of polyunsaturated fatty acids can also occur by a chemical reaction that is catalyzed by cyclooxygenase (Rushing & Selim, 2019). Additionally, the metabolite 8, 9-epoxide enhances and accelerates lipid peroxidation, and this is accompanied by a significant loss of membrane stability and the blockage of the membrane-bound enzyme activity (Xu et al., 2021). AFB1 causes a significant increase in MDA levels in the liver (Naaz et al., 2007) and hepatoma cells (Lee et al., 2005). Additionally, the elevation of lipid peroxide due to AFB1 was shown in the liver (Rastogi et al., 2006), kidney and brain (Madhusudhanan et al., 2004), and integumentary tissue (Rastogi et al., 2007). This disruption was accompanied by a significant increase in the formation of conjugated dienes (Madhusudhanan et al., 2004).

In the present experiments, in the serum of rats in group IV, a significant reduction in the levels of non-enzymatic (glutathione, GSH) and enzymatic (superoxide dismutase, SOD; catalase, CAT; and glutathione reductase, GR) antioxidants was observed after aflatoxin administration. We attribute this reduction to the severe depletion of protein synthesis by the rough endoplasmic reticulum as a direct and/or indirect response to AFB1 accumulated in the liver as observed in the present work. Toxicologically, ROS are highly able to oxidize amino acid side chains and lead to oxidation of amino acid residue side chains, construction of protein–protein cross-linkages, and oxidation of the backbone of proteins, resulting in protein disintegration and leading to the production of abnormal modified proteins that accumulate in cell organelles (Sun et al., 2022). Additionally, the high capacity of AFB1 to generate ROS may induce the ROS-mediated oxidative damage of proteins (Ubagai et al., 2008). In addition, AFB1 had the ability to inhibit and suppress some proteolytic enzymes (Clausen et al., 2002) that control the degradation of proteins, leading to consequent implications in hepatocarcinogenesis (Peng et al., 2007). It has been suggested that several effects of AFB1 can be controlled by its interactions with the proteasome that is responsible for the disintegration of the cellular cytosolic and nuclear proteins. Indeed, AFB1 inhibits the cellular 20S proteasomes, affecting the intracellular resistance against oxidative stress (Amici et al., 2007). The 20S proteasome is the proteolytic mechanism that controls the removal of oxidized proteins (Saeki & Tanaka, 2012), so its inhibition could be attributed to a higher protein carbonyl content observed in cultured hepatoma cell lysates (Amici et al., 2007).

The current results are highly consistent with those reported by several authors. The intraperitoneal administration of AFB1 in rats (2 mg/kg) resulted in a decreased

level of glutathione (GSH) in liver (Nili-Ahmadabadi et al., 2011) and in the culture of hepatic tissue (Alavi et al., 2021). Additionally, the intoxication with AFB1 caused a significant decrease of activity of enzymes involved in the antioxidant defense: succinate dehydrogenase, glucose-6-phosphatase, glutathione peroxidase (GPx) and glutathione reductase (GR), catalase (CAT) superoxide dismutase (SOD) and GST in mice (Adedara et al., 2010) and rats (Rastogi et al., 2001). The decrease of the GSH was accompanied by an increase in the MDA and NO concentrations in the liver and kidney of AFB1-treated chicks (Karaman et al., 2010) or rats (Meki et al., 2004). The AFB1 levels showed a significant direct linear relationship with the levels of MDA and this was associated with a significant positive correlation of +0.99. On the contrary, the levels of GSH and activity of GSPx and GR in the hepatic tissues exhibited significant inverse relationships with the levels of AFB1 accumulated by the liver, and this was associated with a significant negative correlation.

Contrary to our current results, as reported by some authors, the levels of GSH could be elevated as a response to AFB1 administration. (Beers et al., 1992a) reported that the levels of GSH in the kidney were markedly raised after 10 days of intoxication with AFB1. In the same manner, the hepatic GSH content was increased after two and eight hours after a single dose of AFB1, and prolonged to a marked increase after five daily doses of AFB1 (Beers et al., 1992b). Additionally, AFB1 toxicity caused a significant increase in the activity of SOD in Peking ducks exposed to AFB1 (Barraud et al., 2001).

Several studies have been conducted to investigate the beneficial and protective effects of some natural antioxidant extracts in reducing AFB1 toxicity when given before or at the same time (Owumi et al., 2022). In the present data of group V, administration of barley in combination with AFB1 caused the complete disappearance of this toxin from the liver, kidneys, and serum of group V, i.e., the action of barley was focused on the biotransformation of AFB1 in the liver. The fact that AFB1 wasn't detected in the liver, kidneys, and serum of rats in group V could be attributed to the phytochemical components of barley (saponin, tannins, phenols, and coumarins) that play vital bioactivation roles to potentiate and accelerate the phase I and II biotransformation several folds to eliminate AFB1 and, of course, prevent AFB1 accumulation inside the cells (Obadi et al., 2021). Pharmacologically, most of the phytochemical components of barley act as inducers to heavily potentiate and accelerate the key enzymes of Phase I and II to enhance the rate of biotransformation. This, in turn, significantly reduced the half-life time of AFB1 elimination from tissues to outside the body via urine (Choi et al., 2020). The disappearance of AFB1 suggests why the levels of MDA and GSH, as well as the activity of CAT, SOD, and GR, were recovered to their normal levels.

Saponin, as a phytochemical component of barley, is a strong inducer of phase I and II of biotransformation in the hepatic tissues (Alam et al., 2022). This leads to a marked increase in the production of the hepatic lipid droplets that activate, enhance, and accelerate the hepatic biotransformation of AFB1 (Abdel-Latif et al., 2022). In agreement with the present data, the liver of an AFB1-dosed rat did not accumulate any detectable AFB1 traces or its metabolites, and this was associated with a net increase in gene transcripts of the AhR-mediating pathway that may be associated with detoxification of AFB1 (Goodman et al., 2022). Additionally, in vitro, the hepatic lipid droplets can strongly damage and eliminate AFB1 in a short period of time, from one to three days (Choi et al., 2020).

Vegetables and fruits provide a great number of other antioxidant phytochemicals that work against cellular oxidative damage due to their affinity to bind to free radicals and ROS by donating electrons and inhibiting lipooxygenases (Singh et al., 2009; Sirajudeen et al., 2011). Herbal natural extracts from various plants were analyzed and tested to counteract the oxidative stress induced by AFB1 (Bouhlel et al., 2010; Brahmi et al., 2011). The extracts were able to protect cells against the consequences of oxidative stress by inhibiting the formation of intracellular reactive oxygen species, reducing the levels of MDA, decreasing the LPO; increasing the level of glutathione and the PHGPx gene expression (Ravinayagam et al., 2012). Gülçin, (2012) and Zambonin et al., (2012) reported that the phenolic compounds (flavonoids and phenolic acids) are highly able to scavenge ROS and consequently reduce lipid peroxidation through the binding of phenolic compounds to cell membranes (El-Sharaky et al., 2009).

Conclusions

From the current results, we conclude that the barley is an excellent natural agent that has affinity against the AFB1 itself not only against its toxicity. The mechanism of action of barley is based directly on the acceleration the AFB1 biotransformation leading to the elimination of 100% of AFB1 outside the tissues and the body. Consequently the levels of MDA and GSH as well as the activities of CAT, SOD, and GR were recovered to the normal alues. The present findings pave the way in front of the application of barely against AFB1-induced toxicity.

Abbreviations

AFB1: Aflatoxin B1; CAT: Catalase; DMSO: Dimethyl sulfoxide; GSH: Glutathione; LPO: Lipid peroxidation; MDA: Malondialdehyde; PDA: Potato dextrose agar;

ROS: Reactive oxygen species; SOD: Superoxide dismutase; TLC: Thin layer chromatography; YES: Yeast Extract Sucrose.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41936-022-00309-3.

Additional file 1. Table S1. The AFB1 accumulated in the liver, kidney (ng/g), and serum (ng/ml) of normal rats (Group I) and those given, every other day, DMSO (Group II), Barley (Group III), a subacute LD5 of AFB1 alone at 96h (≡ 7.49 mg/kg b. wt., Group IV) and in combination with 2 mg of barley /kg b. wt. (Group V).

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Author contributions
MRE contributes to the animal handling, analytical techniques, data collection, and the statistical analysis. SMA contributes to the experimental design and writing of the manuscript. YHA contributes to the analytical techniques. GMM contributed to the experimental design and data analysis and was major contributor in the writing of manuscript. AAA contributed to the study design, statistical analysis, and writing of the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials
Data obtained by the present study is included in this manuscript. Data are available upon request.

Declarations

Ethics approval and consent to participate
The protocol of the present work was approved by the Ethical Committee of the National Research Centre, Giza, Egypt (Registration No. 15-207). Consent to participate is not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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